ANNUAL REPORTS IN MEDICINAL CHEMISTRY Volume 15

Sponsored by the Division of Medicinal Chemistry of the American Chemical Society

Editor-in-Chief: **HANS-JÜRGEN HESS**

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LESLIE HUMBER · WILLIAM COMER · LESLIE WERBEL
DENIS BAILEY · CHRISTOPHER WALSH · BURT RENFROE

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PREFACE

Following the format of past volumes, Volume 15 is divided into six sections: CNS Agents, Pharmacodynamic Agents, Chemotherapeutic Agents, Metabolic Diseases and Endocrine Function, Topics in Biology, and Topics in Chemistry and Drug Design. The authors, in the ten pages usually allotted to them, strive to give an up-to-date critical account of the important progress that has been made in their fields of expertise and interest. The primary objective remains a quick update of highlights for investigators trying to keep abreast of the important developments peripheral to their area of research. The topics covered in the first four sections are reviewed periodically, while the sections on chemistry and biology contain chapters reviewing special topics, concepts, and trends, often for the first time, that may provide thought for future drug discovery and development.

Extensive bibliographies and a compound and code number index serve to locate quickly a key reference or the structure of a compound.

Because of deadline restrictions to assure early publication, and other commitments facing authors, there may be an occasional deferral of a chapter. Thus, a chapter reviewing the promising developments in the area of recombinant DNA research orginally scheduled for this volume will appear in the next volume.

Dr. Leslie Humber, Dr. Christopher Walsh, and Dr. Burt Renfroe have completed their three-year term of conscientious, invaluable service as section editors. They will be succeeded, respectively, by Dr. John McDermed, Dr. Eugene Cordes, and Dr. Richard Allen.

Again, to the many individuals who contributed their effort and time to this volume, we extend our sincere gratitude and appreciation.

Groton,Connecticut
May 1980

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Section I -CNS Agents

Editor: Leslie G.Humber, Ayerst Laboratories, Montreal, Canada

Chapter 1. Antidepressants

Roger M.Pinder, Organon International, Oss, The Netherlands

Introduction - The safety and cardiotoxicity of tricyclic antidepressants (TCA) remained major concerns throughout 1979 in editorial[1] and review[2-4] comment. The role of TCA in therapeutics was reviewed,[5] with particular reference to plasma levels and clinical response.[6] General reviews appeared on the newer antidepressants,[7] monoamine oxidase inhibitors (MAOI),[8] amoxapine,[9,10] iprindol,[11] maprotiline,[12] mianserin,[11,13] nomifensin,[14] viloxazine[9,15] and electroconvulsive therapy (ECT).[16] Pharmacological[17] and biochemical[18] properties of new antidepressants were reviewed and neuroendocrine aspects of depression reassessed.[19] Sleep, depression and antidepressants were comprehensively reviewed.[20]

In 1979 the first issues appeared of the new quarterly Journal of Affective Disorders, together with monographs on ECT[21] and the psychopharmacology of depression.[22,23] Symposia covered mianserin[24] and TCA plasma level monitoring.[25] Urinary excretion of the principal noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) was used to categorize depressive illness.[26] It was claimed that particular depressive symptoms and response to particular types of antidepressant were associated with high or low MHPG excretion.[27] The role of MHPG as an index of central noradrenergic (NA) activity was reviewed.[28]

TCA and Analogs - Efforts continued in 1979 to develop safer alternatives to traditional TCA. In addition to the often fatal consequences of TCA overdosage,[3] their marked anticholinergic effects at therapeutic dosage[29] are frequently accompanied by cardiotoxicity particularly in the elderly.[30] TCA may also accelerate the natural evolution of bipolar depressive illness by increasing cycle frequency.[31] Epileptic EEG changes occurred already after low doses to cats of maprotiline, clomipramine, imipramine and amitriptyline.[32] TCA did not modify the hypotensive effects of α-methyldopa in healthy humans, in contrast to their reversal of the action of adrenergic neuron blocking agents.[33]

Suggestions that clinical response to tertiary amino TCA depended upon their rate of demethylation[19] were not confirmed for amitriptyline in a double-blind trial.[34] However, the 2-hydroxy metabolites of amitriptyline and imipramine, and their desmethyl analogs, were as potent as the parent TCA in inhibiting uptake of NA and 5-hydroxytryptamine (5-HT) in rat brain,[35] and plasma level measurements in depressed patients supported a role for such metabolites as the major active drug components during antidepressant treatment.[36] Saturation of one or both aromatic rings in imipramine or desipramine did not alter their ability to inhibit 5-HT uptake in rat brain but did abolish the selectivity of desipramine for inhibition of NA uptake.[37]

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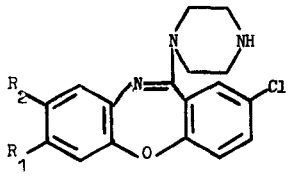
A large multicentre placebo-controlled trial in depressed patients showed a fixed combination of amitriptyline and chlordiazepoxide (Limbitrol^{R}) to be superior to either component given alone.[38] The bridged TCA maprotiline, which is a specific inhibitor of NA uptake,[39] seemed to have quinidine-like antiarrhythmic properties in cardiac patients.[40] In depression, however, its cardiovascular side-effects were similar to imipramine which was also the more effective antidepressant.[41] A rapid onset of action was claimed for doxepin,[42] but its sulfur isostere dothiepin appeared to act more slowly than amitriptyline.[43] Dothiepin caused fewer side-effects than amitriptyline or imipramine, particularly of the anticholinergic type.[43,44] Previous reports of reduced anticholinergic effects with lofepramine, the N-(4-chlorbenzoyl) derivative of desipramine, were not confirmed in a double-blind comparison with amitriptyline.[45]



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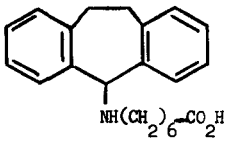

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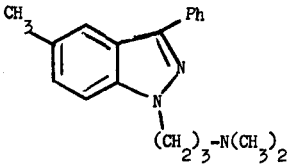

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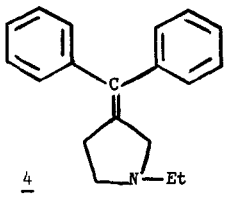
Amoxapine (1a, R_1=R_2=H) is the desmethyl derivative of the antipsychotic drug loxapine, and has both antidepressant and neuroleptic properties in animals.[10,46] Selective inhibition of NA uptake in rat brain was shown by amoxapine and its 7- (1b, R_1=OH, R_2=H) and 8-hydroxy (R_1=H, R_2=OH) metabolites, but only compound 1b bound to neuroleptic receptors *in vitro*.[47] In double-blind trials in depressed patients amoxapine has shown similar efficacy to traditional TCA, but anticholinergic side-effects have been common.[10,48] Amineptine (2) is another TCA with an atypical side-chain. In animals it appeared to be an amphetamine-like central stimulant and a specific inhibitor of dopamine (DA) uptake as well as a releaser of DA.[49] Double-blind trials in depression suggested minimal anticholinergic effects and a more rapid onset of action but similar efficacy to TCA.[50] In these trials, however, dosage ratios have not been optimal, with 150 to 200mg amineptine being compared with only 75mg TCA. FS-32 (3) is an indazol analog, showing imipramine-like effects with central but not peripheral anticholinergic activity.[51] It was less potent than imipramine as an inhibitor of NA uptake, but unlike imipramine it may also release NA.



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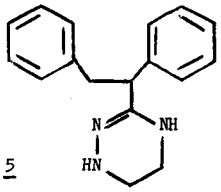

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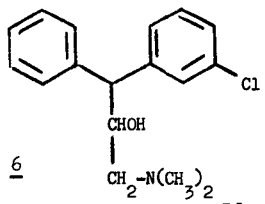

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The psychopharmacological profile of AHR-1118 (4) was reported.[52] This ring-opened TCA showed typical imipramine-like activity in the absence of peripheral anticholinergic activity. In rat brain it was primarily a reuptake blocker , being somewhat more effective against DA than either NA or 5-HT.[53] Other ring-opened TCA analogs included DL-262 (5)[54] and BRL-14342 (6),[55] both of which combine minimal anticholinergic properties with inhibition of NA uptake. Both compounds also inhibit, though to a lesser extent, uptake of DA and 5-HT.

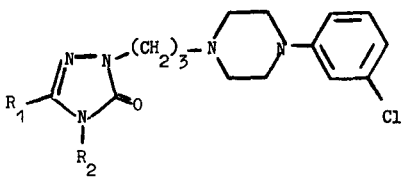
Safer Drugs - Three antidepressants in current clinical practice - mianserin, nomifensin and trazodone - offer substantial advantages over

traditional TCA in terms of fewer side-effects and greater safety. Both mianserin[56] and nomifensin,[57] which were discussed extensively in Volume 14 of this series,[58] appear to be safe in overdosage, but there is as yet little published experience with trazodone. At therapeutic dosage neither mianserin[59,60] nor nomifensin[60,61] affected cardiac conduction or rhythm in depressed patients, and mianserin was consistently less toxic in rabbits than amitriptyline, imipramine or maprotiline.[62] The cardiac safety of trazodone is as yet only fully established in dogs,[63] but in man it had no effect on pressor responses to tyramine or NA.[64]

Further double-blind trials with mianserin established the superiority of the single night-time dose over placebo in depression[65] and of the divided daily dose over diazepam in anxiety[66] and depression.[24] No differences in efficacy but consistently reduced side-effects were evident in numerous comparisons with TCA.[24] In acute or chronic dosage, mianserin reversed the neurochemical and behavioural effects of the α_2-adrenoceptor agonist clonidine as well as itself raising brain levels of NA metabolites; mianserin may combine α_2-antagonism[67,68] with inhibition of NA uptake.[11] At 5-HT receptors, however, the drug blocks post-synaptically rather than pre-synaptically, without effect on uptake.[69]

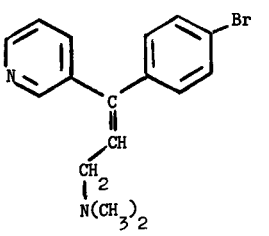
Nomifensin inhibits DA uptake and, to a lesser extent, NA uptake, and may also release DA.[70] These properties are reflected in its inhibition of prolactin release in man.[71] Double-blind trials showed it to lack most amphetamine-like effects[72] and to produce fewer side-effects but equal efficacy to various TCA.[14,73]

Trazodone (7) is a selective inhibitor of 5-HT uptake[74] and may also be a 5-HT antagonist.[75] It did not interact with L-dopa in animals[76] and had no effect on serum prolactin in depressed patients,[77] suggesting a lack of DA effects. Double-blind trials in depression showed trazodone to be superior to placebo and as effective as imipramine, with fewer side-effects.[78]



7, R_1–R_2 = C_4H_4

8, R_1 = R_2 = Et



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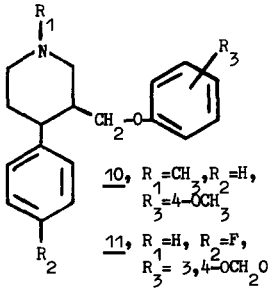


10, R_1=CH_3, R_2=H, R_3=4-OCH_3

11, R_1=H, R_2=F, R_3= 3,4-OCH_2O

5-HT Uptake Inhibitors - Although interest was maintained in 1979 in this type of compound, many of which were reported in Volume 14 of this series,[58] advantages over TCA may be limited since trials so far have failed to establish a correlation between therapeutic outcome and degree of inhibition of 5-HT uptake. Furthermore, the prospect of reduced cardiotoxicity was diminished by reports of serious ECG disturbances in depressed patients receiving fluvoxamine.[79]

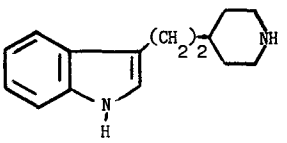
Etoperidone (8) resembles trazodone both structurally and in being more selective but less potent as a 5-HT uptake inhibitor than clomipramine.[80] Like trazodone it lacked cardiotoxicity and anticholinergic effects in animals, and had antidepressant effects in man. 3-Chlorophenyl-piperazine, a metabolite of trazodone[81] and presumably also of etoperidone, is a potent central 5-HT agonist causing anorexia in the rat,[82] properties shared by the isosteric 6-chloro-2-(1-piperazinyl)pyrazine (MK-212).[83]

Neither of these 5-HT agonists has been evaluated in depression.

Both zimelidine (9)[84] and femoxetine (10)[85] are effective antidepressants, and compound 9 causes fewer side-effects than amitriptyline and may lack cardiotoxicity.[60] No correlation was found between the degree of depletion of blood 5-HT levels and therapeutic effect for either drug. Pharmacological studies in dogs and humans showed zimelidine to be equipotent with clomipramine as a 5-HT uptake inhibitor, but to be without anticholinergic activity.[86] It did not potentiate the pressor effects of NA or tyramine. Peak plasma levels were reached in humans about 2 hours after ingestion, but oral bioavailability was only 20%.[86] Zimelidine had no effect on serum prolactin levels in man after single or multiple doses.[87]

Femoxetine (10) and paroxetine (11) are structurally related, but only compound 10 has so far demonstrated antidepressant activity in man.[85] Femoxetine is completely absorbed after oral dosage, but bioavailability is less than 10% because of extensive first-pass metabolism.[88] Like femoxetine, paroxetine virtually depleted whole blood levels of 5-HT in man, but levels returned to normal within 3 to 4 weeks of withdrawal of either drug.[89] The pharmacokinetics of 10 and 11 are almost identical, with slow elimination, almost complete metabolism and urinary excretion.[88,89] Neither drug appears to reverse the antihypertensive action of the adrenergic neuron blocker guanethidine in rats.[90]

Other selective inhibitors of 5-HT uptake reported in 1979 included indalpine (LM 5008, 12), which was 7 times more potent than clomipramine in rat brain[91] and a clinically effective antidepressant without anticholinergic effects.[58] CGP 6085A (13) combines reversible inhibition of MAO-A with an inhibitory potency against 5-HT uptake greater than femoxetine or zimelidine.[92]



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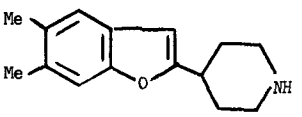

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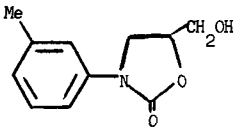

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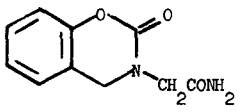
MAOI - These drugs have proven efficacy in neurotic and atypical depressions but their effects in endogenous depression are not conclusive.[8] Suggestions that other types of antidepressant might exert some of their effects through MAO inhibition were not confirmed by reports of unchanged MAO activity during treatment of depressed patients with amitriptyline or zimelidine.[93] Human platelet MAO, which consists exclusively of the B-form of the enzyme, was unaltered during 4 weeks of treatment with the selective MAO-A inhibitor clorgyline but was almost completely inhibited during similar treatment with the MAO-B inhibitor pargyline.[94] Nevertheless, clorgyline produced markedly better antidepressant effects and caused fewer side-effects, suggesting a lack of utility in depression for selective MAO-B inhibitors. However, one such compound, L-deprenyl, was effective in an open trial in depressed patients.[95]

Toloxatone (14) is a clinically effective antidepressant which selectively inhibits MAO-A.[58,96] It is extensively metabolized in man but with retention of the oxazolidinone moiety.[97] Caroxazone (15), which inhibits both forms of MAO, showed typical antidepressant effects in EEG studies in healthy humans, with significant reductions in REM sleep.[98] Ro 11-1163 (16), a new non-hydrazine inhibitor of MAO-A, showed rapid

antidepressant effects in open trials.[99]



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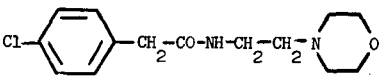

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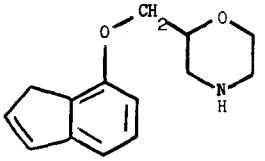

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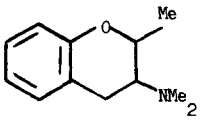
Miscellaneous Drugs - Viloxazine, an established antidepressant[15] and a selective inhibitor of NA uptake,[18,58] is not as safe as its low cardiotoxicity might suggest. A review of viloxazine overdoses showed that 4/64 patients died after ingesting doses from 2.5 to 5g, but the maximum non-fatal dose was about 8g.[100] The new indene derivative YM-08054-7 (17) is a close structural relative of viloxazine but more resembled amitriptyline in its neurochemistry by blocking both NA and 5-HT uptake.[101] Its antidepressant properties in animals excluded anticholinergic effects. Two new chroman derivatives reached the clinic; trebenzomine (18) and tisocromid (19). Trebenzomine combines antipsychotic and antidepressant properties in animals and in man, and was as effective as doxepin in double-blind trials in depression.[102] Tisocromid is the most interesting of a series of sultones from East Germany.[103] It had cholinergic properties and low cardiotoxicity in animals, with weak effects on the uptake and release of NA, DA and 5-HT. Clinical trials showed efficacy comparable to imipramine.



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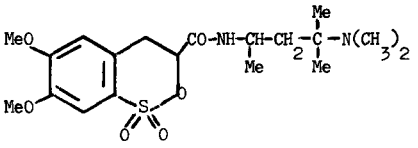

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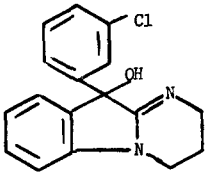

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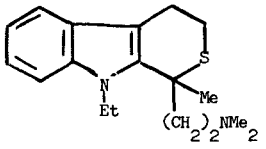

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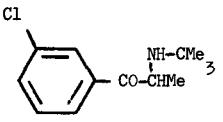

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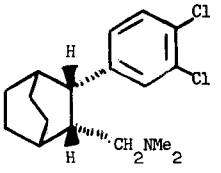

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Ciclazindol (20) shares with its structural analog mazindol anorectic properties and selective inhibition of NA uptake.[58] Its antidepressant properties and lack of anticholinergic effects were confirmed in a double-blind trial versus amitriptyline.[104] Metabolism in man was similar to mazindol, with all major products arising from transformations of the tetrahydropyrimidine ring.[105] Cardiovascular and autonomic effects in animals were minimal,[106] but in humans 20 antagonized pupillary and pressor responses to methoxamine and tyramine suggesting a blocking action on α_1-adrenoceptors as well as NA uptake inhibition.[107,108] Both ciclazindol and tandamine (21) reduced appetite in a placebo-controlled trial in healthy humans.[108] Tandamine is a clinically effective antidepressant and selectively inhibits NA uptake.[58] Its neurochemical and

pharmacological effects, however, are similar to desipramine though with lesser anticholinergic properties.[109] In healthy humans[108] and depressed patients[79] tandamine showed marked anticholinergic side-effects and cardiotoxicity.

The central stimulant effects of bupropion (22) in the rat, and its marked suppression of prolactin secretion in rat and man, appears to be mediated through selective inhibition of DA uptake.[110] In healthy humans, bupropion was devoid of amphetamine-like central stimulant properties and amitriptyline-like sedative effects, and it showed no cardiovascular or anticholinergic side-effects.[111] LR 5182 (23) is one of a series of bicyclo-octanes which showed anti-reserpine activity associated with potent and selective inhibition of DA uptake.[112]

Lithium - Reviews appeared in 1979 on therapeutic and psychopharmacological aspects of the lithium ion,[113] and two more volumes of the lithium bibliography were published.[114] The unique value of lithium as an acute anti-manic agent and as prophylaxis in the longer term treatment of unipolar and bipolar depressive illness is well established, but lithium may also have acute antidepressant effects. In two 3-week double-blind trials lithium was superior to imipramine, and a combination of lithium and tryptophan was superior to tryptophan alone; tryptophan alone was without antidepressant effect.[115]

The mechanism of action of lithium remains uncertain.[113] Like TCA, lithium given chronically to rats produced β-adrenoceptor subsensitivity as measured by decreased receptor density.[116] Concomitant treatment of rats with lithium prevented reserpine-induced supersensitivity of cortical β-adrenoceptors,[117] but failed to affect supersensitivity to 5-HT induced by imipramine or clomipramine.[118] Platelets from depressed patients show a decreased Vmax for 5-HT uptake, an apparent defect that is reversed by lithium (and mianserin) treatment.[120]

Peptides and Aminoacids - Centrally active peptides continued to attract the interest of reviewers in 1979, but controlled trials are rare.[121] In an open trial, 3/6 depressed women became hypomanic after iv injection of 10mg β-endorphin.[121] No antidepressant effects were seen in a double-blind crossover trial of β-endorphin (10mg/70kg) versus placebo.[122] Plasma vasopressin may be low in depressed patients, and improvement occurred in 2/4 patients with bipolar depressive illness who received a synthetic analog 1-desamino-8-D-arginine vasopressin (DDAVP) under double-blind conditions.[123] There were no further reports since last year[58] of the antidepressant effects of melanocyte-stimulating-hormone-releasing factor I (MIF-I) or thyrotrophin-releasing-hormone (TRH).

The role of tryptophan and its 5-hydroxy derivative as anti-depressants is far from established, and evidence to date suggests that L-tryptophan is only effective when combined with a MAOI.[124] In double-blind trials, L-tryptophan was either ineffective[115] or similar in efficacy to imipramine.[125] Unlike most antidepressant treatments including TCA, MAOI, ECT and lithium, all of which tend to suppress REM sleep in humans,[20] L-tryptophan tended to increase REM sleep.[126] The antidepressant effects of DL-phenylalanine, previously observed in open trials,[58] were similar to imipramine in a double-blind trial in depressed patients.[127]

Screening Methods - The failure of tests based upon reversal of reserpine-like effects to detect many new clinically effective antidepressants has spurred research into alternative screening methods. The problems of anti-depressant screening were reviewed,[128] and three standard methods proposed

for general use - antagonism of hypothermia induced by oxotremorine or by high doses of apomorphine, and behavioural despair in rats forced to swim in a restricted space.

The oxotremorine test was developed specifically to detect the activity of β-adrenergic agonists like salbutamol, which have antidepressant properties in man, but it also detects most TCA.[129] Apomorphine-induced hypothermia appears to be more readily reproducible in rats than in mice,[130] but several atypical antidepressants including mianserin and trazodone were not detected.[131] The behavioral despair (acquired immobility) model detects most new antidepressants,[132] but is devalued by its relative insensitivity to drugs affecting central 5-HT mechanisms and by its sensitivity to strain differences and to drugs lacking specific antidepressant properties like caffeine, anticholinergics, pentobarbital and triiodothyronine.[133] The related model of learned helplessness, where rats are exposed to unavoidable trauma, was proposed as a model for depression.[134]

Hyperactivity induced in rats by prolonged social isolation was selectively blocked by clinically effective antidepressants and not by other types of psychotropic drugs.[135] Isolation-induced aggression in mice, a behaviour reversed by most TCA, was reviewed.[136] Another isolation model -which involves separation of infant macaque monkeys from their mothers - produces marked depressive symptomatology similar to the human condition.[137] These methods do not seem suitable for screening antidepressants.

The possibility that mianserin and other antidepressants may be antagonists at α_2-adrenoceptors prompted investigation of models based on the α_2-agonist clonidine. A test involving reversal of clonidine-induced hypothermia in mice was discussed in Volume 14 of this series,[58] and a new test utilises clonidine-induced suppression of exploratory behaviour. The model detects drugs which are selective α_2-antagonists rather than antidepressants *per se*, since mianserin, for example, because of its additional NA uptake inhibitory and post-synaptic activities, did not influence clonidine-induced hypoactivity.[138]

Most antidepressant treatments reduce REM sleep in humans,[20] and a new model utilises EEG in cats for the detection of such activity.[139] At the lowest doses that affected the sleep-wake cycle of cats, most types of antidepressant including TCA, MAOI, mianserin, iprindol and viloxazine selectively depressed REM sleep. Some other types of psychotropic drug also reduced REM sleep but simultaneously affected non-REM stages.

Mechanisms of Action - Biogenic amine hypotheses of depression were reassessed and the evidence implicating a functional deficit of 5-HT in depression reviewed.[140,141] Preliminary evidence suggested that production of tyramine and octopamine is deficient in patients with primary depression.[142] High affinity binding sites for TCA and perhaps other antidepressants were identified in rat cortex and human platelets.[143]

Further evidence accumulated to support the compelling hypothesis that the action of antidepressants involves delayed changes in receptor sensitivity rather than acute events like uptake, perhaps as a result of persistent exposure of the receptors to elevated levels of the transmitter amines.[144] Following chronic but not acute antidepressant treatment of rats there are reports of reduced density of cortical β-adrenoceptors,[145] decreased response of cortical adenylate cyclase to NA stimulation[146,147]

and subsensitivity in atrial α_2-adrenoceptors.[148] These effects have occurred in whole or in part with TCA, MAOI, trazodone, zimelidine, mianserin and repeated ECT,[144] and can be reproduced in vitro with cortical slices.[149] Lithium appears to both reduce β-adrenoceptor density and to increase α-density.[116] The net effect of chronic TCA treatment in rats was antagonistic to NA function as measured by neuronal firing.[150] However, this hypothesis is not supported by studies in man, where the formation of leucocyte adenylate cyclase in response to NA or isoprenaline administration was lower in depressed patients than in normal controls, suggesting NA subsensitivity in depression.[151]

Supersensitivity to 5-HT may also be involved in depression.[152] Evidence was presented that chronic TCA treatment decreases the density of 5-HT receptors in rat brain.[153] However, electrophysiological studies indicated that such treatment produced 5-HT receptor supersensitivity rather than subsensitivity.[118] Changes in DA receptor sensitivity may also play a role, since behavioural responses to apomorphine were modified by chronic treatment with TCA and with mianserin.[154]

References

1. Lancet 2, 511(1979); Drug Ther.Bull. 17, 13(1979).
2. M.W.P.Carney, Brit.J.Psychiat. 134, 637(1979); H.Kopera, Med.Klin. 74, 1339(1979).
3. P.Crome and B.Newman, Postgrad.Med.J. 55, 528(1979); J.Roy.Soc.Med. 72, 649(1979).
4. M.G.Fiori, Curr.Dev.Psychopharmacol. 4, 71(1978); D.G.Spiker, Commun.Psychopharmacol. 2, 419(1978); B.Stimmel, "Cardiovascular Effects of Mood-Altering Drugs", Raven Press, 1979.
5. A.J.Gelenberg, Can.Med.Ass.J. 120, 1377(1979); L.E.Hollister, New Engl.J.Med. 299, 1106, 1168(1978); A.H.Rosenbaum, T.Maruta and E.Richelson, Mayo Clin.Proc. 54, 335(1979).
6. S.C.Risch, L.Y.Huey and D.S.Janowsky, J.Clin.Psychiat. 40, 4, 58(1979).
7. R.A.Lahti, Naturwissenschaften 66, 403(1979); B.E.Leonard, J.Pharmacother. 2, 44 (1979); W.Poldinger, Pharma-Kritik 1, 5(1979); A.Villeneuve, In "Neuropsychopharmacology" (Ed. C.Dumont), Vol.5 of Proc.7th Int.Congr.Pharmacol., Pergamon Press, Oxford, 1979, p 187.
8. F.Quitkin, A.Rifkin and D.F.Klein, Arch.Gen.Psychiat. 36, 749(1979).
9. T.A.Ban, Psychopharmacol.Bull. 15, 22(1979).
10. E.N.Greenblatt, R.A.Hardy and R.G.Kelly, In "Pharmacological and Biochemical Properties of Drug Substances" (Ed. M.E.Goldberg), Vol.2, American Pharmaceutical Association, Washington, D.C., 1979, p 1.
11. A.P.Zis and F.K.Goodwin, Arch.Gen.Psychiat. 36, 1097(1979).
12. J.F.Chevalier, Ann.Med.-Psychol. 137, 360(1979).
13. M.Fink, Psychopharmacol.Bull. 15, 27(1979).
14. R.N.Brogden, R.C.Heel, T.M.Speight and G.S.Avery, Drugs 18, 1(1979).
15. J.F.Chevalier, Ann.Med.-Psychol. 137, 363(1979).
16. T.J.Crow, Psychol.Med. 9, 401(1979).
17. J.Maj, In "Neuropsychopharmacology", see reference 7, p 161.
18. T.J.Crow, In "Neuropsychopharmacology", see reference 7, p 177.
19. N.Matussek, In "Neuropsychopharmacology", see reference 7, p 147; Curr.Med.Res.Opin. 6(Suppl.7), 5(1980).
20. C.N.Chen, Brit.J.Psychiat. 135, 385(1979).
21. M.Fink, "Convulsive Therapy:Theory and Practice", Raven Press, New York, 1979.
22. E.S.Paykel and A.Coppen (Eds.), "Psychopharmacology of Affective Disorders", Oxford University Press, Oxford, 1979.
23. L.L.Iversen, S.D.Iversen and S.H.Snyder (Eds.), "Affective Disorders", Vol.14 of Handbook of Psychopharmacology, Plenum, New York, 1978.
24. Acta Psychiat.Belg. 78, 709-861(1978); Curr.Med.Res.Opin. 6, Suppl.7(1980).
25. Commun.Psychopharmacol. 2, 371-456(1978).
26. J.Schildkraut, P.Orsulak, A.Schatzberg, T.Gudeman, J.Cole, W.Rohde and R.LaBrie, Arch. Gen.Psychiat. 35, 1427, 1436(1978).
27. D.M.Cobbin, B.Requin-Blow, L.R.Williams and W.O.Williams, Arch.Gen.Psychiat. 36, 1111 (1979); I.Modal, A.Apter, M.Golomb and H.Wijsenbeek, Neuropsychobiol. 5, 181(1979); E.Sachetti, E.Allaria, F.Negri, P.A.Biondi, E.Smeraldi and C.Cazzullo, Biol.Psychiat. 14, 473(1979).
28. D.DeMet and A.E.Halaris, Biochem.Pharmacol. 28, 3043(1979).
29. G.Peterson, R.M.Hostetler, R.Kuzma, A.Adolphe and B.Blackwell, Clin.Pharmacol.Ther. 25, 241(1979).
30. M.Rodstein and L.S.Oei, J.Amer.Geriat.Soc., 27, 231(1979).
31. T.Wehr and F.K.Goodwin, Arch.Gen.Psychiat. 36, 555(1979).
32. W.P.Koella, A.Glatt, K.Klebers and T.Durst, Biol. Psychiat. 14, 485(1979).

33. J.L.Reid, A.J.Porsius, C.Zambolis, G.Polak, C.Hamilton and C.R.Dean, Eur.J.Clin.Pharmacol. 16, 75(1979).
34. A.Coppen and V.A.Rama Rao, Lancet 1, 49(1979).
35. J.I.Javaid, J.M.Perel and J.M.Davis, Life Sci. 24, 21(1979).
36. L.Bertilsson, B.Mellstrom and F.Sjoqvist, Life Sci. 25, 1285(1979); W.Z.Potter, H.Calil, A.Zavadil, W.Jusko, T.Sutfin, and J.Rapoport, Clin.Pharmacol.Ther. 25, 242(1979).
37. G.L.Grunewald, T.J.Reitz, J.A.Ruth, S.Vollmer, L.E.Eiden and C.O.Rutledge, Biochem. Pharmacol. 28, 417(1979).
38. J.P.Feighner, Psychopharmacology 61, 217(1979).
39. P.A.Baumann and L.Maitre, J.Int.Med.Res. 7, 391(1979).
40. E.A.Raeder, M.Zinski and R.D.Burckhardt, Brit.Med.J. 2, 104(1979).
41. D.H.Mielke, R.P.Kopeke and J.H.Phillips, Curr.Ther.Res. 25, 738(1979).
42. S.F.Barranco, M.L.Thrash, E.Hackett, J.Frey, J.Ward and E.Norris, J.Clin.Psychiat. 40, 265(1979).
43. J.L.Claghorn, R.J.Mathew and E.E.Johnstone, Psychopharmacol.Bull. 15, 94(1979).
44. U.K.Sheth, T.Paul, N.K.Desai and P.K.Pispati, Brit.J.Clin.Pharmacol. 8, 475(1979).
45. H.A.McClelland, T.A.Kerr, D.A.Stephens and R.W.Howell, Acta Psychiat.Scand. 60, 190(1979).
46. R.Chermat, P.Simon and J.R.Boissier, Arzneim.-Forsch. 29, 814(1979).
47. J.Coupet, C.E.Rauch, V.A.Szues-Myers and L.M.Yunger, Biochem.Pharmacol. 28, 2514(1979).
48. V.N.Bagadia, L.P.Shati, P.V.Pradhari and M.T.Gada, Curr.Ther.Res. 26, 417(1979); K.Fruensgaard, C.E.Hansen, S.Korsgaard, K.Nymgaard and U.H.Vaag, Acta Psychiat.Scand. 59, 502(1979); B.B.Sethi, I.Sharma, H.Singh and U.K.Mehta, Curr.Ther.Res. 25, 726(1979).
49. J.Offermeier, B.Potgieter, H.G.Du Preez and P.J.Meiring, S.Afr.Med.J. 51, 62(1977).
50. Three papers on amineptine, Curr.Med.Res.Opin. 6, 93-110(1979).
51. Y.Ikeda, N.Takano, H.Matsushita, Y.Shiraki, T.Koide, R.Nagashima, Y.Fujimura, M.Shindo, S.Suzuki and T.Iwasaki, Arzneim.-Forsch. 29, 511(1979).
52. M.Bourin, L.Doare, G.Narcise, R.Chermat and P.Simon, J.Pharmacol.(Paris) 10, 205(1979).
53. A.Halaris and E.M.DeMet, Psychopharmacol.Bull. 15, 95(1979).
54. K.Hillier, Drugs Fut. 4, 196(1979).
55. J.A.Clark, M.S.G.Clark, D.V.Gardner, L.M.Gaster, M.S.Hadley, D.Miller and A.Shah, J.Med. Chem. 22, 1375(1979).
56. W.L.Shaw, Curr.Med.Res.Opin. 6(Suppl.7), 44(1980).
57. S.Dawling, R.Braithwaite and P.Crome, Lancet 1, 56(1979).
58. R.M.Pinder, In "Annual Reports in Medicinal Chemistry", Vol. 14, H.-J.Hess ed., Academic Press, New York, 1979, p 1-11.
59. G.D.Burrows, B.Davies, A.Hamer and J.Vohra, Med.J.Austral. 2, 97(1979).
60. C.D.Burgess, S.A.Montgomery, J.Wadsworth and P.Turner, Postgrad.Med.J. 55, 704(1979).
61. P.Dumovic, G.D.Burrows, J.Vohra and S.Freedman, Clin.Exp.Physiol.Pharmacol. 6,229(1979).
62. I.E.Hughes and S.Radwan, Brit.J.Pharmacol. 65, 331(1979).
63. A.W.Gomoll and J.E.Byrne, Eur.J.Pharmacol. 57, 335(1979).
64. P.Larochelle, P.Hamet and M.Enjalbert, Clin.Pharmacol.Ther. 26, 24(1979).
65. R.V.Magnus, Brit.J.Clin.Pract. 33, 251(1979).
66. L.Conti and R.M.Pinder, J.Int.Med.Res. 7,285(1979).
67. J.M.Fludder and B.E.Leonard, Psychopharmacol. 64, 329(1979); Biochem.Pharmacol. 28, 2333 (1979).
68. B.Harper and I.E.Hughes, Brit.J.Pharmacol. 67, 511(1979).
69. F.Cerrito and M.Raiteri, Eur.J.Pharmacol. 57, 427(1979).
70. O.J.Broch, Eur.J.Pharmacol. 58, 419(1979); P.Hunt, J.P.Reynaud, M.Lveen and U.Schacht, Biochem.Pharmacol. 28, 2011(1979).
71. G.Lotti, A.Masala, L.Devilla, S.Alagna, P.Rovasio and G.Delitala, Acta Endocrinol. 91, (Suppl.225), 162(1979).
72. K.Tauber, R.Zapf, W.Rupp and M.Badian, Int.J.Clin.Pharmacol. 17, 32(1979).
73. A.McCawley, Amer.J.Psychiat. 136, 841(1979); B.Woggon and J.Angst, Psychopharmacol.Bull. 15, 29(1979).
74. L.A.Riblet, C.F.Gatewood and R.F.Mayol, Psychopharmacol. 63, 99(1979).
75. L.Baran, J.Maj, Z.Rogoz and G.Skuza, Pol.J.Pharmacol.Pharm. 31, 25(1979).
76. R.Lisciani, A.Baldini and G.B.Ciottoli, Pharmacol.Res.Commun. 11, 265(1979).
77. N.P.V.Nair, S.Hontela and G.Schwarz, IRCS Clin.Pharmacol.Ther. 7, 166(1979).
78. L.F.Fabre, D.M.McLendon and A.Gainey, Curr.Ther.Res. 25, 827(1979); G.A.Trapp, C.R.Handorf and V.Larach, Psychopharmacol.Bull. 15, 25(1979).
79. H.Schanda and B.Saletu, Pharmakopsychiat. 12, 338(1979).
80. M.O.Carruba, M.Parenti, S.Ricciardi, G.B.Piccoti and P.Mantegazza, Pharmacol.Res.Commun. 11, 169(1979); M.Ramacci, O.Ghirardi, F.Maccari, L.Pacifici and P.Sale, Arzneim.-Forsch. 29, 294(1979).
81. M.Melzacka, J.Boksa and J.Maj, J.Pharm.Pharmacol. 31, 855(1979).
82. R.Samanin, T.Mennini, A.Ferraris, C.Bendotti, F.Borsini and S.Garattini, Arch.Pharmacol. 308, 159(1979).
83. B.Clineschmidt, Gen.Pharmacol. 10, 287(1979).
84. A.Coppen, V.A.Rama Rao, C.Swade and K.Wood, Psychopharmacol. 63, 125, 199(1979).
85. C.Borup, I.M.Petersen, P.L.F.Honore and L.Wetterberg, Psychopharmacol. 63, 241(1979).
86. G.Johnson, P.Lundborg and I.Welin-Fogelberg, Acta Pharmacol.Toxicol. 45, 192(1979); K.O.Borg, G.Johnson, L.Jordo, P.L.Lundborg, O.Ronn and I.Welin-Fogelberg, Acta Pharmacol. Toxicol. 45, 198(1979).

87. E.Syvalahti, A.Nagy and H.M.Van Praag, Psychopharmacol. 64, 251(1979).
88. J.Lund, J.A.Christensen, E.Bechgaard, L.Molander and H.Larsson, Acta Pharmacol.Toxicol. 44, 177(1979).
89. J.Lund, B.Lomholt, J.Fabricius, J.A.Christensen and E.Bechgaard, Acta Pharmacol.Toxicol. 44, 289(1979).
90. E.N.Petersen, J.Pharm.Pharmacol. 31, 638(1979).
91. G.Le Fur, M.Kabouche and A.Uzan, Life Sci. 19, 1959(1979).
92. P.C.Waldmeier, P.A.Baumann and L.Maitre, J.Pharmacol.Exp.Ther. 211, 42(1979).
93. M.A.Revely, V.Glover, M.Sandler and A.Coppen, Brit.J.Clin.Pharmacol. 8, 375(1979).
94. S.Lipper, D.L.Murphy, S.Slater and M.S.Buschbaum, Psychopharmacol. 62, 123, 129(1979).
95. J.Mann and S.Gershon, IRCS Clin.Pharmacol.Ther. 7, 450(1979).
96. P.E.Keane, J.P.Kan, N.Sontag and M.Strolin-Benedetti, J.Pharm.Pharmacol. 31, 752(1979).
97. A.Malnoe and M.Strolin-Benedetti, Xenobiotica 9, 281(1979).
98. C.Maggini, Curr.Ther.Res. 25, 671(1979).
99. D.P.Bobon,G.Lepage-Goffioul, P.Rossignol, P.Gilot, A.Adens, G.Plomteux and M.Breulet, Abstr. 15th Int.Congr.Therap., Brussels, Belgium, Sept. 5-9, 1979, p 129.
100. R.P.C.Holland, See reference 99, p 135.
101. M.Harada and H.Maeno, Biochem.Pharmacol. 28, 2645(1979); S.Tachikawa, M.Harada and H.Maeno, Arch.Int.Pharmacodyn. 238, 81(1979).
102. M.Stanley, J.Rotrosen, N.Sculerati, S.Gershon, C.Kuhn and B.M.Cohen, Psychopharmacol. 66, 23(1979); L.A.Borgen, Psychopharmacol.Bull. 15, 92(1979).
103. Nine papers on sultones, Pharmazie 34, 295-305(1979).
104. S.Levine, J.Int.Med.Res. 7, 1(1979).
105. A.J.Swaisland, R.A.Franklin and A.C.White, Brit.J.Clin.Pharmacol. 7, 120(1979).
106. J.F.Waterfall, M.A.Smith, W.H.Gaston, J.Maher and G.Warburton, Arch.Int.Pharmacodyn. 240, 116(1979).
107. F.A.Kerr and E.Szabadi, Brit.J.Clin.Pharmacol. 8, 396P(1979).
108. V.M.S.Oh, R.S.B.Ehsanullah, M.Leighton and M.J.Kirby, Psychopharmacol. 60, 177(1979).
109. T.Pugsley and W.Lippmann, Arch.Pharmacol. 308, 239(1979).
110. H.Canning, D.Goff, M.J.Leach, A.A.Miller, J.E.Tateson and P.L.Wheatley, Brit.J. Pharmacol. 66, 104P(1979); W.C.Stern, J.Rogers, V.Fang and H.Meltzer, Life Sci. 25, 1717(1979).
111. A.W.Peck, C.E.Bye, M.Clubley, T.Hensen and C.Riddington, Brit.J.Clin.Pharmacol. 7, 469(1979).
112. R.W.Fuller, K.W.Perry and H.D.Snoddy, Neuropharmacol. 18, 497(1979).
113. Nineteen papers on lithium, Arch.Gen.Psychiat. 20, 833-914(1979); L.E.Ereshefsky, A.M.Gilderman and C.M.Jewett, Drug Intel.Clin.Pharm. 13, 403, 492(1979); F.N.Johnson, Neurosci.Biobehav. Rev. 3, 15(1979).
114. M.Schou, Neuropsychobiol. 5, 241(1979); 6, 1(1980).
115. E.P.Worrall, J.P.Moody, M.Peet, P.Dick, A.Smith, C.Chambers, M.Adams and G.J.Naylor, Brit.J.Psychiat. 135, 255(1979).
116. J.E.Rosenblatt, C.B.Pert, J.F.Tallman, A.Pert and W.E.Bunney, Brain.Res. 160, 186 (1979).
117. S.Treiser and K.J.Kellar, Eur.J.Pharmacol. 58, 85(1979).
118. D.W.Gallager and W.E.Bunney, Arch.Pharmacol. 307, 129(1979).
119. A.Coppen, C.Swade and K.Wood, Clin.Chim.Acta 87, 165(1978); J.Tuomisto, E.Tukianen and U.G.Ahlfors, Psychopharmacol. 65, 141(1979).
120. G.Grignani, K.Martin and G.V.R.Born, Brit.J.Clin.Pharmacol. 7, 431P(1979).
121. P.A.Berger, S.J.Watson, H.Akil and J.D.Barchas, Psychopharmacol.Bull. 15, 33(1979); A.J.Kastin, R.D.Olson, A.V.Schally and D.H.Coy, Life Sci. 25, 401(1979).
122. D.Catlin, In "Regulation and Function of Neural Peptides" (Proceedings of a Symposium at Gardone Riviera, Italy, Aug.29 - Sept.1, 1979), to be published by Raven Press.
123. P.W.Gold, H.Weingartner, J.C.Ballenger, F.K.Goodwin and R.M.Post, Lancet 2, 992(1979).
124. A.J.Cooper, Psychopharmacol. 61, 97(1979); G.E.Parkes, Drug.Intel.Clin.Pharm. 13, 391(1979).
125. G.Chouinard, S.N.Young, L.Annable and T.L.Sourkes, Acta Psychiat.Scand. 59, 395(1979).
126. A.N.Nicholson and B.M.Stone, EEG Clin.Neurophysiol. 47, 539(1979).
127. H.Beckmann, D.Ather, M.Olteanu and R.Zimmer, Arch.Psychiat.Nervenkr. 227, 49(1979).
128. A.J.Puech, H.Frances, M.Souto, R.Chermat and P.Simon, In "Neuropsychopharmacology", See reference 7, p 171.
129. H.Frances, A.J.Puech, R.Chermat and P.Simon, Pharmacol.Res.Commun. 11, 273(1979).
130. I.P.Lapin and S.Mirzaev, J.Pharmacol.Methods 2, 127(1979).
131. E.Przegalinski, K.Bigajska and J.Siwanowicz, J.Pharm.Pharmacol. 31, 560(1979).
132. R.D.Porsolt, A.Bertin, N.Blavet, M.Deniel and M.Jalfre, Eur.J.Pharmacol. 57, 201(1979).
133. R.G.Browne, Eur.J.Pharmacol. 58, 331(1979); M.D.Schechter and W.T.Chance, Eur.J. Pharmacol. 60, 139(1979).
134. A.D.Sherman, G.L.Allers, F.Petty and F.A.Hern, Neuropharmacol. 18, 891(1979).
135. J.Garzon, J.A.Fuentes and J.Del Rio, Eur.J.Pharmacol. 59, 293(1979).
136. J.B.Malick, Curr.Devel.Psychopharmacol. 5, 1(1979).
137. P.D.Hrdina, P.Van Kolniz and R.Stretch, Psychopharmacol. 64, 89(1979).
138. A.Delini-Stula, P.Baumann and O.Buch, Arch.Pharmacol. 307, 115(1979).
139. R.Polc, J.Schneeberger and W.Haefly, Neuropharmacol. 18, 259(1979).
140. D.L.Garver and J.M.Davis, Life Sci. 24, 383(1979).

141. D.D.Burns and J.Mendels, Curr.Devel.Psychopharmacol. 5, 293(1979).
142. M.Sandler, C.R.J.Ruthven, B.L.Goodwin, G.P.Reynolds, V.A.Rama Rao and A.Coppen, Nature 278, 357(1979).
143. B.Raisman, M.Briely and S.Z.Langer, Nature 281, 148(1979); Eur.J.Pharmacol. 58, 347 (1979).
144. F.Sulser, Trends Pharmacol.Sci. 1, 92(1979).
145. D.A.Bergstrom and K.J.Kellar, Nature 278, 464(1979); J.Pharmacol.Exp.Ther. 209, 256 (1979); G.N.Pandey, W.J.Heine, B.D.Brown and J.M.Davis, Nature 280, 234(1979).
146. M.Dibner and P.B.Molinoff, J.Pharmacol.Exp.Ther. 210, 433(1979).
147. J.Korf, J.B.Sebens and F.Postema, Eur.J.Pharmacol. 59, 23(1979).
148. F.T.Crews and C.B.Smith, Science 202, 322(1978).
149. D.C.U'Prichard and S.J.Enna, Eur.J.Pharmacol. 59, 297(1979).
150. Y.H.Huang, Life Sci. 25, 709(1979).
151. G.N.Pandey, M.W.Dysken, D.L.Garver and J.M.Davis, Amer.J.Psychiat. 136, 675(1979).
152. M.H.Aprison, R.Takahashi and K.Tachiki, In "Neuropharmacology of Behaviour", B.Haber and M.H.Aprison eds., Plenum, New York, 1978, p 23.
153. T.Segawa, T.Mizuta and Y.Nomura, Eur.J.Pharmacol. 58, 75 (1979).
154. A.Delini-Stula and A.Vassout, Eur.J.Pharmacol. 58, 443(1979); G.Serra, A.Argiolas, V.Klimek, F.Fadda and G.L.Gessa, Life Sci. 25, 415(1979).

Chapter 2. Antipsychotic Agents and Dopamine Agonists

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The dopamine (DA) hypothesis of schizophrenia remains the central postulate that governs many of the research strategies concerned with the biochemical, pharmacological and therapeutic understanding and treatment of this disorder. There are, however, numerous other hypotheses of schizophrenia that propose a significant role for such diverse agents as prostaglandins,[1] diet,[2] and endorphins,[3] and, indeed, several recent monographs deal with these topics.[2,4-6] The dopamine theory of schizophrenia, however, is a dynamic theory in its ability to incorporate other hypotheses, and, in the case of the role of endorphins in schizophrenia, a recent proposal has integrated the two into a coherent argument (see below).[7] Nevertheless, the one event clearly associated with virtually all clinically effective antipsychotic agents is modulation of central dopaminergic activity, and this attribute, therefore, forms the basis of this review of antipsychotic agents. Dopamine agonists, on the other hand, are used therapeutically in DA deficient states such as Parkinson's disease. Recent work, however, indicates that DA autoreceptor agonists may have antipsychotic activity.

Dopamine Receptors - Although the therapeutic significance of multiple DA receptors is not yet clear, the subdivision of DA receptors into D-1 and D-2 has delineated certain of the biochemical differences encountered with these receptors. In this classification, D-1 designates DA receptors linked to the activation of adenylate cyclase, while D-2 denotes those receptors not linked to this enzyme.[8] Recent data indicate that both D-1[9] and D-2 receptors exist in the anterior pituitary. The two receptors have been differentiated into the categories shown in Table 1.

Table 1. Properties of Anterior Pituitary DA Receptors

Receptor type	GTP Modulates Agonist Binding	Agonist Binding Affinity (IC_{50})	Control of Prolactin Release
D-1	Yes	μM range	No
D-2	No	nM range	Yes

Thus, the D-1 anterior pituitary receptor is not linked to prolactin release, but guanosine triphosphate (GTP) can modulate DA agonist binding to it.[10] On the other hand, the D-2 receptor located on the mammotrophs controls prolactin release,[11] but GTP does not modulate the affinity of DA agonists for this site.[10] Guanine nucleotides also modulate DA agonist binding to D-1 receptors on striatal cell bodies.[12] This latter finding is based on the observation that destruction of striatal cell bodies with kainic acid causes both the loss of DA activated cyclase[13] and the loss of the ability of GTP to alter DA agonist displacement of ^{3}H-spiroperidol in striatal tissue.[14] Thus, GTP can modulate DA agonist binding to D-1 receptors in both the striatum and the anterior pituitary.

In receptor binding studies, those regions of the brain labeled by ^{3}H-spiro-

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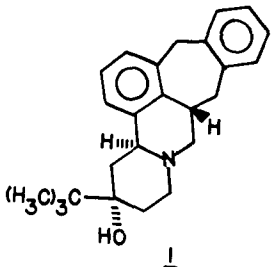
peridol were shown to have little correlation with those regions in which DA stimulates adenylate cyclase.[15] Also, differences in the pre- and postsynaptic loci of D-1 receptors and ^{3}H-spiroperidol binding sites in the striatum[13] and the substantia nigra[16] have been reported recently. ^{3}H-Domperidone (8) appears to be a novel ligand[17] specific for D-2 receptor binding sites in vitro.[18,19] Also, ^{3}H-N-n-propylnorapomorphine was reported to be a highly specific ligand for DA receptors[20,21] and appears preferable to ^{3}H-spiroperidol which can also bind to serotonin receptors.[22] The DA receptors in guinea pig and carp retinas are linked to adenylate cyclase.[18] Since Watling, et al., failed to observe specific binding of ^{3}H-domperidone to these tissues, it implies they contain an apparent homogeneous population of D-1 receptors.[18]

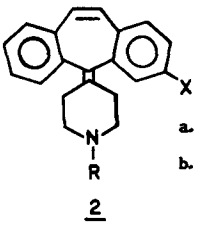
In a study of homovanillic acid (HVA) levels in specific regions of brains obtained post-mortem from schizophrenic patients who had been on long term neuroleptic treatment, Bacopoulos, et al., have reported direct evidence for a regionally specific action of antipsychotic drugs in cingulate, orbital frontal, and perifalciform cortex.[23] Although HVA levels were increased in these areas, implicating them as the sites of action of antipsychotic drugs, no increase in HVA was seen in nucleus accumbens or the putamen.[23] Skirboll, et al., have reported that the DA autoreceptor in the substantia nigra is more sensitive to either DA applied iontophoretically or apomorphine given i.v. than is the postsynaptic DA receptor in the striatum.[24] This important observation is the first direct electrophysiological evidence of the greater sensitivity of the DA autoreceptor and strengthens the heuristic framework that explains the inhibition of motor activity,[25] DA release[26] and synthesis,[27] and alleviation of schizophrenic symptomatology[28] by low doses of apomorphine.

Antipsychotic Agents - McDermed and Miller have reviewed the role of central DA receptor blocking compounds as antipsychotic agents.[29] More recently, a valuable review concerning a systematic study of the pharmacological activities of DA antagonists has appeared.[30]

The first definitive receptor map[31] of the central DA receptor has been proposed by an Ayerst group.[32,33] Detailed analysis of the topographic pharmacophoric patterns[31] of a large number of butaclamol analogues by these scientists has led to a Cartesian coordinate system model of the receptor that contains a planar, lipophilic, primary aromatic binding site, a primary nitrogen binding site with its complementary hydrogen bond donor site, and a lipophilic accessory binding site able to accommodate the tert-butyl group, or similar bulky groups, located in the 3-position of the parent nucleus. Besides accommodating both the rigid and semirigid DA receptor agonist (-)-apomorphine and antagonist (+)-octoclothepin, respectively,[34] this receptor model reflects the observed inherent chirality of the central DA receptor.[33] Moreover, the proposed receptor map may be applicable to multiple DA binding sites rather than just to the classical post-synaptic receptor.[32] Of the analogues prepared during these studies,



1



2

a. $R=CH_3$; X=Br

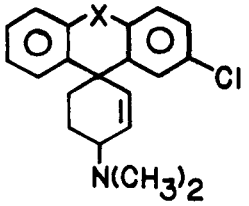
b. $R=CH_2$-c-C_3H_5; $X=SCF_3$

(+)-isobutaclamol (AY-23,396; 1) was found to have a pharmacological profile that was virtually identical to that of (+)-butaclamol.[34] As with its well studied parent, only the dextrorotatory enantiomer of 1, having the 3S, 4aS, 13aS absolute configurations, possesses in vivo and in vitro neuroleptic activity.[34]

A Merck group has shown that the enantioselective interactions of chiral molecules with CNS membrane binding sites is not limited to those molecules that are chiral by virtue of one or more asymmetric centers, but rather that such receptor binding sites recognize chirality arising from molecular asymmetry.[35] Thus, the absolute configuration of (-)-2a, determined by X-ray crystal structure analysis to be $pR_a\ pS_b$, was correlated spectroscopically with seven other pairs of atropisomeric 3-substituted cyproheptadine analogues. Those atropisomers having the $pR_a\ pS_b$ configuration showed uniform stereoselectivity in binding to DA receptors in homogenates of rat caudate, as well as in binding to the central α-adrenergic receptors of calf caudate, while those atropisomers having the opposite absolute configuration showed uniform enantioselectivity in binding to muscarinic cholinergic receptors. For (-)-2b (MK-160), the in vitro DA receptor binding data are consonant with the reported biological and pharmacological data.[36]

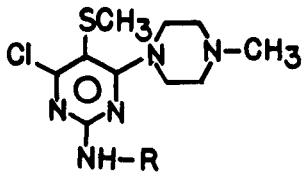
Stereoselective DA receptor blockade has been reported for the rigid tetracyclic spiroamines 3a and 3b.[37] Of the four enantiomers possible in each isomer mixture, only one isomer from each series, namely the cis-levo 3c and 3d, is more active than chlorpromazine and sulpiride in a ^{3}H-spiroperidol binding assay and in the inhibition of apomorphine induced behavior. Both 3c and 3d show preferential binding to limbic, rather than striatal, structures in rat brain. Based on an analysis of the structural features of 3c and 3d, whose absolute configurations were determined by X-ray diffraction, Astra scientists have proposed a hypothesis that chiral, competitive DA receptor antagonists need not have topographical equivalence with DA receptor agonists.[38]

Mezilamine (4a), a pyrimidine derivative not related to any well established class of antipsychotic agents, inhibits DA activation of adenylate cyclase in rat striatum and nucleus accumbens, elevates HVA in rabbit and rat brain, and inhibits ^{3}H-haloperidol binding in rat olfactory tubercle and striatum.[39,40] Mezilamine has been compared to the atypical neuroleptic clozapine since DA turnover and binding studies show it preferentially affects limbic areas as compared to striatum. Related studies of a companion compound 4b (UK 177), however, show a reverse order of preferential activity.[40] The low cataleptogenic activity of 4a in rats is attributed to its α-adrenergic postsynaptic agonist properties since the compound has no anticholinergic or GABA-mimetic activity.[41]



3

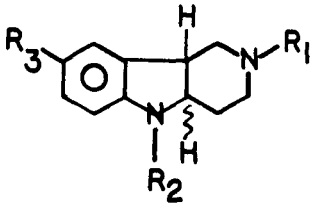
a. X= CH_2-CH_2, A02056
b. X= CH=CH, A02683
c. X= CH_2-CH_2, A23887
d. X= CH=CH, A31472



4

a. R=CH_3
b. R=CH_2-C_6H_5

Neuroleptic-like activity has been previously reported for partially reduced γ-carboline derivatives such as carbidine (cis-5a)[42] and 6a (Abbott 30360).[43] Pfizer scientists have now found that introduction of a 5-aryl group into the tetrahydro-γ-carboline nucleus greatly enhances neuroleptic activity as determined by blockade of amphetamine induced stereotypies in rats and inhibition of striatal ^{3}H-spiroperidol binding.[44] Flutroline (CP 36,584, 6b) is the most interesting member of a group of these 5-aryl carboline compounds, and additional pharmacological data have been reported for this racemate.[45] Crystal structure analysis and comparison with (+)-dexclamol and apomorphine suggests that the DA receptor blocking activity of 6b is due to the presence of a conformationally restricted, extended phenethylamine moiety. Moreover, 6b has an S-shaped arrangement of atoms consistent with the Janssen hypothesis for antipsychotic activity.[44]

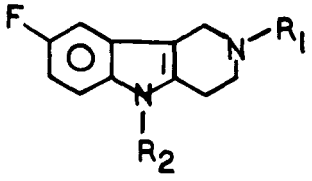


5

a. $R_1=R_3=CH_3$; $R_2=H$

b. $R_1=(CH_2)_3CO$-(4-F-C$_6$H$_4$); $R_2=CH_3$; $R_3=F$

c. $R_1=(CH_2)_3CO$-(4-F-C$_6$H$_4$); $R_2=H$; $R_3=CH_3$



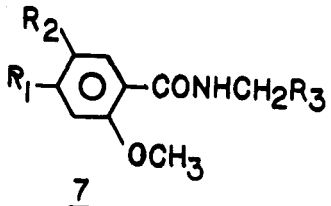
6

a. $R_1=(CH_2)_3CO$-(4-F-C$_6$H$_4$); $R_2=H$

b. $R_1=(CH_2)_3CHOH$-(4-F-C$_6$H$_4$);

$R_2=$-(4-F-C$_6$H$_4$)

A series of 26 cis and trans ring fused hexahydro-γ-carbolines has been reported, of which 5b and 5c are the most interesting.[46] The trans compound 5b approaches the DA receptor blocking potency of chlorpromazine but is less cataleptic, while cis 5c exhibits both apparent neuroleptic and antidepressant activities.



7

a. $R_1=H$; $R_2=SO_2NH_2$; $R_3=$ (1-ethylpyrrolidin-2-yl)

b. $R_1=NH_2$; $R_2=Cl$; $R_3=CH_2N(C_2H_5)_2$

c. $R_1=H$; $R_2=SO_2C_2H_5$; $R_3=$ (1-ethylpyrrolidin-2-yl)

The binding, biochemical, and behavioral actions of the substituted benzamides, a novel class of DA antagonists, are the subject of a recent review by Jenner and Marsden.[47] Also, a monograph on the experimental and clinical pharmacology of sulpiride (7a) and other benzamides has recently appeared.[48] Liebman, et al., have reported that 7a is considerably more effective in inhibiting conditioned avoidance response in the squirrel monkey than in the rat.[49] In a related study, however, Clineschmidt, et al., concluded that conventional neuroleptics, such as haloperidol and chlorpromazine, also show a similar behavioral pattern.[50] There is an interesting report that 7a may be of value in the treatment of autistic children.[51] Based on the dose dependent elevation of the DA metabolite DOPAC in rat striatum, Stanley and Wilk suggested that metoclopramide (7b), previously regarded as lacking antipsychotic

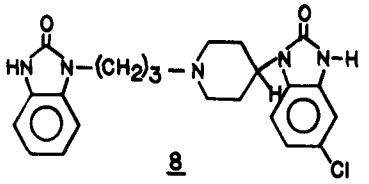
efficacy, would show activity in a dose range comparable to chlorpromazine.[52] Clinical data now support this contention.[53] As in the case of 7a, binding, biochemical, and behavioral studies now indicate that the central pharmacological action of sultopride (7c) resides in the (-)-enantiomer and that this action is mediated via D-2 receptors.[54]

Despite significant advances in the study of endogenously produced opioid peptides, the role of the enkephalins and endorphins in the etiology of and/or treatment of schizophrenia remains, at present, a moot question.[55] The initial report by Kline and his colleagues concerning the beneficial effect of β-endorphin, when administered i.v. to schizophrenic patients, remains to be confirmed.[56] However, des-tyrosine^1-γ-endorphin (DTγE, β-LPH$_{62-77}$), a smaller fragment of the β-endorphin molecule, has been administered to chronic schizophrenic patients refractory to conventional neuroleptic therapy.[57] In a double blind, placebo-controlled crossover study of eight patients, six of whom were maintained on neuroleptic medication, DTγE (1 mg/day; i.m.) rapidly alleviated residual psychotic symptomatology.[57] Two recent studies have shown, however, that DTγE does not act at the neuroleptic receptor labelled by ^{3}H-spiroperidol,[58] nor does it resemble haloperidol in characteristic behavioral response profiles in the rat or in elevating caudate synaptosomal conversion of tyrosine to dopamine.[59] A synthetic analog of met-enkephalin (β-LPH$_{61-65}$), which is itself a smaller fragment of the β-endorphin molecule, H-Tyr-D-Ala-Gly-MePhe-Met(O)-ol (FK-33-824), has been reported to have antipsychotic efficacy in schizophrenic patients,[60] and, as with the opioid peptides, raises plasma prolactin levels in a dose-dependent manner.[61]

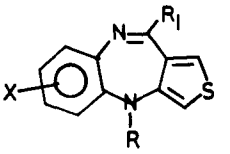
Based on previous findings that a close functional relationship exists between the central dopaminergic and endorphin systems, Volavka, Davis and Ehrlich have linked the dopamine hypothesis of schizophrenia to a theory in which endorphins, acting as neuromodulators or neurotransmitters of the central dopaminergic system, are involved in the etiology of schizophrenia.[7] In this regard, a number of investigators have examined the cerebrospinal fluid (CSF) and hemodialyzates of schizophrenic patients for altered endorphin levels. Domscke, et al., have reported that β-endorphin levels in the CSF of acute schizophrenic patients are ten times higher than those of controls, while the CSF β-endorphin levels in chronic schizophrenics are about 50% of normal.[62] Höllt, et al., however, found no appreciable difference in β-endorphin-like immunoreactivity in CSF of schizophrenics when compared to normal controls.[63] Ross, Berger, and Goldstein have compared the hemodialyzates of 98 schizophrenic patients (paranoid, residual, catatonic, undifferentiated, and schizoaffective) with 42 normal subjects and have found no appreciable differences.[64] Moreover, they could not confirm a previous report[65] that [Leu^5]-β-endorphin is present in the hemodialyzates of schizophrenics. Lewis, et al., also have been unable to confirm the presence of [Leu^5]-β-endorphin in such hemodialyzates.[66] Guidelines for analyzing data to test the endorphin hypothesis of schizophrenia have been suggested by Davis, Buchsbaum, and Bunney.[67]

While the preceding discussion of antipsychotic agents has been concerned with central, rather than peripheral DA receptor antagonists, interest in the latter is growing as evidenced by the recent numerous pharmacological and clinical reports concerning domperidone (8, R 33812).[68] This compound, one of a series of benzimidazoline derivatives that includes halopemide, is a potent DA receptor binding agent. Except at very high doses, or when administered intracerebrally,[69] peripheral administration of 8 affects dopaminergic systems only outside the blood brain barrier, and, thus, the compound has found clinical use as an antiemetic[70] and in certain gastrointestinal disturbances.[71] Furthermore, 8 has been used to control emesis associated with bromocriptine,[72] apomorphine,[73] and L-DOPA[74] treatment of parkinsonian patients without affecting the central DA agonist

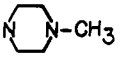
activity of these compounds. Moreover, 8 has no adverse adrenolytic or extrapyramidal effects.[68] In a study of 20 chronic schizophrenic patients being treated with high doses of neuroleptics, 8 gave relief from chronic dyspepsia without exacerbating existing extrapyramidal symptoms or inducing new ones.[75] Domperidone is a potent dopamine antagonist when administered intracerebrally. For example, when given into the nucleus accumbens, it is comparable to fluphenazine in antagonizing amphetamine induced hyperactivity.[69]



8



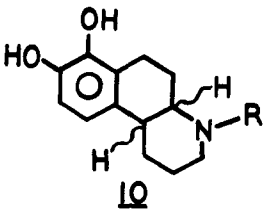
9

a. X=H; R=CH_3; R_1= 

The neuroleptic-like activity of a series of 30 thienobenzodiazepines such as 9 was assessed by inhibition of motor activity in rats and by blockade of (+)-amphetamine lethality in mice.[76] None of the compounds were as active as chlorpromazine. However, those compounds having no nuclear substituents also showed potential antidepressant activity as measured by antagonism of tetrabenazine induced depression in mice, and of those compounds tested in both protocols, 9a was the most interesting. Dual acting antipsychotic-antidepressant drugs are of clinical interest.

Finally, a preliminary report of a conference concerning the role of depot neuroleptics in the treatment of schizophrenic patients concludes there is no difference in safety or efficacy of depot prolonged-acting and oral short-acting forms of antipsychotic drugs.[77]

Dopamine Agonists - Cannon, et al., have presented additional data pertinent to the conformational preference of DA at its receptor sites.[78] A series of 6 cis and trans octahydrobenzo[f]quinolines (10) were evaluated for central and peripheral dopaminergic activities. The trans isomers 10a-c have an α-rotamer DA moiety rigidly held in an extended antiperiplanar manner, believed optimal for receptor interactions, while the corresponding moiety of the cis isomers lacks such conformational integrity. All of the trans isomers 10a-c are at least 100X more potent than apomorphine (APO) in inhibiting the cat cardioaccelerator nerve, and all have emetic activity in dogs (2.7-4.9 x APO). Stereotyped behavior and hyperactivity following s.c. administration to rodents, however, is limited to the trans N-alkyl compounds 10b-c. The cis isomers of 10a-c are virtually devoid of direct dopaminergic activity, as are a series of N-alkyl derivatives of (±)-α-methyldopamine.[79]



10

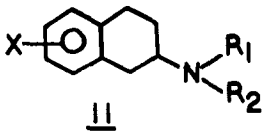
a. R=H

b. R=C_2H_5

c. R=n-C_3H_7

Detailed ^{3}H-DA and ^{3}H-apomorphine binding studies by Tedesco, Seeman and McDermed of the enantiomers of 11a, semirigid analogues of DA in the α-rotamer conformation, have provided the first example of enantioselective dopamine agonist binding to the dopamine receptor.[80] Further comparison of these data with similar binding data for the racemates 11a, 11b, and 11c (the latter analogue having the β-rotamer conformation of dopamine) has afforded evidence for the identity of the ^{3}H-DA and ^{3}H-apomorphine receptor. The greater potency

of (-)-11a over (+)-11a in displacing bound ^{3}H-APO from receptors which are stereospecific for (+)-butaclamol, supports the hypothesis that the neuroleptic receptor is the same as that which binds ^{3}H-APO. As noted by these authors, however, other data do not support this controversial hypothesis.[81] Not only is the lone electron pair of the tertiary nitrogen atom of apomorphine required for ligand-receptor interaction, but stereospecific orientation of this lone electron pair also appears to be a critical factor involved in receptor binding.[82] In conjunction with previously reported crystallographic data for R-(-)-apomorphine, Tedesco, et al., have now defined the most probable absolute conformation of dopamine, including the nitrogen lone electron pair, at the ^{3}H-APO receptor.[80]



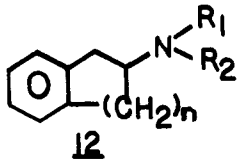
11

a. X=5-OH; R_1=R_2=n-C_3H_7

b. X=6-OH; R_1=R_2=n-C_3H_7

c. X=7-OH; R_1=R_2=n-C_3H_7

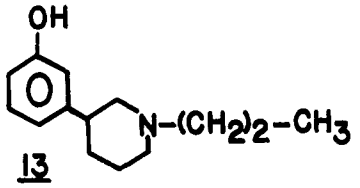
Rusterholz, et al., have compared the dopaminergic properties of several non-hydroxylated 2-aminotetralins (12, n=2) with analogous compounds having a five (12, n=1) or seven (12, n=3) membered alicyclic ring rather than a cyclohexane ring.[83] Although much weaker agonists than apomorphine, 12a (n=2), 12b (n=2), and 12b (n=1) inhibited prolactin release, induced emesis in dogs, and caused rotational behavior in 6-OHDA unilaterally lesioned rats (0.05 x APO). Metabolic activation via aromatic hydroxylation has been suggested as a mechanism whereby these compounds exert their direct acting dopaminergic effects.



12

a. R_1=CH_3, R_2=n-C_3H_7

b. R_1=R_2=n-C_3H_7



13

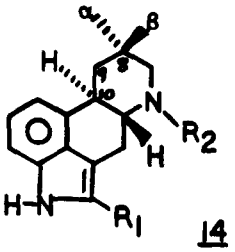
Forty-four 5-hydroxy and 5-methoxy-N-alkyl and N,N-dialkyl-2-aminotetralins 11 (X=5-OH and 5-OCH_3) were evaluated both for their ability to reduce the DOPA synthesis rate in presynaptic neurones (autoreceptor stimulation) as well as for their ability to stimulate locomotor activity (postsynaptic stimulation).[84] The active compounds of this series showed no apparent specificity with regard to pre- and postsynaptic dopaminergic stimulation, and there was no specificity for the limbic system over striatum. However, a preliminary report by these same authors indicates that compound 13 is a selective agonist at the DA autoreceptor.[85]

Ginos, et al., have presented additional data pertinent to the dopamine agonism of N,N-disubstituted dopamines and N,N-disubstituted 2-amino-6,7-dihydroxytetralin derivatives 11 (X=6,7-$(OH)_2$).[86] This study has confirmed their previous report that an N-n-propyl substituent confers optimum dopaminergic activity in these groups of compounds.

Many ergot alkaloids and their derivatives show mixed agonist-antagonist activity with respect to both pre- and postsynaptic DA receptors, but one of the principal pharmacological attributes of these compounds is postsynaptic D-2 receptor agonist activity.[87,88,89] The clinical use of bromocriptine (CB 154), the best known of these compounds, in neurological disorders such as Parkinson's disease,[90] and in endocrinological disorders that involve pathologically elevated prolactin levels, [91] is the subject of recent reviews.[92,93]

Lilly scientists have reported both animal and human pharmacological studies on a new ergoline derivative, pergolide (14a).[94] Lew and his associates have

compared 14a with its analogues 14b and 14c for relative binding affinities to striatal membrane ^{3}H-DA binding sites, and for their abilities to elicit rotation in 6-OHDA lesioned rats and to relieve surgically induced tremor in monkeys.[95] In all of these tests, 14a was the most active compound. Pergolide is 10X more potent than the structurally related lergotrile (14d) as a dopamine agonist.[94] Moreover, 14a is reported to have less effect than 14d on other monoaminergic systems.[94] In normal male subjects, 14a (100-400 µg; p.o.) results in a dose dependent inhibition of prolactin for more than 24 hours.[96] Pergolide is also a potent antihypertensive agent lowering blood pressure and heart rate in hyper-and normotensive rats.[97] This antihypertensive activity is completely antagonized by haloperidol. Initial observations on a small number of patients suggest that 14a is a potent antiparkinson drug.[93]



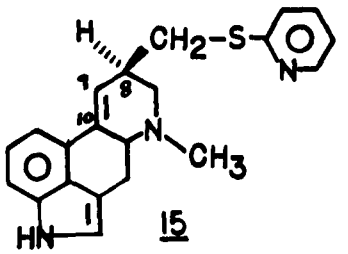
14

a. R_1=H, R_2=n-C_3H_7; β=CH_2SCH_3

b. R_1=H, R_2=CH_3; β=CH_2SCH_3

c. R_1=H, R_2=C_2H_5; β=CH_2SCH_3

d. R_1=Cl, R_2=CH_3; β=CH_2CN



15

In a preliminary report, Wong and Bymaster have shown that the relative binding affinities of ergot related drugs for membrane sites specifically binding ^{3}H-DA and ^{3}H-spiroperidol are functions of the size of the substituent in the 8-position as well as the location of the double bond, 8,9 vs. 9,10, that occurs in the clavine and ergolene type alkaloids.[98] Cassady has also noted that the size and stereochemistry of the substituted amino group in 8α and 8β-aminoergoline derivatives influence binding of the compounds at the prolactin-inhibiting factor receptor.[99]

Recent clinical reports indicate that 15 (CF 25-397) is not effective in the treatment of parkinsonism,[100] and that hepatotoxicity is one of the adverse effects of 14d.[93]

References

1. D. F. Horrobin, Lancet, 1, 529 (1979).
2. "The Biological Basis of Schizophrenia", G. Hemmings and W. A. Hemmings, Eds., MTP Press Ltd., Lancaster, U. K., 1978.
3. W. E. Bunney, Jr. (Moderator), Ann. Intern. Med., 91, 239 (1979).
4. "Schizophrenia, Science and Practice", J. C. Shershow, Ed., Harvard University Press, Cambridge, Mass., 1978.
5. "The Nature of Schizophrenia: New Approaches to Research and Treatment", L. C. Wynne, R. L. Cromwell and S. Matthysse, Eds., John Wiley and Sons, New York, 1978.
6. "Neurochemical and Immunological Components in Schizophrenia", D. Bergsma and A. L. Goldstein, Eds., Alan R. Liss, Inc., New York, 1978.
7. J. Volavka, L. G. Davis and Y. H. Ehrlich, Schizophr. Bull., 5, (2), 227 (1979).
8. J. W. Kebabian and D. B. Calne, Nature, 277, 93 (1979).
9. H. S. Ahn, E. Gardner and M. H. Makman, Eur. J. Pharmacol., 53, 313 (1979).
10. D. R. Sibley and I. Creese, Eur. J. Pharmacol., 55, 341 (1979).
11. M. G. Caron, M. Beaulieu, V. Raymond, B. Gagne, J. Drouin, R. Lefkowitz and F. Labrie, J. Biol. Chem., 253, 2244 (1978).
12. I. Creese, T. B. Usdin and S. H. Snyder, Mol. Pharmacol., 16, 69 (1979).
13. R. Schwarcz, I. Creese, J. T. Coyle, and S. H. Snyder, Nature, 271, 766 (1978).
14. I. Creese, T. Usdin and S. H. Snyder, Nature, 278, 577 (1979).
15. M. Quik and L. L. Iversen, Eur. J. Pharmacol., 56, 323 (1979).
16. M. Quik, P. C. Emson, and E. Joyce, Brain Res., 167, 355 (1979).

17. M. Baudry, M. P. Martres, and J. C. Schwartz, Naunyn-Schmiedeberg's Arch. Pharmacol., 308, 231 (1979).
18. K. J. Watling, J. E. Dowling and L. L. Iversen, Nature, 281, 578 (1979).
19. P. M. Laduron and J. E. Leysen, Biochem. Pharmacol., 28, 2161 (1979).
20. M. Titeler and P. Seeman, Eur. J. Pharmacol., 56, 291 (1979).
21. I. Creese, L. Padgett, E. Fazzini and F. Lopez, Eur. J. Pharmacol., 56, 411 (1979).
22. J. E. Leysen, C. J. E. Niemegeers, J. P. Tollenaere and P. M. Laduron, Nature, 272, 168 (1978).
23. N. C. Bacopoulos, E. G. Spokes, E. D. Bird and R. H. Roth, Science, 205, 1405 (1979).
24. L. R. Skirboll, A. A. Grace, and B. S. Bunney, Science, 206, 80 (1979).
25. A. Carlsson in "Pre and Postsynaptic Receptors", E. Usdin and W. E. Bunney, Jr., Eds., Marcel Dekker, New York, 1975, p. 49.
26. L.-O Farnebo and B. Hamberger, Acta. Physiol. Scand., (Suppl.), 371, 35 (1971).
27. T. C. Westfall, M.-J. Besson, M.-F. Georguieff and J. Glowinski, Naunyn-Schmiedeberg's Arch. Pharmacol., 292, 279 (1976).
28. C. A. Tamminga, M. H. Schaeffer, R. C. Smith and J. M. Davis, Science, 200, 567 (1978).
29. J. McDermed and R. J. Miller in "Annual Reports in Medicinal Chemistry", Vol. 14, H.-J. Hess, Ed., Academic Press, N. Y. 1979, p. 12.
30. C. J. E. Niemegeers and P. A. J. Janssen, Life Sci., 24, 2201 (1979).
31. P. Gund in "Annual Reports in Medicinal Chemistry", Vol. 14, H.-J. Hess, Ed., Academic Press, N. Y., 1979, p. 299.
32. L. G. Humber, F. T. Bruderlein, A. H. Philipp, M. Götz, and K. Voith, J. Med. Chem., 22, 761 (1979).
33. A. H. Philipp, L. G. Humber, and K. Voith, J. Med. Chem., 22, 768 (1979).
34. L. G. Humber, A. H. Philipp, K. Voith, T. Pugsley, W. Lippmann, F. R. Ahmed, M. Przybylska, J. Med. Chem., 22, 899 (1979).
35. W. C. Randall, P. S. Anderson, E. L. Cresson, C. A. Hunt, T. F. Lyon, K. E. Rittle, D. C. Remy, J. P. Springer, J. M. Hirshfield, K. Hoogsteen, M. Williams, E. A. Risley and J. A Totaro, J. Med. Chem., 22, 1222 (1979).
36. B. V. Clineschmidt, M. A. McKendry, N. L. Papp, A. B. Pflueger, C. A. Stone, J. A. Totaro, and M. Williams, J. Pharmacol. Exper. Ther., 208, 460 (1979).
37. S.-O. Ögren, H. Hall, and C. Köhler, Life Sci., 23, 1769 (1978).
38. B. Carnmalm, L. Johansson, S. Rämsby, N. E. Stjernström, and A. Wägner, Acta Pharm. Suecica, 16, 239 (1979).
39. A. Uzan, G. LeFur, N. Mitrani, M. Kabouche and A.-M. Donadieu, Life Sci., 23, 261 (1978).
40. G. LeFur, M.-C. Burgevin, C. Malgouris and A. Uzan, Neuropharmacol., 18, 591 (1979).
41. G. LeFur, J. Mizoule, J. Rataud, and A. Uzan, Eur. J. Pharmacol., 58, 359 (1979).
42. W. M. Herrmann and J. Fabricius, Dis. Nerv. Syst., 35, 28 (1974).
43. E. T. Kimura, P. W. Dodge, P. R. Young and R. P. Johnson, Arch. Int. Pharmacodyn. Ther., 190, 124 (1971).
44. C. A. Harbert, J. J. Plattner, W. M. Welch, A. Weissman, and B. K. Koe, Mol. Pharmacol., in press (1980).
45. B. K. Koe, A. Weissman, J. J. Plattner, C. A. Harbert and W. M. Welch, Pharmacologist, 21, 180 (1979).
46. Y. Nagai, A. Irie, Y. Masuda, M. Oka and H. Uno, J. Med. Chem., 22, 677 (1979).
47. P. Jenner and C. D. Marsden, Life Sci., 25, 479 (1979).
48. "Sulpiride and Other Benzamides. Experimental and Clinical Pharmacology", P. F. Spano, M. Trabucchi, G. U. Corsini and G. L. Gessa, Eds., Raven Press, New York, 1979.
49. J. Liebman, R. Neale, and N. J. Moen, Eur. J. Pharmacol., 50, 377 (1978).
50. B. V. Clineschmidt and J. J. Witoslawski, Comm. Psychopharmacol., in press (1980).
51. B. Miller and H. Wallis, Muench. Med. Wochenschr., 121, 667 (1979).
52. M. Stanley and S. Wilk, Life Sci., 24, 1907 (1979).
53. M. Stanley, A. Lautin, J. Rotrosen, and S. Gershon, IRCS Clin. Pharmacol. Ther., 7, 322 (1979).
54. A. Clow, P. Jenner, C. D. Marsden, C. Reavill, and A. Theodorou, Brit. J. Pharmacol., 67, 433 P (1979).
55. J. Ananth and T. S. Callanan, Comprehensive Psychiatry, 20, 246 (1979).
56. N. S. Kline, C. H. Li, H. E. Lehmann, A. Lajtha, E. Laski, and T. Cooper, Arch. Gen. Psychiatry, 34, 1111 (1977).
57. W. M. A. Verhoeven, H. M. van Praag, J. M. van Ree and D. deWied, Arch. Gen. Psychiatry, 36, 294 (1979).
58. N. W. Pedigo, N. C. Ling, T. D. Reisine and H. I. Yamamura, Life Sci., 24, 1645 (1979).
59. S. B. Weinberger, A. Arnstein and D. S. Segal, Life Sci., 24, 1637 (1979).
60 N. Nedopil and E. Ruether, Pharmakopsychiat. Neuro-Psychopharmakol., 12, 277 (1979).
61. B. von Graffenried, E. del Pozo, J. Roubicek, E. Krebs, W. Poldinger, P. Burmeister, and L. Kerp, Nature, 272, 729 (1978).
62. W. Domschke, A. Dickschas and P. Mitznegg, Lancet, 1, 1024 (1979).
63. V. Höllt, H. M. Emrich, O. A. Muller and R. Fahlbusch in "Characteristics and Function of Opioids, J. M. Van Ree and L. Terenius, Eds., Elsevier, N. Y., 1978, p. 279.
64. M. Ross, P. A. Berger and A. Goldstein, Science, 205, 1163 (1979).
65. R. M. Palmour and F. R. Ervin, 7th Ann. Mtg. Soc. Neurosci., Anaheim, Calif., 1977, Abst., Vol. 3, #1029.

66. R. V. Lewis, L. D. Gerber, S. Stein, R. L. Stephen, B. I. Grosser, S. F. Velick and S. Udenfriend, Arch. Gen. Psychiatry, 36, 237 (1979).
67. G. C. Davis, M. S. Buchsbaum and W. E. Bunney, Jr., Schizophr. Bull., 5 (2), 244 (1979).
68. A. J. Reyntjens, C. J. E. Niemegeers, J. M. Van Nueten, P. Laduron, J. Heykants, K. H. L. Schellekens, R. Marsboom, A. Jageneau, A. Brockaert, and P. A. J. Janssen, Arzneim. Forsch., 28, (II), 1194 (1978).
69. B. Costall, D. H. Fortune and R. J. Naylor, J. Pharm. Pharmacol., 31, 344 (1979).
70. A. Reyntjens, Postgraduate Med. J., 55, (Suppl. 1), 50 (1979).
71. A. De Schepper, F. Wollaert and A. Reyntjens, Arzneim. Forsch., 28 (II), 1196 (1978).
72. Y. Agid, A. M. Bonnet, P. Pollak and J. L. Signoret, Lancet, 1, 570 (1979).
73. G. U. Corsini, G. L. Gessa, M. del Zompo and A. Mangoni, Lancet, 1, 954 (1979).
74. M. Bogaerts, M. Braems and C. Martens, Postgraduate Med. J., 55 (Suppl. 1), 51 (1979).
75. R. Deberdt, Postgrad. Med. J., 55 (Suppl. 1), 48 (1979).
76. J. B. Press, C. M. Hofmann, N. H. Eudy, W. J. Fanshawe, I. P. Day, E. N. Greenblatt and S. R. Safir, J. Med. Chem., 22, 725 (1979).
77. J. Levine, N. R. Schooler and G. B. Cassano, Psychological Med., 9, 383 (1979).
78. J. G. Cannon, C. Suarez-Gutierrez, T. Lee, J. P. Long, B. Costall, D. H. Fortune, and R. J. Naylor, J. Med. Chem., 22, 341 (1979).
79. J. G. Cannon, Z. Perez, J. P. Long, D. B. Rusterholz, J. R. Flynn, B. Costall, D. H. Fortune, and R. J. Naylor, J. Med. Chem., 22, 901 (1979).
80. J. L. Tedesco, P. Seeman and J. D. McDermed, Mol. Pharmacol., 16, 369 (1979).
81. P. Seeman, K. Westman, M. Protiva, J. Jilek, P. C. Jain, A. K. Saxena, N. Anand, L. Humber and A. Philipp, Eur. J. Pharmacol., 56, 247 (1979).
82. J. L. Tedesco and P. Seeman, Soc. Neurosci., 4, Abst. 1679, 522 (1978).
83. D. B. Rusterholz, J. P. Long, J. R. Flynn, J. G. Cannon, T. Lee, J. P. Pease, J. A. Clemens, D. T. Wong and F. P. Bymaster, Eur. J. Pharmacol., 55, 73 (1979).
84. U. Hacksell, U. Svensson, J. L. G. Nilsson, S. Hjorth, A. Carlsson, H. Wikström, P. Lindberg, and D. Sanchez, J. Med. Chem., 22, 1469 (1979).
85. S. Hjorth, A. Carlsson, P. Lindberg, D. Sanchez, H. Wikström, L.-E. Arvidsson, U. Hacksell, J. L. G. Nilsson and U. Svensson, 18th Ann. Mtg. Am. Coll. of Neuropsychopharmacol., San Juan, Puerto Rico, Dec. 12-14, 1979.
86. J. Z. Ginos, J. M. Stevens and D. E. Nichols, J. Med. Chem., 22, 1323 (1979).
87. J. Y. Lew, F. Hata, T. Ohashi and M. Goldstein, J. Neural Trans., 41, 109 (1977).
88. M. Goldstein, J. Y. Lew, S. Nakamura, A. F. Battista, A. Lieberman and K. Fuxe, Fed. Proc., 37, 2202 (1978).
89. K. Fuxe, B. B. Fredholm, S.-O. Ögren, L. F. Agnati, T. Hökfelt, and J.-A. Gustafsson, Fed. Proc., 37, 2181 (1978).
90. A. N. Lieberman, M. Kupersmith, G. Gopinathan, E. Estey, A. Goodgold and M. Goldstein, Neurology, 29, 363 (1979).
91. A. E. Mehta and G. Tolis, Drugs, 17, 313 (1979).
92. J. D. Parkes, Drugs, 17, 365 (1979).
93. A. Lieberman, A. Neophytides, M. Kupersmith, I. Casson, R. Durso, S. H. Foo, M. Khayali, T. Tartaro and M. Goldstein, Am. J. Med. Sci., 278, 65 (1979).
94. R. W. Fuller, J. A. Clemens, E. C. Kornfeld, H. D. Snoddy, E. B. Smalstig, and N. J. Bach, Life Sci., 24, 375 (1979).
95. J. Y. Lew, S. Nakamura, A. F. Battista and M. Goldstein, Comm. Psychopharmacol., 3, 179 (1979).
96. L. Lemberger and R. E. Crabtree, Science, 205, 1151 (1979).
97. T. T. Yen, N. B. Stamm, and J. A. Clemens, Life Sci., 25, 209 (1979).
98. D. T. Wong and F. P. Bymaster, Joint Central-Gr. Lakes Regional Mtg., Am. Chem. Soc., Indianapolis, Ind., May 24-26, 1978, Abst., MEDI. #25, p. 111.
99. A. M. Crider, C. K. Lu, H. G. Floss, J. M. Cassady and J. A. Clemens, J. Med. Chem., 22, 32 (1979).
100. P. F. Teychenne, R. Pfeiffer, S. M. Bern and D. B. Calne, Neurology, 27, 1140 (1977).

Chapter 3. Anti-Anxiety Agents, Anticonvulsants, and Sedative-Hypnotics

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In the past year, work related to benzodiazepine (BZ) receptors has accelerated. BZ receptors have been confirmed in mammalian brain by autoradiographic methods[1] and positive emission tomography.[2] A reduction in the number of BZ receptors was confirmed in "nervous" mice with degenerated Purkinje cells,[3,4] on whose dendrites BZ receptors are localized.[5,6]

The intimate relationship between BZ sites and some, but not all,[7] GABA binding sites has been studied and reviewed.[8-17] Two distinct types of BZ receptors appear to exist.[18,19] Type I anxiolytic sites are not coupled to GABA sites or a chloride conductance system,[20-22] whereas Type II sites are coupled and probably mediate sedation and muscle relaxation.[19,23] Non-BZ ligands,[23] apparently selective for Type I receptors, as well as some BZ's[24] have reduced sedative action.

Changes in kinetics of BZ receptor binding have been shown to occur after acute diazepam administration,[25] spontaneous seizures,[26] electroshock-induced seizures,[27] conflict and footshock,[28] denervation,[29] and in Huntington's Chorea.[30] However, in one study, only minimal changes in BZ binding occured after several different experimental stresses.[31] Conflicting results on changes in BZ receptor binding in response to chronic treatment with BZ's have been reported.[32-34] A critique on these findings has appeared.[35]

The subject of endogenous substrates for the BZ receptor has been reviewed.[36] Identified as endogenous BZ receptor ligands with BZ-like properties are the purines inosine and hypoxanthine,[37-40] related purines,[37,41] and nicotinamide.[42] Other ligands include high-molecular weight peptides isolated from several animal species,[43-45] and a low-molecular weight factor from human urine.[46]

The synthetic BZ irazepene (1n) was found to be a noncompetitive, irreversible inhibitor of diazepam (1b) binding, thus rendering this ligand of potential utility in BZ receptor isolation, characterization, and localization.[47]

The blockade by physostigmine of BZ-induced sleep or coma has been attributed to blockade of BZ receptors, and not to an anticholinergic effect.[48] Pentylenetetrazole (PTZ) was found to inhibit BZ binding,[41] suggesting that the anti-convulsant effects of BZ's and the convulsant effects of PTZ may be mediated in part through the BZ receptor.[49]

A molecular orbital study showed a highly significant correlation between the anti-PTZ activity of a series of BZ's and electron density in the Py orbital at the aromatic carbon adjacent to the amide nitrogen.[50]

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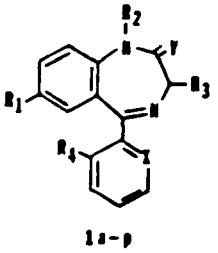
Novel animal tests for the pharmacological characterization of anti-anxiety agents,[51,52] as well as a model for study of BZ physical dependence[53] have been described. Reviews on the mechanism of action of BZ's,[54,55] and the chemistry, activity profiles, clinical applications, evaluation, pharmacology, metabolism, and pharmacokinetics of anxiolytics[56] have appeared.



1a-p

	R_1	R_2	R_3	R_4	X	Y
1a	Br	H	H	H	N	O
1b	Cl	CH_3	H	H	CH	O
1c	Cl	H	H	Cl	CH	O
1d	Cl	CH_3	$OCON(CH_3)_2$	H	CH	O
1e	Cl	H	$CO_2^-K^+$	H	CH	O
1f	Cl	CH_2CF_3	H	H	CH	O
1g	Cl	H	OH	Cl	CH	O
1h	Cl	CH_2-cyclopropyl	H	H	CH	O
1i	Cl	CH_3	OH	Cl	CH	O
1j	NO_2	CH_3	H	F	CH	O
1k	Cl	$(CH_2)_2N(Et)_2$	H	F	CH	O
1l	NO_2	H	H	Cl	CH	O
1m	Cl	CH_2-cyclopropyl	H	F	CH	O
1n	Cl	$(CH_2)_2NCS$	H	F	CH	O
1o	Cl	$CH_2{\equiv}CH$	H	H	CH	O
1p	Cl	CH_2CF_3	H	F	CH	S

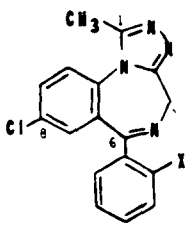
Benzodiazepines and Related Compounds

Anxiolytic Agents - Clinical studies on the treatment of various anxiety states with bromazepam (1a),[57-59] alprazolam (2a)[60,61] chlordesmethyldiazepam (1c),[62] camazepam (1d),[63] clorazepate (1e),[64] halazepam (1f),[65] ketazolam (4),[66] lorazepam (1g),[67-70] prazepam (1h),[71,72] tofisopam (5),[73,74] and ORF 8063 (3b),[75] have been published. A pharmacokinetic study on lorazepam has appeared,[76,77] as has a detailed review of the chemistry, pharmacology, metabolism, pharmacokinetics, and clinical studies on clobazam (3a).[78]

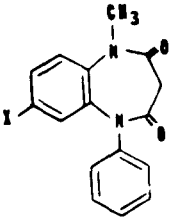
A SAR study with triazolobenzodiazepines showed that enhanced anticonflict activity in rodents results upon substitution of electron-releasing substituents at C-1 (cf. structure 2) and with introduction of o-Cl into the C-6 phenyl substituent. Electron withdrawing groups in the 1-position or removal of the 8-Cl reduce or eliminate activity.[79] A series of ethers and esters of 3-hydroxy-1,4-benzodiazepine-2-ones was prepared to determine if lipophilic character influenced CNS effects. No correlation was found between octanol-water partition coefficient and activity in rodent screens indicative of potential anxiolytic activity.[80]

Sedative Hypnotics - Compounds found active as sedative-hypnotics include lormetazepam (1i) in a double blind study with preoperative inpatients[81]; lorazepam (1g) in post-operative patients[82] and in

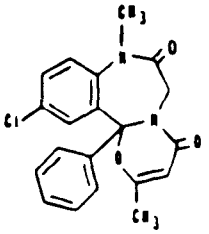
acute alcoholics;[83] pinazepam (1o) in normal subjects;[84] and quazepam (Sch-16134, 1p) in chronic insomniacs.[85]



2a. X=N
2b. X=Cl



3a. X=Cl
3b. X=CF_3



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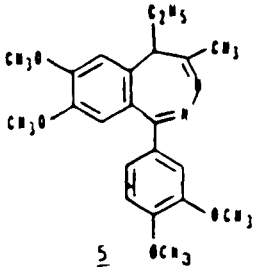


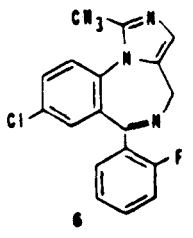
5

Used as i.v. anesthetics, midazolam (6)[86] and flunitrazepam (1j)[87] compared favorably to thiopental. The anesthesia produced by midazolam[88] may be due to synaptic accumulation of GABA resulting from blockade of GABA uptake.[89]

In EEG studies in man, WE-941 (7) in single oral doses of 0.3 mg was an effective sleep-inducer[90] found to be equivalent to flurazepam at 30 mg in a double-blind study.[91] Additional studies with flurazepam (1k) in normal subjects have been reported.[92] In a repeated dose study, triazolam (2b) at 0.6 mg/night, p.o. and flurazepam (1k) at 30 mg were equieffective on the first two and last two nights of a seven-day double-blind study in chronic insomniacs.[93]

Reported side effects with benzodiazepines as hypnotics include anterograde amnesia in normal subjects with flunitrazepam at 2 mg p.o.,[94] and persistent cumulative decrements in daytime mental and physical acuity with flurazepam at 30 mg p.o. in double-blind studies with insomniacs.[95-97] Several articles have appeared dealing with clinical[98,99] and neurochemical[100] aspects of the "rebound" phenomenon associated with withdrawal of benzodiazepines. A summary on the value of sleep-lab studies in the clinical evaluation of hypnotics has also appeared.[101]

Pharmacological and EEG studies in rodents suggested hypnotic properties for RU 31158 (8).[102]



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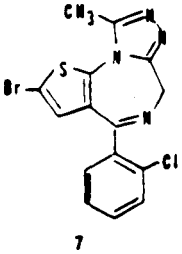

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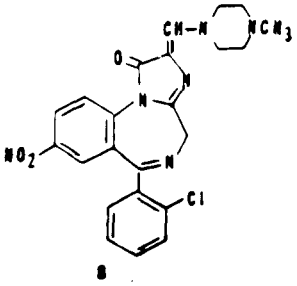
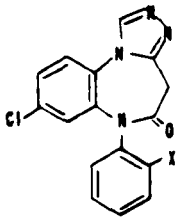


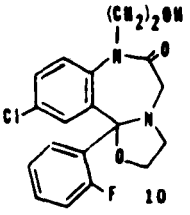
8

Anticonvulsants - Clonazepam (1l) was found useful in the treatment of epilepsy in a crossover comparison with valproate sodium.[103] A study on the influence of this drug on the spontaneous EEG in rats has been published.[104] A pilot study found clorazepate (1e) to be as effective as phenobarbital as secondary anticonvulsants in combination with phenytoin, but with fewer side effects.[105]

Clobazam (3a)[106] and two new 1,5-benzodiazepines, BAU 426 (9a) and BAU 500 (9b)[107] protected photosensitive baboons against epileptic responses. The new compounds KB 509 (1m)[108] and MS-4101 (10)[109] showed anticonvulsant, sedative, and antiaggressive activity in rodents.



9a. X=Cl
9b. X=CF_3



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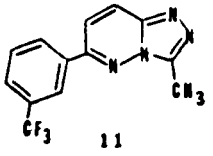

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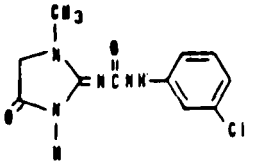
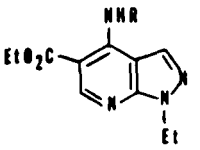


12

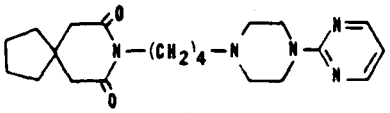
Non-Benzodiazepines

Anxiolytic Agents - CL 218,872 (11) is the prototype of a new series of triazolopyridazines[110] found to be capable of displacing ^{3}H-diazepam from its binding sites with a potency comparable to that of BZ's. Analysis of binding kinetics shows a Hill coefficient of less than unity, suggesting selective interaction with a subpopulation of BZ receptors.[18,23,111] Pharmacologically, this compound was active in tests predictive of anxiolytic activity, but only weakly active in tests which measure muscular incoordination and sedation.[111] A similar separation of anxiolytic and side effects has also been claimed for fenobam (McN-3377, 12).[112]

The pyrazolopyridines SQ 65396 (cartazolate, 13a) and SQ 20009 (13b), active as anxiolytics clinically and in animal models, were found to produce an enhancement of ^{3}H-diazepam binding to rat brain membranes.[113,114] These drugs may exert their anxiolytic properties by altering the affinity of an endogenous ligand for the BZ receptor.

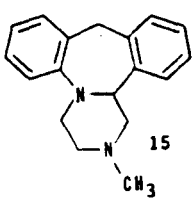


13a. R=n-C_4H_9
13b. R=N=C(CH$_3$)$_2$



14

A double-blind study indicated that buspirone (14) at 20 mg daily was equivalent to diazepam at 19 mg as an anxiolytic.[115] In a mixed double-blind, single-blind study, mianserin (15) at 40 mg/day appeared superior as an anxiolytic to 22 mg/day of diazepam.[116] A double-blind controlled study in geriatric psychiatric patients with doxepin (16) indicated possible utility as an anxiolytic agent.[117]



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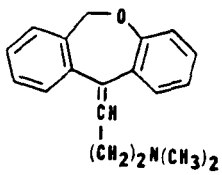
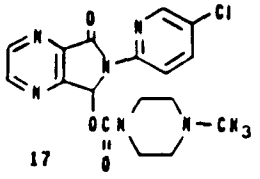


16

Sedative-Hypnotics - In vivo and in vitro studies have shown the clinically effective [118] hypnotic, zopiclone (17), to be comparable to BZ's in blocking the binding of ^{3}H-diazepam and ^{3}H-flunitrazepam to brain receptors. [119] The pharmacological profile of taglutimide (K-2004, 18) has been reported. [120] EMD-16923 (19), which was shown to be clinically effective in anxiety, produced an EEG-pattern in rabbits indicative of drowsiness, yet behaviorally, produced excitement.[121] A three-month study indicated that chlormethiazole (20) retained its hypnotic effectiveness in geriatric patients during prolonged-continuous use. [122]



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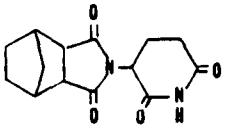

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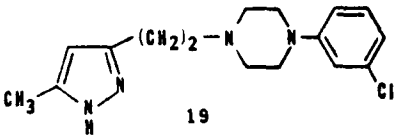

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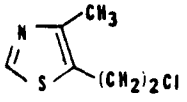
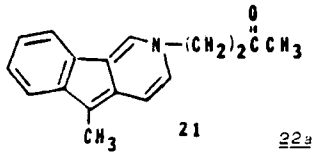


20

Anticonvulsants - A review on methodology in anticonvulsant drug screening has appeared.[123] The amygdaloid kindled rat has been proposed as a sensitive screen for anticonvulsant drugs.[124] QSAR studies on anticonvulsants have been published relating anti-electroshock (MES) activity of 15 phenylsuccinimide derivatives with total lipophilicity,[125] and on 13 various non-BZ's relating MES with both log P and log MW, and anti-PTZ activity with log P.[126] A possible relationship between stereochemical features and activity of anticonvulsant drugs may exist.[127]



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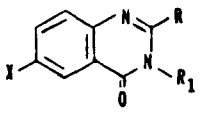


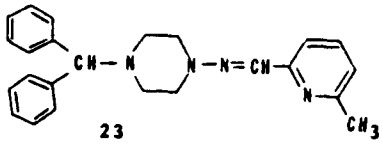
22a, R = CH_3; R_1 = 2-$CH_3C_6H_4$; X = H

22b, R = CH_2F; R_1 = 2-CH_3-3-ClC_6H_3; X = H

22c, R = CH_2F; R_1 = 2-$CH_3C_6H_4$; X = NH_2

22d, R = C_6H_5; R_1 = CH_2CONH_2; X = H

22e, R = C_6H_5; R_1 = CH_2CON⟨⟩; X = H

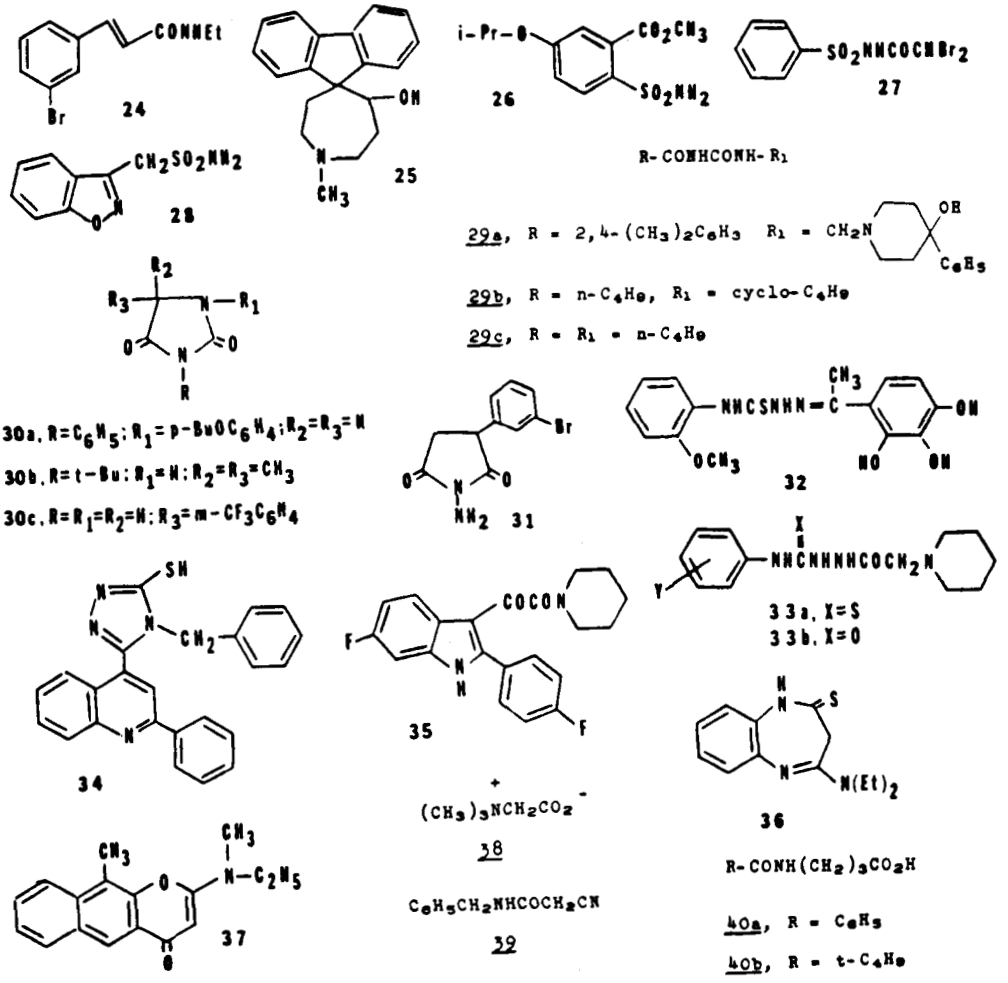


23

The indenopyridine YG 19-256 (21) protected photosensitive baboons against photically-induced seizures.[128]

Several methaqualone (22a) analogs, 22b-c, were found to be more active vs. PTZ and MES induced convulsions and less toxic than the parent compound.[129] The related quinazolones 22d[130] and 22e[131] protected against PTZ convulsions. Propizine (SC-13504, 23) was reported to be equipotent with phenobarbital in protection against MES, but ineffective against chemically induced seizures.[132] However, another group was able to observe anti-PTZ activity for this drug using a slightly modified test procedure.[133] Cinromide (24) was found potent vs. MES in rodents, although somewhat less effective vs. PTZ. The drug is currently being evaluated in man.[134] Compounds reported active

against MES and/or PTZ-induced convulsions in rodents are the azepine derivative 25,[135] sulfonamides 26,[136] 27,[137] and 28,[138] acylureas 29a,[139] b,[140] and c,[141] hydantoins 30a,[142] b,[143] and c,[144] succinimide 31,[145] thiosemicarbazone 32,[146] thiosemicarbazide 33a,[147] and semicarbazide 33b,[148] triazole 34,[149] indole 35,[150] 1,5-benzodiazepine 36,[151] naphthapyran 37,[152] betaine 38,[153] and cyanoacetamide 39.[154]



Increased GABA-ergic activity may also play a role in the action of anticonvulsants. Thus the GABA analogs 40a-b easily cross the blood-brain barrier, are enzymatically unmasked, and show anti-PTZ and anti-bicuculline activity.[155] The GABA agonist muscimol which crosses the blood-brain barrier, was also effective in these tests.[156,157] The GABA-reuptake inhibitor, L-2,4-diaminobutyric acid, was found effective in inhibiting convulsions induced by the GABA antagonists picrotoxin and 3-mercaptopropionate,[158] and the GABA-transaminase inhibitors γ-acetylenic and γ-vinyl-GABA protected photosensitive baboons against photically-induced seizures.[159]

References

1. W. S. Young, III, and M. J. Kuhar, Nature, 280, 389 (1979).
2. D. Comar, M. Maziere. J. M. Godot, G. Berger, F. Soussaline, C. Menini, G. Arfel, and R. Naquet, Nature, 280, 329 (1979).
3. P. Skolnick, P. J. Syapin, B. A. Paugh, and S. M. Paul, ibid., 277, 397 (1979).
4. C. Braestrup, M. Nielsen, G. Biggio, and R. F. Squires, Neuroscience Letters, 13, 219 (1979).
5. R. C. Speth and H. I. Yamamura, Eur. J. Pharmacol., 54, 397 (1979).
6. N. Bresolin, R. C. Speth, T. T. McManus, H. I. Yamamura, and L. Z. Stern, Ann. Neurology, 6, 160 (1979).
7. C. Braestrup, M. Nielsen, P. Krogsgaard-Larsen, and E. Falch, Nature, 280, 331 (1979).
8. E. Costa and A. Guidotti, Ann. Rev. Pharmacol. Toxicol., 19, 531 (1979).
9. I. L. Martin and J. M. Candy, Neuropharmacology, 17, 993 (1978).
10. P. G. Montaralo, F. Raschi, and P. Strata, Brain Res., 162, 358 (1979).
11. T. H. Chiu and H. C. Rosenberg, Eur. J. Pharmacol., 56, 337 (1979).
12. D. W. Gallagher, J. W. Thomas, and J. F. Tallman, Biochem. Pharmacol., 27, 2745 (1978).
13. M. Karobath, P. Placheta, M. Lippitsch, and P. Krogsgaard-Larsen, Nature, 278, 748 (1979).
14. M. Karobath and G. Sperk, Proc. Natl. Acad. Sci. U.S.A., 76, 1004 (1979).
15. M. Karobath and P. Placheta, Naunyn-Schmiedbergs Arch. Pharm., 307, 62 (1979).
16. M. Karobath and M. Lippitsch, Eur. J. Pharmacol., 58, 485 (1979).
17. A. Guidotti, M. Baraldi, J. P. Schwartz, and E. Costa, Pharmacol. Biochem. Behav., 10, 803 (1979).
18. R. F. Squires, D. I. Benson, C. Braestrup, J. Coupet, C. A. Klepner, V. Meyers, and B. Beer, ibid., 10, 825 (1979).
19. C. A. Klepner, A. S. Lippa, D. I. Benson, M. C. Sano, and B. Beer, ibid., 11, (1979).
20. J. F. Tallman and D. W. Gallagher, ibid., 10, 809 (1979).
21. J. M. Candy and I. L. Martin, Nature, 280, 172 (1979).
22. T. Costa, D. Rodbard, C. Pert, ibid., 277, 316 (1979).
23. A. S. Lippa, D. Critchett, M. C. Sano, C. A. Klepner, E. N. Greenblatt, J. Coupet, and B. Beer, Pharmacol. Biochem. Behav., 10, 825 (1979).
24. M. Babbini, M. Gaiardi, and M. Bartoletti, Life Sci., 25, 15 (1979).
25. R. C. Speth, N. Bresolin, and H. I. Yamamura, Eur. J. Pharmacol., 59, 159 (1979).
26. R. Squires, R. Naquet, D. Riche, and C. Braestrup, Epilepsia, 20, 215 (1979).
27. S. M. Paul and P. Skolnick, Science, 202, 892 (1978).
28. A. S. Lippa, C. A. Klepner, L. Yunger, M. C. Sano, W. V. Smith, and B. Beer, Pharmacol. Biochem. Behav., 9, 853 (1978).
29. G. Biggio, M. G. Corda, C. Lamberti, and G. L. Gessa, Eur. J. Pharmacol., 58, 215 (1979).
30. T. D. Reisine, G. J. Wastek, R. C. Speth, E. D. Bird, and H. I. Yamamura, Brain Res., 165, 183 (1979).
31. C. Braestrup, M. Nielsen, E. B. Nielson, and M. Lyon, Psychopharmacology, 65, 273 (1979).
32. H. C. Rosenberg and T. H. Chiu, Life Sci., 24, 803 (1979).
33. C. Braestrup, M. Nielsen, and R. F. Squires, ibid., 24, 347 (1979).
34. P. DiStefano, K. R. Case, G. D. Colello, and H. B. Bosmann, Cell. Biol. Int. Rep., 3, 163 (1979).
35. D. H. Overstreet and H. I. Yamamura, Life Sci., 25, 1865 (1979).
36. P. J. Marangos, S. M. Paul, F. K. Goodwin, and P. Skolnick, Life Sci., 25, 1093 (1979).
37. H. W. Damm, W. E. Muller, and U. Wollert, Eur. J. Pharmacol., 55, 331 (1979).
38. T. Asano and S. Spector, Proc. Natl. Acad. Sci. U.S.A., 76, 977 (1979).
39. P. Skolnick, P. J. Syapin, B. A. Paugh, V. Moncada, P. J. Maragos, and S. M. Paul, Proc. Natl. Acad. Sci. U.S.A., 76, 1515 (1979).
40. P. Skolnick, P. J. Marangos, P. Syapin, F. K. Goodwin, and S. M. Paul, Pharmac. Biochem. Behav., 10, 815 (1979).
41. P. J. Marangos, S. M. Paul, A. M. Parma, F. K. Goodwin, P. Syapin, and P. Skolnick, Life Sci., 24, 851 (1979).
42. H. Mohler, P. Polc, R. Cumin, L. Pieri, and R. Kettler, Nature, 278, 563 (1979).
43. G. D. Colello, D. M. Hockenbery, H. B. Bosmann, S. Fuchs, and K. Folkers, Proc. Natl. Acad. Sci. U.S.A., 75, 6319 (1978).
44. A. Guidotti, G. Toffano, and E. Costa, Nature, 275, 242 (1979).
45. J. C. Nixon and J. H. Woolf, Pharmacology, 21, 242 (1979).
46. M. Nielsen, O. Gredal, and C. Braestrup, Life Sci., 25, 679 (1979).
47. K. C. Rice, A. Brossi, J. Tallman, S. M. Paul, and P. Skolnick, Nature, 278, 854 (1979).
48. K. V. Speeg, Jr., S. Wang, G. R. Swant, M. L. Berman, and S. Schenker, Life Sci., 24, 1345 (1979).

49. S. M. Paul, P. J. Syapin, B. A. Paugh, V. Moncada, and P. Skolnick, Nature, 281, 688 (1979).
50. R. W. Lucek, W. A. Garland, and W. Dairman, Fed. Proc., 38, 541 (1979).
51. M. Davis, Psychopharmacology, 62, 1 (1979).
52. S. F. File and J. R. G. Hyde, Pharmac. Biochem. Behav., 11, 65 (1979).
53. G. P. Ryan and N. R. Boisse, Pharmacology, 21, 152, (1979).
54. M. Karobath, Trends Neur., 2, 166 (1979).
55. W. Schallek, W. D. Horst, and W. Schlosser in "Advances in Pharmacology and Chemotherapy", Vol. 16., Academic Press, 1979.
56. "Anxiolytics," S. Fielding and H. Lal, Eds., Futura Publishing Co., Mt. Kisco, N. Y., 1979.
57. K. Podiwinsky and K. Jellinger, Wien. Klin, Wochenschr., 91, 240 (1979).
58. Z. Rydzynski, A. Araszkiewicz and W. Gruszczynski, Acta. Nerv. Super. (Praha). 20, 248 (1978).
59. H. B. Lin and C. C. Chen, J. Formosan Med. Assoc., 78, 267 (1979).
60. L. F. Fabre and D. M. McLendon, Curr. Ther. Res., 25, 519 (1979).
61. D. M. McLendon and L. F. Fabre, ibid., 26, 430 (1979).
62. A. Castellani, M. Colafelice, M. Forini, and D. Perbellini, Clin. Ther., 2, 62 (1978).
63. W. Petro, Ther. Gegw., 118, (1979).
64. D. Wheatly, Psychosomatics, 20, 195 (1979).
65. S. Zisook, P. J. Rogers, T. R. Faschingbauer, and R. A. Devaub, J. Clin. Psychiatry, 39, 683 (1978).
66. L. F. Fabre, Jr., D. M. McLendon, Curr. Ther. Res., 25, 710 (1979).
67. B. M. Saxena, A. N. Singh, J. L. Nelson, and G. Mahutte, ibid., 25, 150 (1979).
68. A. N. Singh and B. Saxena, ibid., 26, (1979).
69. L. McCurdy, Am. J. Psychiatry, 136, 187 (1979).
70. W. R. Porter and W. J. Lancee, Clin. Ther., 2, 31 (1978).
71. L. F. Fabre, D. M. McLendon, and A. Mallette, Curr. Ther. Res., 25, 527 (1979).
72. J. H. Weir, J. Clin. Psychiatry, 39, 841 (1978).
73. H. L. Goldberg and R. J. Finnerty, Am. J. Psychiatry, 136, 196 (1979).
74. R. Sladka, J. Dostalova, V. Haskovcova, M. Jarosova, J. Slanska, F. Faltus, and V. Filip, Acta. Nerv. Super. (Praha), 20, 245 (1978).
75. J. T. Kelly, R. L. Zimmerman, and B. C. Schiele, Neuropsychobiology, 4, 283 (1978).
76. D. J. Greenblatt, M. D. Allen, S. Lochniskar, J. S. Harmatz, and R. I. Shader, Clin. Pharmacol.Ther., 26, 103 (1979).
77. D. J. Greenblatt, R. I. Shader, K. Franke, D. S. MacLaughlin, J. S. Harmatz, M. D. Allen, A. Werner, and E. Woo, J. Pharm. Sci., 68, 59 (1979).
78. Articles in Br. J. Clin. Pharmacol., 7, Suppl. 1 (1979).
79. J. B. Hester, Jr. and P. von Voightlander, J. Med. Chem., 22, 1390 (1979).
80. T. Kovac, F. Kajfez, V. Sienjic, N. Blazevic, and D. Kolbah, ibid., 22, 1093 (1979).
81. H. Ott, A. Doenicke, C. Abress, R. Fischl, K. G. Hemmerling, and K. Fichte, Anaesthesist, 28, 29 (1979).
82. F. Camu, Acta Anaesthesiol. Belg., 29, 191 (1978).
83. I. N. Hosein, R. de Freitas, and M H. Beaubrun, West Indian Med. J., 18, 45 (1979).
84. C. Maggini, M. Guazzelli, M. Mauri, G. F. deLiso, and E. Bernardi, Riv. Neurol.,
85. T. I. Roth, E. I. Tietz, M. Kramer, and M. Kaffeman, J. Int. Med. Res., 7, 583 (1979).
86. J. G. Reves, R. Vinik, A. M. Hirschfield, R. Halcomb, and S. Strong, Can. Anaesth. Soc. J., 26, 42 (1979).
87. J. L. Marti Viano, A. Nunez-Cacho de Lis, M. C. Llombart Rosa, and C. Bellvert-Ortiz, Rev. Espanola Anaest. Reanim. 25, 380 (1978).
88. C. R. Brown, F. H. Sarnquist, C. A. Canup, and T. A. Pedley, Anesthesiology, 50,. 467 (1979).
89. E. O. Bixler, M. B. Scharf, C. R. Soldatos, D. J. Mitsky and A. Kales, Life Sci., 25, 1379 (1979).
90. S. Kubicki, Z. EEG-EMG, 10, 95 (1979).
91. B. Saletu, J. Greenberger, J. Volavka, and P. Bernes, Arzneim.-Forsch., 29,(I), 700 (1979).
92. I. Feinberg, G. Fein, J. M. Walker, L. J. Prince, T. C. Floyd, and J. D. March, Arch. Gen. Psychiatry, 36, 95 (1979).
93. P. R. Sundaresan, W. M. Wardell, M. Weintraub, and L. Lasagna, Clin. Pharmacol. Ther., 25, 391 (1979).
94. S. C. Cheng and E. A. Brunner, Anesthesiology, 51, 543 (1979).
95. I. Oswald, Br. Med. J., 1, 1167 (1979).
96. M. W. Church, and L. C. Johnson, Psychopharmacology, 61, 309 (1979).
97. J. G. Gillen, W. B. Mendelson, W. C. Dement, and F. Solomon, Science, 205, 954 (1979).
98. A. Kales, M. B. Scharf, J. D. Kales, and C. R. Soldatos, J. Amer. Med. Assoc., 241, 1692 (1979).
99. Lancet, 1, 196 (1979).
100. R. B. Rastogi, Y. D. Lapierre, and R. L. Singhal, Prog. Neuro-Psychopharmac., 2, 43 (1978).

101. A. Kales, M. B. Scharf, C. R. Soldatos, and E. O. Bixler, J. Clin. Pharmacol., 19, 329 (1979).
102. T. G. Johns, D. C. Piper and G. W. L. Walls, Arch. Int. Pharmacodyn., 240, 53 (1979).
103. R. A. Shakir, R. N. Nanda, D. G. Lambie, and R. H. Johnson, Arch. Neurol., 36, 301 (1979).
104. I. Heidler, J. Mares, and S. Trijan, Acta. Nerv. Super. (Praha), 21, 13 (1979).
105. A. S. Troupin, P. Friel, A. J. Wilensky, L. Moretti-Ojemann, R. H. Levy, and P. Feigl, Neurology, 29, 458 (1979).
106. A. G. Chapman, R. W. Horton, and B. S. Meldrum, Epilepsia, 19, 293 (1979).
107. B. S. Meldrum and R. W. Horton, Psychopharmacology, 60, 277 (1979).
108. T. Sukamoto, K. Ito, and T. Nose, Jap. J. Pharmacol., 28 (Suppl.), 33P (1978).
109. T. Mitshushima and S. Ueki Nippon Yakurigaku Zasshi, 78, 959 (1978). [Chem. Abstr., 90, 180083u (1979)].
110. J. D. Albright, R. I. Trust, and D. B. Moran, Abstracts of Papers 177th National Meeting ACS, Honolulu, Hawaii, April, 1979, MEDI 10.
111. A. S. Lippa, J. Coupet, E. N. Greenblatt, C. A. Klepner, and B. Beer, Pharmac. Biochem. Behav., 11, 99 (1979).
112. C. R. Rasmussen and J. F. Gardocki, Abstracts of Papers, 178th National Meeting ACS, Washington, D. C., September 1979, MEDI 24.
113. B. Beer, C. A. Klepner, A. S. Lippa, and R. F. Squires, Pharmac. Biochem. Behav., 9, (849) 1978.
114. M. Williams and E. A. Risley, Life Sci., 24, 833 (1979).
115. H. L. Goldberg, Psychopharmacol. Bull., 15, 90 (1979).
116. L. Conti and R. M. Pinder, J. Int. Med. Res., 7, 285 (1979).
117. J. V. Ananath, J. H. Sohn, T. A. Ban and H. E. Lehmann, Curr. Ther. Res., 25, 133 (1979).
118. R. Duriez, C. Barthelemy, H. Rives, J. Courjaret, J. Gregoire, Therapie, 34, 317 (1979).
119. J. C. Blanchard, A. Boireau, C. Ganet, and L. Julou, Life Sci., 24, 2417 (1979).
120. W. G. Schutzenberger, N. Kolassa, H. Wiener, O. Kraupp, and E. Tuisal, Arzneim.-Forsch., 29,(II), 1146 (1979).
121. S. Watanabe, H. Kawasaki, and S. Ueki, ibid., 29(I), 274 (1979).
122. O. Dehlin, T. Falkheden, R. Gatzinska, and P. Nordquist, Clin. Ther., 2, 41 (1978).
123. R. L. Krall, J. K. Penny, B. G. White, H. J. Kupferberg, and E. A. Swinyard, Epilepsia, 19, 409 (1978).
124. D. Ashton and A. Wauquier, Psychopharmacology, 65, 7 (1979).
125. J. Lapszewicz, J. Lange, S. Rump, and K. Walczyna, Eur. J. Med. Chem., 13, 4654 (1978).
126. E. J. Lien, R. C. H. Liao, and H. G. Shinouda, J. Pharm. Sci., 68, 463 (1979).
127. A. Camerman and N. Camerman, Acta Crystallographica, Sec. A., 34, 581 (1978).
128. B. S. Meldrum and R. W. Horton, Experientia, 35, 796 (1979).
129. J. Tani, Y. Yamada, T. Oine, T. Ochiai, R. Ishida, and I. Inoue, J. Med. Chem., 22, 95 (1979).
130. V. S. Misra and R. N. Pandey, J. Indian Chem. Soc., 55, 1046 (1978).
131. V. S. Misra, R. N. Pandey and P. R. Dua, Pol. J. Pharmacol. Pharm., 30, 573 (1978).
132. H. L. Edmonds, Jr., L. G. Stark, D. M. Stark, C. R. McCormack, D. M. Sylvester, and S. I. Bellin, J. Pharmacol. Exp. Ther., 208, 236 (1979).
133. G. D. Novack, L. G. Stark, and S. L. Peterson, Neuropharmacology, 17, 659 (1978); idem., J. Pharmacol. Exp. Ther., 208, 480 (1979).
134. F. E. Soroko, E. M. Grivsky, B. T. Kenny, R. E. Bache, and R. A. Maxwell, Fed. Proc., 38, 753 (1979).
135. Y. Nagai and H. Uno, Chem. Pharm. Bull., 27, 2056 (1979).
136. V. A. Shkulev, L. S. Abovyan, I. A. Dzhagatspanyan, N. E. Akopyan and D. L. Mundzhoyan, Khim.-Farm. Zhr., 13(2), 36 (1979). [C.A., 90, 203623m (1979)].
137. H. Uno, M. Kurokawa, U. Masuda, and H. Nishimura, J. Med. Chem., 22, 180 (1979).
138. G. E. Batrak, M. T. Plotnikova, E. T. Zlenko, S. I. Khrustalev. and M M. Kremlev, Khim.-Farm. Zhr., 13(1), 32 (1979). [C.A., 91, 13649x (1979)].
139. B. R. Pandey, A. K. Gupta, K. Raman, and S. S. Parmar, Res. Comm. Chem. Path. Pharmacol., 23, 349 (1979).
140. K. A. Zirvi and T. Fakouhi, Il Farmaco (Ed. Sci.), 34, 170 (1979).
141. M. S. Dar, K. Zirvi, and T. Fakouhi, ibid., 34, 936 (1979).
142. P. C. Joshi, S. S. Parmar, and V. K. Rastogi, J. Heterocyclic Chem., 16, 607 (1979).
143. S. Chiu, L. Keifer, and J. W. Timberlake, J. Med. Chem., 22, 746 (1979).
144. C. A. Risinger and N. B. Mehta, Abstracts of Papers, 177th National Meeting ACS, Honolulu, Hawaii, April, 1979, MEDI 15.
145. S. Rump, I. Ilczuk and K. Walczyna, Anzneim.-Forsch., 29(I), 290 (1979).
146. S. Tripathi, B. R. Pandey, K. Raman, J. P. Barthival, K. Kislion, and K. P. Bhargava, Res. Comm. Chem. Pathol. Pharmacol. 22, 291 (1978).

147. R. S. Misra, S. S. Parmar, V. Kishore, B. Ali, and T. K. Gupta, J. Heterocyclic Chem., 16, 613 (1979).
148. R. S. Misra, C. Drivedi, S. S. Parmar, and S. P. Singh, ibid., 15, 681 (1978).
149. A.S.B. Hazzaa, N. S. Halib, S. M. El-Khawass, T. T. Daabees, F. M. Sharahi, and G. G. Tawil, Sci. Pharm., 46, 298 (1978).
150. K. C. Joshi, V. N. Patshak, and P. Chand, Agric. Biol. Chem. 42, 1723 (1978).
151. G. Roma, E. Vigevani, A. Balbi, and A. Emili, Il Farmaco (Ed. Sci.), 34, 62 (1979).
152. G. Roma, E. Vigevani, M. Mazzei, A. Emili, A. Ambrosini, and N. Passerini, ibid., 33, 822 (1978).
153. W. J. Freed, J. C. Gillin, and R. J. Wyatt, Epilepsia, 20, 209 (1979).
154. C. M. Darling and P. Pryor, J. Pharm. Sci., 68, 108 (1979).
155. L. Galzigna, L. Garbin, M. Bianchi, and A. Marzotto, Arch. Int. Pharmacodyn., 235, 73 (1978).
156. R. C. Collins, Neurology, 29, 603 (1979).
157. W. D. Matthews and G. P. McCafferty, Neuropharmacology, 18, 885 (1979).
158. P. V. Taberner and F. Roberts, Eur. J. Pharmacol., 52, 281 (1978).
159. R. W. Horton and B. S. Meldrum, Br. J. Pharmacol., 63, 390P (1978).

Chapter 4. Analgesics, Endorphins, and the Opiate Receptor

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Introduction - The endorphin system has appeared to become more and more complex since the discovery of the enkephalins in 1975. During 1979 additional developments have included the discovery of several novel endorphins - particularly dynorphin and its congeners - and the addition of new sub-classifications of opioid receptors. Relevant reviews published during 1979 include those on biology of opioid peptides;[1] peptide neurotransmitters;[2] opiates, opioid peptides and single neurones;[3] the relation of opioid peptides and morphine to neuroendocrine function;[4] basic and clinical studies of endorphins;[5] and endorphins: new gut peptides.[6] Other summaries of relevant research include "clinical relevance of the opioid receptor and opioid receptor research",[7] "opioid peptides and their relatives",[8] "pain, enkephalin and acupuncture",[9] and "is substance P a transmitter of pain signals".[10] The proceedings of the 1979 International Narcotic Research Conference have recently been published.[11]

Enkephalins and Endorphins

While the occurrence of the Met^5-enkephalin sequence at the N-terminus of β-endorphin indicates a biosynthetic origin of Met^5-enkephalin, the lack of identification of an analogous Leu^5-enkephalin precursor implies that the endorphin system is complex. As early as 1976, Goldstein suggested that most of the endorphin activity of the pituitary gland was not attributable to β-LPH related peptides.[12] Recently, this group has described the properties of the tridecapeptide 1 isolated from porcine melanotropin concentrate, which they suggest corresponds to the N-terminal sequence of dynorphin, a novel pituitary endorphin. Dynorphin, if it is identical to "slow reversing endorphin" has a molecular weight of approximately 1750 daltons.[14] Dynorphin (1-13) (1) has a molecular weight of 1604 daltons indicating that the parent peptide has a total sequence of 14-15 residues. The structure of dynorphin (1-13) is particularly interesting as the Leu^5-enkephalin C-terminus is followed by an Arg-Arg sequence, potentially allowing facile release of Leu^5-enkephalin. In the isolated guinea-pig ileum preparation, 1 is 700 times more potent than Leu^5-enkephalin, while in the mouse vas deferens, it is 3 times more potent. Differences between 1 and other endorphins are also apparent in its receptor kinetics, naloxone reversibility, and enzymic stability. Another endorphin related to Leu^5-enkephalin, α neo-endorphin, 2, has been described by Japanese workers.[15] It can be seen that this peptide, isolated from porcine hypothalami, is similar in amino acid composition and partial sequence to 1. The rigorous characterization and comparison of these peptides should clarify their relationship to each other.

$$\overset{5}{\text{Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys}}\overset{10}{\phantom{\text{Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys}}} \qquad 1$$

The isolation of a number of putative precursors of Met^5-enkephalin has been reported. Interestingly, the sequences of these peptides appear

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unrelated to β-endorphin and suggest a precursor of the "dynorphin" type - i.e., a basic residue adjacent to the C-terminus of the enkephalin pentapeptide. Huang et al[16] have reported the isolation of the hexapeptide 3 from porcine hypothalami. Several peptides with opioid (radio-receptor) activity have been discovered in bovine adrenal medulla.[17] One of these has been characterized as the heptapeptide 4. This heptapeptide has also been found in beef striatum in amounts comparable to Leu-enkephalin.[18]

Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Arg-(Pro,Gly,Tyr_2,Lys,Arg) - 2
H-Tyr-Gly-Gly-Phe-Met-(O)-Arg-OH - 3
H-Tyr-Gly-Gly-Phe-Met-Arg-PheOH - 4

The identification of N-acetyl porcine β-LPH_{61-91} and N-acetyl β-LPH_{61-87} has led to the suggestion that endorphins may be stored as "biologically inactive" forms.[19] The fact that the N-acetyl peptides retain full immunoreactivity with antisera directed against the parent peptide provides further evidence of the potential danger of "characterisation" by immunoassay methods. The distribution of β-LPH_{61-91}, NAc β-LPH_{61-91}, β-LPH_{61-87} and NAc β-LPH_{61-87} have been studied in rat pituitary and brain.[20] Whereas the 61-87-derived peptides predominate in the pituitary, β-LPH_{61-91} was the major component in the hypothalamus and midbrain. These results provide further proof that the processing of lipotropin is under differential control in anatomically distinct regions of the central nervous system. Interestingly, salmon "endorphin" has recently been characterised as an N-α-acetyl peptide.[21] This twenty-nine residue peptide includes the Met-enkephalin sequence in the 13 positions with which it shows homology with mammalian endorphins. The search for a Leu^5-analogue of β-endorphin continues: a peptide isolated from the hemodialysate of a psychotic patient has been claimed to behave like synthetic Leu^5-β-endorphin in several biological and radio-immunoassays.[22] However, it has been shown that total endorphin immunoreactivity in plasma does not differ greatly between groups of schizophrenic patients and normal subjects.[23] In another report, levels of Met^5- or Leu^5-β-endorphin did not exceed 30 pmole L^{-1} in the dialysates of schizophrenics or controls.[39] The dipeptide HTyr-ArgOH (Kyotorphin) has been isolated and identified in bovine brain by its ability to induce antinociception in mice (tail pinch method) following central administration.[24] The effects of the dipeptide in vivo are reversible by naloxone, but the compound does not inhibit the electrically-induced contractions of the guinea-pig ileum. More recently, it has been suggested that Tyr-Arg is a possible Met-enkephalin "releaser", as the dipeptide increases the release of Met-enkephalin from superfused guinea-pig striatal slices.[25]

The so-called "exorphins" have been detected in pepsin hydrolysates of wheat gluten and α-casein.[26] The heptapeptide β-casomorphin, 5, has been isolated from an enzymatic digest of bovine β-casein.[27] The N-terminal pentapeptide, obtained by carboxypeptidase Y digestion showed a higher opioid activity on the isolated guinea-pig ileum.

Several reports on novel approaches to synthetic analogues of the enkephalins/endorphins have been published. A series of amine (e.g. 6) and amine oxide (e.g. 7) analogues related to "descarboxy" Leu^5-enkephalin (8) have been described.[28]

Tyr-Pro-Phe-Pro-Gly-Pro-Ile-OH - 5
Tyr-D-Ala-Gly-MePhe-NH-R

R = $CH_2CH_2NMe_2$ - 6 ; R = $CH_2CH_2N(\rightarrow O)Me_2$ - 7; R = $CH_2CH_2CHMe_2$ - 8

Typically, the amine and amine oxide series are 4-10 times more potent than morphine in the rat tail flick assay. The amine oxide series is unusual, however, in that certain members of the series show extremely high potency (0.00002-0.0000008 mg kg^{-1}) in the mouse writhing assay. More detailed descriptions of structure-activity relationships in the Sandoz "sulphoxide" series have appeared.[29,30] The synthesis and biological activity of C-terminally modified "retro-inverso" analogues of enkephalin have been reported.[31] More results are necessary, however, before the general applicability of the "retro-inverso" concept of reversal of peptide bonds can be established. It must be remembered that the enkephalin analogues described to date retain intact those structural features essential for activity at opioid receptors.

Debate has continued on the enzyme(s) responsible for the degradation of enkephalin. It is now thought that enkephalinase is unlikely to be identical to angiotensin converting enzyme.[3,33,34] However, the influence, if any, of the angiotensin converting enzyme inhibitor captopril on the actions of opioids is unclear. In a number of well controlled experiments in vitro and in vivo it has been shown that captopril does not exert any influence on the actions of exogenous or endogenous enkephalins.[35] In contrast, some interaction between captopril and morphine has been demonstrated in mice.[36] The latter results cannot be explained by simple potentiation of the effects of either exogenous morphine or endogenous opioids. Purification of enkephalin degrading activity from a solubilised membrane preparation of rat brain has yielded fractions which give rise to free tyrosine (aminopeptidase action), an enzyme designated enkephalinase A which generates Tyr-Gly-Gly and a newly reported enkephalinase B which generates a Tyr-Gly fragment.[37] The role of these enzymes in the synaptic inactivation of the enkephalins is not clear. Data on the degradation of leucine^5-enkephalin by intact N4TG1 neuroblastoma cells strongly suggest that there is no correlation between receptor occupancy and the rate of enkephalin degradation.[38]

Physiological Role and Clinical Studies

There has been considerable interest in the possible roles of various endorphins in a variety of physiological and pharmacological processes. Many studies have used antagonism by naloxone as the criterion for implicating endogenous opiates in a process, but the specificity of action of naloxone has now been questioned.[40] It is confirmed that endogenous opiates and morphine do not effect spontaneous release of prolactin in vitro, but block dopamine inhibition of prolactin secretion.[41-43] It is further confirmed that naloxone (0.06-0.6 mg kg^{-1}) reverses morphine-induced increases in prolactin (PRL) secretion in rats,[44] and naloxone (0.2 mg kg^{-1}) lowers basal prolactin levels (60 mins post-administration) in man,[45] but similar effects in female patients (0.8-20 mg kg^{-1})[46] and in sexually unspecified psychiatric patients[47] on growth hormone and prolactin secretion were absent. Naloxone (2.5 mg kg^{-1} s.c.) leads to increases in serum luteinising hormone in the prepubertal female,[49] but not in prepubertal male rats, possibly implicating opioid peptide modulation of luteinising hormone secretion during sexual maturation. Naloxone also enhances sexual performance in male rats.[50] Post-menopausal flushing in women has been found to be invariably associated with large increases in LH, small increases in follicle stimulating hormone but no change in prolactin levels.[51] No significant changes in dopamine, noradrenaline and adrenaline levels were observed, suggesting non-involvement of peripheral adrenergic mechanisms in this flushing. Naloxone was shown to inhibit post-menopausal flushing in a number of women.[52] Naloxone is reported to

inhibit both chlorpropamide induced flushing in diabetics and that produced by FK 33-824.[53]

A combination of fluorescence and immunocytochemical techniques[54] and lesion studies[55] show complementary distributions of enkephalins and catecholamines in rat brain. Morphine's effects may be mediated by the initial release of adenosine.[56]

Further studies with morphine and D-Ala^2, D-Leu^5-enkephalin suggest 5-hydroxytryptamine involvement in modulation of antinociceptive effects of opiates, but again the precise nature of this is ill-defined.[57] Direct evidence has emerged to implicate spinal 5-hydroxytryptamine and noradrenaline terminals in mediation of spinal antinociceptive effects of morphine in the periaqueductal gray.[58]

The issue of endorphinergic involvement in the aetiology of mental disorders has become more complex.[59-62] Previous reports of inactivation of enkephalin type materials in CSF of schizophrenics have been shown unlikely to be due to degradation of β-endorphin related peptides in CSF.[63]

Previous open studies hypothesizing endorphin involvement in psychotic symptoms were again not confirmed by treatment of schizophrenics with naloxone (1.6 mg).[64] Nevertheless, the synthetic enkephalin FK 33-824 (0.5-1.5 mg/patient) is reported to show some antipsychotic activity of short duration.[66,67]

Although Des-Tyrosine^1-γ-endorphin showed no haloperidol-like neuroleptic activity in the rat,[68] evidence of activity was seen in a subset of neuroleptic-resistant schizophrenics.[69]

Several reports confirm that naloxone (1.2-3.5 mg kg^{-1} i.v.) successfully reverses some effects of alcohol intoxication in man,[70-72] alone and in combination with diazepam, lithium, methaqualone and phenobarbitone,[73] although attempted antagonism of the effects of diazepam in rats with naloxone (100-150 mg kg^{-1}) or naltrexone (172 mg kg^{-1}) show limited effects.[74] The antidiarrhoeal activity of loperamide, presumed to be due to effects on smooth muscle contraction is antagonized by naloxone and thus may be opiate receptor-mediated.[75] SKF 525-A (structurally related to propoxyphene) shows antinociceptive action in mice (ED_{50} 60 mg kg^{-1} i.p. vs. 24 mg kg^{-1} i.p., mouse tail flick) partially reversible by naloxone (4 mg kg^{-1}).[76]

(-)-Naloxone (20 μg ml^{-1} i.c.v.) has been shown to reverse cardiovascular side effects (arterial hypotension and heart rate) in halothane anaesthetised dogs, whereas (+)-naloxone was without significant effect.[77] Naloxone (0.2-2 mg kg^{-1}) also inhibits or reverses the hypertension in unanaesthetised spontaneously hypertensive rats, suggesting the possibility of an endorphinergic component in control of sympathetic tone.[78]

Recent reports implicate both endogenous[79,80] and exogenous[26] peptides in regulation of obesity. The overall picture is complex,[81] high levels of pituitary endorphin in ob/ob mice appear more a consequence of obesity than a cause,[82] and even anorexic/behavioral effects caused by overcrowding in rats can be reversed by naltrexone.[83] Further reports confirm reduction in food intake by naloxone.[84,85] The hypotension and pulse pressure drop in acutely bled conscious rats is reversed sustainably by naloxone, inferring an endorphinergic role in the pathophysiology of hypovolemic shock.[86]

Opiate Receptors

A wide variety of drugs were examined for their ability to induce transfer of (^{3}H)-cerebroside sulphate (CS) used as a model receptor, from an aqueous to a non-aqueous phase in order to examine the postulate that agonist and antagonist actions are determined by the physiochemical difference in properties between drug-receptor complexes.[87] These transfers and their inhibitions by various antagonists, correlated closely with their analgetic potencies in man, suggesting a similarity between opiate-CS and opiate-receptor interactions. The drug-CS complexes appear to be of two types, hydrophobic and hydrophilic, and it is proposed that the hydrophilicity (and hence agonist/antagonist character) of the drug-CS complex is associated with degree of hydration rather than ionic bond strength.

Low concentration binding studies of ^{125}I-(D-Ala^2,-D-Leu^5) enkephalin, (^{3}H)-naloxone and (^{3}H)-dihydromorphine to rat brain preparations enable clear differentiation between at least two receptor sites of differing affinities,[88] and data suggests the hydrophobic group of the enkephalin phenylalanine residue could be responsible for the receptor differentiation between morphine and enkephalin. Other detailed studies show regional distribution of a multiplicity of receptors in rat brain preparations.[89] Image intensified fluorescence microscopy has been used to study binding of a bioactive enkephalin analogue, Tyr-D-Ala-Gly-Phe-Leu-Lys-rhodamine to N4TG1 neuroblastoma cells.[90] Reactive sulphydryl and disulphide groups appear essential for clustering of these cells (but not binding). Sulphydryl reagents (10^{-3}M iodoacetate, 10^{-4}M iodoacetamide or 10^{-3}M dithiothreitol) seem to dissociate between binding and cluster formation.

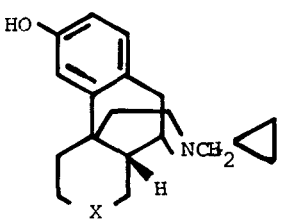
Quantum-mechanical conformation (PCILO) calculations for a series of oripavine derivatives show low energy conformers of carbinol substituents on C_7-C_{19}-R_1R_2OH with and without intramolecular H-bonding to the C_6-OCH_3 groups.[91] These results are consistent with NMR, IR and crystallographic data, and emphasize the potential role of a lipophilic binding site for C_{19} carbinols to explain the differing activities of diastereoisomers at C_{19}.

Analgetics

Morphine - Morphinans - The role of the phenolic group in opiate receptor binding and antinociception has been examined in a series of morphine derivatives.[92] 3-Deoxydihydromorphinone and 3,6-dideoxydihydromorphine were approximately equipotent with morphine in the hot-plate assay. The 3-hydroxy analogue in each case was more potent, but binding (rat brain, 0.32 m sucrose, 0.01 M Tris, pH 8.0, 37°) in all cases was significantly reduced.

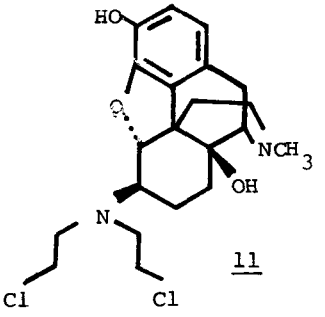
The N-(2-cyanoethyl) moiety substituted in various classes of opioids causes paradoxical pharmacological effects. Large increases in antinociceptive potency (hot-plate, Nilsen assay) for the 3-hydroxymorphinan, (-)-normetazocine,[93] and 9-α-ethyl-5-methyl-6,7-benzomorphan analogues[94] were seen. This substitution apparently causes lowered physical dependence capacity in rhesus monkeys whilst suppression does not produce a typical morphine-like withdrawal syndrome.[95] The same substitution on morphine, codeine, oxymorphone,[93] and ketobemidone and prodine analogues[94] did not improve antinociceptive potency. Enantiomer differentiation by the opiate receptor appeared to increase with this substitution in the 3-hydroxymorphinan and normetazocine analogues.

The 6-oxamorphinan 9, whilst being an opioid antagonist approximately equipotent with naloxone, but of longer duration, is also an agonist in the hot-plate test (ED_{50} 0.32 mg kg^{-1}).[96] UM1150, 10, is a potent long-acting (4.0 hours in monkey) antagonist retaining hot-plate activity (ED_{50} 17.2 mg kg^{-1}).[96] Chloroxymorphamine (COA), 11, the 6 β-N-mustard derivative of oxymorphone is a potent nonequilibrium narcotic agonist in the guinea pig ileum preparation.[97] The nonequilibrium behavior of COA is relevant to the mode of agonist/receptor interaction, since it is receptor occupancy, rather than rate of receptor interaction which is important for agonist activity of COA.



9, X = O

10, X = C = O



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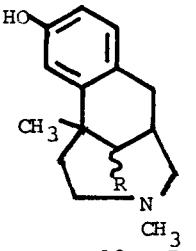


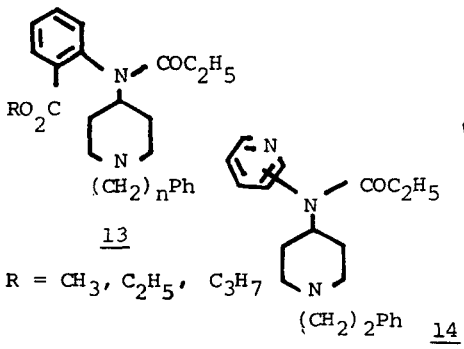
12a, R = H

b, R = αCH3

c, R = βCH3

Benzomorphans - The medicinal chemistry of the important series of 5-phenyl-6,7-benzomorphans,[98] and the full papers related to two other 6,7-benzomorphan series referred to last year, have been published.[99,100] Two examples of the class of 2,6-methano-3-benzazocin-11β-yl alkanones have been shown not to substitute for morphine.[95]

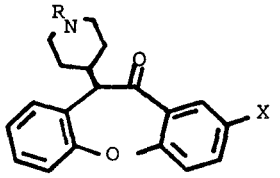
Resolution of the previously reported diastereoisomeric mixtures 12 has been accomplished.[101] Predictably, activity resides in the levo-isomer



13

R = CH_3, C_2H_5, C_3H_7

14



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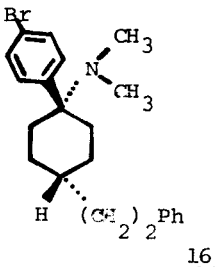


16

for 12b, c,- but surprisingly little difference was seen for isomers of 12 a. Although active in the mouse tail pressure test (0.7-26.5 mg kg^{-1} s.c. and in the hot-plate (0.49-17.9 mg kg^{-1} s.c.), they do not suppress morphine withdrawal symptoms in the precipitated withdrawal test.[96] Behavioural responses accompanied by self-administration are seen with some of these drugs.[96]

Substituted Piperidines - Weakly active (hot-plate) analogues of fentanyl bearing carbalkoxy substituents, 13,[102] and the three isomeric N-(pyridyl) analogues 14,[103] have been reported. The 2-pyridyl analogue was marginally

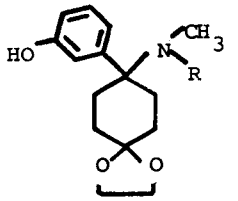
less potent than fentanyl (hot-plate, 0.14 vs 0.09 mg kg^{-1} s.c.), the others (3-, and 4-pyridyl) being both less potent (hot-plate, 0.26 and 4.1 mg kg^{-1} s.c.) and less toxic (LD_{50}'s of 66, 78.5 and 110 mg kg^{-1} s.c. for the three isomers, respectively). Like fentanyl, all three isomers showed rapid onset and durations of activity of up to 90 minutes. A series of 10,11-dihydro-11-oxospiro(dibenz(bis)oxepin-10,4-piperidine) derivatives 15, derived by combination of a psychotropic tricyclic nucleus with a piperidine moiety, have only weak activity in the rat tail flick test (1.6-7.0 mg kg^{-1}) and in the phenylquinone writhing test.[104] Lack of antagonism by naloxone for one analogue may indicate non-opioid-mediated analgesia.

Miscellaneous - A structurally novel series of 4-aryl-4-aminocyclohexane derivatives have both interesting agonist and antagonist properties. Compound 16 is 10,000 times as potent as morphine in the mouse tail flick and the HCl writhing assay (0.0001 and 0.0001 mg kg^{-1} s.c. vs. 1.5 and 0.5 mg kg^{-1} s.c. for morphine in the same assay). These effects are antagonized by naloxone.[105] Compound 17a displayed weak (13 vs. 1.5 mg kg^{-1} for naloxone) antagonism of the effects of morphine (6.3 mg kg^{-1} morphine sulphate).[106] One example, 17b (at 4 mg kg^{-1}), has been reported to only partially substitute for morphine,[95] and to neither suppress nor precipitate abstinence signs in monkeys.[96]

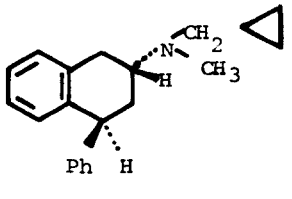
Two derivatives of 1-phenyl-3-aminotetralin showed only weak antinociceptive potency (ED_{50} 17.8 mg kg^{-1} s.c. hot-plate) or weak antagonism of morphine (AD_{50} 33.5 mg kg^{-1} s.c. vs. 6.5 mg kg^{-1} morphine), respectively.[107] One example, UM1153, 18, at 5 mg kg^{-1} neither suppressed nor precipitated the signs of morphine dependence.[96]

In a series of tropanes 19, the 3β-substituted analogues show greater antinociceptive potency than the 3α ones (1.80-0.047 vs. 35.2-2.22 mg kg^{-1} s.c., tail flick),[108] and greatest potency within the series is found in compounds substituted with benzyl or phenethyl on the tropane nitrogen atom.

A limited series of Δ^9-tetrahydrocannabinol derivatives 20 are devoid of significant analgesic activity (hot-plate).[109] 1,2-Diphenyl-2-(4-substituted 1-piperazinyl)ethanol analogues 21, showed only moderate activity up to about 3 times that of codeine (in the D'Amour Smith test).[110] A further series of substituted piperazine analogues appeared only weakly active (Haffner test) and quite toxic.[111]



17a, R = CH_3
b, R = C_2H_5



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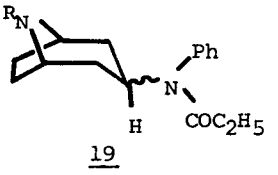

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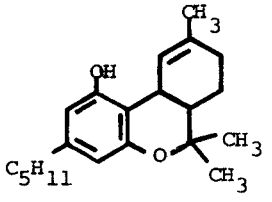

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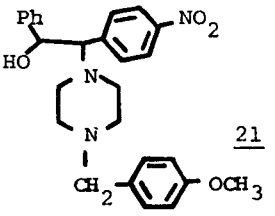

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References

1. A. Beaumont, J. Hughes, Ann. Rev. Pharmacol.Toxicol., 19,245 (1979).
2. S.H. Snyder, R.B. Innis, Ann. Rev. Biochem., 48, 755 (1979).
3. R.A. North, Life Sciences, 24, 1527 (1979).
4. J. Meites, J.F. Bruni, D.A. Van Vugt, A.F. Smith, Life Sci., 24, 1325 (1979).
5. W.E. Bunney Jr., C.B. Pert, W. Klee, E. Costa, A. Pert and G.C. Davis, Ann. Internal. Med., 91, 239 (1979).
6. R.F. Ambinder and M.M. Schuster, Gastroenterology, 77, 1132 (1979).
7. S.H. Snyder, Nature, 279, 13 (1979).
8. J. Hughes, Nature, 278, 394 (1979).
9. S.H. Chung and A. Dickinson, Nature, 283, 243 (1979).
10. J.L. Marx, Science, 205, 886 (1979).
11. "Endogenous and Exogenous Opiate Agonists and Antagonists", E.L. Way (Ed.), Pergamon Press, New York (1980).
12. S. Gentlemen, M. Ross, L.I. Lowney, B.M. Cox and A. Goldstein in "Opiates and Endogenous Opioid Peptides", H.W. Kosterlitz(Ed.) Elsevier North Holland Biomedical Press Amsterdam, 1976, page 27.
13. A. Goldstein, S. Tachibana, L.I. Lowney, M. Hunkapiller and L. Hood, Proc. Nat. Acad. Sci., U.S.A. ,76, 6666 (1979).
14. L.I. Lowney, S.B. Gentlemem and A. Goldstein, Life Sciences, 24, 2377 (1979).
15. K. Kangawa, H. Matsuo and M. Igarishi, Biochem. Biophys. Res. Commun., 86, 153 (1979).
16. W.Y. Huang, R.C.C. Cheng, A.J. Kastin, D.H. Coy and A.V. Schally, Proc. Natl. Acad. Sci., U.S.A., 76, 6177 (1979).
17. R.V. Lewis, A.S. Stern, J. Rossier, S. Stein and S. Udenfriend, Biochem. Biophys. Res. Commun., 89, 822 (1979).
18. A.S. Stern, R.V. Lewis, S. Kimura, J. Rossier, L.D. Gerber, L. Brink, S. Stein and S. Udenfriend, Proc. Natl. Acad. Sci., U.S.A., 76, 6680 (1979).
19. D.G. Smyth, D.E. Massey, S. Zakarian and M.D.A. Finnie, Nature, 279, 252 (1979).
20. S. Zakarian and D. Smyth, Proc. Natl., Acad. Sci., U.S.A., 76, 5972 (1979).
21. H. Kawaachi, M. Tubokawa and K. Muramoto, Biochem. Biophys. Res. Commun., 88, 1249 (1979).
22. B.M. Cox, M. Ross, A. Goldstein and R.M. Palmour, Brain Research, 165, 311 (1979).
23. M. Ross, P.A. Berger, and A. Goldstein, Science, 205, 1163 (1979).
24. H. Takagi, H. Schiomi, H. Ueda and H. Amano, Eur. J. Pharmacol., 55, 109 (1979).
25. H. Takagi, H. Schiomi,H. Ueda and H. Amano, Nature, 282, 410 (1979).
26. C. Zioudrou, R.A. Steaty and W.A. Klee, J. Biol. Chem., 251, 2446 (1979).
27. A. Henschen, V. Brantl, H. Teschemacher and F. Lottspeich, in ref. 11, page 223.
28. J.D. Bower, B.K. Handa, A.C. Lane, J.A.H. Lord, G. Metcalf, B.A. Morgan, M.J. Rance, P.M. Richards and C.F.C. Smith, in ref. 11, page 29.
29. J. Pless,W. Bauer, F. Cardinaux, A. Closse, D. Hauser, R. Huguenin, D. Roemer, H.H. Buescher and R.C. Hill, Helv. Chim. Acta., 62, 398 (1979).
30. D. Roemer and J. Pless, Life Sciences, 24, 621 (1979).
31. M. Chorev, R. Chavitz, M. Goodman, S. Minick and R. Guillemin, Science, 204, 1210 (1979).
32. J.P.Swerts, R. Perdrisot, B. Malfroy and J.C. Schwartz, Eur. J. Pharmacol., 53, 209 (1979).
33. J.P. Swerts, R. Perdrisot, G. Patey, S. De la Baume and J.C. Schwartz, Eur. J. Pharmacol., 57, 274 (1979).
34. A. Arregui, G.M. Lee, P.C. Emson and L.L. Iversen, Eur. J. Pharmacol., 59, 141 (1979).
35. W.R. Buckett, Eur. J. Pharmacol., 57, 267 (1979).
36. R.K. Türker, M. Ilhan and Z.S. Ercan, Eur. J. Pharmacol., 58, 99 (1979).
37. C. Gorenstein and S.H. Snyder, Life Sciences, 25, 2065, (1979).
38. E. Hazum, K.J. Chang and P. Cuatrecasas, Life Sciences, 24. 137 (1979).
39. R.V. Lewis, L.D. Gerber, S. Stein R.L. Stephen, B.I. Grosser, S.F. Velick and S. Undenfriend, Arch. Gen. Psychiatry, 36, 237 (1979).
40. J. Sawynok, C. Pinsky and F.S. LaBella, Life Sciences, 25, 1621 (1979).
41. K.M. Foley, I.O. Kourides, C.E. Inturrisi, R.F. Kaiko, C.G. Zaroulis, J.N. Posner, R.W. Houde and C.H. Li, Proc. Natl. Acad. Sci., 76, 5377 (1979).
42. A. Enjalbert, M. Rubert, L. Fiore, S. Arancibia, M. Priam and C. Kordon, Eur. J. Pharmacol., 53, 211 (1979).
43. A. Enjalbert, M. Ruberg, S. Arancibia, M. Priam and C. Kordon, Nature, 280, 595 (1979).
44. J.I. Koenig, M.A. Mayfield, S.M. McCann and L. Krulich, Life Sciences, 25, 853 (1979).
45. P. Rubin, S. Swezey and T. Blaschke, Lancet, 1293 (1979).
46. J.B. Martin, G. Tolis, I. Woods and H. Guyda, Brain Research, 168, 210 (1979).
47. D. Janowsky, L. Judd, L. Huey, N. Roitman and D. Parker, Psychopharmacology, 65, 95 (1979).
48. E.L. Lien, A. Morrison and W. Dvonch, Life Sciences, 25, 1709 (1979).
49. M.S. Blank, A.E. Panerai and H.G. Friesen, Science, 203, 1129 (1979).
50. R.M. Myers and M.J. Baum, Pharmacol.Biochem. and Behaviour, 10, 615 (1979).
51. R.F. Casper, S.S.C. Yen and M.M. Wilkes, Science, 205, 823 (1979).
52. S.L. Libhtman and H.S. Jacobs, Lancet, 1071 (1979).
53. R.D.G. Leslie, D.A. Pyke and W.A. Stubbs, Lancet, 341 (1979).
54. R.P. Johnson, M. Sar and W.E. Stumpf, Neuroscience Letters, 14, 321 (1979).
55. C. Llorens-Cortes, H. Pollard and J.C. Schwartz, Neuroscience Letters, 12, 165 (1979).
56. T.W. Stone and M.N. Perkins, Nature, 281, 227 (1979).

57. R.L. Lee, R.D.E. Sewell and P.J.S. Spencer, Neuropharmacology, 18, 711 (1979).
58. T.L. Yaksh, Brain Research, 160, 180 (1979).
59. L.M. Gunne, L. Lindström and E. Widerlöv, in: Endorphins in Mental Health Research, London, MacMillan Press, page 547 (1979).
60. H.M. Emrich, V. Höllt, M. Kissling, M. Fischler, H. Laspe, H. Heinemann, D.V. Zerrsen and A. Herz, Pharmakopsychiat., 12, 269 (1979).
61. W. Domschke, A. Dickschas and P. Mitznegg, Lancet, 1024 (1979).
62. T.J. Crow, Brit. Med. J., 66 (1979).
63. J.P.H. Burbach, J.G. Loeber, J. Verhoef, E.R. de Kloet, J.M. van Ree and D. de Wied, Lancet, 480, (1979).
64. J. Lipinski, R. Meyer, C. Kornetsky and B.M. Cohen, Lancet, 1292 (1979).
65. H. Lehmann, N.P.V. Nair and N.S. Kline, Am. J. Psychiatry, 136, 762 (1979).
66. N. Nedopil and E. Ruther, Pharmakopsychiat., 12, 277 (1979).
67. E. Krebs and J. Roubicek, Pharmakopsycniat., 12, 86 (1979).
68. S.B. Weinberger, A. Arnsten and D.S. Segal, Life Sciences, 24, 1637 (1979).
69. W.M.A. Verhoeven, H.M. van Praag, J.M. van Ree and D. de Wied, Arch. Gen. Psychiatry, 36, 297 (1979).
70. A.I. Mackenzie, Lancet, 733 (1979).
71. S.C. Sørensen, Lancet, 688 (1979).
72. W.J. Jeffcoate, M.H. Cullen, M. Herbert, A.G. Hastings and C.P. Walder, Lancet, 1157 (1979).
73. A.K.S. Ho and C.C. Ho, Pharmacol. Biochem. and Behaviour, 11, 111 (1979).
74. M.A. Walz and W.M. Davis, Drug and Chemical Toxicology, 2, 257 (1979).
75. M.F. Piercey and M.J. Ruwart, Br. J. Pharmacol., 66, 373 (1979).
76. T. Lehman and G.R. Peterson, Psychopharmacology, 59, 305 (1978).
77. J.O. Arndt and E. Freye, Nature, 277, 399 (1979).
78. C. Farsang and G. Kunos, Br. J. Pharmac., 67, 161 (1979).
79. J. McCloy and E.F. McCloy, Lancet, 156 (1979).
80. H. Kather and B. Simon, Lancet, 905 (1979).
81. S. Inoue and G.A. Bray, Life Sciences, 25, 561 (1979).
82. J. Rossier, J. Rogers, T. Shibasaki, R. Guillemin and F.E. Bloom, Proc. Natl. Acad. Sci. 76, 2077 (1979).
83. S. Amir, H.Z. Galina and Z. Amit, Neuropharmacology, 18, 905, (1979).
84. B. Brands , Life Sciences, 24, 1773 (1979).
85. S.G. Holtzman, Life Sciences, 24, 219 (1979).
86. A.I. Faden and J.W. Holaday, Science, 205, 317 (1979).
87. T.M. Cho, J.S. Cho and H.H. Loh, Mol. Pharmacol., 16, 393 (1979).
88. K.J. Cheng and P. Cuatrecasas, J. Biol., Chem., 254, 2610 (1979).
89. K.J. Cheng, B.R. Cooper, E. Hazum and P. Cuatrecasas, Mol. Pharmacol., 16, 91 (1979).
90. E. Hazum, K.J. Cheng and P. Cuatrecasas, Nature, 282, 626 (1979).
91. G.H. Loew and D.S. Berkowitz, J. Med. Chem., 22, 603 (1979).
92. J. Reden, M.F.Reich, K.C. Rice, A.E. Jacobson, A. Brossi, R.A. Streaty and W.A. Klee, J. Med. Chem., 22, 256 (1979).
93. A.E. Jacobson, K.C. Rice, J. Reden, L. Lupinacci, A. Brossi, R.A. Streaty and W.A. Klee, J. Med. Chem., 22, 328 (1979).
94. I.M. Uwaydah, M.K. Waddle and M.E. Rogers, J. Med. Chem., 22, 889 (1979).
95. M.D. Aceto, L.S. Harris, W.L. Dewey and E.L. May, Dependence Studies of New Compounds in the Rhesus Monkey, Medical College of Virginia (1979).
96. H.H. Swain, J.H. Woods, F. Medzihradsky, C.B. Smith and C.L. Fly, Evaluation of New Compounds for Opioid Activity, University of Michigan (1979).
97. T.P. Caruso, A.E. Takemori, D.L. Larson and P.S. Portoghese, Science, 204, 318 (1979).
98. N. Yokayama, P.I. Almaula, F.B. Block, F.R. Granat, N. Gottfied, R.T.Hill, E.H. McMahon W.F. Munch, H. Rachlin, J.K. Saelens, M.G. Siegel, H.C. Tomaselli, and F.H. Clarke, J. Med. Chem.. 22, 537 (1979).
99. W.R. Michne, T.R. Lewis, S.J. Michalac, A.K. Pierson and F.J. Rosenberg, J. Med. Chem., 22, 1158 (1979).
100. H. Merz and K. Stockhaus, J. Med. Chem., 22, 1475 (1979).
101. S. Shiotani, T. Kometani, T. Nozawa, A. Kurobe and O. Futsukaichi, J. Med. Chem., 22, 1558 (1979).
102. A. Burkartsmaier and E. Mutschler, Arch. Pharm., 311, 843 (1978).
103. S. Grossmann, U. Moser and E. Mutschler, Arch. Pharm., 311, 1010 (1978).
104. H.H. Ong, J.A. Profitt, T.C. Spaulding and J.C. Wilker, J. Med. Chem., 22, 834 (1979).
105. D.Lednicer and P.F. Von Voigtlandler, J. Med. Chem., 22, 1157 (1979).
106. P.F. von Voigtlander, D. Lednicer, R.A. Lewis and D.D. Gay, International Narcotics Research Conference (1979).
107. W.G. Reifenrath and D.S. Fries, J. Med. Chem., 22, 204, (1979).
108. T.N. Riley and J.R. Bagley, J. Med. Chem., 22, 1167 (1979).
109. W.A. Skinner, G. Rackur and E. Uyeno, J. Pharm. Sci., 68, 330 (1979).
110. N. Shimokawa, H. Nakamura, K. Shimakawa, H. Minami and H. Nishimura, J. Med. Chem., 22, 58 (1979).
111. J. Okada and M. Shimabayashi, J. Pharm. Soc. Japan, 98, 1619 (1979).

Chapter 5. GABA Agonists and Antagonists

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Introduction - Imbalance between γ-aminobutyric acid (GABA) (1) and other central neurotransmitters contributes to the development of Huntington's chorea,[1] Parkinson's disease,[2,3] and probably spasticity and epilepsy.[5,6] The hypothesis of a correlation between GABA dysfunction and schizophrenia [7,8] is supported by the discovery of decreased GABA activity in certain brain regions of schizophrenic patients.[9] Decreased GABA activity apparently contributes to the pathogenesis of these disorders, and consequently the object of pharmacological interventions in GABA synaptic mechanisms must be stimulation of GABA neurotransmission. The processes and functions illustrated in Fig. 1 may be particularly susceptible to pharmacological manipulation, for example; the presynaptic autoreceptor (A) assumed to regulate GABA release; GABA-T, which catalyzes the conversion of GABA into succinic semialdehyde (SSA); the GABA uptake processes in glia cells (B) and in nerve terminals (C); and the postsynaptic receptor or the adjacent modulator mechanism and Cl^--ionophore.

This chapter is a review of recent developments in the field of GABA agonists and antagonists, including a summary of our present knowledge of the GABA receptors in the mammalian central nervous system (CNS). Various aspects of the central GABA system have recently been discussed in books [10–13] and in review articles.[14–19]

GABA Receptors in the Mammalian CNS - GABA receptors have been extensively studied.[19,20] These studies have revealed the existence of multiple GABA receptors in mammalian CNS, but our knowledge of their structure and function is still very incomplete. "Presynaptic" (axo-axonic)[22,23] and in particular postsynaptic (axo-somatic or axo-dendritic)[23] GABA receptors, which produce depolarization and hyperpolarization of the respective postsynaptic membranes have been studied *in vivo* using electrophysiological techniques. These actions of GABA apparently result in increased membrane permeability to Cl^-, indicating that both types of postsynaptic receptors are coupled to Cl^--ionophores. The development of receptor affinity binding techniques [24,25] has made it possible to study GABA receptors or receptor binding sites on synaptic membrane fragments *in vitro*.

Our present knowledge of the location and function of receptors in GABA-mediated synapses is summarized in Fig. 1. The high(D)- and low(E)-affinity binding sites for GABA on the postsynaptic receptors may represent two distinct macromolecules or they may be part of the same molecular entity.[26] On crude synaptic membranes the high-affinity GABA binding sites are occupied by a substance assumed to be an endogenous inhibitor or a modulator of the GABA receptors. This inhibitor, which can be removed from isolated membranes by detergents [26,27] or rapid freezing,[28] appears to be an acidic protein,[29,30] although the activity concerned may in part be associated with phospholipids [31] or with GABA itself.[32] Since the GABA

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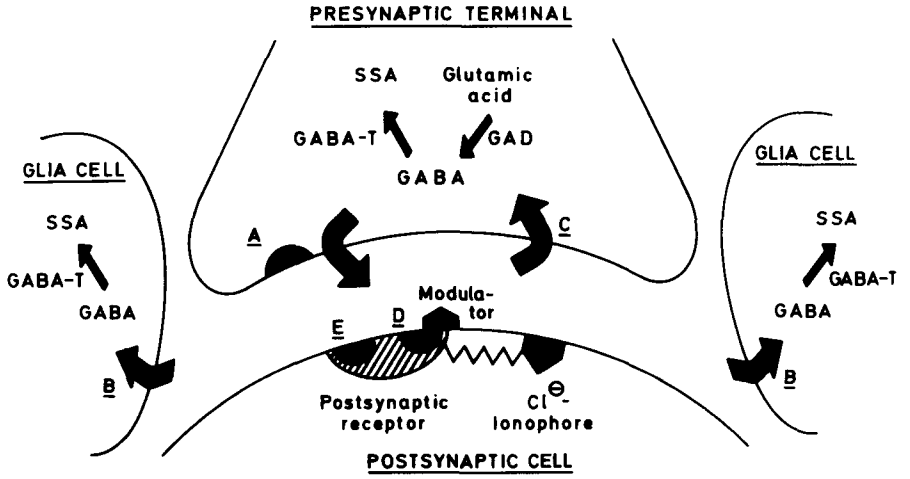
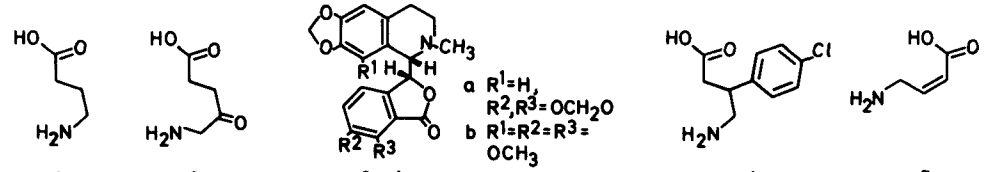


Figure 1

receptors in different regions of the human brain seem to be masked to a dissimilar extent,[33] the development of GABA agonists with selective effects on restricted brain areas is a possibility. The binding site of the GABA receptor has recently been solubilized,[34-36] and the conformation of the receptor protein apparently is maintained during solubilization. Isolation of this GABA receptor recognition site, which reveals two populations of binding sites with ligand specificities similar to those of the receptors on synaptic membranes,[34-36] may open up the prospect of studying GABA agonist-receptor interactions at the molecular level.

Studies *in vitro* have revealed the existence of presynaptic GABA autoreceptors [37-39] (A in Fig. 1). GABA and GABA agonists inhibit the release of ^{3}H-GABA accumulated in synaptosomes, and δ-aminolaevulinic acid (2) appears to be a selective agonist for this population of GABA receptors.[39] These findings suggest that the physiological release of GABA is subject to negative feedback control through presynaptic autoreceptors, which are sensitive to the GABA antagonist (+)-(1*S*,9*R*)-bicuculline (BIC) (3a).[38,39]



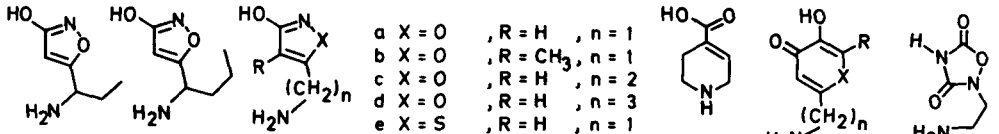
The GABA analogue baclofen (4) has a BIC-insensitive depressant effect on the firing of cat spinal neurones.[40] Baclofen appears to be a selective agonist for a population of GABA receptors on sympathetic nerve terminals.[41] Activation of these receptors apparently inhibits transmitter release.[41] Whereas baclofen (4) is a very weak and non-stereoselective inhibitor of the binding of ^{3}H-GABA to rat brain membranes,[42]

(-)-(4) is more than 100 times more potent than the (+)-isomer in the present system.[41] Like baclofen (4), *cis*-4-aminocrotonic acid (5) [43] and the muscimol analogues, (6) and (7),[44] are BIC-insensitive neuronal depressants, and these compounds may also be agonists for this proposed novel type of presynaptic GABA receptor.

The "active conformation" of GABA on its postsynaptic receptors is different from the conformation in which GABA is bound by the transport carriers.[44,45] It is possible that GABA also adopts different conformations during the interaction with its different receptors. Consequently, elucidation of the "receptor-active conformation" of GABA may be an important step in attempts to develop therapeutically useful GABA agonists on a rational basis.

GABA Agonists

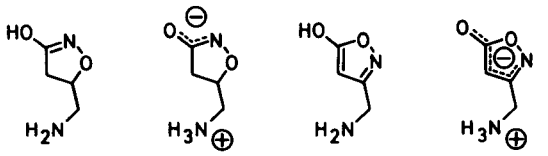
Agonists for Postsynaptic GABA Receptors - Electrophysiological techniques offer the most direct approach to studies of agonist-receptor interactions. The intrinsic activity of GABA agonists *in vivo* can be measured by microelectrophoretic application of the compounds on central neurones, frequently on cat spinal neurones.[46] A GABA agonist is traditionally defined as a BIC-sensitive, strychnine-insensitive neuronal depressant,[46] a definition with obvious limitations. Receptor affinity binding techniques are most conveniently used for the determination of the affinity of compounds for GABA receptors *in vitro*. So far ^{3}H-GABA,[24,27] ^{3}H-muscimol (8a),[37,47,48] and ^{3}H-isoguvacine (9) [49] have been utilized as ligands for studies of GABA agonist-receptor interactions. Electrophysiological as well as receptor binding techniques have inherent limitations, but in combination they constitute an important step in the development of GABA agonists. With a few exceptions there is a good agreement between the relative potencies of GABA agonists as BIC-sensitive depressants of feline spinal neurones and as inhibitors of the binding of ^{3}H-GABA to receptors on rat brain membranes.[45,49-51]



6 7 8 a-g 9 10 a-d 11

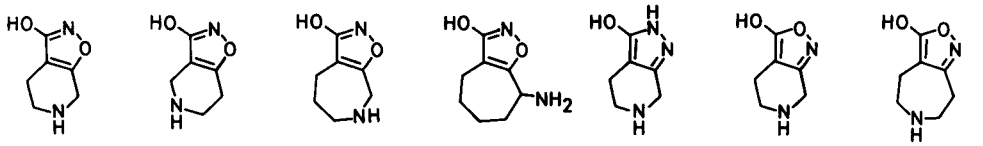
Muscimol (8a), a powerful GABA agonist,[52] is also a substrate for the neuronal GABA uptake system [53] and an inhibitor of glial GABA uptake.[54] Other heterocyclic compounds, like kojic amine (10a) [55] and quisqualamine (11),[56] are putative GABA agonists. In contrast to GABA,[57] muscimol [37,58] and kojic amine [55] can penetrate the blood-brain barrier (BBB) and may be useful lead structures for the development of GABA agonists of therapeutic value. Even minor structural alterations of muscimol, however, result in considerable loss of GABA agonist activity.[45,49,50] Thus, the muscimol analogues (8b-d) are 3-4 orders of magnitude weaker than muscimol, and the related derivatives (10b-d) of kojic amine are inactive in animal experiments.[55]

The effects of alterations of the ring of muscimol have been studied. (*RS*)-4,5-Dihydromuscimol (DHM) (12) and thiomuscimol (8e) are approximately equipotent with muscimol (8a) as GABA agonists, whereas isomuscimol (13) and the analogues (8f,g) are very weak.[28] There is no simple correlation between the protolytic properties of these compounds



12 13

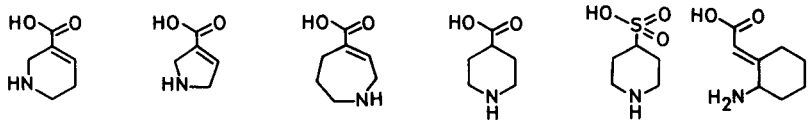
and their effects on the GABA receptors.[28] The degree of delocalization of their negative charges, however, seems to be a factor of importance for GABA agonist activity as exemplified by DHM (12) and isomuscimol (13).[28] The charge distribution in the former compound is similar to that in GABA. In the weak GABA agonist isomuscimol (13) the negative charge is highly delocalized, and muscimol has a charge distribution similar to that illustrated for DHM (12).[59]



14 15 16 17 18 19 20

Among a number of bicyclic muscimol analogues only THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol) (14) is a potent and specific GABA agonist.[28,60,61] The related compounds (15-17) have little or no receptor affinity, and (15) is an inhibitor of GABA uptake.[62] Alterations of the 5-membered ring of THIP (14) also strongly affect the biological activity. Compound (18) and closely related analogues are inactive,[63] whereas isoTHIP (4,5,6,7-tetrahydroisoxazolo[3,4-*c*]pyridin-3-ol) (19) and isoTHAZ (5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*d*]azepin-3-ol) (20) have GABA antagonistic properties.[64] The 3-isoxazolol nucleus can be regarded as a masked carboxyl group, and accordingly the amino acid isoguvacine (9), related to THIP (14), is also a specific and very potent GABA agonist.[28,60] Structure-activity studies on isoguvacine analogues emphasize the "ligand specificity" of the GABA receptors. Guvacine (21) has no receptor affinity, but it is a potent inhibitor of neuronal and glial GABA uptake,[45,54] and (22) [54] and (23) [63] are 2-3 orders of magnitude weaker than isoguvacine (9). Isonipecotic acid (24) [60] and piperidine-4-sulphonic acid (P4S) (25) [65] are specific GABA agonists, P4S being equipotent with isoguvacine. Compound (26), on the other hand, has no receptor affinity [66] in spite of the fact that its "GABA structure element" is locked in a conformation similar to that in isoguvacine (9).

Structure-activity studies on chiral GABA agonists of known configuration may provide information about the molecular aspects of GABA-recep-



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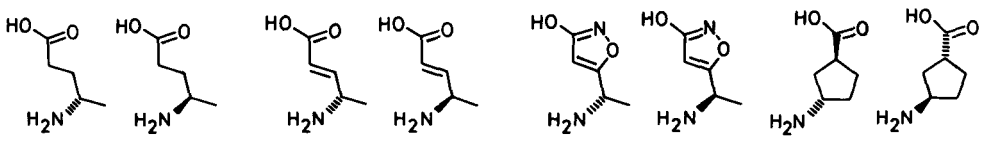


S R S R S R 1S,3S 1R,3R

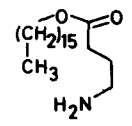
27 28 29 30

tor interactions. (*S*)- and (*R*)-4-aminovaleric acid (27) are equipotent as inhibitors of GABA binding, whereas the (*S*)-forms of the more rigid GABA analogues (28) and (29) are 20-30 times more potent than the (*R*)-isomers.[50,54] Similarly (1*S*,3*S*)-(30) is a more powerful GABA agonist than its optical antipode.[67] With respect to inhibition of neuronal and glial GABA uptake the (*R*)-isomers of (27) and (28) are, however, more potent than the corresponding (*S*)-forms,[54] emphasizing the different "active conformations" of GABA on the receptor and transport carrier.

GABA Agonist-Benzodiazepine Interactions - At least some of the therapeutic effects of the benzodiazepines may be the result of facilitation of GABA receptor function,[68,69] although these drugs are not acting as GABA agonists.[70] GABA [71,72] and the relatively flexible GABA agonists muscimol (8a), thiomuscimol (8e), and DHM (12) stimulate the binding of radioactive diazepam (^{3}H-DZ) to isolated synaptic membranes.[72-75] In contrast, the more rigid GABA agonists isoguvacine (9), THIP (14), and P4S (25), which have secondary amino groups, are inactive as activators of ^{3}H-DZ binding,[73-75] and they are capable of reversing muscimol-stimulated ^{3}H-DZ binding.[75] Nevertheless, structure-activity studies including the (*S*)- and (*R*)-isomers of (27)-(29) indicate that BIC-sensitive postsynaptic GABA receptors are mediating the stimulation of ^{3}H-DZ binding *in vitro*.[49,75] The binding site for the GABA receptor modulator (Fig. 1) apparently is identical with the "benzodiazepine receptor",[29,49] and the stimulation of ^{3}H-DZ binding by GABA and by flexible, but not more rigid, GABA agonists suggests that conformational changes play a vital role in the regulation of GABA receptor functions.[49]

Intranigral Injections - Unilateral injections of GABA or GABA agonists into pars reticulata of the substantia nigra (SN) in rats induce a contralateral turning behaviour as a result of activation of GABA receptors.[76-79] The turning response is dopamine-independent but sensitive to GABA antagonists.[76,80] Since (+)- and (-)-baclofen (4) are equipotent in this model,[42] GABA receptors on sympathetic nerve terminals are apparently not involved (cf. above). There is generally a good correlation between the intensity and duration of the turning response and GABA agonist activity.[76,81] This animal model seems to be a useful test system for GABA agonists and antagonists.[64]

Pro-drugs - In contrast to GABA and isoguvacine (9), muscimol (8a), THIP (14), and kojic amine (10a) are centrally active after systemic administration (SA). While THIP does not interact with GABA-T *in vitro*,[28] muscimol is rapidly metabolized in the periphery, probably by transamination,[58] and metabolites may contribute to the central effects observed after SA of muscimol. For these reasons, there is a need for pro-drugs of GABA and potent GABA agonists like muscimol and isoguvacine. GABA cetyl ester (31) is centrally active after SA,[82] and SL 76002 (32), a GABA-mimetic compound,[83] may decompose in brain tissue to yield GABA.[84] Some acyloxymethyl esters of isoguvacine, including (33), which are hydrolyzed to



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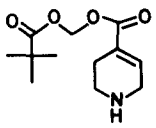

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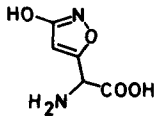


34

yield isoguvacine under approximate physiological conditions,[85] have weak anticonvulsant effects after SA. The centrally active amino acid ibotenic acid (34) decarboxylates in CNS tissue to give muscimol (8a).[86] However, the excitatory effects of (34) on central neurones [86] make it inapplicable as a muscimol pro-drug.

Anticonvulsant Effects - Muscimol (8a) protects animals against audiogenic convulsions,[87] as well as those induced by electro-shock,[88] metrazole,[88] and isoniazide.[82,89] Muscimol can block seizures induced by low doses of BIC,[90] but it does not alter the effects of strychnine [82,89] and higher doses of BIC.[90] Isoguvacine (9) is partially effective against audiogenic seizures after i.c.v. but not after i.p. administration.[87] Baclofen (4) [82,89] and kojic amine (10a) [55] have anticonvulsant properties after p.o. administration. Rather high doses of SL 76002 (32) protect animals against convulsions in a variety of test systems.[83]

Effects on Morphine Analgesia - Morphine analgesia may involve the GABA system by an as yet unclarified mechanism.[91-93] Muscimol (i.v.) is reported to potentiate morphine analgesia in rats.[94] Whereas these effects have not been confirmed in more recent studies, muscimol was shown to antagonize morphine-induced hypermotility.[95]

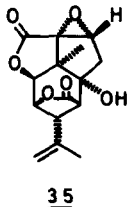
Effects on Blood Pressure and Heart Rate - Injections of GABA and muscimol into cats (i.c.v., but not i.v.) cause reductions in blood pressure and heart rate.[96-98] Similar effects were provoked by baclofen (4) (i.c.v.) and kojic amine (10a) (i.c.v.),[98] and GABA is assumed to inhibit centrally evoked cardiovascular responses by preventing increases in sympathetic outflow.[96,97] Injections of BIC into nucleus ambiguus of the brainstem in cats, however, depress blood pressure and heart rate.[99] This effect of BIC can be reversed by muscimol, which alone has no effect.[99] Nucleus ambiguus apparently is the site where GABA regulates parasympathetic cardiac functions. Cerebral blood vessels contain GABA receptors.[100,101] Dilation of cerebral arteries from cats, dogs, and humans *in vitro* by GABA and GABA agonists can be blocked by BIC.[101]

Interactions with Dopamine and Neuroleptics - The antipsychotic effects of neuroleptics are normally related to their antagonism of dopamine (DA)-induced stereotypic behaviour in animals.[102,103] Muscimol (8a) [104-107] and THIP (14) [107] potentiate DA-provoked stereotypic gnawing, and in acute experiments they reverse the antagonistic effects of neuroleptics on this behaviour.[107] The anti-stereotypic effects of the butyrophenones are more easily antagonized by GABA agonists than, for example, the phenothiazines,[107] supporting the proposal that more than one type of DA receptor exists in the brain.[107-110] Muscimol inhibits DA-dependent locomotor activity,[110,111] suggesting that limbic DA mechanisms are preferentially inhibited by GABA agonists.[110] Since the antipsychotic activity of neuroleptics may be primarily related to blockade of limbic DA receptors, combined therapy using neuroleptics and GABA agonists may improve the treatment of schizophrenia.[110]

Clinical Studies - Muscimol (8a) and SL 76002 (32) have been subjected to preliminary clinical studies. Muscimol was found ineffective in the treatment of Huntington's chorea,[112] whereas it reduced the involuntary movements of schizophrenic patients with tardive dyskinesia.[113] However, muscimol worsened the symptoms of patients with chronic schizophrenia,[113] and SL 76002 did not provide relief.[84] However, SL 76002 did eliminate seizures in epileptic patients,[84] and it reduced the symptoms of patients having Huntington's disease for less than 4 years. Clinical studies in progress with THIP (14), which is well tolerated by rats, dogs, and baboons, are expected to provide more information about the therapeutic usefulness of GABA agonists.

GABA Antagonists

A prerequisite for satisfactory characterization of the multiple GABA receptors in the mammalian brain is the development of specific antagonists for each population of receptors. The "classical" GABA antagonists [46,52] BIC (3a) and picrotoxinin (35) have different mechanisms of action.[16,17,114] BIC binds to the GABA receptor, possibly to an "antagonist conformational state".[115,116] The site of action of picrotoxinin seems to be the Cl^--ionophore associated with the GABA receptor.[117] BIC methiodide (BMI) has GABA antagonistic properties similar to those of BIC,[118] and ^{3}H-BMI is a tool for mechanistic studies of GABA receptor antagonists.[119] Dihydropicrotoxinin (DHP) has a mechanism of action similar to that of picrotoxinin (35).[117] Using ^{3}H-DHP as a ligand certain convulsant compounds have been shown to interact with the Cl^--ionophore concerned.[120]



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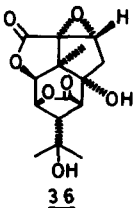

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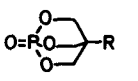
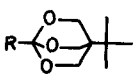


a R=CH_3
b R=C_2H_5
c R=$CH(CH_3)_2$

37a-c



38a-c

Receptor Antagonists - Although BIC and BMI are generally accepted as selective GABA antagonists, their value as tools for GABA receptor studies *in vivo* is limited,[121] and the molecular mechanisms of BIC and BMI are enigmatic. The GABA receptors exhibit stereoselectivity with respect to antagonists of the phthaleideisoquinoline type. Thus (-)-(1*R*,9*S*)-BIC is much weaker than BIC (3a) *in vivo* and as an inhibitor of ^{3}B-BMI binding,[115,116] and (+)-(1*S*,9*R*)-narcotine (3b) is much more potent than its optical isomers.[122,123]

Cl^--Ionophore and Other Antagonists - GABA and BIC (3a) do not interfere with the binding of ^{3}H-DHP to synaptic membranes,[120] indicating that the GABA receptor and the Cl^--ionophore are distinct structural units. In contrast to picrotine (36), the hydrated analogue of picrotoxinin (35), DHP and (35) are potent inhibitors of ^{3}H-DHP binding.[120] A number of cyclic phosphates and orthocarboxylates like (37a-c) and (38a-c) are inhibitors of ^{3}H-DHP binding, suggesting that these convulsants are capable of blocking Cl^--ionophores.[120,123] The convulsant barbiturates are much more potent than the sedative barbiturates as inhibitors of ^{3}H-DHP binding.[120] As mentioned above isoTHIP (19) and isoTHAZ (20) have GABA antagonistic properties,[64] but their site of action is not known.

References

1. "Huntington's Disease," T.N. Chase, N.S. Wexler and A. Barbeau, Eds., Adv. Neurol. Vol. 23, Raven Press, New York, 1979.
2. O. Hornykiewicz, K.G. Lloyd and L. Davidson, in "GABA in Nervous System Function," E. Roberts, T.N. Chase and D.B. Tower, Eds., Raven Press, New York, 1976, p. 479.
3. K.G. Lloyd, S. Dreksler, L. Shemen and L. Davidson, in "GABA-Biochemistry and CNS Functions," P. Mandel and F.V. DeFeudis, Eds., Adv. Exp. Med. Biol., Vol. 123, Plenum Press, New York, 1979, p. 399.
4. N.E. Naftchi, W. Schlosser and W.D. Horst, in "GABA-Biochemistry and CNS Functions," P. Mandel and F.V. DeFeudis, Eds., Adv. Exp. Med. Biol., Vol. 123, Plenum Press, New York, 1979, p. 431.
5. B.S. Meldrum, Int. Rev. Neurobiol., 17, 1 (1975).
6. B.S. Meldrum, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 390.
7. E. Roberts, Biochem. Pharmacol., 23, 2637 (1974).
8. D.P. Van Kammen, Am. J. Psychiatry, 134, 138 (1977).
9. T.L. Perry, J. Buchanan, S.J. Kish and S. Hansen, Lancet i, 237 (1979).
10. "GABA in Nervous System Function," E. Roberts, T.N. Chase and D.B. Tower, Eds., Raven Press, New York, 1976.
11. "Amino Acids as Chemical Transmitters," F. Fonnum, Ed., Plenum Press, New York, 1978.
12. "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979.
13. "GABA-Biochemistry and CNS Functions," P. Mandel and F.V. DeFeudis, Eds., Adv. Exp. Med. Biol., Vol. 123, Plenum Press, New York, 1979.
14. I.A. Sytinsky, A.T. Soldatenkov and A. Lajtha, Prog. Neurobiol. (Oxford), 10, 89 (1978).
15. S.J. Enna and A. Maggi, Life Sci., 24, 1727 (1979).
16. G.A.R. Johnston, Annu. Rev. Pharmacol., 18, 269 (1978).
17. P.R. Andrews and G.A.R. Johnston, Biochem. Pharmacol., 28, 2697 (1979).
18. B.W. Metcalf, Biochem. Pharmacol., 28, 1705 (1979).
19. A. Nistry and A. Constanti, Prog. Neurobiol. (Oxford), 13, 117 (1979).
20. F.V. DeFeudis, Prog. Neurobiol. (Oxford), 9, 123 (1977).
21. B.R. Lester and E.J. Peck, Jr., Brain Res., 161, 79 (1979).
22. D.R. Curtis, in "Amino Acids as Chemical Transmitters," F. Fonnum, Ed., Plenum Press, New York, 1978, p. 55.
23. K. Krnjević, in "GABA-Biochemistry and CNS Functions," P. Mandel and F.V. DeFeudis, Eds., Adv. Exp. Med. Biol., Vol. 123, Plenum Press, New York, 1979, p. 271.
24. S.J. Enna and S.H. Snyder, Brain Res., 100, 81 (1975).
25. S.H. Snyder and J.P. Bennett, Annu. Rev. Physiol., 38, 153 (1976).
26. J.S. Horng and D.T. Wong, J. Neurochem., 32, 1379 (1979).
27. S.J. Enna and S.H. Snyder, Mol. Pharmacol., 13, 442 (1977).
28. P. Krogsgaard-Larsen, H. Hjeds, D.R. Curtis, D. Lodge and G.A.R. Johnston, J. Neurochem., 32, 1717 (1979).
29. A. Guidotti, G. Toffano and E. Costa, Nature (London), 275, 553 (1978).
30. G. Toffano, A. Guidotti and E. Costa, Proc. Natl. Acad. Sci. USA, 75, 4024 (1978).
31. G.A.R. Johnston and S.M.E. Kennedy, in "Amino Acids as Chemical Transmitters," F. Fonnum, Ed., Plenum Press, New York, 1978, p. 507.
32. D.V. Greenlee, P.C. Van Ness and R.W. Olsen, Life Sci., 22, 1653 (1978).
33. S.J. Enna, J.W. Ferkany and P. Krogsgaard-Larsen, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 191.
34. M. Gavish, R.S.L. Chang and S.H. Snyder, Life Sci., 25, 783 (1979).
35. D.V. Greenlee and R.W. Olsen, Biochem. Biophys. Res. Commun., 88, 380 (1979).
36. O. Chude, J. Neurochem., 33, 621 (1979).
37. S.R. Snodgrass, Nature (London), 274, 392 (1978).
38. P.R. Mitchell and I.L. Martin, Nature (London), 274, 904 (1978).
39. M.J.W. Brennan and R.C. Cantrill, Nature (London), 280, 514 (1979).
40. D.R. Curtis, C.J.A. Game, G.A.R. Johnston and R.M. McCulloch, Brain Res., 70, 493 (1974).
41. N.G. Bowery, D.R. Hill, A.L. Hudson, A. Doble, D.N. Middlemiss, J. Shaw and M. Turnbull, Nature (London), 283, 92 (1980).
42. J.L. Waddington and A.L. Cross, Neurosci. Lett., 14, 123 (1979).
43. G.A.R. Johnston, D.R. Curtis, P.M. Beart, C.J.A. Game, R.M. McCulloch and B. Twitchin, J. Neurochem., 24, 157 (1975).
44. P. Krogsgaard-Larsen, G.A.R. Johnston, D.R. Curtis, C.J.A. Game and R.M. McCulloch, J. Neurochem., 25, 803 (1975).
45. P. Krogsgaard-Larsen, in "Amino Acids as Chemical Transmitters," F. Fonnum, Ed., Plenum Press, New York, 1978, p. 305.
46. D.R. Curtis and G.A.R. Johnston, Ergeb. Physiol. Biol. Chem. Exp. Pharmakol., 69, 97 (1974).
47. K. Beaumont, W.S. Chilton, H.I. Yamamura and S.J. Enna, Brain Res., 148, 153 (1978).
48. Y.-J. Wang, P. Salvaterra and E. Roberts, Biochem. Pharmacol., 28, 1123 (1979).

49. P. Krogsgaard-Larsen and J. Arnt, in "Proceedings of the International Symposium on GABA and Other Inhibitory Transmitters," H. Lal and S. Fielding, Eds., Myrtle Beach, South Carolina, 6.-11. November 1979, in press.
50. P. Krogsgaard-Larsen, T. Honoré and K. Thyssen, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 201.
51. P. Krogsgaard-Larsen and J. Arnt, in "GABA-Biochemistry and CNS Functions," P. Mandel and F.V. DeFeudis, Eds., Adv. Exp. Med. Biol., Vol. 123, Plenum Press, New York, 1979, p. 303.
52. D.R. Curtis, A.W. Duggan, D. Felix and G.A.R. Johnston, Brain Res., 32, 69 (1971).
53. G.A.R. Johnston, S.M.E. Kennedy and D. Lodge, J. Neurochem., 31, 1519 (1978).
54. A. Schousboe, P. Thorbek, L. Hertz and P. Krogsgaard-Larsen, J. Neurochem., 33, 181 (1979).
55. J.G. Atkinson, Y. Girard, J. Rokach and C.S. Rooney, J. Med. Chem., 22, 99 (1979).
56. R.H. Evans, A.A. Francis, K. Hunt, M.R. Martin and J.C. Watkins, J. Pharm. Pharmacol. 30, 364 (1978).
57. R.D. Myers, in "Handbook of Psychopharmacology," Vol. 2, L.L. Iversen, S.D. Iversen and S.H. Snyder, Eds., Plenum Press, New York, 1975, p. 1.
58. M. Baraldi, L. Grandison and A. Guidotti, Neuropharmacology, 18, 57 (1979).
59. T. Honoré and L. Brehm, Acta Crystallogr., B34, 3417 (1978).
60. P. Krogsgaard-Larsen, G.A.R. Johnston, D. Lodge and D.R. Curtis, Nature (London), 268, 53 (1977).
61. P. Krogsgaard-Larsen and G.A.R. Johnston, J. Neurochem., 30, 1377 (1978).
62. P. Krogsgaard-Larsen and G.A.R. Johnston, J. Neurochem., 25, 797 (1975).
63. P. Krogsgaard-Larsen and T.R. Christiansen, Eur. J. Med. Chem. Chim. Ther., 14, 157 (1979).
64. J. Arnt and P. Krogsgaard-Larsen, Brain Res., 177, 395 (1979).
65. P. Krogsgaard-Larsen, E. Falch, A. Schousboe, D.R. Curtis and D. Lodge, J. Neurochem., 34, 1980, in press.
66. G.A.R. Johnston, R.D. Allan, S.M.E. Kennedy and B. Twitchin, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 149.
67. G.A.R. Johnston, R.D. Allan, P.R. Andrews, S.M.E. Kennedy and B. Twitchin, in "Adv. Pharmacol. Chemother.", Vol. 2, P. Simon, Ed., Pergamon Press, Oxford, 1978, p. 11.
68. "Adv. Biochem. Psychopharmacol.," Vol. 14, E. Costa and P. Greengard, Eds., Raven Press, New York, 1975.
69. W. Haefely, P. Polc, R. Schaffner, H.H. Keller, L. Pieri and H. Möhler, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 357.
70. D.R. Curtis, D. Lodge, G.A.R. Johnston and S.J. Brand, Brain Res., 118, 344 (1976).
71. I.L. Martin and J.M. Candy, Neuropharmacology, 17, 993 (1978).
72. J.F. Tallman, J.W. Thomas and D.W. Gallager, Nature (London), 274, 383 (1978).
73. R. Maurer, Neurosci. Lett., 12, 65 (1978).
74. M. Karobath, P. Placheta, M. Lippitsch and P. Krogsgaard-Larsen, Nature (London), 278, 748 (1979).
75. C. Braestrup, M. Nielsen, P. Krogsgaard-Larsen and E. Falch, Nature (London), 280, 331 (1979).
76. J. Scheel-Krüger, J. Arnt and G. Magelund, Neurosci. Lett., 4, 351 (1977).
77. H.R. Olpe, H. Schellenberg and W.P. Koella, Eur. J. Pharmacol., 45, 291 (1977).
78. M.C. Olianas, G.M. DeMontis, G. Mulas and A. Tagliamonte, Eur. J. Pharmacol., 49, 233 (1978).
79. A. Dray and D.W. Straughan, J. Pharm. Pharmacol., 28, 400 (1976).
80. J. Arnt and J. Scheel-Krüger, Psychopharmacology, 62, 267 (1979).
81. J. Arnt, J. Scheel-Krüger, G. Magelund and P. Krogsgaard-Larsen, J. Pharm. Pharmacol., 31, 306 (1979).
82. A. Delini-Stula, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 482.
83. K.G. Lloyd, P. Worms, H. Depoortere and G. Bartholini, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 308.
84. G. Bartholini, B. Scatton, B. Zivkovic and K.G. Lloyd, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 326.
85. E. Falch and P. Krogsgaard-Larsen, in "Receptors for Neurotransmitters and Peptide Hormones," M. Kuhar, S.J. Enna and G. Pepeu, Eds., Raven Press, New York, 1980, in press.
86. D.R. Curtis, D. Lodge and H. McLennan, J. Physiol. (London), 291, 19 (1979).
87. G. Anlezark, J. Collins and B. Meldrum, Neurosci. Lett., 7, 337 (1977).
88. H.-H. Frey, C. Popp and W. Löscher, Neuropharmacology, 18, 581 (1979).
89. S.R. Naik, A. Guidotti and E. Costa, Neuropharmacology, 15, 479 (1976).
90. P. Worms, H. Depoortere and K.G. Lloyd, Life Sci., 25, 607 (1979).
91. I.K. Ho, H.H. Loh and E.L. Way, Life Sci., 18, 1111 (1976).
92. I. Kääriäinen and P. Wikberg, Acta Pharmacol. Toxicol., 39, 536 (1976).
93. Y. Yoneda, S. Takashima and K. Kuriyama, Biochem. Pharmacol., 25, 2669 (1976).

94. G. Biggio, D. Della Bella, V. Frigeni and A. Guidotti, Neuropharmacology, 16, 149 (1977).
95. A.V. Christensen, J. Arnt and J. Scheel-Krüger, Eur. J. Pharmacol., 48, 459 (1978).
96. M.J. Antonaccio, L. Kerwin and D.G. Taylor, Neuropharmacology, 17, 597 (1978).
97. M.J. Antonaccio, L. Kerwin and D.G. Taylor, Neuropharmacology, 17, 783 (1978).
98. C.S. Sweet, H.C. Wenger and D.M. Gross, Can. J. Physiol. Pharmacol., 57, 600 (1979).
99. J.A. DiMicco, K. Gale, B. Hamilton and R.A. Gillis, Science, 204, 1106 (1979).
100. M. Fujiwara, I. Muramatsu and S. Shibata, Br. J. Pharmacol., 55, 561 (1975).
101. L. Edvinsson and D.N. Krause, Brain Res., 173, 89 (1979).
102. A.V. Christensen, B. Fjalland and I.M. Nielsen, Psychopharmacology, 48, 1 (1976).
103. V. Petersen and A.V. Christensen, Acta Pharmacol. Toxicol., 31, 488 (1972).
104. H. Scheel-Krüger, A.V. Christensen and J. Arnt, Life Sci., 22, 75 (1978).
105. J. Arnt, A.V. Christensen and J. Scheel-Krüger, J. Pharm. Pharmacol., 31, 56 (1979).
106. R.M. Ridley, P.R. Scraggs and H.F. Baker, Psychopharmacology, 64, 197 (1979).
107. A.V. Christensen, J. Arnt and J. Scheel-Krüger, Life Sci., 24, 1395 (1979).
108. A.R. Cools, Life Sci., 25, 2475 (1979).
109. L. Garau, S. Govoni, E. Stefanini, M. Trabucchi and P.F. Spano, Life Sci., 23, 1745 (1978).
110. K. Fuxe, K. Andersson, S.-O. Ögren, M. Perez De La Mora, R. Schwarcz, T. Hökfelt, P. Eneroth, J.-Å. Gustafsson and P. Skett, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 74.
111. J. Scheel-Krüger, A.R. Cools and W. Honig, Eur. J. Pharmacol., 42, 311 (1977).
112. I. Shoulson, D. Goldblatt, M. Charlton and R.J. Joynt, Ann. Neurol., 4. 279 (1978).
113. C.A. Taminga, J.W. Crayton and T.N. Chase, Am. J. Psychiatry, 135, 746 (1978).
114. G.A.R. Johnston, in "GABA in Nervous System Function," E. Roberts, T.N. Chase and D.B. Tower, Eds., Raven Press, New York, 1976, p. 395.
115. H. Möhler and T. Okada, Nature (London), 267, 65 (1977).
116. H. Möhler and T. Okada, Mol. Pharmacol., 14, 256 (1978).
117. R.W. Olsen, D. Greenlee, P. Van Ness and M.K. Ticku, in "Amino Acids as Chemical Transmitters," F. Fonnum, Ed., Plenum Press, New York, 1978, p. 467.
118. G.A.R. Johnston, P.M. Beart, D.R. Curtis, C.J.A. Game, R.M. McCulloch and R.M. MacLachlan, Nature (London) New Biol., 240, 219 (1972).
119. H. Möhler, in "GABA-Biochemistry and CNS Functions," P. Mandel and F.V. DeFeudis, Eds., Adv. Exp. Med. Biol., Vol. 123, Plenum Press, New York, 1979, p. 355.
120. R.W. Olsen, M.K. Ticku, D. Greenlee and P. Van Ness, in "GABA-Neurotransmitters," P. Krogsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Eds., Munksgaard, Copenhagen, 1979, p. 165.
121. R.G. Hill, M.A. Simmonds and D.W. Straughan, Br. J. Pharmacol., 56, 9 (1976).
122. S.J. Enna, J.F. Collins and S.H. Snyder, Brain Res., 124, 185 (1977).
123. N.G. Bowery, J.F. Collins, G. Cryer, T.D. Inch and N.J. McLaughlin, in "GABA-Biochemistry and CNS Functions," P. Mandel and F.V. DeFeudis, Eds., Adv. Exp. Med. Biol., Vol. 123, Plenum Press, New York, 1979, p. 339.

Chapter 6. Interoceptive Discriminative Stimuli in the Development of CNS Drugs and a Case of an Animal Model of Anxiety

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Introduction - In order to develop new drugs, we continuously need novel animal models that are reliable, efficacious and rapid in predicting the clinical usefulness of new chemicals. Towards the search for such new models, the recently recognized paradigm of drug discrimination deserves perusal since this method offers an innovative approach in the development of animal models which employ quantitative behavioral measures to identify both desirable and undesirable properties of potential drug substances. However, unlike classical procedures of behavioral pharmacology, the drug discrimination approach does not focus on the drug effects on behavior as such, but rather, a behavioral measure is used to bioassay the animal's perceptual realization that the drug is acting in the body. The drug actions that are the basis of this realization are defined as interoceptive stimuli. Recent surveys[1,2] showed that a wide variety of drugs produce interoceptive stimuli. Among them are many psychotropic drugs that have been found useful in providing new animal models.

Stimuli are conventionally defined as events that are reliably perceived by the subject and are taken into account for causing a specific change in behavior. The stimulus events that are primarily initiated from within the body, such as drug-induced neuronal events, are termed interoceptive stimuli, in contrast to exteroceptive stimuli (such as light, sound) which primarily originate in the external environment. Although stimuli of both categories have been recognized to be discriminable for many years, it is only recently that convincing evidence has been presented to indicate that drug actions can indeed produce internally discriminable stimuli. It is because of the availability of this wealth of recent evidence that the discriminative stimulus properties of drugs have become the focus of interest in developing new bioassays for drug development.

Discriminative properties are those characteristics of a stimulus that are reliably employed to set that stimulus apart from other stimuli to which a subject may be concurrently exposed. It is now well established that a number of drugs are capable of producing interoceptive discriminative stimuli (IDS). Some IDS provide distinct standards for comparison to identify new chemicals that produce similar IDS. If the IDS selected for a bioassay is representative of a clinically relevant action, then the procedure of identifying new drugs on the basis of the IDS can serve as a tool of drug development.

Methodological Considerations - Laboratory procedures employed to measure IDS are many. The usual ones were described in a recent review[3] and will not be re-elaborated here. It is sufficient to say that IDS can be easily established in a wide variety of animals and experimental paradigms. For the purpose of drug development, it is convenient to train rats to press one lever after an injection of a drug and another lever after a vehicle injection. When the appropriate lever is selected, a food pellet is delivered after each 10 responses. In this way, the first nine responses are

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meant to provide no feedback for the appropriate lever selection. Responses on the other lever are recorded but are not reinforced. A drug or the drug vehicle is injected at a predetermined time before the rat is placed in the Skinner Box and allowed to select the appropriate lever. Care is taken to allow no other cue, signal or hint for lever selection to be present.

Drugs known for certain pharmacological actions are selected to produce specific IDS so that they are suitable to provide a standard for subsequent comparisons. The drug thus selected is allowed to produce biochemical changes in the body that are characteristic of the drug. These changes form a physiological stimulus complex. When the nervous system is excited directly or indirectly through this stimulus complex, a new state is organized within the nervous system and certain components of that state can be consciously perceived, some dominantly and others minimally. In the beginning of training, the subject attends to dominant components of the stimulus complex. The other components are effectively ignored as irrelevant at that time. If the presence of the dominant stimulus component (cue) is found reliably associated with the response selection, it is adopted for discrimination learning. However, if a reliable association is not found with the dominant stimulus, one of the other cues is selected for discrimination.

The animal does not usually respond to the interoceptive stimuli at the beginning of a discrimination training. Rather, the subject first responds to more prominent stimuli present in its environment. The obvious ones include position of the operandum such as a lever, sequence of the training session, olfactory sensations or many other sensory cues which have been previously found more relevant in the natural environment. It is only when the external stimuli do not work that the distinct IDS associated with the drug action becomes the focus of discrimination learning. With most psychotropic drugs, 8-12 training sessions of 15 min each are needed before the subject begins to attend to IDS and 15-30 additional sessions are required to train for good discrimination. Once the rat learns to reliably identify a specific IDS, it can be employed in the bioassay procedures often for 6-12 months. This long period of usefulness following the initial training makes this method to be the most economical of the behavioral pharmacological methods available for drug development.

Following training, the rats can be used in a variety of ways. To simply categorize a new compound with regard to pharmacological classification, the unknown compound is injected and the rat is placed in the Skinner box for testing. If the rat selects the drug appropriate lever, the unknown is considered to be similar to the training drug with respect to IDS and perhaps other pharmacological properties. If the selected lever is the vehicle appropriate lever, the unknown drug is different with respect to IDS. Here, selection of the appropriate lever is treated as an all or none response in order to calculate the effective dose.

There are four types of discrimination found applicable in drug development. The most often used discrimination employs a drug versus vehicle distinction which has been found very useful to define specific IDS associated with any drug class and to identify new drugs for pharmacological classification. The second type of discrimination utilizes two doses of the same drug. This discrimination can provide distinction between quantitative and qualitative aspects of two different IDS produced by the same drug. For example, whereas small doses of amphetamine produce IDS related to the psychostimulant properties, high doses produce IDS related to psychotomimetic actions. Still another dose of amphetamine is found related to the cardiovascular effects of amphetamine. The third type of discrimination experiments involve discrimination between two drugs. This type is particularly

useful in obtaining information on the differences between the pharmacological properties of two drugs belonging to the same class. For example, many neuroleptics are also anticholinergics. If an animal can discriminate between two neuroleptics such as haloperidol and clozapine, one of which is devoid of anticholinergic properties, the discrimination between the two may provide a measure of anticholinergic side effects. In the fourth type, a standard drug is compared concurrently with vehicle and a number of other drugs during training itself. This discrimination is very useful to identify one specific IDS among many IDS produced by the same drug. For example, mixed agonist-antagonist type analgesics often produce undesirable psychotomimetic dysphoria. In the latter respect, they differ from pure agonist analgesics. In order to identify dysphoria as a dominant IDS, the mixed type drug may be discriminated against a combination of a placebo and a narcotic agonist, each given on the vehicle days alternatively or associated with third lever. The rat will be trained to select the drug lever after the mixed drug, and the vehicle lever after vehicle injection, or the injection of a pure agonist. This method of presenting contrasts to the animal promotes faster and sharper learning of the targeted IDS.

Interoceptive Stimulus Properties of Psychoactive Drugs - The IDS produced by psychoactive drugs are many as is illustrated by the drug classes listed in Table 1.

Table 1. Psychotropic Drugs Known to Produce Interoceptive Discriminable Stimuli

Drug	Proposed Nature of Dominant IDS
Amphetamines (low dose)	Psychostimulant arousal
Amphetamines (high dose)	Psychotomimetic euphoria
Antidepressants	Not known
Apomorphine	Central dopaminergic stimulation
Aspirin	Peripheral analgesia
Barbiturates	Sedation
Bemegride	Anxiety states (?), preconvulsive arousal (?)
Benzodiazepines	Anxiolytic, anticonvulsant (?)
Cannabinoids	Euphoria, dysphoria (?)
Clonidine	Central adrenergic, anxiolytic (?), dopaminergic (?) activity
Cocaine	Euphoria, anxiety (?)
Ethanol	Sedation
Hallucinogens	Psychotomimetic effects, serotonergic stimulation
Muscarinics -antimuscarinics	Central muscarinics stimulation/inhibition
Narcotics	Euphoria, dysphoria (?)
Narcotic antagonists	Dysphoria (?)
Neuroleptics	Not known
Nicotine	Central nicotinic stimulation
Pentylenetetrazol	Anxiety states, preconvulsive arousal (?)
Phenylbenzoquinone	Visceral pain
Quipazine	Psychotomimetic, central serotonergic stimulation
Steroids	Not known
Thyrotropin-releasing hormone	Not known

Each one of the psychoactive drugs (or drug classes) produces its own IDS with intriguing characteristics offering new potential for drug development. Because a comprehensive critique of each IDS is not the objective of this review, we chose to describe the recently identified IDS produced by pentylenetetrazol (PTZ) as an illustration of what can be done with IDS as tools for drug development. Many other IDS have been discussed in a recent monograph.[1]

Anxiety-Provoking Action of Pentylenetetrazol as IDS - Pentylenetetrazol induced IDS was originally investigated to develop a laboratory model of a preconvulsive neuronal excitation (aura). But, as the work progressed, it became evident that pentylenetetrazol induced IDS may actually be related to an anxiety state produced by this drug. Pentylenetetrazol has been known to cause intense anxiety in patients.[4] Since there were no chemical agents known to induce anxiety in the laboratory animal, this IDS was persued as the model of anxiety so that a new research tool might be established to investigate both the neurophysiological mechanisms of anxiety, as well as antianxiety drugs.

In a two-lever choice situation, laboratory rats treated with pentylenetetrazol readily learn to choose that lever specifically designated to be reinforced only when pentylenetetrazol is producing a cue. This talent is acquired gradually in a learning situation as is illustrated in Fig. 1 and is described in the previous section of this review. The animals first

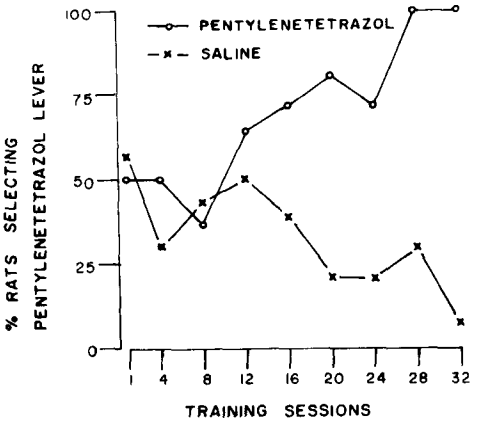

Fig. 1 - Acquisition of pentylenetetrazol versus saline discrimination. Values are the percentage of rats selecting the pentylenetetrazol lever on the indicated training session when a test injection of pentylenetetrazol or saline was given.[5] Data are based upon 14 rats at each point.

press two levers randomly. Gradually, the hit and trial responding is replaced by logical choices which increases in frequency until all choices are exercised only upon recognition of the IDS induced by pentylenetetrazol. The choice procedure is so designed that chance selections are not allowed towards reaching the learning criteria. If the drug is not acting in the body, as is the case after vehicle injection or an injection of a powerful antagonist before the training drug, another positively identified choice for non-drug has to be made in order to obtain food reinforcement. If the unknown drug is tested in doses too toxic, no choice is exercised, rather than reverting back to random responding. Once the ability to recognize drug IDS in order to make correct response selection is acquired, the rats continue to exhibit perfect discrimination, with a little practice, for the remainder of their life. The only limitation on the long-term testing is not the trained rat but the nature of the drugs tested. Some drugs may not be tolerated if the injections are not spaced appropriately. Others may show tolerance with time. With pentylenetetrazol, any effect determinental to the IDS-type bioassay can be avoided for over a year by spacing the

pentylenetetrazol injections to not more than twice a week. On this schedule, the rats do not develop pentylenetetrazol-related myoclonus until after a year or so. Even then, the dose-response curves of IDS induced by pentylenetetrazol are not changed, and the rats do not necessarily have to be discarded.[6]

Research in the past few years has provided overwhelming evidence, as has been discussed elsewhere,[5,7-11] that pentylenetetrazol-induced IDS represents an anxiety state which can be measured qualitatively and quantitatively. Anxiolytic drugs antagonize the pentylenetetrazol-induced IDS in a predictive manner and dose dependently. The blockade of pentylenetetrazol-induced IDS with anxiolytic drugs selected from different chemical classes is illustrated in Fig. 2.

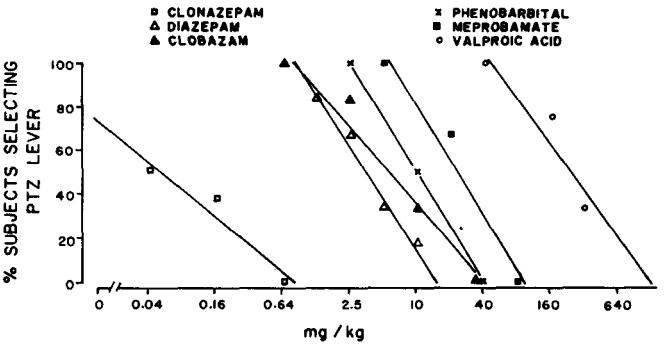

Fig. 2 - Dose-dependent antagonism by various anxiolytics of the anxiomimetic IDS produced by pentylenetetrazol in rats trained to discriminate pentylenetetrazol from saline. Rats were injected with pentylenetetrazol 30 min after each test drug and tested 15 min later. Percent of rats selecting pentylenetetrazol designated lever is given against the log test dose.

Irrespective of their chemical class and other distinguishing properties, if the test drugs are known to be anxiolytics, they are also active against pentylenetetrazol-induced IDS. This relationship has been found to be true in all of the drugs tested to date. In addition, a cursory look at the dose-response curves points out a practical advantage of this method for evaluating new anxiolytic drugs. The most often employed method to identify new anxiolytics was that developed by Geller and Seifter.[12] This method is based upon an antagonism of response suppression caused by punishment for responding in a conflict paradigm. Prior to drug testing, the rats are trained to learn the conflict-induced suppression. Although very reliable in detecting anxiolytic activity, the Geller and Seifter procedure, fails to produce linear dose responses necessary to bioassay drug potency, and the data obtained through this procedure are not directly suitable for ED_{50} calculation.[13] Moreover, the Geller and Seifter procedure requires use of electric shock applied to the animal's feet, which may be contraindicated in many situations. For example, intense stress related to painful electric shock is known to cause marked neuroendocrinological changes that may significantly interact with the new drug being tested. In contrast, the IDS procedure uses a chemical agent, which is neither painful nor pathological, to produce the anxiogenic effect, and the data obtained from IDS experiments provide more linear dose-response relationships. To pharmacologists engaged in the discovery and development of new drugs, this type of simplicity and sensitivity is a welcome feature. Additionally, the anxiolytic doses obtained from the pentylenetetrazol discrimination bioassay are highly correlated with those obtained in the conflict procedure (Fig. 3) of measuring anxiety and with clinically effective doses (Fig. 4).

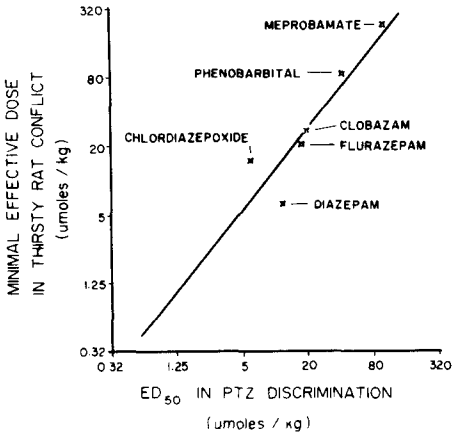

Fig. 3 - Correlation between the effective doses of anxiolytic drugs in antagonizing the anxiomimetic IDS of pentylenetetrazol with their effective doses in the conflict test.

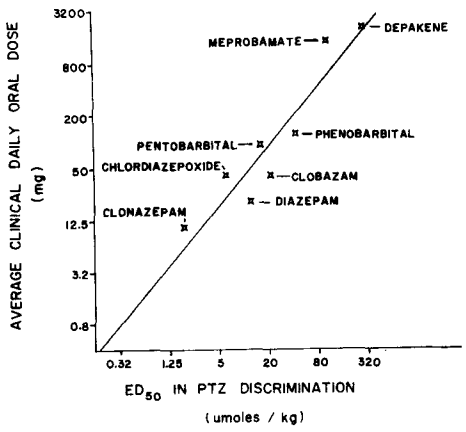

Fig. 4 - Correlation between the effective doses of anxiolytic drugs in antagonizing the anxiomimetic IDS of pentylenetetrazol with their effective clinical doses.

Whereas the data from known anxiolytics are confirmatory in nature, results from valproic acid testing provided an objective occasion to test the predictive validity of this new bioassay. Shearman and Lal[5] found that valproic acid antagonizes the pentylenetetrazol induced IDS. This observation permitted an a priori prediction that valproic acid would possess anxiolytic activity. Valproic acid was known to be an anticonvulsant drug whose antianxiety properties had not been tested in the clinic.[14] In order to test the validity of the above prediction, valproic acid was recently tested in the Geller-Seifter test of anxiety and found to be an active anxiolytic.[15]

There is some evidence that the Geller-Seifter test may be related to "fear and stress" anxieties of corticosteroid releasing type and may not really be a model of anxiety most often encountered clinically. Data with clonidine provides interesting insight in this regard. In spite of its sedative actions, clonidine is not considered an anxiolytic by the clinicians except in special circumstances where anxiety is caused by heroin[16] or alcohol[17] withdrawal. In view of the clonidine's specificity for panic anxiety, clonidine was recently tested in both the Geller-Seifter conflict test and in the pentylenetetrazol discrimination test. Clonidine was found very active in the Geller-Seifter test[18] but totally inactive in the pentylenetetrazol discrimination test,[6] suggesting that whereas the Geller-Seifter paradigm may provide a model of anxiety related to panic and stress, the pentylenetetrazol IDS may provide a model for neurogenic anxiety most often encountered in the clinical practice.

Pentylenetetrazol, in high doses, induces convulsions leading to death. The anticonvulsive property of drugs against pentylenetetrazol has been proposed as a bioassay to evaluate anxiolytic drugs.[19] Are the pentylenetetrazol convulsions an extension of the neurophysiological processes that underly pentylenetetrazol induced IDS? Although an answer cannot be provided until the neurophysiological processes underlying anxiety and convulsions are identified, there are indirect data that suggest some overlaps and some distinctions. If both of the actions of pentylenetetrazol are the

same, then any drug active in blocking one action should also block the other. This is not the case. It is true that most of the anxiolytic drugs block pentylenetetrazol-induced convulsions. However, those anxiolytics are also anticonvulsants besides being anxiolytic. Whether pentylenetetrazol seizure antagonism reflects the anticonvulsant or anxiolytic property of these drugs has not been determined. There are a number of anticonvulsant drugs, however, that are not anxiolytics. Testing of these drugs in both animal tests should provide indication of separation if there is any. Among the potent antiepileptic drugs are included, etomidate, ethosuximide and trimethadione which readily antagonize pentylenetetrazol convulsions. But these drugs are neither anxiolytic nor antagonists of the pentylenetetrazol-induced IDS.[5] These data and the experiments with clonidine suggest that, whereas either the antagonism of pentylenetetrazol convulsions or disinhibition of Geller-Seifter responses can often be taken as predictors of anxiolytic action, most of the time they are not always reliable in their prediction of antianxiety action because they produce false positives. To date, there have not been any false positives encountered in the pentylenetetrazol discrimination test of anxiety.

Besides the predictive value of the pentylenetetrazol-induced IDS for identifying new anxiolytics, this paradigm can also be used to predict anxiogenic side effects of new drugs. Anxiogenic effects of drugs are currently not recognized by any animal experiment. For example, psychiatrists have known for many years that cocaine produces acute anxiety in the abusers who take this drug in high doses.[20-22] However, to date, no animal data have been available to predict this action of cocaine. When cocaine was tested in the pentylenetetrazol-saline discrimination paradigm, it was found to generalize with pentylenetrazol,[9] suggesting that cocaine can be anxiogenic. It is interesting to note that the anxiogenic property of cocaine is not the dominant IDS produced by this drug and is thus not recognized in the animals that are not specifically trained to attend to the anxiomimetic effect. Animals trained only to discriminate cocaine from saline learn to perceive a more predominant IDS that is related to euphoria and which can be readily blocked by dopamine antagonist drugs. In contrast, cocaine generalization to pentylenetetrazol in animals trained to discriminate pentylenetetrazol from saline is not blocked by haloperidol as is the case with human anxiety, but instead the anxiogenic IDS of cocaine is antagonized by diazepam.

Conclusions - New animal procedures have been identified to quantitatively measure IDS produced by many psychoactive and other drugs. These economical and reliable procedures lend themselves to many problems of drug development including, but not limited to, the identification of new drugs. This was illustrated by describing IDS produced by pentylenetetrazol and its applications in identifying anxiolytic drugs as well as anxiogenic side effects of new chemicals. Other examples are cited in which drug-induced IDS are employed to develop animal models of several disease states; to classify new drugs; to predict abuse potential of a new drug; to study mechanisms of drug action; and to develop chemical agents which provide warning signals against an oncoming medical crisis.[2]

References

1. H. Lal in "Discriminative Stimulus Properties of Drugs," H. Lal, Ed., Plenum Press, New York, 1977.
2. H. Lal, Drug Develop. Ind. Pharmacol., 5, 133 (1979).
3. H. Lal, G. Gianutsos and S. Miksic in "Discriminative Stimulus Properties of Drugs," H. Lal, Ed., Plenum Press, New York, 1977, pp. 23-45.
4. W.D. Winter and M.B. Wallach in "Psychomimetic Drugs," D.H. Efron, Ed., Raven Press, New York, 1969, pp. 193-228.
5. G.T. Shearman and H. Lal, Neuropharmacol., in press (1980).

6. G.T. Shearman and H. Lal, unpublished data.
7. G.T. Shearman and H. Lal, in "Stimulus Properties of Drugs: Ten Years of Progress," F.C. Colpaert and J.A. Rosecrans, Eds., Elsevier, Holland, 1978, pp. 181-199.
8. G.T. Shearman and H. Lal, Fedn. Proc., 38, 256 (1979).
9. G. Shearman and H. Lal, Psychopharmacol., 64, 315 (1979).
10. G.T. Shearman, S. Miksic and H. Lal, Pharmacol. Biochem. Behav., 10, 795 (1979).
11. G.T. Shearman and H. Lal, Pharmacol., 21, 267 (1979).
12. I. Geller and J. Seifter, Psychopharmacol., 1, 482 (1960).
13. G.T. Pollard and J.L. Howard, Psychopharmacol., 62, 117 (1979).
14. A.T. Dren, personal communication.
15. H. Lal, G.T. Shearman, S. Fielding, R. Dunn, H. Kruse and K. Theurer, Brain Res. Bull., 4, 711 (1979).
16. M.S. Gold, D.E. Redmond, Jr. and H.D. Klebar, Lancet, 1, 929 (1978).
17. S.E. Bjorkqvist, Acta Psych. Scand., 52, 256 (1975).
18. S.A. Bullock, H. Kruse and S. Fielding, Pharmacol., 20, 223 (1978).
19. A.S. Lippa, P.A. Nash and E.N. Greenblatt in "The Anxiolytics," S. Fielding and H. Lal, Eds., Futura Publishing Co., New York, 1979, pp. 41-81.
20. S. Cohen, J.A.M.A., 231, 245 (1975).
21. R.K. Siegel in "Cocaine: 1977," R.C. Petersen and R.C. Stillman, Eds., N.I.D.A., Washington, D.C., 1977, pp. 119-136.
22. D.R. Wesson and D.E. Smith in "Cocaine: 1977," R.C. Petersen and R.C. Stillman, Eds., N.I.D.A., Washington, D.C., 1977, pp. 137-152.

Section II - Pharmacodynamic Agents

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Chapter 7. Pulmonary and Antiallergy Drugs

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Introduction - The variable nature and complex etiology of allergic asthma has been re-emphasized,[1,2] and a review of the drugs currently in use in this indication has appeared.[3] The inability to effectively extrapolate the pharmacological behavior of newer therapeutic agents to that observed in the clinic remains a concern. The utility of bronchoprovocation as a standard technique was assessed in a recent workshop.[4]

General - Reviews of the present understanding of the production and control of IgE[5,6] and its interaction with antigen[6,7] have appeared. Preparative isolations of the cell receptor have been reported.[8,9] Cross-linking of these receptors increases membrane permeability and thereby effects calcium influx.[10] The involvement of protein phosphorylation in this process has been suggested.[11] The dramatic and rapid turnover of specific phospholipids in concanavalin A induced degranulation of rat peritoneal mast cells has been reported[12] to involve breakdown to diacylglycerol (DAG), followed by phospholipid resynthesis. The site and significance of these phospholipid alterations and the intracellular function of DAG remain to be established. In another report,[13] the increase in phosphatidylinositol turnover was suggested as evidence of a regulatory role in the formation of membrane Ca^{+2} channels. Turnover of phosphatidylserine (PS) was not observed in the above studies; however, this phospholipid specifically potentiates concanavalin A or antigen-induced histamine release.[14] N-substituted derivatives of PS (e.g. N-acetyl PS) inhibit this potentiation,[15] whereas the fragment lysophosphatidylserine is 50 to 1000 x more potent than PS as an enhancer.[14,16]

Inhibitors of Mediator Release - The regulation of protein phosphorylation has been suggested as the mode of action of disodium cromoglycate (DSCG).[11] Phosphodiesterase (PDE) inhibition is not a major effect since the concentrations of DSCG and of M & B 22948 (1) required to inhibit rat mast cell c-AMP PDE were found in both instances to be more than ten times that necessary for the inhibition of histamine release.[17] In chopped human lung, DSCG has been reported[18] to not only inhibit histamine release induced by anti-IgE, but also to potentiate that induced by anti-IgG. This dichotomy has been offered as an explanation for the ubiquitous bell-shaped dose response in *in vitro* assays and for the unpredictability of DSCG in the clinical situation.

Sodium nivimedone (BRL-10833, 2) at 100mg p.o. was found "almost as effective" as inhaled DSCG in exercise-induced bronchoconstriction,[19] beneficial at 200mg (t.i.d.) in asthmatics,[20] but ineffective in ulcerative colitis.[21] Evidence of malignancy in rats has resulted in the withdrawal of 2 from further clinical trials.[20] Bufrolin (ICI-74917, 3),

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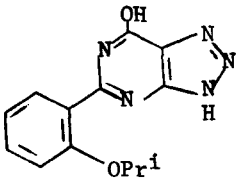

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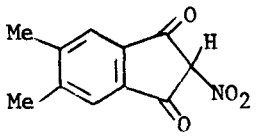

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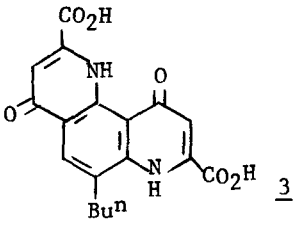

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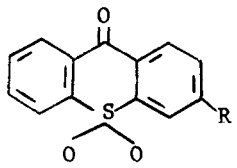
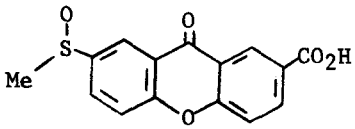


4, R = tetrazole
5, R = CO_2H



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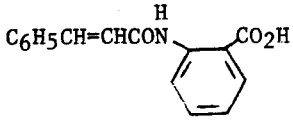

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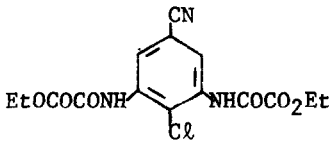

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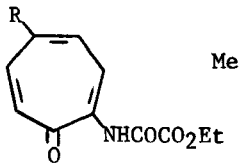
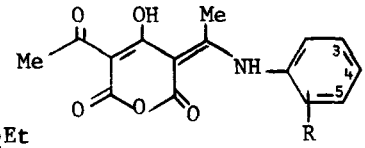


9, R = H
10, R = $NHCOCO_2Et$

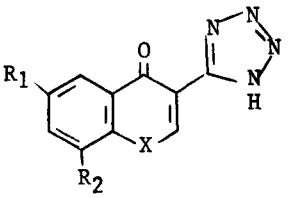


11

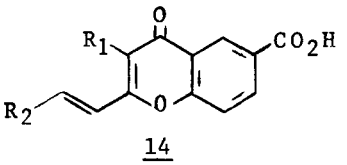
was equivalent to DSCG in a controlled trial with thirty-two asthmatics.[22] A comparison of doxantrazole (4) with DSCG in fourteen asthmatics favored DSCG although neither drug was significantly better than placebo;[23] 4 has been withdrawn from further study.[24] The carboxylic acid analog BW437C (5) was shown[25] to be more effective than either 4 or DSCG in inhibiting antigen-induced histamine release in human leukocytes. The xanthone RS-7540 (6) as a wet aerosol (20mg) was judged effective in exercise-induced asthma; no significant side effects were observed.[26] The anthranilic acid N-5[1] (7) was evaluated (5-10mg/kg/day p.o.) in 284 asthmatic children;[27] more than 60% of the patients improved; and no significant side effects were observed at 5mg/kg.

Lodoxamide ethyl (U-42718, 8) had ID_{50}'s of 0.07 and 1mg/kg p.o. in the rat PCA and primate ascaris lung assay, respectively;[28] however, in guinea pig aerosol lung anaphylaxis, an IgG based model, 50mg/kg p.o. was required for effective protection. Inhibition by orally administered AY-25674(9) of passive anaphylaxis in the rat hind paw[29] was 0.4 x as potent as 2, whereas intraperitoneally in the same model, 9 was 4 times as effective as DSCG. Ring substitution generally reduced activity,[30] although an analog incorporating a second oxamate grouping (10) was equivalent to 9. Toxicological findings discouraged clinical development of 9.[29]

The evaluation of a large series of pyranenamines (11) in rat and primate models has been described.[31] Optimum i.v. activity (PCA ID_{50} 0.9mg/kg) was found with 3,5-bis-glyceramoyl substitution,



12, R_1 = Et, R_2 = H, X = O
13, R_1 = H, R_2 = Cℓ, X = NH



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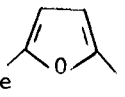
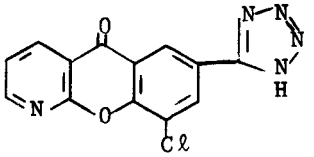


15, R_1 = Pr^n, R_2 = Me



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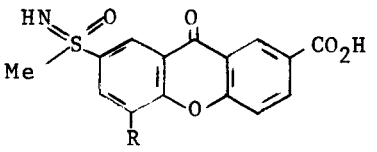


17

whereas p.o., the 3-hydroxy-4-amino analog (ID_{50} 0.8mg/kg) and the corresponding propionylamide (ID_{50} 0.6mg/kg) were most potent. The QSAR trends in this series indicate hydrophilicity as an important property of the aryl substituents.[32]

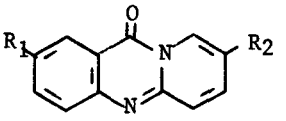
The chromone AA-344 (12) was effective in inhibiting homologous PCA and passive systemic anaphylaxis in the guinea pig but was only slightly effective in the Arthus reaction.[33] DSCG was ineffective in these models. The structures of the major metabolites of 12 have been described;[34] oxidation at both carbons of the ethyl substituent and glucuronic acid attachment to the tetrazole are the primary processes. 3-(Tetrazol-5-yl) quinolines have been reported as moderate inhibitors of rat PCA by i.p. and p.o. administration.[35] The incorporation of a 4-oxo-substituent significantly enhanced activity. Compound 13, the most potent of the series, was 33 x DSCG (i.p.) and 32 x 4 (p.o.).

In the series 14, the vinyl moiety, an alkyl substituent at C-3 (R_1) and a heteroaryl grouping at R_2 are prerequisites for optimum activity; 15 was most potent (rat PCA ID_{50}'s 0.25mg/kg i.p. and 2mg/kg p.o.).[36]

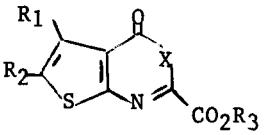
The benzopyranopyridine Y-12141 (16), was orally active in the rat PCA (ID_{50} 2.5mg/kg)[37] and inhibited IgG-mediated experimental asthma in the guinea pig.[38] In the novel series of xanthone-2-carboxylic acids (17) optimum PCA activity was found with RU-31156 (17, R=C_6H_{13}, ID_{50} 0.005 mg/kg i.v. and 0.2mg/kg p.o.);[39] crosstachyphylaxis with DSCG was reported in a detailed pharmacological profile of RU-31156.[40]

Oral activity in the rat PCA and allergic bronchospasm models was observed with a series of 11-oxo-11H-pyrido(2,1-b)quinazoline-8-carboxylic acids;[41] the 2-methyl analog (18) was 3 x DSCG i.v. and ∿ 5 x 4 p.o. In an independent extension of this series,[42] 19 was found to be 300 x more potent than 18 in the rat PCA (ID_{50} 0.02mg/kg p.o.); the 8-isopropyl-2-carboxylic acid (20) was also very potent in this model (ID_{50} 0.45mg/kg p.o.).

Moderate i.v. and p.o. activities in the rat PCA and allergic



18, R_1 = Me, R_2 = CO_2H
19, R_1 = Pr^i, R_2 = CO_2H
20, R_1 = CO_2H, R_2 = Pr^i



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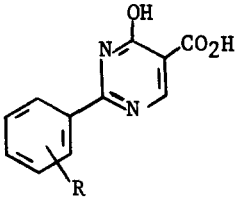
22, R_1 = H, R_2 = R_3 = Et, X = NH



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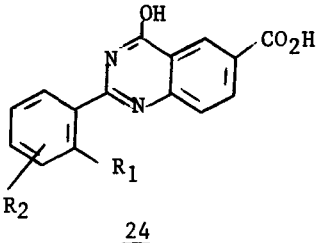

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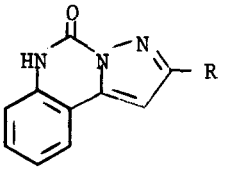


25, R = CH_2OH
26, R = CO_2H

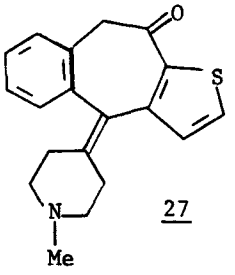
bronchospasm models have been reported[43] for the novel heterocyclic series of general formula 21. Optimum PCA activity p.o. (ID_{50} 2.4mg/kg) was observed with 22; the oxazines (21, X = O) were in general less active than the pyrimidine (21, X = NH) analogs.

Structure-activity relationships in the series 23[44] and 24[45] parallel those observed in the development of 1.[46] The most potent representatives were 23 (R = 2-c-PrCH_2O-, ID_{50} 0.1mg/kg p.o.) and 24 (R_1 = OEt, R_2 = 3-OCH_3, ID_{50} 1mg/kg p.o.) in the rat PCA assay.

Oral PCA activity (rat, ID_{50} 2-4mg/kg) has been claimed[47] for SQ-13847 (25). The corresponding carboxylic acid SQ-11903 (26) was only weakly active p.o., but 100 x 25 on i.v. administration; rapid in vivo metabolism of 25 to 26 was reported. Crosstachyphylaxis of 26 with DSCG was observed.

The remaining compounds reviewed here do inhibit mediator release but this activity is generally secondary to the antagonism of mediator effects or other activities. Ketotifen (HC-20511, 27) was orally effective in bronchial provocation in asthmatic adults with specific antigen,[48,49] histamine[48,50,51] and aspirin,[52] but not with acetylcholine[48] or methacholine.[53] In one study,[54] 27 was ineffective in adults in exercise-induced asthma but, in another[48] was judged equal to DSCG. In controlled therapeutic trials, 27 was ineffective in children,[55,56] but in adults was equivalent to DSCG.[48]

The phthalazinone (azelastine, A-5610, 28), equivalent to mepyramine as an H_1 antagonist, inhibited 48/80 histamine release in rat mesentery pieces by pretreatment at concentrations of 10^{-3} to 10^{-4}g/ml. At higher concentrations, 28 alone released histamine in a dose-dependent manner.[57] Tiaramide (29) was equivalent to DSCG in the inhibition of mast cell degranulation,[58] but much more effective (ID_{50} 3.3mg/kg i.v., 36mg/kg p.o.) in active systemic anaphylaxis[59] in the rat. Cinnarizine (30) was found to be clinically effective in the treatment of twelve asthmatics by oral administration (75mg t.i.d.); no significant side effects were observed.[60]



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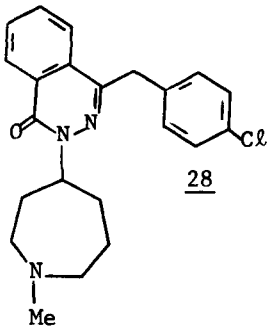

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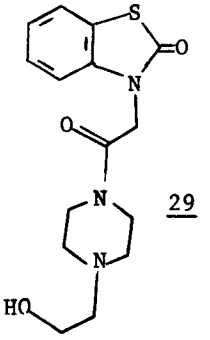

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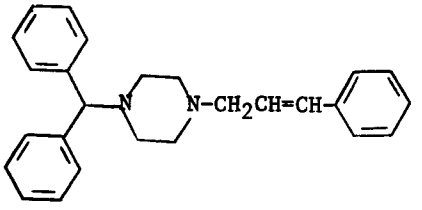

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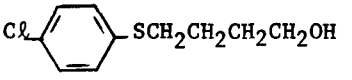


31

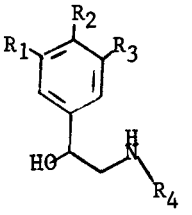
DSCG and W-2719 (31) were equivalent in their ability to inhibit rat mast cell degranulation, but in chopped rabbit lung 31 was 100 x more potent in preventing histamine release.[61] On oral administration in man, 31 is not detectable in the plasma or urine; rapid metabolism to p-chlorophenylthioacetic acid is a primary process.[62] This metabolite is claimed to be responsible for the in vivo antihistamine effects of 31.

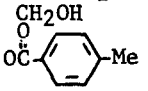
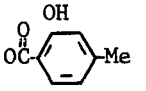
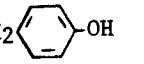
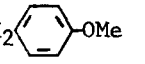
β_2-Adrenergic Stimulation of Adenyl Cyclase (AC) - Binding of the β-adrenergic receptor to isoproterenol (32) in reticulocyte membranes increases phospholipid methylation which subsequently enhances membrane fluidity.[63] This event has been suggested to stimulate AC by increasing the lateral movement of the activated β-receptor complex and providing a greater opportunity for coupling with the enzyme system. The mechanism whereby this coupling results in the activation of AC has been reviewed.[64]

The selective β_2 stimulatory activity of clenbuterol (NAB 365, 33) was confirmed by a comparison of its effects with those of 32 on bronchial, cardiac and hypothalamic adenyl cyclase.[65] Clinically 33 (20μg i.v.) was equivalent to terbutaline (34, 250μg i.v.) in exercise-induced bronchospasm;[66] cardiovascular, cerebral and skeletal muscular side effects were seen with 34, but absent with 33.

Carbuterol (35) was found to be more effective than ephedrine[67] or salbutamol (36)[68] in adult asthmatics; mild tachycardia was observed with both drugs in the latter study.

Bitolterol (37), an ester prodrug to colterol (38), was less potent than 32 or 38 in dogs, but had a much longer duration of action.[69] The bronchodilator/cardiovascular dose separation with 37 was superior to that of 32, 36, or 38 and is explained as a consequence of greater deposition of 37 in lung, rather than heart tissue, and by the fact that lung esterase activity is greater than that of the heart.



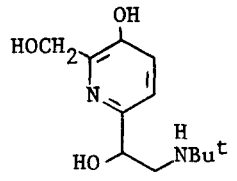
	R_1	R_2	R_3	R_4
32	OH	OH	H	Pr^i
33	Cℓ	NH_2	Cℓ	But
34	OH	H	OH	But
35	$NHCONH_2$	OH	H	But
36	CH_2OH	OH	H	But
37			H	But
38	OH	OH	H	But
39	OH	H	OH	
40	CH_2OMe	OH	H	
41	OH	H	OH	CMe_2CH_2OH

In asthmatics,[70] fenoterol (39) provided a bronchodilator effect exceeding that of 34 in magnitude and duration, although in another study[71] 34 was claimed to be of greater benefit. Enhancement of mucociliary clearance was demonstrated clinically[72] with 39 in bronchial asthma.

Replacement of the hydroxymethyl group of 36 by methoxymethyl improved bronchodilating activity in the guinea pig significantly. From a series with this modification, 40 was most potent.[73] The racemic diastereomers 40a (mp 168-170°C) and 40b (mp 125-126°C) differed in activity by a ratio (a:b) of 3:1. That of 40a was 4 x 36; the levo isomer was 2 x the racemate, whereas the dextro isomer was inactive.

In human lung fragments, 36, but not DSCG, was a potent inhibitor of anaphylactic release of histamine and SRS-A; however in rat mast cells only DSCG was effective.[74] The bronchodilating potency of KWD-2131 (41) was 0.02-0.03 x 34, but 0.15 x as effective in inhibiting histamine release from human lung tissue.[75] In eight atopic subjects, 34 intradermally provided a dose-dependent inhibition of allergen-induced skin reaction;[76] the flare responses to histamine, 48/80 or trypsin were not inhibited.

In isolated guinea pig tracheal muscle and atria, pirbuterol (42) was found to possess 9 times the β_2-selectivity of 36.[77] In a multiple dose evaluation,[78] 42 was judged effective and free of cardiovascular side effects; tremor was observed in 7 out of 21 patients. Metabolism of procaterol (43) in dogs[79] proceeds primarily via the glucuronide; unchanged 43 was excreted in 10-40% on i.v. and 10-30% on p.o. administration. The ratio of increases in blood lactate and insulin to that of free fatty acids in dogs was utilized as a measure of β_2-selectivity (43 ∿ 36 >> 32).[80]



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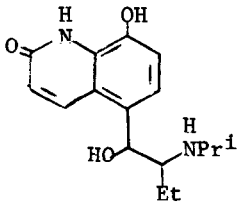

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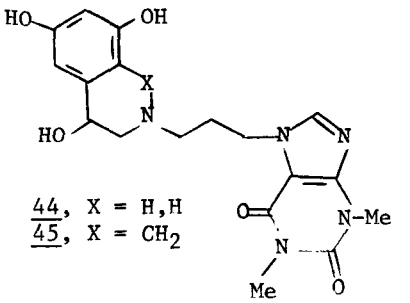
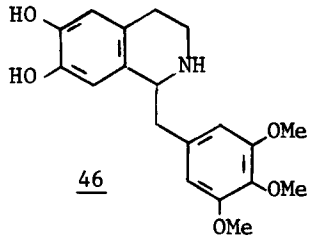


44, X = H,H
45, X = CH_2



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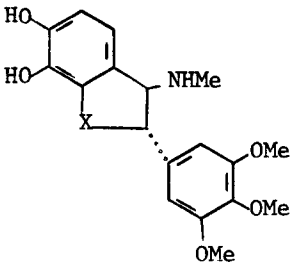
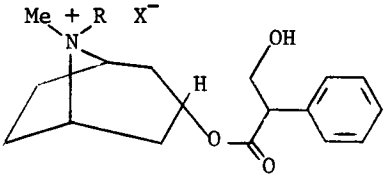


47

Reproterol (44) was found effective in the treatment of bronchospasm in infants and children.[81] The structure and synthesis of the primary metabolite of 44 in animals and man (45) has been reported;[82] no β-mimetic activity was observed.

In a series of benzylamine derivatives[83] modeled after trimetoquinol (46), the β-mimetic activity of the tetrahydronaphthalene (47: X = CH_2CH_2), while being 500 x less than that of 46, was 2 x more potent than the corresponding 1,2-diarylethylamine (47: X = H,H) and 10 x more potent than the cis tetrahydronaphthalene. The indane (47: X = CH_2) was inactive.

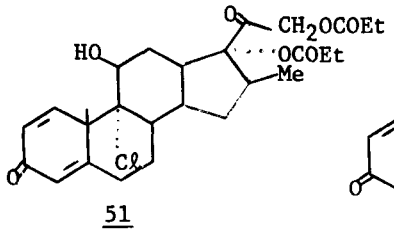
Anticholinergics - The potential utility of anticholinergic drugs in the treatment of bronchial asthma has become more evident, both experimentally and in the clinic. The developments in this field have been extensively reviewed.[2,84] A review of the clinical performance of ipratropium bromide (Sch 1000, 48) has also appeared;[85] 48 was judged effective prophylactically against general airways irritation and free of significant side effects. The specific indication of 48 for prophylaxis rather than as a bronchospasmolytic has been further emphasized.[86] Inhibition of nasal methacholine provocation by 48 has been reported.[87] The ethyl analog of 48, oxitropium bromide (49, Ba253) was found safe and effective in chronic non-specific obstructive lung disease;[88] equivalence to 48 was claimed although a direct comparison was not made.



48, R = Pr^i , X = Br
49, R = Et , X = Br
50, R = Me , X = NO_3

Atropine methonitrate (50) was equivalent to 36 in 18 asthmatics in bronchodilator effect but of longer duration; the combination of 50 and 36 was more effective than either agent alone. Dryness of mouth and mild visual blurring was observed with 50.[89]

Corticosteroids - A review of the multiplicity of effects of corticosteroids on immune processes has appeared.[90] This class of drugs provides effective and often essential therapy, although the mechanism of action in allergic disorders remains uncertain. Systemic side effects are their primary drawback, although inhaled preparations have alleviated this problem somewhat. Efficacy was generally maintained throughout a 3-5 year study with beclomethasone dipropionate (51) aerosol in asthmatic children.[91] No influence on growth rate or the development of monilial infection was observed.



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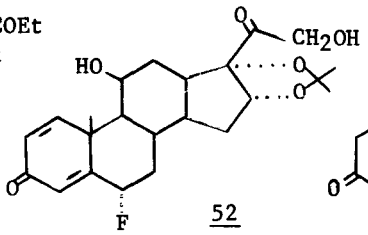

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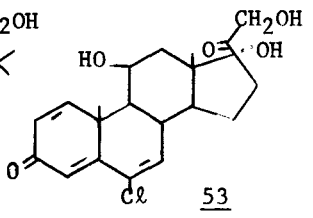
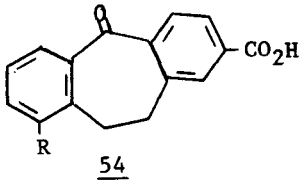


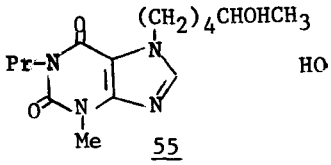
53

Flunisolide (52) was judged topically effective in bronchial asthma[92] and allergic rhinitis;[93] side effects were minor. Cloprednol (53) in single daily doses (p.o.) was more effective than alternate day prednisolone in asthmatic children;[94] partial pituitary-adrenal suppression was observed in both instances. In a twelve month study,[95] seventeen steroid-dependent children were switched from daily prednisone to 53. Improvement in symptoms was observed with less pituitary-adrenal axis dysfunction; pulmonary function was unchanged.

Miscellaneous - A series of 5H-dibenzo [a,d] cyclohepten-5-one carboxylic acids having bronchodilator activity has been described;[96] the most potent representatives (54, R=Me or Cl) were equivalent (i.p.) to theophyline in histamine aerosol challenge in guinea pigs. The xanthine (55) was a more potent bronchodilator than theophylline and was significantly more selective for the bronchopulmonary cAMP PDE in guinea pigs.[97]



54



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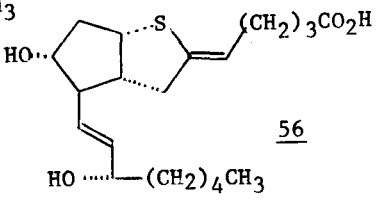

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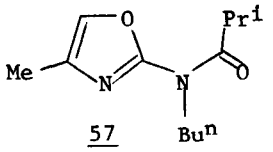

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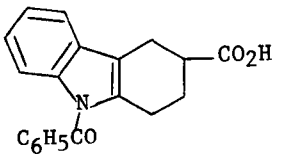

58

The stable 6,9-thia-analog of PGI_2 (56) inhibited rat PCA (58%) at 30µg/kg i.v., but was less potent than PGI_2 or PGE_2 at the same dose (77% and 76% respectively).[98] The significance of H_1 and H_2 receptors and the utility of their antagonists in immediate hypersensitivity has been reviewed.[99]

Isamoxole (57) is one member of a series of 2-acylalkylamino oxazoles[100] shown to selectively inhibit SRS-A release (93% at 10µg/ml) from passively sensitized chopped human lung. Orally (2 x 100mg/kg) 57 was ineffective in the rat PCA but was equivalent to 27 (2mg/kg) in the inhibition of antigen-induced bronchospasm in guinea pigs. Oxarbazole (58) was effective in guinea pigs in the inhibition of bronchoprovocation induced by SRS-A but not by histamine or $PGF_{2\alpha}$.[101] A review of pharmacological effects of the SRS-A antagonist FPL-55712 has appeared.[102]

References

1. J.C. Hogg, P.D. Paré, R.C. Boucher, M.C. Michoud, Can. Med. Assoc. J., 121, 409 (1979).
2. Scandinavian Symposium on Chronic Airways Disease, N. Svedmyr and M.S. Nilsson, Ed., Scand. J. Resp. Dis., Suppl. 103 (1979).
3. N. Svedmyr and B.G. Simonsson, Pharmac. Ther. B., 3, 397 (1978).
4. Bronchoprovocation Techniques for the Evaluation of Asthma, R.R. Rosenthal, Ed., J. Allergy Clin. Immunol., 64, 561 (1979).
5. W.Y. Lee and A.H. Sehon, Immunological Rev., 41, 200 (1978).
6. K. Ishizaka and T. Ishizaka, ibid., 41, 109 (1978).
7. H. Metzger, ibid., 41, 186 (1978).
8. A. Kulczycki and C.W. Parker, J. Biol. Chem., 254, 3187 (1979).
9. J. Kanellopoulos, G. Rossi and H. Metzger, ibid., 254 7691 (1979).
10. T. Ishizaka, J.C. Foreman, A.R. Sterk, K. Ishizaka, Proc. Nat. Acad. Sci, 76, 5858 (1979).
11. T.C. Theoharides, W. Sieghart, P. Greengard and W.W. Douglas, Science, 207 80 (1980).
12. D.A. Kennerly, T.J. Sullivan and C.W. Parker, J. Immunol., 122, 152 (1979).
13. S. Cockcroft and B.D. Gomperts, Biochem. J., 178, 681 (1979).
14. T.W. Martin and D. Lagunoff, Nature, 279, 250 (1979).
15. T.W. Martin and D. Lagunoff, Science, 204, 631 (1979).
16. G.A. Smith, T.R. Hesketh, R.W. Plumb, J.C. Metcalfe, FEBS Letters, 105, 58 (1979).
17. H. Bergstrand, B. Lundquist and A. Schurmann, Mol. Pharmacol., 14, 848 (1978).
18. M.K. Church and C.F. Gradidge, Life Sci., 23, 1899 (1978).
19. W. Lenney, A.D. Milner and R.M. Tyler, Brit. J. Dis. Chest, 72, 225 (1978).
20. E.M. Lumb, G.J.R. McHardy and A.B. Kay, Brit. J. Clin. Pharmacol., 8, 65 (1979).
21. P.S. Davies, J. Rhodes, B. Counsell, R.V. Heatley and R.G. Newcombe, Clin. Allergy, 9, 373 (1979).
22. J. Moxham and M. McAllen. Clin. Allergy, 9, 61 (1979).
23. H.R. Gribbin, J.E. Harvey and A.E. Tattersfield, Brit. Med. J., 92 (1979).
24. Scrip., p. 15 (Feb. 3, 1979).
25. J.L. Pinnas, T.M. Chen and J.G. Perkins, Clin. Res., 26, 293A (1978).
26. B. Stenius, Y. Salorinne and D. Parrott, Scand. J. Resp. Dis., 59, 75 (1978).
27. H. Shioda and Collaborators, Multicenter Study, Allergy, 34, 213 (1979).
28. H.G. Johnson, R.L. Griffin, J.B. Wright, Agents and Actions, 9, 235 (1979).
29. R.R. Martel, J. Klicius and J. Pinski, Can. J. Physiol. Pharmacol., 56. 1005 (1978).
30. J.F. Bagli, T. Bogri, B. Palameta, R. Martel, W. Robinson, T. Pugsley and W. Lippmann, J. Med. Chem., 22, 1186 (1979).
31. K.M. Snader, L.W. Chakrin, R.D. Cramer, Y.M. Gelernt, C.K. Miao, D.H. Shah, J.W. Venslavsky, C.R. Willis and B.M. Sutton, ibid., 22, 706 (1979).
32. R.D. Cramer, K.M. Snader, C.R. Willis, L.W. Chakrin, J. Thomas, and B.M. Sutton, ibid., 22, 714 (1979).
33. H. Kuriki, T. Saijo, Y. Ashida and Y. Maki, Japan J. Pharmacol., 29, 733 (1979).
34. A. Nohara, H. Kuriki, T. Ishiguro, T. Saijo, K. Ukawa, Y. Maki and Y. Sanno, J. Med. Chem., 22, 290 (1979).
35. E.H. Erickson, C.F. Hainline, L.S. Lenon, C.J. Matson, T.K. Rice, K.F. Swingle and M. Van Winkle, ibid., 22, 816 (1979).
36. G. Doria, C. Romeo, A. Forgione, P. Sberze, N. Tibolla, M.L. Corno, G. Cruzzola and G. Cadelli , Eur. J. Med. Chem., 14, 347 (1979).
37. K. Goto, M. Terasawa and Y. Maruyama, Int. Archs. Allergy Appl. Immunol., 59, 13 (1979).
38. M. Terasawa, K. Goto and Y. Maruyama, Jap. J. Pharmacol., 28 suppl., 85p (1978).
39. A.C. Barnes, P.W. Hairsine, S.S. Matharu, P.J. Ramm and J.B. Taylor, J. Med. Chem., 22, 418 (1979).
40. P. Miller and G.W.L. James, Arch. Int. Pharmacodyn., 231, 328 (1978).
41. C.F. Schwender, B.R. Sunday, D.J. Herzig, E.K. Kusner, P.R. Schumann and D.L. Gawlak, J. Med. Chem., 22, 748 (1979).
42. J.W. Tilley, R.A. LeMahieu, M. Carson, R.W. Kierstead, H.W. Baruth and B. Yaremko, ibid., 23, 92 (1980).
43. D.L. Temple, J.P. Yevich, R.R. Covington, C.A. Hanning, R.J. Seidehamel, H.K. Mackey and M.J. Bartek, ibid., 22, 505 (1979).
44. P.F. Juby, T.W. Hudyma, M. Brown, J.M. Essery, R.A. Partyka, ibid., 22, 263 (1979).
45. G. Doria, C. Romeo, P. Sberze, M. Tibolla, M.-L. Corno and G. Cadelli, Eur. J. Med. Chem., 14, 247 (1979).
46. B.J. Broughton, P. Chaplen, P. Knowles, E. Lunt, S.M. Marshall, D.L. Pain and K.R.H. Wooldridge, J. Med. Chem., 18, 1117 (1975).
47. C.A. Free, F.B. Casey, B.E. Abboa-Offei, L.E. Hall, J. Marretta and S. Starkweather, Fed. Proc., 38, 523 (1979).
48. L. Craps, C. Greenwood and P. Radielovic, Clin. Allergy, 8, 373 (1978).
49. V. Hartmann, H. Magnussen, and J.P. Holle, Prax. Pneumol., 33, 309 (1979).
50. K. Mattson, H. Poppius and R. Nikander-Hurme, Clin. Allergy, 9, 411 (1979).
51. H.M. Beumer, Respiration, 37, 271 (1979).
52. B. Wüthrich, ibid., 37, 224 (1979).
53. K. Mattson, H. Poppius and R. Hurme, Clin. Allergy, 9, 495 (1979).
54. H. Gmür and M. Scherrer, Schweiz. Med. Wschr., 109, 881 (1979).
55. B. Taylor and R. Ford, Clin. Allergy, 9, 241 (1979).

56. O. Østerballe and E.A.L. Nielsen, Allergy, 34, 125 (1979).
57. K. Tasaka and M. Akagi, Arzneim.-Forsch., 29, 488 (1979).
58. M.L. Renoux, B.J. Weill, D. Wallach and E. Chwetzoff, Allergy, 33, 76 (1978).
59. T. Takashima, T. Ono, M. Ohtsuka, J. Mori and S. Kumada, Arzneim.-Forsch., 29, 903 (1979).
60. M.B. Emanuel, J.A. Chamberlain, S. Whiting, B.G. Rigden and A.H. Craven, Brit. J. Clin. Pharmac., 7, 189 (1979).
61. G.M. Fukui, B.J. Ludwig, R.D. Sofia, D.B. Reisner, and F.M. Berger, Arzneim.-Forsch., 29, 912 (1979).
62. R.G. Meeks, B.M. Bayer, R.D. Sofia, J.R. Bianchine, D. Couri, ibid., 29, 1756 (1979).
63. F. Hirata, W.J. Strittmatter and J. Axelrod, Proc. Nat. Acad. Sci., 76, 368 (1979).
64. R.J. Lefkowitz, Ann. Int. Med., 91, 450 (1979).
65. E. Marmo, A.P. Caputi, G.M. Nistico, F. Rossi and E. Lampa, Res. Commun. Chem. Path. Pharmacol., 23, 3 (1979).
66. N. Del Bono, F. Quartieri and C. Vibelli, Clin. Allergy, 9, 277 (1979).
67. T.D. James and H.A. Lyons, J. Am. Med. Assoc., 241, 704 (1979).
68. J.S. Guleria, K.V. Vasu and J.N. Pande, Ann. Allergy, 43, 123 (1979).
69. H. Minatoya, J. Pharmacol. Exp. Ther., 206, 515 (1978).
70. G. Anderson, E. Wilkins and A.G. Jariwalla, Brit. J. Dis. Chest, 73, 81 (1979).
71. P.W. Trembath, J.K. Greenacre, M. Anderson, S. Dimmock, L. Mansfield, J. Wadsworth and M. Green, J. Allergy Clin. Immunol., 63 395 (1979).
72. R. Felix, J.P. Hedde, H.J. Zwicker and C. Winkler, Prax. Pneumol. 32, 777 (1978).
73. S. Sohda, M. Fujimoto, T. Tamegai and N. Hirose, J. Med. Chem., 22, 279 (1979).
74. P.R. Butchers, J.R. Fullarton, I.F. Skidmore, L.E. Thompson, C.J. Vardey and A. Wheeldon, Brit. J. Pharmacol., 67, 23 (1979).
75. K. Strandberg, K.-O. Pegelow, C.G.A. Persson and L. Sörenby, Allergy, 34, 221 (1979).
76. R. Grönneberg, K. Strandberg and Ö. Hägermark, ibid., 34, 303 (1979).
77. P.F. Moore, J.W. Constantine and W.E. Barth, J. Pharmacol., Exp. Ther., 207, 410 (1978).
78. H.M. Beumer, Int. J. Clin. Pharmacol. Biopharm., 17, 18 (1979).
79. Y. Yasuda, N. Fujisawa, S. Morita and H. Kohri, Arzneim.-Forsch., 29, 261 (1979).
80. Y. Irie, J. Igawa, J. Hosokawa and Y. Saitoh, Eur. J. Pharmacol., 53, 351 (1979).
81. D. Cămprag, V. Dovat, B. Bogdanov, B. Čonkić, D. Tabori and R. Aurich, Med. Welt, 30, 897 (1979).
82. G. Niebch, K.H. Klingler, G. Eikelmann, N. Kucharczyk, Arzneim-Forsch., 28, 765 (1978).
83. S. Yamamura, K. Oda, T. Mizoguchi, S. Saito, Y. Iwasawa, M. Ohashi and A. Kiyomoto, Chem. Pharm. Bull., 26, 3613 (1978).
84. C.W. Hertz, Scand. J. Resp. Dis., Suppl. 103, 105 (1979).
85. B.G. Simonsson, ibid., Suppl. 103, 130 (1979).
86. G. Schultze-Werninghaus, E. Gonsior, J. Meier-Sydow, Dtsch. Med. Wschr., 104, 1099 (1979).
87. P. Borum, F.S. Larsen and N. Mygind, Acta Otolaryngol., Suppl. 360, 35 (1979).
88. E. Flohr and K.O. Bishoff, Respiration, 38, 98 (1979).
89. R.J. Pierce, C.J. Allen and A.H. Campbell, Thorax, 34, 45 (1979).
90. J.E. Parrillo and A.S. Fauci, Ann. Rev. Pharmacol. Toxicol., 19, 179 (1979).
91. S. Godfrey, L. Balfour-Lynn and M. Tooley, J. Allergy Clin. Immunol., 62, 335 (1978).
93. D.R. Webb, M.F. Mullarkey and M.I. Freeman, Ann. Allergy, 42, 80 (1979).
93. J.N. Sahay, S.S. Chatterjee and C. Engler, Clin. Allergy, 9, 17 (1979).
94. E.F. Ellis, H.G. Morris, F. Kiechel, J.D. Pollock, L.J. Strand, J. Clin. Pharmacol., 19, 675 (1979).
95. G.G. Shapiro, D.S. Tattoni, V.C. Kelley, W.E. Pierson, C.S. Dorsett and C.W. Bierman, Pediatrics, 63, 747 (1979).
96. J.P. Dunn, D.M. Green, I.T. Harrison, P.H. Nelson, J.R. Pfister, A.P. Roszkowski and K.G. Untch, J. Med. Chem., 22, 1357 (1979).
97. V. Stefanovich and E. Porsche, Arzneim.-Forsch., 29, 917 (1979).
98. K. Komoriya, H. Ohmori, A. Azuma, S. Kurozumi, Y. Hashimoto and K.C. Nicolaou, Japan, J. Pharmacol., 29, 811 (1979).
99. M. Plaut, J. Allergy Clin. Immunol., 63, 371 (1979).
100. W.J. Ross, R.C. Harrison, M.R.J. Jolley, M.C. Neville, A. Todd, J.P. Verge, W. Dawson and W.J.F. Sweatman, J. Med. Chem., 22, 412 (1979).
101. Z.E. Mielens, Pharmacol., 17, 323 (1978).
102. N. Chand, Agents and Actions, 9, 133 (1979).

Chapter 8. Slow-Reacting Substances

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Introduction - Since the observation by Kellaway and Trethewie[1] that cobra venom released a slow-reacting substance (SRS) from guinea-pig lung, a number of SRSs have been described, the most interesting of which is the immunologically generated SRS-A.[2] Although the biological activity of partially purified material has been described, many attempts to elucidate the structure of the various SRSs have been unsuccessful, mainly due to the impure material. The various SRSs have very potent biological actions but, particularly in the case of the immunological material, they are released in small quantities. Combined with difficulties in purification, the small amount available has delayed successful structural elucidation of the SRSs until very recently. The use of reverse phase HPLC by Morris et al for the purification of SRS-A[3,4] was a major advance and has led to the preparation of chemically pure SRS-A.[5] Up to the present time SRS/SRS-A have been quantitated by bioassay using guinea-pig ileum (in the presence of mepyramine and hyoscine or atropine) and expressed in terms of units. Unfortunately, different groups use units based on different measurements thus making direct comparisons of the various preparations of SRS/SRS-A difficult. However, accurate determination of the molecular weight of an SRS from RBL-1 cells by mass spectrometry[5] heralds the precise quantitation of the SRSs on a weight basis.

Due to similarity in the biological actions of SRS-A and the non-immunologically generated SRSs, a number of authors describe them all as SRS-A, but until the structures of the various SRS-As have been determined precisely this resemblance of structure should not be assumed.

Release and Preparation of SRS-A and SRSs - Many of the early attempts to determine the structures of these substances were carried out on immunologically generated material (from guinea-pig lung or rat peritoneum)[6] or on SRS released from cat paw by histamine releasers.[7] The original purification techniques were lengthy and involved numerous chromatography procedures. The development of the HPLC techniques has led to rapid and efficient purification of these unstable substances.[3,5]

Several groups have used a non-immunological stimulus, the calcium ionophore A23187, to generate SRSs from tissues.[8,9] The use of dispersed cell systems to produce SRSs has simplified their purification since the crude SRS from cells suspended in buffer is far less contaminated than material immunologically released in vivo or in vitro. As shown in Table 1, the non-immunologically generated SRSs are indistinguishable from SRS-A in a number of biological tests. It was also impossible to demonstrate qualitative differences between the SRSs after a single HPLC step,[10] but subtle structural differences may be revealed after further purification.

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Table 1. Comparison of biological properties of SRSs

	Guinea-Pig	Human SRS-A	Rat† SRS	RBL-1 SRS	MCT SRS
Contracts guinea-pig ileum	+(2)	+(2)	+(2)	+(2)	+(1)
Contracts human bronchus, guinea-pig trachea	+(1)	+(1)	+(1)	+(1)*	+(1)*
Releases PGs, Tx	+(2)	+(1)	+(1)	+(2)	+(1)
Antagonized by FPL 55712	+(2)	+(2)	+(2)	+(2)	+(1)
Inactivated by arylsulphatase	+(2)	+(1)	+(1)	+(2)	+(1)
Inactivated by soybean lipoxygenase	+(2)	+(1)	+(1)	+(1)	+(1)

(1) - ex-charcoal material used
(2) - material from all stages of purification used
* - not tested on human bronchus
Rat† - A23187-induced SRS from rat peritoneum
RBL-1 rat - basophil leukemia cells
MCT - murine mastocytoma cells

SRS-A has been described as an acid lipid,[11] and Walker showed that its release was connected with arachidonic acid metabolism when indomethacin potentiated the release of SRS-A from human lung.[12] A similar effect was seen during immunologic challenge of guinea-pig lung[13] (Fig. 1); we found the same modulation of A23187-induced SRS release from lung tissue,[14] and other groups have found identical effects of indomethacin on release of SRS (-A) in other systems.[15] In platelets[16] the products of lipoxygenase were increased during inhibition of cyclo-oxygenase showing the re-direction of arachidonic acid metabolism and thus providing an explanation for the potentiation of SRS-A release. Fatty acids which are substrates for lipoxygenase[17,18] potentiated the release of SRS-A and this potentiation was increased still further in the presence of indomethacin. Together with evidence from other groups,[19] this suggested that immunologically generated SRS-A was a product of a lipoxygenase and

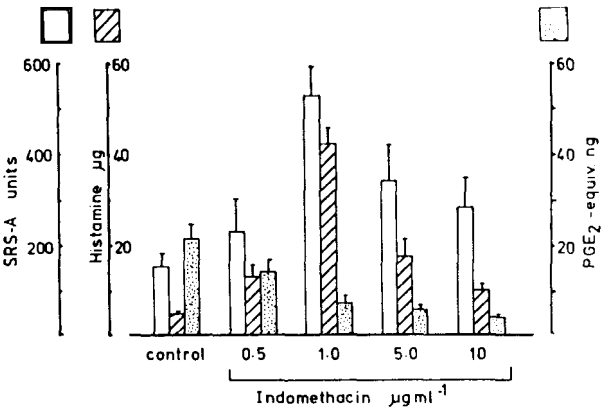


Figure 1. The effect of indomethacin on the release of SRS-A, histamine and prostaglandins during anaphylaxis in guinea-pig isolated perfused lungs.

probably a metabolite of arachidonic acid (Fig. 2). Similar experiments in rat basophil leukemia (RBL-1) cells showed non-immunologically induced SRS to be a lipoxygenase product,[20] and the incorporation of ^{14}C-arachidonic acid into SRS confirmed its precursor role.The inhibition of SRS-A release by compounds which inhibit lipoxygenase, such as eicosatetraynoic acid (ETA), nordihydroguaiaretic acid (NDGA) or BW577c,[18,21] provided further evidence for the role of lipoxygenase. This was confirmed using degradative techniques when the first structure of an SRS from murine mastocytoma cells, leucotriene C (LTC), was proposed by Samuelsson's group.[22] Following the study of arachidonic acid metabolism in rabbit polymorphonuclear leucocytes (PMNs),[23] LTC was shown to be a metabolite of arachidonic acid produced by a lipoxygenase which catalysed different reactions from the platelet lipoxygenase.

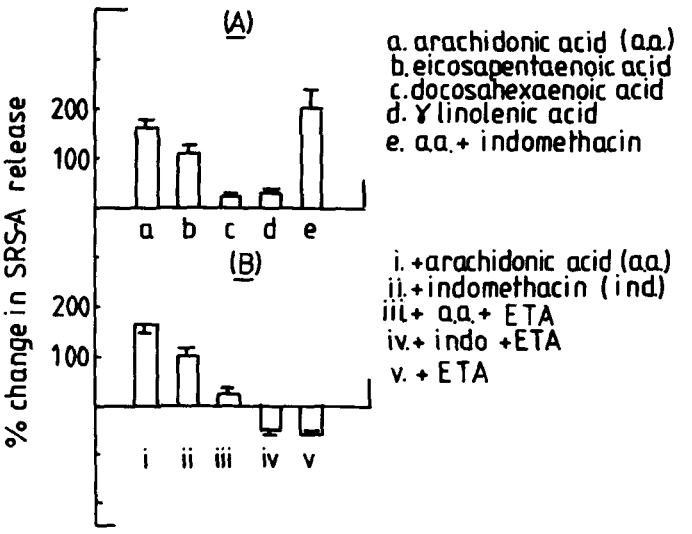


Figure 2. (A). The effect of substrates for lipoxygenase (a-d) and indomethacin on release of SRS-A from guinea-pig chopped lung.

(B). Inhibition by eicosatetraynoic acid (ETA) of SRS-A release potentiated by a.a. or indo.

The cell-type which generates SRS-A in lung tissue is unknown but SRS-A is released from human mast cells and basophils.[24,25] The calcium ionophore A23187 stimulates SRS release from a number of cell types; for instance, human PMNs,[26] rat peritoneal cells,[27] monocytes[9] and RBL-1 cells,[8] and murine mastocytoma cells[22] and peritoneal cells.[28]

Structural Studies - (i) Chemical Tests - The stability of SRS-A and other SRSs under various chemical conditions has been investigated to throw light on the structures under examination. Stability was measured in terms of the preservation or destruction of the biological activity (Tables 2,3). The observation that SRS-A was destroyed by incubation with arylsulphatase[29] led to the suggestion that SRS-A/SRS was a sulphate ester; however, stability of highly purified material did not suggest the presence of a sulphate moiety[30] and this was finally discounted (in the case of RBL-1 SRS) by mass spectrometric data.[5] Sulphur is, however, present in SRSs but as a thioether linkage to the acid lipid skeleton.

Table 2. Conditions under which SRSs are stable

	Guinea-pig SRS-A	Human SRS-A	Rat SRS	RBL-1 SRS	MCT SRS
Boiling 60 min	+(1)	+(1)	+(1)	+(1)	+(1)
Base 0.1M NaOH RT 30 min	+(1)	+(1)	+(1)	+(1)	+(1)
$NaBH_4$ RT 30 min	+(2)	NT	NT	NT	NT
Ether pH 3.0	+(2)	+(2)	+(2)	NT	NT
Water pH 7.0	+(3)	+(3)	+(3)	+(3)	+(3)

(1) ex-charcoal material used
(2) ex-sephadex G15
(3) material at all stages of purification

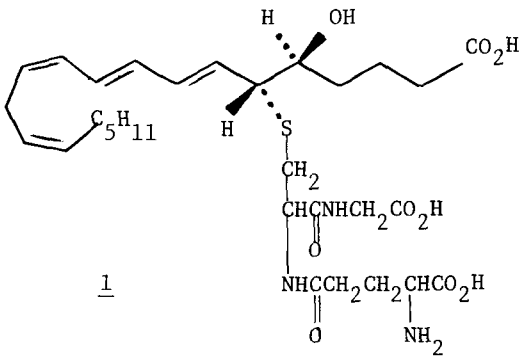
Table 3. Conditions under which biological activity of SRS is destroyed

	Possible chemical groups indicated
1. HCl 0.1M RT 30 min	
2. Acetylation	
(i) methanol acetic anhydride 4:1 v/v. RT 1 min	α amino group
(ii) pyridine acetic anhydride 1:10 v/v RT 30 min	amino/hydroxyl groups, double bonds
Fluram treatment	amino groups
3. Methylation	
(i) CH_2N_2 RT 30 min	
(ii) Methanol/HCl RT 30 min	free carboxyl groups
(iii) Methanol/BF_3 37°C 30 min	
4. Catalytic hydrogenation Ni_2B RT and 55°C in Methanol	unsaturated/(thioether linkage)
5. CNBr treatment	thioether linkage
6. Arylsulphatase 1 mg/ml 1 h	sulphate
Soybean lipoxygenase 50 μg/ml 1 h	cis,cis-1,4-pentadiene

The observation that pure SRS-A shows a uv absorbance of λmax at 280 nm in MeOH and has a uv spectrum characteristic of modified conjugated trienes[3] was a major step forward and enabled SRS-A and other SRSs to be classified as trienes. Destruction of biological activity by soybean lipoxygenase showed that SRS-A contains a cis,cis-1,4-pentadiene sequence.[10]

The use of protein chemistry techniques showed the presence of an α-amino group(s), and other tests indicated the presence of hydroxyl and carboxy groups.[4]

Using degradative techniques, Samuelsson's group made the first suggestion for the structure of an SRS derived from murine mastocytoma cells by A23187.[22,31] This SRS was described as leukotriene C (LTC)(1). LTC is derived from arachidonic acid by the action of a lipoxygenase which stimulates the incorporation of oxygen and the formation of 5-hydroperoxy-eicosapentaenoic acid followed by an unstable intermediate 5,6-epoxy-eicosatetraenoic acid (leukotriene-A). Like SRS-A, LTC has a λmax in MeOH at 280 nm, is a triene, and is inactivated by lipoxygenase. The structure of LTC was originally reported as 5-hydroxy-6-cysteinyl-7,9,11,14-eicosatetraenoic acid.[32] Later, by comparison with synthetic compounds, the structure of LTC was revised and described as 5-hydroxy-6-γ-glutamylcysteinylglycinyl-7,9,11,14-eicosatetraenoic acid.[33] The final stereochemistry of LTC was determined by Corey, et al.[34]



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(ii) Mass Spectrometric Data - After two stages of HPLC, four uv absorbing compounds (I-IV) can be separated in SRS-A from guinea-pig lung, (Fig. 3).[4] Compounds I and II are related by a 2-3 nm shift in MeOH (λmax 280 nm, 278 nm, respectively) as are compounds III and IV (λmax 270 nm, 268 nm, respectively). Compound I fulfills all the criteria for SRS-A, compound II has less activity on the guinea-pig ileum, and the hypsochromic shift of 2-3 nm is consistent with compound II being a cis/trans or other closely related isomer of SRS-A. Compounds III and IV have no activity on the guinea-pig ileum. Compound III has a uv spectrum consistent with a cis,trans,trans or similar triene (λmax 270 nm) whereas compound IV possibly corresponds to the all-trans triene.[35] Mass spectrometric evidence (of the trimethylsilyl derivative of the carboxylic methyl ester) shows that compound III is 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid,[4] a metabolite of arachidonic acid originally detected in rabbit PMNs.[23] This was the first demonstration that this metabolite is generated during immuno-

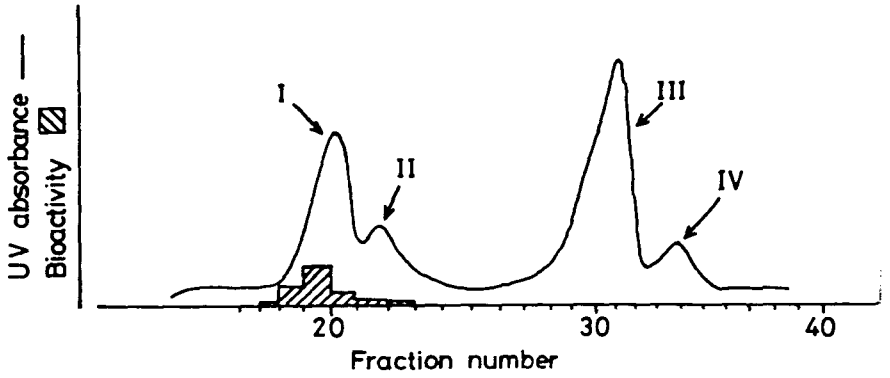
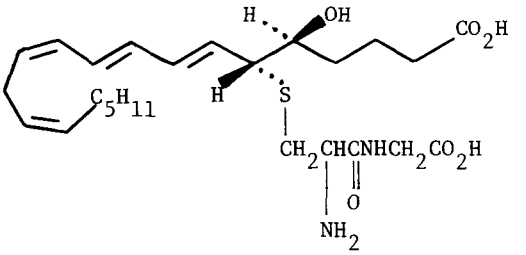


Figure 3. Elution profile of guinea-pig SRS-A (μ Bondapak C_{15} column) after second HPLC step in n-propanol: acetic acid: water gradient. Of the four uv absorbing compounds, compound I is SRS-A.

logical challenge of guinea-pig lung tissue and shows that the 5,6-epoxy-eicosatetraenoic acid must be formed during anaphylaxis.

Using SRS derived non-immunologically from RBL-1 cells as a source of compounds for the study of SRS-A, Morris et al[5] obtained the first mass spectrometric evidence for the structure of the major biologically active species of an SRS by examining the intact molecule as a derivative. The exact structure of the side chain linked by a thioether at C6 was determined by amino-acid analysis and sequence determination; the RBL-1 SRS is a peptidolipid, 5-hydroxy-6-cysteinyl-glycinyl-7,9,11,14-eicosatetraenoic acid (2). This work was an important break-through in the field of structure elucidation of SRSs since the structure was determined on the naturally occurring SRS and not on synthetic molecules which possess SRS-like biological activity. Similarly, by the use of protein chemistry techniques and mass spectrometric analysis of a derivative of the intact molecule of SRS-A from guinea-pig lung, Morris et al[54] showed the structure of this SRS-A to be identical with that of RBL-1 SRS.



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RBL-1 SRS has the characteristic triene chromophore originally identified in SRS-A from guinea-pig lung[3] and has biological actions indistinguishable from those of SRS-A. After HPLC at least three uv absorbing compounds can be separated in RBL-1 SRS; compound I is the major biologically active species, compound II also has biological activity, while compound III is inactive on guinea-pig ileum (Fig. 4). As in guinea-pig SRS-A, compound II is probably an isomer of compound I (2), whereas compound III is probably 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid.

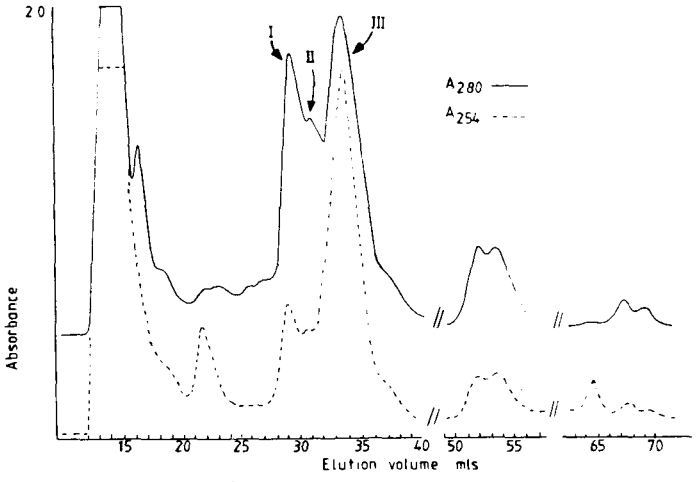


Figure 4. Elution profile of RBL-1 SRS after HPLC (as in Fig. 3). Biological activity resides in peaks I and II.

SRSs with Related Structures - Metabolism of arachidonic acid by cyclo-oxygenase gives rise to a number of products including prostaglandins E_2, D_2, $F_{2\alpha}$. These prostaglandins have minor differences in structure which produce marked differences in their pharmacologic actions. It seems likely that metabolism of arachidonic acid by lipoxygenase (possibly in different cells where the epoxide could undergo attack by different nucleophiles) could form SRSs of different structures.

The difference in structure of the SRSs from RBL-1 cells and murine mastocytoma cells may be the first evidence of a "family" of SRSs. A variety of mercaptans stimulate production of SRS from mononuclear cells.[36] The mercaptans may be incorporated into SRS(s) and, instead of stimulating SRS release, may actually give rise to SRSs with varying thioether-peptide side chains at C6 which vary in potency. By analogy, SRSs may also be derived from fatty acids other than arachidonic acid since 5,8,11,14-eicosapentaenoic acid potentiated release of SRS-A from guinea-pig lung.[18]

Pharmacology - Most of the biological actions of the SRSs described in the literature have been obtained using partially purified material and require repeating with pure materials. However, in comparative studies using immunologically generated SRS-As from human and guinea-pig lung and non-immunological SRSs from rat peritoneum, RBL-1 cells and murine mastocytoma cells, all SRSs showed the same biological activity in the tests carried out (Table 1)[10,37] but no quantitative measurements of relative potencies were carried out. SRS-A from guinea-pig lung and SRS from RBL-1 cells can be separated into a number of uv absorbing compounds.[4,5] Although some of these compounds are inactive on the guinea-pig ileum, it is not known whether they are active in other systems or whether they potentiate or inhibit the actions of the major biologically active species. Other groups have found multiple active peaks in SRSs.[38,39] The two peaks of activity obtained during purification of SRS generated by A23187 have different relative potencies on guinea-pig parenchyma lung strips,[39] which is the first demonstration of differing activities of SRSs, suggesting that there may be complex interactions between the different components of SRSs.

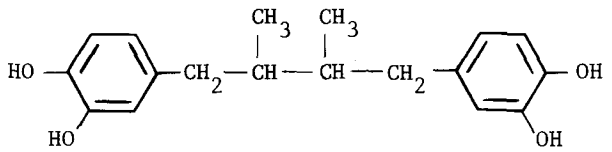
Since SRS-A is thought to be an important bronchoconstrictor in asthma, most of the pharmacologic investigations of the SRSs have centered on the lungs. The fact that A23187 stimulates release of SRS from human lung tissue[40] suggests that this substance(s) has a role in intrinsic as well as extrinsic asthma. However, pure SRS-A has actions in the microvasculature of guinea-pig skin.[41] Pure SRS-A, like bradykinin or histamine, caused exudation of plasma, confirming initial observations with partially purified material.[42] This action was potentiated by vasodilator prostaglandins. Unlike the vasodilators, bradykinin, or histamine, SRS-A caused reduction in blood flow which was not blocked by indomethacin. These findings suggest that SRS-A may not only contribute to edema and swelling in the airways but may also play a role in inflammation. Indeed other products of lipoxygenase have been shown to be chemotactic,[43] a property which might be shared by the SRSs.

SRSs stimulate metabolism of arachidonic acid in guinea-pig lung and cause release of TxA_2 and other cyclo-oxygenase products.[44] This action of SRSs is limited to guinea-pig lung tissue and does not occur in human or rat lung.[45,46] The release of TxA_2 from human lung was stimulated mechanically but not by antigen challenge, and it therefore seems unlikely that this action of SRS-A is important in asthma.

Release of TxA_2 by SRS-A is inhibited by cyclo-oxygenase inhibitors, such as indomethacin, glucocorticoids[44] and mepacrine, which suggests a phospholipase A-dependent step in this action of SRS-A/SRS.

Modulation of Release - The release of SRS-A and SRSs can be modified by drugs which interfere with arachidonic acid metabolism. The output of these materials is greatly increased by cyclo-oxygenase inhibitors[12,13,14,20] due to the re-direction of arachidonic acid metabolism via the lipoxygenase pathway.[47] Inhibition of synthesis of thromboxanes (with imidazole) or prostacyclin (with 15-hydroperoxy-arachidonic acid, 15-HPAA) also increases release of SRS-A from guinea-pig lung. A combination of both of these inhibitors caused the greatest potentiation of SRS-A release.[48] However, 15-HPAA may have stimulated output of SRS-A by a mechanism other than inhibition of synthesis of prostacyclin.[19] SRS(-A) release may be increased directly by arachidonic acid[17,20] or eicosapentaenoic acid.[17] These exogenous fatty acids may be incorporated into SRS[20] or act by displacing endogenous arachidonic acid.[23]

Compounds which inhibit the action of lipoxygenases also block the release of SRS-A/SRS; output is prevented by NDGA (3), eicosatetraynoic acid (4),[4] or BW577c.[21] Re-direction of arachidonic acid metabolism away from lipoxygenase via cyclo-oxygenase may be seen with NDGA as with diethylcarbamazine.[37,49] These observations suggest that drugs causing inhibition of lipoxygenase, possibly together with cyclo-oxygenase, may be of therapeutic use in asthma.



3

$CH_3(CH_2)_4(C{\equiv}CCH_2)_4CH_2CH_2CO_2H$

4

Enzymic Degradation - The biological activity of a number of SRSs is destroyed by soybean lipoxygenase and arylsulphatase.[10] Lipoxygenase oxidizes the cis,cis-1,4-pentadiene of the SRS molecule, a structural feature which is essential for biological activity. The digestion of SRSs by arylsulphatase is more difficult to explain because at least the SRS from RBL-1 cells does not contain a sulphate moiety.[5] It is feasible that the molecule could be misrecognized in terms of binding the conjugated triene to the aromatic binding site on the enzyme, the sulphur and oxygen atoms then possibly being in the correct position to lead to cleavage of the sulphur side chain. This suggestion, together with the destruction of the thioether linkage by cyanogenbromide[4] causing a loss of SRS-A activity, indicates the intact thioether is critical for biological activity. It is interesting that molecules with at least two different peptides attached to the thioether possess SRS-like bioactivity.[5,33]

Conclusion - In the last two years, since it became possible to completely purify small quantities of SRSs,[3] scientific interest has focused on these products of arachidonic acid metabolism by a lipoxygenase. There is probably a family of related substances all possessing SRS-like biological activity but with different molecular structure. The structure of an SRS may depend on its cell of origin or

on the releasing stimulus. In the lung a variety of stimuli cause arachidonic acid to be metabolized via the cyclo-oxygenase pathway resulting in the formation of prostaglandins, thromboxanes and prostacyclin.[50] There is evidence that prostaglandins are generated in the smooth muscle of the large airways,[51] thromboxane A_2 in the lung parenchyma,[52] while prostacyclin is probably secreted in the pulmonary endothelium.[53] During anaphylactic shock or stimulation by A23187, arachidonic acid metabolism is triggered but the balance between cyclo-oxygenase and lipoxygenase products is altered, perhaps by calcium-dependent mechanisms, with the appearance of at least SRS-A or SRS and 5,12-dihydroxy-eicosatetraenoic acid. SRSs interact with prostaglandins and thromboxanes in terms of release and pharmacologic actions and may interact with prostacyclin (via their action on blood vessels)[41] and perhaps with biologically active peptides.

Recent discoveries predict the timely replacement of the biological assay of naturally occurring SRSs expressed in activity units by accurate mass spectrometric and ultraviolet quantitation. Together with the availability of pure materials these will be valuable assets in this fast-moving field.

References

1. C. H. Kellaway and E. R. Trethewie, Q. J. Exp. Physiol., 30, 121 (1940).
2. W. E. Brocklehurst, J. Physiol., 151, 416 (1960).
3. H. R. Morris, G. W. Taylor, P. J. Piper, P. Sirois and J. R. Tippins, FEBS Lett., 87, 203 (1978).
4. H. R. Morris, G. W. Taylor, P. J. Piper and J. R. Tippins, Agents and Actions Supplement 6, Prostaglandins and Inflammation, K. D. Ramsford and A. W. Ford-Hutchinson, Ed., Birkhauser Verlag Basel, 1979, p. 27.
5. H. R. Morris, G. W. Taylor, P. J. Piper, M. N. Samhoun and J. R. Tippins, Prostaglandins, 19, 185 (1980).
6. R. P. Orange, M. D. Valentine and K. D. Austen, J. Exp. Med., 127, 767 (1968).
7. E. Anggard, V. Bergqvist, B. Högberg, K. Johansson, 1. L. Thon and B. Uvnas, Acta Physiol. Scand., 59, 97 (1963).
8. B. A. Jakschik, A. Kuczycki, H. H. MacDonald and C. W. Parker, J. Immunol., 119, 618 (1977).
9. M. K. Bach and J. R. Brashler, J. Immunol., 120, 998 (1978).
10. H. R. Morris, P. J. Piper, G. W. Taylor and J. R. Tippins, Br. J. Pharmacol., 67, 179 (1979).
11. R. P. Orange, R. C. Murphy, M. L. Karnovsky and K. F. Austen, J. Immunol., 110, 760 (1973).
12. J. L. Walker, in "Advances in the Biosciences," Vol. 9, S. Bergstrom and S. Bernhard, Ed., Braunschweig: Pergamon Press, Vieweg, 1972, p. 235.
13. D. M. Engineer, U. Niederhauser, P. J. Piper and P. Sirois, Br. J. Pharmacol., 62, 61 (1978).
14. P. J. Piper and J. P. Seale, Br. J. Pharmacol., 67, 67 (1979).
15. L. D. Yecies, S. M. Johnson, H. J. Wedner and C. W. Parker, J. Immunol., 122, 2090 (1979).
16. M. Hamberg, Biochem. et. Biophys. Acta, 431, 651 (1976).
17. D. H. Nugteren, Biochem. Biophys. Acta, 380, 299 (1975).
18. P. J. Piper, J. R. Tippins, H. R. Morris and G. W. Taylor, in "Arachidonic Acid Metabolism in Inflammation and Thrombosis," Vol. 4, K. Brune and M. Baggiolini, Ed., AAS Birkhäuser Verlag Basel, 1979, p. 37.
19. J. J. Adcock, L. G. Garland, S. Moncada and J. A. Salmon, Prostaglandins, 16, 179 (1978).
20. B. A. Jakschik, S. Falkenhein and C. W. Parker, Proc. Natl. Acad. Sci., 74, 4577 (1977).
21. J. F. Burka and R. J. Flower, Br. J. Pharmacol., 65, 35 (1979).
22. B. Samuelsson, P. Borgeat, S. Hammarström and R. C. Murphy, Prostaglandins, 17, 785 (1979).
23. P. Borgeat and B. Samuelsson, Proc. Natl. Acad. Sci., U.S.A., 76, 2148 (1979).
24. N.A.M. Paterson, S. I. Wasserman, J. W. Said and K. F. Austen, J. Immunol., 117, 1356 (1976).
25. M. C. Conroy, R. P. Orange and L. M. Lichtenstein, J. Immunol., 116, 1677 (1976).
26. O. Radmark, C. Malmsten and B. Samuelsson, FEBS Lett., 110, 213 (1980).
27. M. K. Bach and J. R. Brashler, J. Immunol., 113, 2040 (1974).

28. P. Sirois, D. M. Engineer, P. J. Piper and E. G. Moore, Experientia, 35, 361 (1979).
29. R. P. Orange, R. C. Murphy and K. F. Austen, J. Immunol., 113, 316 (1974).
30. C. W. Parker, M. G. Huber and S. Falkenhein, Clin. Res., 27, 473A (1979).
31. R. C. Murphy, S. Hammarstrom and B. Samuelsson, Proc. Natl. Acad. Sci., 76, 4275 (1979).
32. J. L. Fox, Chem. Eng. News., 57 (24), 19 (1979).
33. S. Hammarstrom, R. C. Murphy, B. Samuelsson, D. A. Clark, C. Mioskowski and E. J. Corey, Biochem. Biophys. Res. Commun., 91, 1266 (1979).
34. E. J. Corey, D. A. Clark, G. Goto, A. Marfat, C. Mioskowski, B. Samuelsson, and S. Hammarstrom, J. Am. Chem. Soc., 102, 1436 (1980).
35. G. S. Bild, C. S. Ramadoss, S. Lim and B. Axelrod, Biochem. Biophys. Res. Commun., 74, 949 (1977).
36. M. K. Bach and J. R. Brashler, Life Sci., 23, 2119 (1978).
37. P. J. Piper, in "Proceedings of Xth Congress of Allergology," Jerusalem, 1980.
38. C. W. Parker, B. A. Jakschik, M. G. Huber and S. F. Falkenhein, Biochem. Biophys. Res. Commun., 89, 1186 (1979).
39. M. K. Bach, J. R. Brashler, M. A. Johnson and J. M. Drazen, in "Proceedings of Xth Congress of Allergology," Jerusalem, 1980.
40. J. P. Seale and P. J. Piper, Lancet, II, 1265 (1978).
41. P. J. Piper and T. J. Williams, Prostaglandins, in press (1980).
42. W. E. Brockelhurst, in "Third International Symposium on Vasoactive Polypeptides." Bradykinin and related kinins., Pergamon Press., San Paulo, 1966, p. 189.
43. E. J. Goetzl, H. R. Hill and R. R. Gorman, Prostaglandins, 19, 71 (1980).
44. D. M. Engineer, H. R. Morris, P. J. Piper and P. Sirois, Br. J. Pharmacol., 64, 211 (1978).
45. P. J. Piper and J. L. Walker, Br. J. Pharmacol., 47, 291 (1973).
46. F. AL-Ubaidi and Y. S. Bakhle, Eur. J. Pharmacol., in press. (1980).
47. M. Hamberg, Biochem. Biophys. Acta, 431, 651 (1976).
48. D. M. Engineer, P. J. Jose, P. J. Piper and J. R. Tippins, J. Physiol., 281, 42P, (1978).
49. P. J. Piper, J. R. Tippins, H. R. Morris and G. W. Taylor, Prostaglandins, 19, in press (1980).
50. R. J. Gryglewski, in "Prostacyclin," J. R. Vane and S. Bergstrom, Ed., Raven Press, New York, N.Y., 1979, p. 275.
51. J. Orehek, J. S. Douglas, A. J. Lewis and A. Bouhuys, Nature (New. Biol.), 245, 84 (1973).
52. Y. Kapanci, P. Mo Costabello and G. Gabbiani, in "Lung Cells in Disease," A. Bouhuys, Ed., North Holland Publishing Co., Amsterdam, 1976, p. 69.
53. S. Moncada, R. Korbut, S. Bunting and J. R. Vane, Nature, 273, 767 (1978).
54. H. R. Morris, G. W. Taylor, P. J. Piper and J. R. Tippins, Nature, in press, (1980).

Chapter 9. Antihypertensive Agents

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General - Considerable effort is being devoted to assessing the benefit of treating mild hypertension.[1] The Australian Blood Pressure Study has shown that treatment of patients with a diastolic blood pressure (DBP) $\geqslant$ 100 mm Hg. reduces the incidence of stroke,[1,2] whilst the Hypertension Detection and Follow-up Program indicates that effective management of milder hypertension (DBP 90-104 mm Hg.) reduces mortality.[3] However, this latter study was not placebo-controlled and may reflect the benefit of overall medical care rather than drug treatment.[4] Other large studies currently in progress may clarify this aspect as well as define the relative merits of different types of therapy.[1,5,6]

The report from the WHO Expert Committee on Hypertension reflects the growing practice of initiating therapy with β-blockers rather than diuretics.[7] A flexible approach seems justified since hypertension is not a uniform disease.[8,9] Women appear to tolerate diuretics better than do men, whereas the reverse applies to β-blockers.[5] In addition, the old benefit more from diuretics than do the young.[10,11] Some 40% of patients do not respond adequately to diuretics due to compensatory activation of the renin-angiotensin system.[12]

Renin-Angiotensin System - Accumulating evidence points to a complex interaction between the renin-angiotensin, kallikrein-kinin and prostaglandin systems in controlling renal function and vascular tone. It appears that kallikrein activates prorenin, and thus kinins and angiotensins may be generated through a common initial pathway, which also activates the clotting and fibrinolytic systems.[13,14] Evidence has been presented that angiotensin II (AII) releases PGI_2 into the systemic circulation.[15] Additionally, the steroidogenic action of AII and angiotensin III is potentiated or possibly mediated by prostaglandins.[16] On the other hand, PGI_2 may stimulate renin release,[17] and PGE_2 and PGI_2 are implicated in the vasodilator action of bradykinin.[18] Blood pressure and fluid balance may be further controlled by an independent renin-angiotensin system in the brain.[19]

The etiology and management of low-renin hypertension, which characterises some 20-30% of hypertensives, has been reviewed.[20] The existence of a pressor, ouabain-like, humoral agent has also been proposed in this volume-expanded state.[21] In fact, a low molecular weight substance with some of the properties of cardiac glycosides has been isolated from guinea-pig brain[22] and bovine hypothalamus.[23] This may be similar to the factor, obtained from plasma of hypertensive dogs, which potentiates the pressor response to AII and noradrenaline (NA).[24] Interestingly, in erythrocytes from patients with essential hypertension the ratio of Na^+/K^+ net fluxes is depressed[25] and Na^+ concentration raised whilst ouabain sensitive active Na^+ efflux is reduced.[26]

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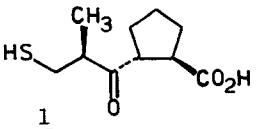
A review of captopril in the treatment of hypertension has been published.[27] Attainment of blood pressure control varies widely (35 - 90%),[28-31] and development of tolerance has been described in a small group of patients.[32] However, most of those who do not respond adequately appear to do so on addition of a thiazide.[28,33] This combination seems particularly useful as captopril represses the rise in AII and aldosterone levels, as well as the hypokalemia induced by the diuretic.[34] Following an initial depressor response with captopril, some investigators report a plateau phase or even a rise in blood pressure before the full antihypertensive effect is achieved after 8-10 days treatment.[29,35] The compound reduces peripheral resistance with little effect on heart rate,[29] and although cardiac output is unaffected acutely, it appears to rise on chronic treatment.[36] The most commonly reported side effects are the development of a rash,[28-31,34,37] which may be due to potentiation of kinin-mediated skin reactions,[38] and loss of taste.[34,37]

The mode of action of captopril in man requires further clarification.[28-30] However, supression of AII formation, augmentation of kinin levels, reduction in aldosterone secretion and altered prostaglandin levels, consequent to angiotensin converting enzyme (ACE) inhibition, all seem to contribute. In man the activity of captopril is attenuated by indomethacin.[39] Venous bradykinin levels do not appear to rise.[34] However, measurement of circulating components of the angiotensin/kinin systems may be misleading as the action of captopril at vascular sites could be more important.[27,29,34] It has been shown to increase renin activity in rat vascular tissue.[40] Other modes of action than ACE inhibition are still not excluded, as for instance captopril, but not SQ-20,881, attenuates the vascular response to NA.[41]

The carbocyclic analogue (1) of captopril retains significant ACE inhibitory activity, confirming that the nitrogen atom in the prototype plays little part in its interaction with the enzyme.[42] The 2-phosphono-pyrrolidine, 2, is some eight times less potent than the corresponding proline analogue.[43]



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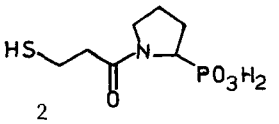

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Centrally Acting Drugs - Although clonidine is reported to reduce renovascular resistance chronically,[44] a reduction in renal blood flow, associated with an increase in plasma renin concentration, has been noted in some patients.[45] In a multicentre comparison, guanabenz, in contrast to α-methyldopa, did not cause fluid retention.[46] In patients, tiamenidine has produced substantial falls in blood pressure with only small changes in heart rate, and on compound withdrawal, plasma NA did not exceed pretreatment levels.[47]

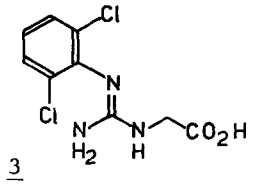
Both α_1- and α_2-adrenoceptor binding sites have been characterised in membrane fractions from brain homogenates using a variety of ligands.[48-51] Neither site appears to be localised on noradrenergic neurones[49] and their number and relative proportions vary in different brain areas.[50] A detailed examination of the kinetics of ^{3}H-clonidine binding to α_2-adrenoceptors has shown a biphasic pattern of dissociation, suggesting an interaction with two receptor subtypes whose relative population also appears to exhibit regional variation.[52] Studies in the rat indicate that the Nucleus Tractus Solitarius (NTS), an area richly populated with α_2-adrenoceptors,[53] is either a principal site or relay point in the antihypertensive action, though not the sedative effect, of clonidine.[54] However, stimulation of central α_1-adrenoceptors may also be involved in

the pathway through which clonidine exerts its cardiovascular activity since this is inhibited by prazosin.[55,56] Furthermore, a comparison of ICI-101,187 with two analogues has shown a better relationship between hypotensive activity and peripheral postsynaptic than with presynaptic adrenoceptor agonist activity, whilst the reverse applied to sedation.[57] Interpretation of these data, however, is complicated by the fact that there are both postsynaptic α_1- and α_2-like adrenoceptors.[58,59]

FLA-136 does not displace clonidine in ligand binding experiments,[60] supporting the suggestion that its antihypertensive activity is due to a metabolite.[61] Studies with 3-O-methyl-α-methyldopa, which lowers blood pressure in spontaneously hypertensive rats (SHR), suggest that 3-O-methylated metabolites may participate in the action of α-methyldopa.[62] Urapidil, thought to have a central component of action, appears to be an antagonist and partial agonist at α_1- and α_2-adrenoceptors.[63] Orthostatic collapse has been reported with urapidil in a multicentre study.[64]

Cimetidine and metiamide, applied intracerebroventricularly (ICV) to the rat, antagonise the cardiovascular effects of clonidine.[65] However, a direct interaction between clonidine and these compounds at either α_2-adrenoceptors or H_2-receptors is unlikely, since they are virtually ineffective in displacing specifically bound ^{3}H-clonidine.[51,66] It has been suggested that histamine and clonidine interact at an 'imidazole receptor' which is also activated by imidazole acetic acid.[67] The latter compound, however, interacts with GABA receptors,[68] and in the rat GABA (ICV) lowers blood pressure, heart rate and plasma renin activity (PRA).[69] Interestingly, STH-2330 (3), which incorporates the key features of GABA, has been identified as a metabolite of clonidine in man.[70]

Opioid peptides may be involved in the central regulation of cardiovascular function,[71] and this is further supported by the observation that naloxone can block the antihypertensive activity of clonidine in SHR.[72] The interaction does not appear to occur at the same receptor, but some brain areas, such as the NTS, contain a high density of opioid receptors as well as α_2-adrenoceptors.[53] Enkephalinase-A and brain ACE have been reported to be different.[73]



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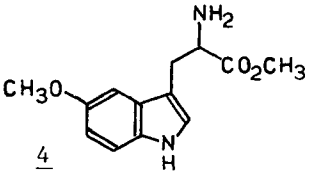

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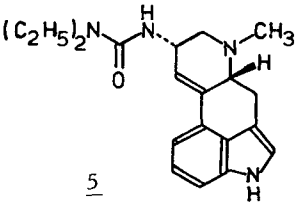

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It seems likely that serotonin (5-HT) is involved in the central regulation of blood pressure and an angiotensin/5-HT axis has been suggested.[74] A central site of action is proposed for the antihypertensive activity observed in animals with the 5-HT agonist, TR-3369 (4),[75] and the combination of fluoxetine (an inhibitor of 5-HT uptake) and 5-hydroxytryptophane.[76] Inhibitory presynaptic 5-HT receptors have been identified on peripheral noradrenergic neurones and these differ from classical D-receptors.[77] The 5-HT content of platelets is reduced in hypertensives, possibly reflecting a 5-HT deficiency in the brain.[78]

Lisuride (5)[79] and pergolide[80] lower blood pressure and heart rate in SHR. Both effects are antagonised by haloperidol, and additional studies with liseride have supported the involvement of a central dopaminergic mechanism.[79] Surprisingly, lisuride also has significant

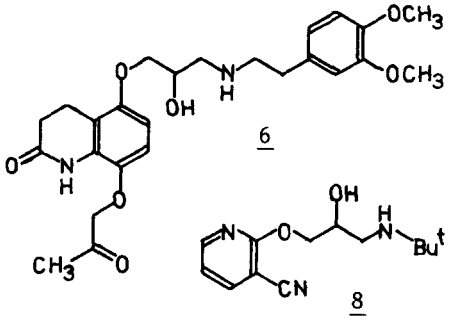
β-blocking activity aside from its known affinity for α-adrenergic- and 5-HT-receptors.[81] Some of the antihypertensive activity of bromocryptine may be due to inhibition of peripheral sympathetic nerve function through presynaptic dopamine receptor stimulation.[82] However, the compound can also stimulate α_2- and block α_1-adrenoceptors.[83]

Adrenergic Blocking Agents - The clinical pharmacology and therapeutic use of both α- and β-blockers have been reviewed.[84] Nadolol (Corgard®) and atenolol (Tenormin®) have been approved by the FDA for use in hypertension, both drugs offering the advantage of once-a-day dosage.[85,86] In contrast to other β-blockers, nadolol increases renal blood flow in man,[87] a property which may provide additional therapeutic benefit. Although it has been suggested that propranolol is "the drug of first and only choice for hypertension,"[88] a plethora of other β-blockers is available in Europe with selection usually based on cost[89] and potential side effect profile.[90] Thus, a non-selective agent may be safer in subjects with compromised cardiac function,[91] β_1-selective compounds may be more appropriate for diabetics[92] and smokers,[93] while preference with respect to long-term effects on plasma triglycerides is still not clarified.[94,95] β-Antagonists appear to be less effective in the elderly,[11] possibly due to reduced β-receptor sensitivity[96] or exaggerated expansion of extracellular volume.[97] The deleterious effects following sudden withdrawal of β-antagonists[98] may be due to both enhanced receptor sensitivity[99] and increased release of catecholamines.[100]

Current hypotheses on the mechanisms by which β-blockers lower blood pressure have been summarised[101] but little progress has been made, possibly because different factors may operate after acute and chronic administration. The lack of an acute effect of propranolol on blood pressure, despite reductions in heart rate and PRA, may be due to enhanced baroreceptor reflex activity,[102] corticosteroid release,[103] or increases in circulating catecholamines.[104] However, baroreflex sensitivity does not change in man following chronic propranolol treatment.[105] In SHR, no correlation was found between the reduction in heart rate produced by various β-blockers and their ability to prevent the development of hypertension.[106] In anaesthetised cats, both atenolol and metoprolol have similar acute effects on blood pressure and heart rate, despite attaining quite different concentrations in CSF and in the brain,[107] thus suggesting a peripheral site of action. However, uptake of atenolol into rat brain is enhanced on chronic treatment, and central mechanisms could be involved on prolonged therapy.[108] The role of the renin/angiotensin system in the antihypertensive effects of β-blockers is still not clear.[109] For example, in contrast to propranolol, the maximum antihypertensive response to atenolol is associated with only small reductions in PRA, and concomitant administration of a diuretic may actually be less effective than atenolol alone. Thus, it has been suggested that atenolol may be preferred in low/normal-renin cases whilst propranolol would be more appropriate in high-renin cases and in combination with diuretics or vasodilators.[110]

OPC-1427 (6) and sulfinalol (Win 40808-7) show acute antihypertensive activity in SHR and thus may have an advantage over other β-blockers by possessing additional vasodilator properties.[111,112] Sulfinalol is also active in the dog and has a pharmacological half-life of some 10 hrs. Further developments in the imidazolylphenoxypropanolamine series have been reported and 7 lowers blood pressure in SHR, although direct vasodilator and β_2-agonist activities are not prominent.[113] Extension of this "symbiotic approach to drug design" has produced the β-blocker/vasodilator MK761 (8) which is a potent antihypertensive agent in several animal

models,[114,115] with mechanistic differences from an earlier prototype 9:[116] Structure-activity relationships around 8 are narrow and the only variation which maintains adequate dual activity is replacement of -CN by $-CF_3$. However, MK761 was teratogenic in rabbits.[114] A similar conceptual approach appears to have been employed in the design of SK&F 92,657 (10), which also displays vasodilator and β-blocking activity.[117]



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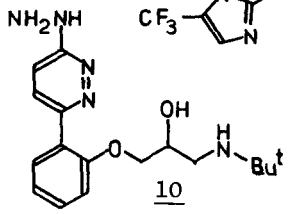

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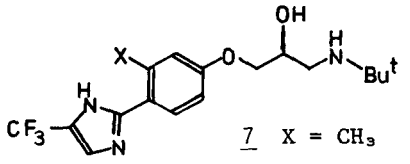
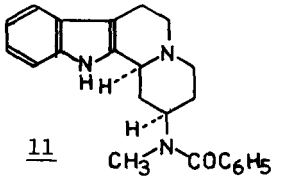


7 X = CH_3

9 X = H

The combined α- and β-blocking activity of labetalol results in a reduction in peripheral resistance with little effect on cardiac output and this haemodynamic profile is maintained during long term administration.[118] Proceedings of the 2nd Labetalol Symposium have appeared.[119] The antihypertensive activity of nylidrin in the rat is due mainly to β_2-mediated vasodilatation[120] but interaction with α-adrenoceptors may also be relevant.[121]

Selective postsynaptic α-antagonists such as prazosin are effective antihypertensive agents.[122] Debate continues over the reasons for the lack of reflex tachycardia[123] and renin release.[124] For example, in man, prazosin does not modulate either the sympathetic discharge due to central stimulation or responses mediated via β-adrenoceptors,[125] although in the dog a direct action at cardiac "chronotropic" receptors has been suggested.[126] In the rat, the acute hypotensive effect of prazosin is closely related to its α-blocking properties,[127] no changes in aortic or cardiac cAMP and cGMP were detected[128] and unlike phenoxybenzamine, cardiac dopamine-β-hydroxylase activity was not decreased.[129] Tiodazosin (BL-5111A)[130] is not as potent as prazosin but like E-643[131] may have some direct vascular activity.[132] In man, trimazosin lowers blood pressure via a reduction in peripheral resistance with a concomitant improvement in renal haemodynamics.[133] In the dog, systemic vasodilatation is observed without blockade of the NA pressor response.[134] Indoramin has a similar overall profile to prazosin in man but is some ten times less potent and produces central side effects.[135] Cyclised analogues such as 11, which also have a structural resemblance to reserpine, retain α-receptor affinity and lower blood pressure in SHR.[136]



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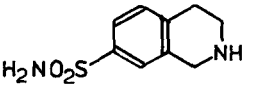

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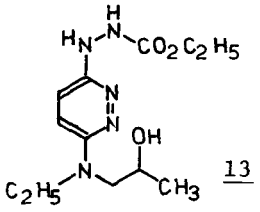

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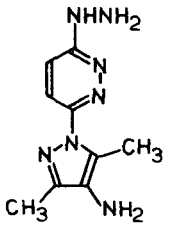
Although modulation of the adrenergic system is also possible via manipulation of neurotransmitter biosynthesis, administration of the phenylethanolamine-N-methyl transferase (PNMT) inhibitor SK&F 64139 to resting volunteers produced no haemodynamic effects.[137] However, compounds of this type may be useful in stress-induced hypertension where

both the synthesis and release of adrenaline may be enhanced. SK&F 29,661 (12) which does not enter the brain and has no α- or β-blocking properties, may help to define the physiological consequences of peripheral PNMT inhibition.[138] Dihydralazine lowered blood pressure in young SHR but PNMT activity in the brain did not change.[139] Thus, the previously reported increase in PNMT levels in this model may be the cause, rather than the consequence, of the hypertensive process. DL-α-monofluoromethyldopa is a potent, enzyme-activated, irreversible inhibitor of aromatic aminoacid decarboxylase which depletes biogenic amines in both brain and peripheral tissues[140] and lowers blood pressure in SHR after oral administration.[141]

Vasodilators - Hydrazine has been detected in the urine of patients undergoing treatment with hydralazine[142] but the significance of this observation requires further clarification. The hypotensive effect of hydralazine in the anaesthetised dog appears to be mediated via vasodilator prostaglandins but this may not be the case in the conscious state or in other species.[143] The reflex increase in heart rate, plasma NA and PRA due to hydralazine therapy can be attenuated in man with oxprenolol but no additional fall in blood pressure was observed.[144] Both oxdralazine (L6150) and ISF 2469 (13) are effective in man when combined with a β-blocker and diuretic.[145,146] Endralazine (BQ 22-708) is the most potent compound in a series of tetrahydropyrido(4,3-c)pyridazines and produces less tachycardia in rat and dog than dihydralazine.[147,148] The pyrazolylpyridazine derivative 14 shows similar activity to hydralazine in lowering blood pressure in SHR and in inhibiting prostaglandin A isomerase in vitro.[149]

Calcium antagonists are being increasingly studied in hypertension and nifedipine effectively lowers blood pressure in man producing only a small increase in heart rate on chronic therapy.[150,151] Unlike other vasodilators, there is little change in PRA, or fluid retention, suggesting an additional renal action. Modification of both the ester function and nitro-group in this dihydropyridine series has a marked effect on vasodilator activity[152,153] and niludipine has a longer duration of antihypertensive action than nifedipine in the dog.[154]



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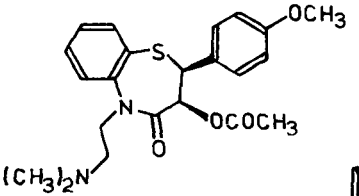

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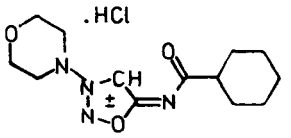

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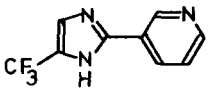

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Nifedipine is more effective in relaxing vessels from SHR than normal animals, suggesting an increased reliance on extracellular calcium for vascular contraction in this hypertensive model.[155] Treatment of young SHR for 12 months with verapamil lowered blood pressure and there was also a trend towards "normalisation" of the calcium dependency.[156] Although diltiazem (15) lowered blood pressure in DOCA/saline hypertensive rats, the pressor responses to both NA and AII were not affected.[157] Curiously, verapamil appears to be a more effective antihypertensive agent in Zulus than in Caucasians.[158]

Combination of the sydnone vasodilator, PR-G 138 (16), with propranolol lowered blood pressure in patients with no increase in heart

rate or PRA.[159] Tolmesoxide alone was moderately effective in volunteers after oral administration (400 mg) and produced reflex tachycardia.[160] MK534 (17) is a potent, long-acting antihypertensive agent in SHR, but the compound caused myocardial necrosis in dogs.[161]

References

1. J.M. Walker and D.G. Beevers, Drugs, 18, 312 (1979).
2. Australian Therapeutic Trial in Mild Hypertension, Clin.Sci., 57, Suppl. 5, 449s (1979).
3. H.D.F.P. Cooperative Group, J.Am.Med.Assoc., 242, 2562 and 2572 (1979).
4. W.S. Peart and E.W. Miall, Lancet, 1, 104 (1980).
5. Report of M.R.C. Working Party on Mild to Moderate Hypertension, Br.Med.J., 1, 1437 (1977).
6. L. Wilhelmsen, G. Berglund, R. Sannerstedt, L. Hansson, O. Andersson, R. Sievertsson and J. Wikstrand, Br.J.Clin.Pharmacol., 7, 261s (1979).
7. E.D. Frohlich, Hypertension, 1, 547 (1979).
8. M. Mendlowitz, ibid., 1, 435 (1979).
9. M.C. Khosla, I.H. Page and F.M. Bumpus, Biochem.Pharmacol., 28, 2867 (1979).
10. O. Lederballe Pedersen and E. Mikkelsen, Eur.J.Clin.Pharmacol., 16, 311 (1979).
11. K.O. Stumpe and A. Overlack, Br.J.Clin.Pharmacol., 7, 189s (1979).
12. H. Ibsen, A. Leth, H. Hollnagel, A.M. Kappelgaard, M. Damkjaer Nielsen, N.J. Christensen and J. Giese, Acta.Med.Scand., 205, 547 (1979).
13. J.E. Sealey, S.A. Atlas, J.H. Laragh, M. Silverberg and A.P. Kaplan,Proc.Natl.Acad. Sci., 76, 5914 (1979).
14. F.H.M. Derkx, B.N. Bouma, M.P.A. Schalekamp and M.A.D.H. Schalekamp, Nature, 280, 315 (1979).
15. R.J. Gryglewski, Biochem.Pharmacol., 28, 3161 (1979).
16. W.B. Campbell, C.E. Gomez-Sanchez, B.V. Adams, J.M. Schmitz and H.D. Itskovitz, J.Clin.Invest., 64, 1552 (1979).
17. J.A. Oates, A.R. Whorton, J.F. Gerkens, R.A. Branch, J.W. Hollifield and J.C. Frolich, Fed.Proc., 38, 72 (1979).
18. A. Nasjletti and K.U. Malik, Life Sci., 25, 99 (1979).
19. M.I. Phillips, J. Weyhenmeyer, D. Felix, D. Ganten, and W.E. Hoffman, Fed.Proc., 38, 2260 (1979).
20. A. Ganguly and M.H. Weinberger, Am.Heart J., 98, 642 (1979).
21. F.J. Haddy, M.B. Pamnani and D.L. Clough, Life Sci., 24, 2105 (1979).
22. M.C. Fishman, Proc.Natl.Acad.Sci., 76, 4661 (1979).
23. G.T. Haupert and J.M. Sancho, ibid., 76, 4658 (1979).
24. C.T. Huang, R. Cardona and A.M. Michelakis, Am.J.Physiol., 234, E25 (1978).
25. R.P. Garay and P. Meyer, Lancet, 1, 349 (1979).
26. A. Fadeke Aderounmu and L.A. Salako, Eur.J.Clin.Invest., 9, 369 (1979).
27. A.B. Atkinson and J.I.S. Robertson, Lancet, 2, 836 (1979).
28. P. Larochelle, J. Genest, O. Kuchel, R. Boucher, Y. Gutkowska and D.McKinstry, Canad.Med.Ass.J., 121, 309 (1979).
29. J.M. Sullivan, B.A. Ginsburg, T.E. Ratts, J.G. Johnson, B.R. Barton, D.H. Kraus, D.N. McKinstry and E. Muirhead, Hypertension, 1, 397 (1979).
30. E.L. Bravo and R.C. Tarazi, ibid., 1, 39 (1979).
31. D.H. Friedlander, New Zealand Med.J., 90, 146 (1979).
32. M. Saragoca, R.C. Tarazi, E.L. Bravo and F.M. Fouad, Clin.Res., 27, 317A (1979).
33. H.R. Brunner, H. Gavras, B. Waeber, G.A. Turini, D.N. McKinstry, R.A. Vukovich and I. Gravas, Br.J.Clin.Pharmacol., 7, 205s (1979).
34. C.I. Johnston, B.P. McGrath, J.A. Millar and P.G. Matthews, Lancet, 2, 493 (1979).
35. S.A. Atlas, D.B. Case, J.E. Sealey, J.H. Laragh and D.N. McKinstry, Hypertension, 1, 274 (1979).
36. R. Fagard, A. Amery, P. Lijnen and T.Reybrouck, Clin.Sci., 57, 131s (1979).
37. G.A. MacGregor, N.D. Markandu, J.E. Roulston and J.C. Jones, Br.Med.J., 2, 1106 (1979).
38. J.K. Wilkin, J.J. Hammond and W.M. Kirkendall, Pharmacologist, 21, 178 (1979).
39. F.R. Crantz, S.L. Swartz, N.K. Hollenberg, T.J. Moore, R.G. Dluhy, L. Levine and G.H. Williams, Clin.Res., 27, 592A (1979).
40. M.M. Asaad and M.J. Antonaccio, Pharmacologist, 21, 212 (1979).
41. T. Okuno, K. Kondo, K. Konishi, T. Saruta and E. Kato, Life Sci., 25, 1343 (1979).
42. A. Sugie and J. Katsube, Chem.Pharm.Bull. 27, 1708 (1979).
43. E.W. Petrillo and E.R. Spitzmiller, Tet.Letters, 4929 (1979).
44. I.M. Cohen, D.T. O'Connor, R.A. Preston and R.A. Stone, Clin.Pharmacol.Ther., 26, 572 (1979).
45. C. Thananopavarn, P. Eggena, J.D. Barrett, M.S. Golub and M.P. Sambhi, Clin.Res., 27, 546A (1979).
46. B.R. Walker, M. Geczy and J.A. Gold, Clin.Pharmacol.Ther., 25, 252 (1979).

47. C. Zamboulis, V. Hossmann, C.T. Dollery and H. Eckert, Br.J.Clin.Pharmacol., 8, 390P (1979).
48. P. Greengrass and R. Bremner, Eur.J.Pharmacol., 55, 323 (1979).
49. D.C. U'Prichard and S.H. Snyder, Life Sci., 24, 79 (1979).
50. S.J. Peroutka, D.A. Greenberg, D.C. U'Prichard and S.H. Snyder, Mol.Pharmacol., 14, 403 (1978).
51. B.R. Rouot and S.H. Snyder, Life Sci., 25, 769 (1979).
52. D.C. U'Prichard, W.D. Bechtel, B.M. Rouot and S.H. Snyder, Mol.Pharmacol., 16, 47 (1979).
53. W. Scott Young and M.J. Kuhar, Eur.J.Pharmacol., 59, 317 (1979).
54. R.W. Rockhold and R.W. Caldwell, Neuropharmacol., 18, 347 (1979).
55. P.B.M.W.M. Timmermans, E. Lam and P.A. van Zwieten, Eur.J.Pharmacol., 55, 57 (1979).
56. I. Cavero and A.G. Roach, Br.J.Pharmacol., 62, 468P (1978).
57. P. Birch, D.P. Clough, R. Hatton and D.J. Wheatley, ibid., 68, 107P (1980).
58. G.M. Drew and S.B. Whiting, ibid., 67, 207 (1979).
59. P.B.M.W.M. Timmermans, H.Y. Kwa and P.A. van Zwieten, Naunyn-Schmiedeberg's Arch. Pharmacol., 310, 189 (1979).
60. B. Jarrott, W.J. Louis and R.J. Summers, Biochem.Pharmacol., 28, 141 (1979).
61. P.B.M.W.M. Timmermans, E. Lam and P.A. van Zwieten, Naunyn-Schmiedeberg's Arch.Pharmacol., 306, 127 (1979).
62. F.G. Zavisca, A.P. Breau and R.J. Wurtman, Circulation Res., 45, 684 (1979).
63. M. Eltze, Eur.J. Pharmacol., 59, 1 (1979).
64. E.G. Bruckschen, F. Henze and G. Michael, Arzneim.Forsch., 28, 1176 (1978).
65. K.R. Borkowski and L. Finch, J.Pharm.Pharmacol., 31, 16 (1979).
66. A. Pilc, K. Gołembiowska-Nikitin and J. Vetulani, Eur.J.Pharmacol., 56, 177 (1979).
67. I. Paakkari, H. Karppanen and P. Paakkari, Acta.Med.Scand., Suppl. 625, 81 (1979).
68. C.Braestrup, M. Nielsen, P. Krogsgaard-Larsen and E. Falch, Nature, 280, 331 (1979).
69. C.J. Wallis and M.P. Printz, Circulation, 60, II-177 (1979).
70. C.J. Struck, S. Darda, H. Stähle, H.J. Förster and D.Arndts, Naunyn-Schmiedeberg's Arch.Phamracol., 308, Suppl., R22 (1979).
71. P. Bolme, K. Fuxe, L.F. Agnati, R. Bradley and J. Smythies, Eur.J.Pharmacol., 48, 319 (1978).
72. C. Farsang and G. Kunos, Br.J.Pharmacol., 67, 161 (1979).
73. C. Gorenstein and S.H. Snyder, Life Sci., 25, 2065 (1979).
74. V.E. Nahmod, S. Finkielman, E.E.Benarroch and C.J. Pirola, Science, 202, 1091 (1978).
75. E. Hong, R. Rión and P. Nava-Félix, Pharmacologist, 21, 254 (1979).
76. R.W. Fuller, D.R. Holland, T.T. Yen, K.G. Bemis and N.B. Stamm, Life Sci., 25, 1237 (1979).
77. W. Feniuk, P.P.A. Humphrey and A.D. Watts, Br.J.Pharmacol., 67, 247 and 423P (1979).
78. K.P. Bhargava, N. Raina, N. Misra, K. Shanker and S. Vrat, Life Sci., 25, 195 (1979).
79. G. Mannesmann, M. Haberey, B. Müller and H. Goedecke, Naunyn-Schmiedeberg's Arch.Pharmacol., 308, Suppl., R18 (1979).
80. T.T. Yen, N.B. Stamm and J.A. Clemens, Life Sci., 25, 209 (1979).
81. T. Cote, M. Munemura and J. Kebabian, Eur.J.Pharmacol., 59, 303 (1979).
82. M.F. Lokhandwala, ibid., 56, 253 (1979).
83. A. Gibson and M. Samini, J.Pharm.Pharmacol., 31, 826 (1979).
84. D.G. McDevitt, Drugs, 17, 267 (1979).
85. L. Volicer, C-S. Liang, H. Gavras, C.P. Tifft, G.R. Kershaw, I. Gavras, D.L.Griffith, R. Vukovitch and H.R. Brunner, J.Clin.Pharmacol., 19, 137 (1979).
86. O.R. Nilsson, B.E. Karlberg, O. Ohlsson, T. Thulin and K. Tolagen, Acta.Med.Scand., 206, 303 (1979).
87. N.K. Hollenberg, D.F. Adams, D.N. McKinstry, G.H. Williams, L.J. Borucki and J.M. Sullivan, Br.J.Clin.Pharmacol., 7, 219s (1979).
88. R.P. Ahlquist, Am.Heart J., 97, 137 (1979).
89. O. Lyngstam and L. Rydén, Lancet, 2, 634 (1979).
90. H.J. Waal-Manning, Drugs, 17, 129 (1979).
91. F.H.H. Leenen, Br.J.Clin.Pharmacol., 7, 173s (1979).
92. I. Lager, G. Blohmé and U. Smith, Lancet, 1, 458 (1979).
93. J. Trap-Jensen, J.E. Carlsen, T.L. Svendsen and N.J. Christensen, Eur.J.Clin.Invest., 9, 181 (1979).
94. J.L. Day, N. Simpson, J. Metcalfe and R.L. Page, Br.Med.J., 1, 77 (1979).
95. I.W. Beinart, D.G. Cramp, R.M. Pearson and C.W.H. Havard, Postgrad.Med.J., 55, 709 (1979).
96. R.E. Vestal, A.J.J. Wood and D.G. Shand, Clin.Pharmacol.Ther., 26, 181 (1979).
97. S. Rasmussen and K. Rasmussen, Eur.J.Clin.Pharmacol., 15, 305 (1979).
98. S.B. Garbus, M.A. Weber, R.T. Priest, D.D. Brewer and F.A. Hubbell, J.Clin. Pharmacol., 19, 476 (1979).
99. O. Lederballe Pedersen, E. Mikkelsen, J. Lanng Nielsen and N.J. Christensen, Eur.J.Clin.Pharmacol., 15, 215 (1979).
100. S. Nattel, R.E. Rangno and G. van Loon, Circulation, 59, 1158 (1979).
101. A. Scriabine in "Ann.Rev.Pharmacol. and Toxicol.", 19, 269 (1979).
102. H.A.J. Struyker-Boudier, J.F. Smits and H. van Essen, Clin.Sci., 56, 163 (1979).
103. F.P. Nijkamp, R. Van den Bosch and W. De Jong, Eur.J.Pharmacol., 56, 187 (1979).

104. A. Morganti, T.G. Pickering, J.A. Lopez-Ovejero and J.H. Laragh, Am.Heart J., 98, 490 (1979).
105. R.T. Krediet and A.J. Dunning, Br.Heart J., 41, 106 (1979).
106. C. Richer, N. Venturini-Souto and J.F. Giudicelli, Experientia, 35, 656 (1979).
107. P.A. van Zwieten and P.B.M.W.M. Timmermans, J. Cardiovas. Pharmacol., 1, 85 (1979).
108. J.A. Street, B.A. Hemsworth, A.G. Roach and M.D. Day, Arch.Int.Pharmacodyn.Ther., 237, 180 (1979).
109. I. Gavras, H. Gavras, H.R. Brunner and C-S. Liang, Br.J.Clin.Pharmacol. 7, 213s (1979).
110. A.H. Teeuw, F.H.H. Leenen, G.G. Geyskes and P. Boer, Clin.Pharmacol.Ther., 25, 294 (1979).
111. K. Sugawara, N. Takami and M. Ozaki, Arch.Int.Pharmacodyn.Ther., 240, 294 (1979).
112. P.H. Hernandez, H.E. Lape and R.E. Philion, Fed.Proc., 38, 738 (1979).
113. J.J. Baldwin, E.L. Engelhardt, R. Hirschmann, G.F. Lundell, G.S. Ponticello, C.T. Ludden, C.S. Sweet, A. Scriabine, N.N. Share and R. Hall, J.Med.Chem., 22, 687 (1979).
114. J.J. Baldwin, W.C. Lumma, Jr., G.F. Lundell, G.S. Ponticello, A.W. Raab, E.L. Engelhardt, R. Hirschmann, C.S. Sweet and A. Scriabine, ibid., 22, 1284 (1979).
115. C.S. Sweet, A. Scriabine, D. Weitz, C.T. Ludden, D.H. Minsker and C.A. Stone, J.Pharmacol.Exp.Ther., 211, 200 (1979).
116. A. Scriabine, C.T. Ludden, G. Morgan and J.J. Baldwin, Experientia, 35, 1634 (1979).
117. E.M. Taylor, A.M. Roe, and R.A. Slater, Clin.Sci., 57, Suppl.5, 433s (1979).
118. G. Koch, Br.Heart J., 41, 192 (1979).
119. D.A. Richards, B.N.C. Prichard, Eds., Br.J.Clin.Pharmacol., 8, Suppl. 2 (1979).
120. T.T. Yen and D.V. Pearson, Res.Commun.Chem.Pathol.Pharmacol., 23, 11 (1979).
121. M. Aggerbeck, G. Guellaën and J. Hanoune, Br.J.Pharmacol., 65, 155 (1979).
122. R. Pieske, Therapiewoche, 29, 5612 (1979).
123. D.W. Hardey and M.F. Lokhandwala, Eur.J.Pharmacol., 57. 251 (1979).
124. R.M. Graham and W.A. Pettinger, N.Eng.J.Med., 300, 232 (1979).
125. L.A. Buzzeo, J.M. Steele and J. Lowenstein, J.Pharmacol.Exp.Ther., 211, 345 (1979).
126. A. Taylor, W. Fennell, T. Brandon, G. Hopkins, R. Miller and J. Mitchell, Pharmacologist, 21, 177 (1979).
127. H.F. Oates, Arch.Int.Pharmacodyn.Ther., 237, 282 (1979).
128. H. Sands and R. Jorgensen, Biochem.Pharmacol., 28, 685 (1979).
129. F.L. Atkins and G.L. Nicolosi, ibid., 28, 1233 (1979).
130. L.E. Roebel, A.P. Florczyk and J.P. Buyniski, Res.Commun.Chem.Pathol.Pharmacol., 23, 29 (1979).
131. S. Fujino and K. Hoshi, Experientia, 35, 634 (1979).
132. H.F. Oates and L.M. Stoker, Arch.Int.Pharmacodyn.Ther., 240, 305 (1979).
133. S.G. Chrysant, T.M. Luu and K. Danisa, Clin.Pharmacol.Ther., 25, 217 (1979).
134. P. Macho and S. Vatner, Clin.Res., 27, 565A (1979).
135. G.S. Stokes, G.W. Frost, R.M. Graham and E.P. MacCarthy, Clin.Pharmacol.Ther., 25, 783 (1979).
136. S.S. Klioze, F.J. Ehrgott, J.C. Wilker and D.L. Woodward, J.Med.Chem., 22, 1497 (1979).
137. J.W. Dubb, R.M. Stote, F. Alexander, A.P. Intoccia, M. Geczy and R.G. Pendleton, Clin.Pharmacol.Ther., 25, 837 (1979).
138. R.G. Pendleton, G. Gessner, G. Weiner, B. Jenkins, J. Sawyer, W. Bondinell and A. Intoccia, J.Pharmacol.Exp.Ther., 208, 24 (1979).
139. L. Denoroy, B. Renaud, M. Vincent, J. Sacquet and J. Sassard, Eur.J.Pharmacol., 58, 207 (1979).
140. M.J. Jung, M.G. Palfreyman, G. Ribereau-Gayon, J. Wagner and M. Zraïka, Br.J.Pharmacol., 67, 460P (1979).
141. J.R. Fozard, M.G. Palfreyman, M. Spedding, J. Wagner and J.K. Woodward, ibid, 67, 461P (1979).
142. J.A. Timbrell and S.J. Harland, Clin.Pharmacol.Ther., 26, 81 (1979).
143. G. Haeusler and M. Gerold, Naunyn-Schmiedeberg's Arch.Pharmacol., 310, 155 (1979).
144. I.B. Davies, P.S. Sever and T. Rosenthal, Br.J.Clin.Pharmacol., 8, 49 (1979).
145. A. Salvadeo, G. Villa, S. Segagni and D.Criscuolo, Arzneim.Forsch., 29, 1753 (1979).
146. P. van Brummelen, F.R. Bühler, W. Kiowski, P. Bolli and O. Bertel, Int.J.Clin.Pharmacol.Biopharm., 17, 380 (1979).
147. E. Schenker and R. Salzmann, Arzneim.Forsch., 29, 1835 (1979).
148. R. Salzmann, H. Bürki, D. Chu, B.Clark, P. Marbach, R. Markstein, H. Reinert, H. Siegl and R. Waite, ibid., 29, 1843 (1979).
149. G. Szilagyi, E. Kasztreiner, L. Tardos, L. Jaszlits, E. Kósa, G. Cseh, P. Tolnay and I. Kovacs-Szabó, Eur.J.Med.Chem., 14, 439 (1979).
150. O. Lederballe Pedersen, E. Mikkelsen, N.J. Christensen, H.J. Kornerup and E.B. Pedersen, Eur.J.Clin.Pharmacol., 15, 235 (1979).
151. M.T. Olivari, C. Bartorelli, A. Polese, C. Fiorentini, P. Moruzzi and M.D. Guazzi, Circulation, 59, 1056 (1979).
152. F. Bossert, H. Horstmann, H. Meyer and W. Vater, Arzneim.Forsch., 29, 226 (1979).
153. M. Iwanami, T. Shibanuma, M. Fujimoto, R. Kawai, K. Tamazawa, T. Takenaka, K. Takahashi and M. Murakami, Chem.Pharm.Bull., 27, 1426 (1979).

154. M. Hiwatari and N. Taira, Arzneim.Forsch., 29, 1373 (1979).
155. O. Lederballe Pedersen, E. Mikkelsen and K.-E. Andersson, Acta.Pharmacol.Toxicol., 43, 137 (1978).
156. O. Lederballe Pedersen, Arch.Int.Pharmacodyn.Ther., 239, 208 (1979).
157. I. Yamaguchi, K. Ikezawa, S. Murata, H. Narita, T. Ikeo and M. Sato, Nippon Yakurigaku Zasshi, 75, 191 (1979). C.Abs. 91, 83165K (1979).
158. W.P. Leary and A.C. Asmal, Curr.Ther.Res., 25, 747 (1979).
159. J. Vos and E.J. Dorhout Mees, Br.J.Clin.Pharmacol., 8, 155 (1979).
160. J.A. Buylla, J.M. Clifford and R.D. Wynne, ibid, 8, 402P (1979).
161. A. Scriabine, C.T. Ludden, L.S. Watson, J.M. Stavorski, G. Morgan and J.J. Baldwin, Experientia, 35, 653 (1979).

Chapter 10. Agents for the Treatment of Ischemic Heart Disease

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Introduction - Ischemic heart disease[1] results when the coronary blood supply is not sufficient to provide oxygen and substrate, or to remove metabolic end products at a rate which meets the needs of the functioning heart muscle. The consequences of myocardial ischemia may manifest themselves as the clinical syndromes of angina pectoris, cardiac arrhythmias, pump failure, infarction or sudden death. In recent years, there have been many important advances in our understanding of the genesis and pathophysiology of myocardial ischemia including angina pectoris,[2] arrhythmias,[3] myocardial infarction,[4] the regulation and distribution of coronary flow,[5] collateral circulation in the heart,[6] adrenergic effects on coronary flow,[7] atherosclerosis,[8] the incidence and significance of coronary vasospasm,[9] platelet aggregation and thrombus formation,[10] adrenergic responses and oxygen demand[11] and metabolism in normal and ischemic myocardium.[12] Equally significant advances have occurred in the medical treatment of ischemic heart disease, particularly the development of agents which relieve angina,[13] as well as drugs which preserve the viability and function of jeopardized myocardium after an acute infarct,[14] and an increased understanding of how and why these drugs work.[15]

There are a number of therapeutic approaches to restoring a favorable oxygen supply/demand balance to ischemic myocardium and thereby relieving angina pectoris or reducing ischemic damage.[16] These include:

a)altering physiologic determinants of myocardial oxygen demand.
b)improving blood supply and distribution to ischemic myocardium.
c)shifting intracellular metabolic pathways toward anaerobic mechanisms of energy supply.
d)reducing autolytic processes in ischemic tissues.

A variety of drugs have been shown to have beneficial effects in experimental and clinical ischemic episodes. These include agents which alter heart rate, contractility, peripheral resistance, diastolic interval and other hemodynamic parameters; anticoagulants; drugs which inhibit platelet aggregation; coronary vasodilators; drugs which prevent or reverse coronary artery vasospasm; agents which stimulate glycolysis and/or reduce fatty acid metabolism; drugs which enhance translocase and other enzyme activities; agents which reduce tissue swelling and local edema and those which supress autolytic cell damage.

The relationship between the known effects of these drugs and the reduction of ischemia has not been clearly defined. Many agents affect several parameters concurrently, and it is not clear which are the more critical mechanisms. We have grouped these drugs into categories based on their most prominent pharmacologic properties, and we review the recent chemical, experimental and clinical findings as they relate to the effects of these drugs on myocardial ischemia.

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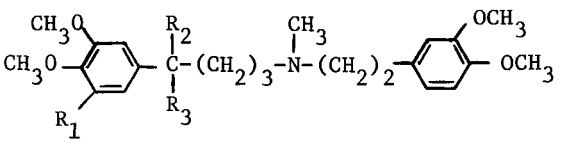
Calcium Channel Blockers - Calcium is intimately related to oxygen utilization in the myocardium because of its role in pacemaker activity and heart rate, conduction, arrhythmogenesis, excitation-contraction coupling, contractility and vascular resistance.[17] Cellular energy (ATP) is used up in reactions involved in intracellular calcium binding and release, calcium efflux from cells and in mitochondrial reactions involving calcium. Elevated intracellular levels of calcium are thought to contribute in various ways to myocardial cell damage and necrosis during ischemia.[18] Since calcium is also related to smooth muscle contractility, it plays a direct role in coronary resistance and perhaps coronary artery vasospasm.[19]

A number of drugs which appear to act primarily by inhibiting the movement of calcium into myocardial and smooth muscle cells through membrane "slow" channels have been investigated in ischemic heart disease, and the clinical pharmacology of these agents has recently been reviewed.[20-22] The major structural types of these drugs are represented by verapamil (1), nifedipine (4) and diltiazem (10). These drugs have been shown to reduce the cellular influx of calcium and reduce myocardial ischemia in a variety of animal models.[21] Some of the demonstrated pharmacologic effects of these drugs which are considered to contribute to their beneficial actions are: decreased oxygen consumption of the functioning heart, lowered arterial blood pressure, decreased peripheral resistance, improved cardiac output, decreased tension-time index, lowered heart rate, reduced contractility, inhibition of oxidative metabolism and other metabolic effects, coronary vasodilation and increased coronary flow.[23]



1 R_1=H, R_2=iPr, R_3=CN

2 R_1=CH_3O, R_2=iPr, R_3=CN

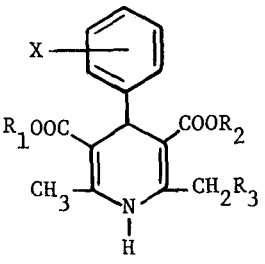
3 R_1=H, R_2R_3=$SO_2(CH_2)_3SO_2$

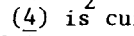
The pharmacologic and therapeutic uses of verapamil have been reviewed.[22,24] Verapamil has been found to be significantly better than placebo and equi-effective to propranolol as an antianginal agent in a recent, carefully controlled study.[25] Although verapamil was originally introduced as a coronary vasodilator for use in angina, it is now more frequently used for its conduction slowing and antiarrhythmic properties.[26] The R(+) isomer of verapamil is 10X more potent than the S(-) isomer in depressing A-V conduction, and 3X as potent in decreasing sinus node automaticity.[27] In contrast, the negative inotropic actions are mainly due to the S(-) enantiomer.[27] There has been concern that decreased contractility by verapamil may seriously compromise myocardial function in patients with coronary artery disease. However, the potent vasodilating properties of verapamil compensate for the intrinsic decrease in left ventricular contractility, and verapamil is reported to improve cardiac function in such patients.[28]

In a series of verapamil analogues, including D600 (2), the negative inotropic potency in isolated cat papillary muscles correlated well with a combination of the Hammett σ and molar volume of substituents on the benzene ring attached to the chiral center.[29] A quaternary benzylic carbon atom in the verapamil structure appears to be essential for activity; however, the cyano and isopropyl groups may be replaced without loss of calcium blocking activity.[30] These structure-activity relationships have led to the synthesis of an achiral analogue of verapamil, dimeditiapramine (Ro 11-1781, 3),[30]

which slows A-V conduction and causes coronary vasodilation[31] similar to verapamil.[32,33]

After oral administration in man, verapamil undergoes extensive first-pass metabolism and only 3-4% of unchanged drug is excreted. The major metabolites are formed by N-alkyl cleavage of the homoveratryl (~32%) and methyl (~10%) groups and by O-demethylation.[34] Some of these metabolites are biologically active.[34] Dimeditiapramine (3) undergoes similar metabolic dealkylation in dogs.[30]



	X	R_1	R_2	R_3
4	2-NO_2	Me	Me	H
5	2-CF_3	Et	Et	H
6	3-NO_2	$nPrOCH_2CH_2$	$nPrOCH_2CH_2$	H
7	3-NO_2	iPr	$MeOCH_2CH_2$	H
8	3-NO_2	Et	Et	OH
9	3-NO_2	Me		H

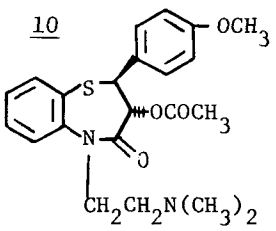
The calcium channel blocker nifedipine (4) is currently attracting much clinical interest for the treatment of ischemia and hypertension. It has been shown to be of therapeutic benefit in patients with stable angina,[35] exercise-induced angina[36] and Prinzmetal's variant angina.[20] The pharmacology of nifedipine and its use in angina has been reviewed[37,38] and discussed at a symposium.[39]

Nifedipine is a more potent inhibitor of transmembrane calcium influx than verapamil and it has a more pronounced effect on coronary artery smooth muscle than on myocardial tissue.[40] It dilates large coronary arteries and arterioles and increases total coronary flow. Its major beneficial action in exertional angina, however, may be through peripheral arteriolar dilation, which unloads the ischemic left ventricle. Its potent antispasmodic actions account for its effectiveness in angina due to coronary artery spasm. In contrast to verapamil, nifedipine has been found to have no important effects on electrophysiologic properties of the human heart at therapeutic doses.[41] It prolongs A-V conduction time in isolated heart preparations[40] as well as in some animal models[42] but not in humans. In isolated blood-perfused A-V nodal preparations, verapamil increases both blood-flow through the A-V nodal artery and A-V conduction time, in the same dose range. In contrast, blood flow is about ten times more sensitive to nifedipine than conduction.[43] Dose selection is critical for studies with nifedipine, since excessive blood pressure reduction may be harmful to the ischemic myocardium.[44]

In addition to nifedipine (4), a number of closely related dihydropyridines are under development. SKF-24,260 (5)[45] and niludipine (Bay A-7168, 6)[46,47] appear very similar to nifedipine in their animal pharmacology. Little information is available, yet, on nimodipine (Bay e-9736, 7)[48] or FR-7534 (8).[49] Nicardipine, (YC-93, 9) is a potent, water-soluble vasodilator with selectivity for cerebral and coronary vascular beds in animals and humans.[50,51] Chronic administration of YC-93 to dogs causes typical calcium antagonistic actions on the heart muscle, with bradycardia and heart block at high doses.[52] However, this agent is also a potent inhibitor of cyclic AMP phosphodiesterase which may contribute to its vasodilating actions.[53] The chemistry of 9, and related amino-substituted dihydropyridines, and the structural parameters which allow optimal vasodilating activity have been described.[54]

The antianginal drug diltiazem (10) has been marketed in Japan since 1974. Although a structurally novel inhibitor of calcium influx into coronary and vascular smooth muscle cells,[55] this drug has much in common pharmacologically with nifedipine and verapamil. It dilates large coronary arteries[56] and exhibits a negative inotropic action on the myocardium of patients with coronary artery disease.[57,58]

Several additional anti-ischemic drugs have calcium antagonist properties, including perhexiline (11), mecinarone (12), bepridil (28), prenylamine (29), fendiline (30), and droprenylamine (31), but their actions do not appear as specific as the drugs discussed earlier.[59-61] It is not certain to what extent calcium antagonism contributes to the overall effects of these drugs.



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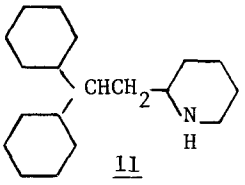

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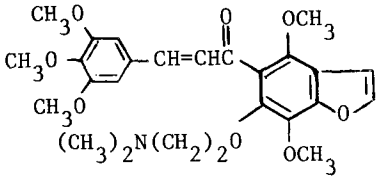
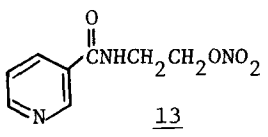


12

Vasodilator Drugs - The most obvious beneficial effect in ischemic heart disease of drugs which decrease vascular tone and enhance blood flow is that of improving coronary flow to the ischemic myocardium. In addition to increasing myocardial blood supply, however, agents which dilate peripheral vessels may also reduce the oxygen demand of the heart by lowering peripheral resistance (afterload) or decreasing venous return (preload). Thus, in the continuing investigation into the mode of action of nitroglycerin, it has been shown that this drug does not decrease coronary artery resistance, increase coronary blood flow or alter coronary flow distribution. In a study in patients with coronary artery disease, it was shown that nitroglycerin caused a peripheral pooling of blood volume, thus affecting both a systolic and diastolic unloading of the heart and a decrease in myocardial oxygen consumption.[62] Following acute coronary artery occlusion in the dog, nitroglycerin did not decrease the area of ischemia in the heart.[63,64] In a group of patients treated with nitroglycerin during the post-infarction hospital stay, however, the electrocardiographic indices of infarct size extension were decreased and the incidence of in-hospital mortality was significantly lower than that for untreated post-infarct patients.[65] The use of various nitrates, orally and sublingually, in the treatment of angina pectoris has been reviewed[66] and the pharmacologic properties of a new orally active nitrate SG-75 (2-nicotinamidoethyl nitrate) (13) have been reported.[67,68]



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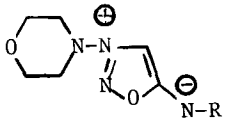


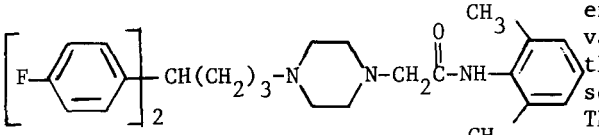
14 R=COOEt

15 R=H

The hemodynamic actions of molsidomin (14) are similar to those of the nitrates in the dog[69] and in man.[70] In contrast to the nitrates, however, this drug has a prolonged action after oral administration.[69,71] It improves epicardial blood flow in the dog after coronary artery occlusion more effectively than nitroglycerin.[72] It has been used to successfully treat patients with ischemic heart disease.[73,74] Molsidomin is a carbamate, but its pharmacologic effects appear to be

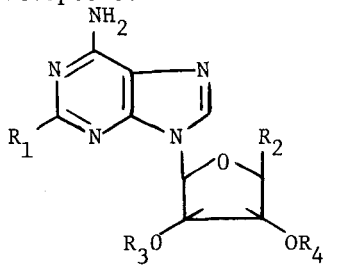
dependent upon its conversion to the sydnonimine (15) by hepatic metabolism and nonenzymatically to N-nitroso-N-morpholino aminoacetonitrile.[69]

Most of the classical coronary dilators (i.e., drugs which selectively increase coronary blood flow in normal vessels) have not proven to be particularly useful in the treatment of ischemic heart disease. These agents may in fact aggravate ischemia by a "steal" effect whereby peripheral blood flow or flow to non-ischemic myocardium is increased at the expense of flow to ischemic areas of the heart.[75] Some drugs of this type, however, including dipyridamole and lidoflazine (16), continue to be widely used for angina. Lidoflazine has been known for over 15 years and is available as an antianginal agent in most European countries.[76] It is presently under clinical investigation in the U.S. and has been recommended for NDA approval. Lidoflazine exerts a number of beneficial effects on the ischemic heart. In addition to its coronary vasodilating action, it has been shown to decrease cardiac work by decreasing heart rate, contractility, stroke volume and by lowering peripheral resistance.[77] Lidoflazine increases oxygen delivery to the myocardium during physical exercise[77] and increases the exercise capacity of patients with angina pectoris as well as post-infarct patients.[76,78] Upon long-term administration, lidoflazine enhances the proliferation of vascular cells and promotes the growth of collateral vessels in the ischemic heart.[79] This property has also been demonstrated for the coronary vasodilator dipyridamole.[80]



16

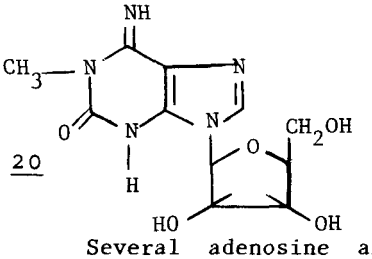
One of the physiologic factors involved in coronary vasodilation during ischemia is the release of adenosine from ischemic tissues. Adenosine (17) is a naturally occurring substance which dilates coronary arteries[5] and probably plays an important function in the regulation of coronary blood flow by its action on localized adenosine receptors.[81]



17 $R_1 = R_3 = R_4 = H$, $R_2 = CH_2OH$

18 $R_1 = 4\text{-}CH_3OC_6H_4$, $R_2 = CH_2OH$, $R_3 = R_4 = H$

19 $R_1 = H$, $R_2 = CONHEt$, $R_3R_4 = (CH_3)_2C$



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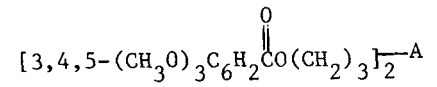
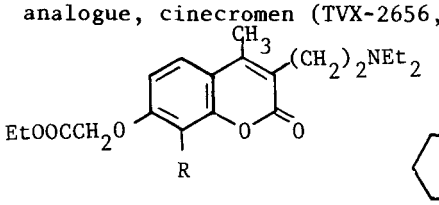


21 A= 1,4-piperazinyl

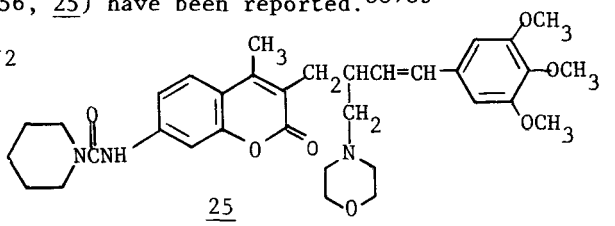
22 A= $N(CH_2)_2N$ (with CH_3 on each N)

Several adenosine analogues, doridosine (20), Gulden-Lomberg 744-98 (18) and CV-1674 (19), are reported to have potent coronary dilating activity of long duration,[82-84] and the structure-activity relationships of an extensive series of adenosine analogues has been

described.[85] In addition to a direct action on adenosine receptors, drugs may potentiate the actions of adenosine by preventing its uptake into cardiac cells and subsequent deamination. Dipyridamole, dilazep (21), hexobendine (22), lidoflazine (16) and papaverine inhibit the uptake of adenosine into ischemic myocardial cells, thus making more adenosine available to cause vasodilation.[86] Nitroglycerin and the coronary vasodilators aminophylline and carbochromen (23) had no effect on adenosine uptake. None of these agents were found to affect adenosine release. The mechanism of carbochromen induced coronary vasodilation is unclear, but does not involve calcium antagonism. In fact, this drug reverses verapamil-induced depression of conduction in the SA- and AV-node.[87] Properties of the 6-chloro derivative of carbochromen, AD-6 (24) and a new, orally-active analogue, cinecromen (TVX-2656, 25) have been reported.[88,89]



23 R=H 24 R=Cl



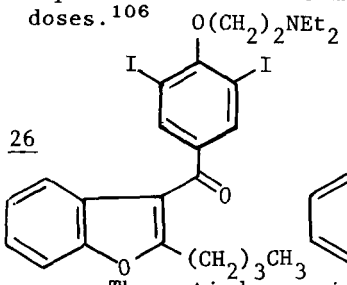
25

Another factor thought to be important in the local autoregulation of coronary blood flow is the synthesis and release of prostaglandins.[5,90] The chemical and metabolic relationships among the naturally occurring prostaglandins have been reviewed.[81] The endogenous substances PGH_2, $PGF_2\alpha$, TXA_2 and TXB_2 have been shown to be vasoconstrictors, whereas PGE_1 and PGI_2 (prostacyclin) are potent coronary vasodilators.[92] The role of PGE_2 is not well defined, as it has been shown to have no effect on rabbit coronary circulation, to be a vasodilator in the guinea pig heart and to constrict cat coronary arteries.[93] Following acute coronary artery occlusion in the cat, $PGF_2\alpha$ worsened the ischemic condition, while PGE_1, PGE_2 and PGI_2 reduced myocardial ischemia.[90,94] The beneficial actions of these prostaglandins are not limited to coronary artery dilation. These agents also decrease peripheral resistance, blood pressure, cardiac output and cardiac work, stabilize lysosomes in ischemic tissues, prevent platelet aggregation and inhibit the production of TXA_2.[90,94,95] The potent coronary vasodilator bradykinin is known to exert its effects in part through the release of PGI_2 in the heart.[96] Other coronary dilators may share this mechanism of action as well.[97]

"Anti-Adrenergic" Vasodilators. A group of anti-ischemic agents improve the myocardial oxygen supply by dilating coronary vessels, and decrease oxygen consumption by non-specific antiadrenergic effects ("adrenomodulation").[98] This latter action leads to decreased cardiac responsiveness to various sympathetic stimuli, reduction in heart rate and less exercise-induced tachycardia, without depression of cardiac contractility.

Perhexiline (11) may be considered to be of this type. It is marketed for angina in many countries and an NDA was filed in the U.S. in 1978. This drug is reported to be particularly valuable for treating intractable angina when β-blockers are contraindicated or ineffective;[99] it is also effective in anginal patients with angiographically normal coronary arteries.[100] However, side-effects are common and hepatotoxicity and peripheral neuropathy may occur on long-term use.[101,102] The exact mode of action of perhexiline in reducing exercise-induced tachycardia and angina is not clear.[20,99]

Amiodarone (26) causes dilation of peripheral and coronary arteries,[103] and many clinical studies have shown antianginal efficacy for amiodarone.[98] However, side-effects (corneal deposits, skin pigmentation and thyroid effects) limit the chronic use of this drug.[104] A structurally related agent, butoprozine (27) has very similar pharmacological properties, hopefully without amiodarone's adverse effects.[104,105] Bepridil (28) is another drug of this type, but it is reported to cause some decrease in cardiac contractility at high doses.[106]



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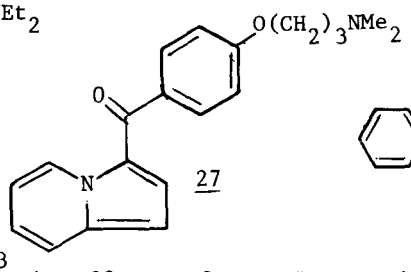

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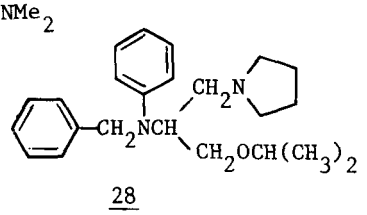
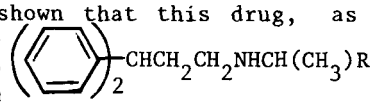


28

The antiadrenergic effects of prenylamine (29) may be due to its partial depletion of tissue catecholamine stores and to its sedative action.[107] Recently it has been shown that this drug, as well as the close analogues fendiline (30) and droprenylamine (31), also inhibit transmembrane calcium influx.[108-110] The mode of action of these agents is complex and their use in ischemic disease has not been clearly established.



29 R= benzyl

30 R= phenyl

31 R= cyclohexyl-CH_2

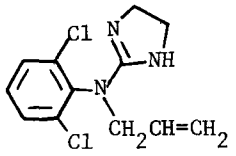
Beta-receptor Blocking Drugs - The neurohumoral factors involved in myocardial ischemia and the therapeutic benefits of the beta blocking drugs have been the most extensively studied aspects of ischemic heart disease.[13,111] These drugs and their well known pharmacologic properties have been discussed,[112] including a review in Annual Reports[113].

In addition, the effects of the beta-blocking drugs on coronary blood flow and distribution have been investigated. The small distal arteries and arterioles of the coronary bed contain almost exclusively beta-adrenergic receptors, while the larger proximal arteries are mediated by both alpha and beta receptors.[114] Alpha receptor stimulation causes vasoconstriction, while beta receptor stimulation results in relaxation.[7,114] However, in the partially ischemic heart, beta stimulation leads to a greater decrease in resistance in those vessels serving non-ischemic tissue than in those supplying ischemic areas, thus creating an unfavorable distribution of blood flow away from the ischemic areas.[115] The beta receptor blocking drugs, therefore cause a redistribution of blood flow which increases flow through ischemic tissues.[116,117]

Numerous clinical trials have supported the efficacy of these drugs in patients with angina pectoris.[13,118] The role of the beta-blockers in post-infarct therapy is less well established but has been the subject of several investigations.[111,117,119,120]

Miscellaneous Agents - Drugs which delay or reduce the cytolytic processes which are triggered in ischemic myocardium have been shown to reduce infarct severity in experimental animals and man. Among those which have been shown to be effective are cobra venom factor,[121] ibuprofen[122] and hyaluronidase.[123] Streptokinase, which prevents

platelet aggregation, has been found to be beneficial in some post-infarct patients.[124] ST 567 (N-allyl clonidine) (32) was shown to reduce myocardial oxygen uptake, principally by reducing heart rate, and to protect the ischemic myocardium of experimental animals.[125] Creatinol O-phosphate (33) has been the subject of recent investigations. This drug protects the rat heart from isoproterenol-induced necrosis,[126] increases the survival time of hypoxic isolated heart preparations,[127] and has been shown to have anti-ischemic effects in animals and in man.[128,129] It is reported to be without any significant side effects.[128,130] The mechanism of action of creatinol O-phosphate has not been clearly demonstrated. It is not a beta-blocker or a calcium antagonist;[127] however, it apparently affects the membrane transport of ions, as it prevents the accumulation of intracellular calcium and loss of magnesium from ischemic myocardial cells.[131]



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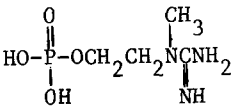

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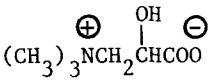

34

The beneficial effects of drugs which reduce myocardial oxygen requirements by altering metabolic patterns have been investigated in ischemic heart disease. The ability of glucose-insulin-potassium combinations to enhance glycolytic (anaerobic) metabolism in the heart and benefit anginal and post-infarct patients have been studied.[132] The most beneficial interventions of this type, however, may be those which involve the metabolism of fatty acids. Fatty acid metabolism proceeds via the oxidative cycles in cell mitochondria and thus requires oxygen. The fatty acids themselves produce several deleterious effects in ischemic myocardium.[133] The role of fatty acids in myocardial metabolism has been reviewed.[134] The drug UK 25842 (L-4-hydroxyphenylglycine) reduces the uptake of free fatty acids by myocardial cells.[135] Administration of UK 25842 during myocardial ischemia has been shown to enhance glucose utilization, reduce fatty acid uptake, increase the respiratory quotient of the myocardium and reduce myocardial oxygen consumption in the dog[136] and in man.[135] The drug did not produce any measurable hemodynamic changes. It was additionally shown to prevent injury in the hypoxic rat and dog heart[136] and to delay the onset of angina in patients.[135,137] Another drug which has recently been investigated in this area is carnitine (34). Carnitine is an endogenous substance which plays a role in intracellular fatty acid transport as well as ATP and ADP movement across the mitochondrial membrane.[133] Carnitine is lost from ischemic cells, and the administration of carnitine has been shown to be beneficial during myocardial ischemia.[138] The l-isomer of carnitine appears to exhibit the best therapeutic effectiveness.[133]

References

1. Joint International Society and Federation of Cardiology/World Health Organization Task Force on Standardization of Clinical Nomenclature, Circulation, 59, 607 (1979).
2. A. Oberman in "Coronary Artery Disease: Recognition and Management", C. E. Rackley and R. O. Russell, Eds., Futura Pub. Co., Mount Kisco, N.Y., 1979, p 1.
3. L. S. Driefus, S. Ogawa, P. N. Shenoy and K. R. Chandry in "Advances in the Management of Clinical Heart Disease", Vol. 3, J. I. Haft and C. P. Bailey, Eds., Futura Pub. Co., Mount Kisco, N.Y., 1978, p 5.

4. M. Fishbein in "The Treatment of Acute Myocardial Ischemia: An Integrated Medical/Surgical Approach", L. H. Cohn, Ed., Futura Pub. Co., Mount Kisco, N.Y., 1979, p 11.
5. F. L. Belloni, Cardiovasc. Res., 13, 63 (1979).
6. W. Flameng, F. Schwarz, F. Hehrlein and A. Boel, Basic Res. Cardiol., 73, 188 (1978).
7. M. Gerova, E. Barta and J. Gero, Circ. Res., 44, 459 (1979).
8. N. Woolf, Postgrad. Med. J., 54, 156 (1978).
9. L. D. Hillis and E. Braunwald, N. Engl. J. Med., 299, 695 (1978).
10. E. Lichstein in "Acute Myocardial. Infarction", Vol. 4, E. Donoso and J. Lipski, Eds., Stratton Intercont. Med. Book Corp. New York, N.Y., 1978, p 194.
11. R. P. Karlsberg, P. A. Penkoske, P. E. Cryer, P. B. Carr and R. Roberts, Cardiovasc. Res., 13, 523 (1979).
12. L. H. Opie, Jpn. Circ. J., 42, 1223 (1978).
13. N. V. Kaverina and V. B. Chumburidze, Pharmacol. Therap. 4, 109 (1979).
14. W. J. Untereker in "Acute Myocardial. Infarction", Vol. 4, E. Donoso and J. Lipski, Eds., Stratton Intercont. Med. Book Corp., New York, N.Y., 1978, p 60.
15. L. Szekeres, Basic Res. Cardiol., 73, 133 (1978).
16. J. Schener and M. V. Cohen in "Acute Myocardiol. Infarction", Vol. 4, E. Donoso and J. Lipski, Eds., Stratton Intercont. Med. Book Corp., New York, N.Y., 1978, p 37.
17. R. Kaufmann in "The Action of Drugs on Calcium Metabolism", P. A. van Zwieten and E. Schonbaum, Eds., Gustav Fischer Verlag, New York, N.Y., 1978, p 25.
18. A. M. Katz and H. Reuter, Am. J. Cardiol., 44, 188 (1979).
19. R. J. Luchi, R. A. Chahine and A. E. Raizner, Ann. Intern. Med., 91, 441 (1979).
20. P. D. Henry, Pract. Cardiol., 5, 145 (June, 1979).
21. K. Landmark and H. Refsum, Acta Pharmacol. Toxicol., 43, Supp. 1, 15 (1978).
22. B. H. Singh, G. E. Ellrodt and C. T. Peter, Drugs, 15, 169 (1978).
23. R. Gross, H. Kirchheim and K. von Olshausen, Arzneim. Forsch., 29, 1361 (1979).
24. J. K. Vohra, Drugs, 13, 219 (1977).
25. S. Jaishankar, M. P. Gupta and S. Padmavati, Clin. Trials J., 15, 174 (1978).
26. W. S. Aronow, D. Landa, G. Plasencia, R. Wong, R. P. Karlsberg and J. Ferlinz, Clin. Pharmacol. Ther., 26, 578 (1979).
27. G. A. Lupi, F. Urthaler and T. N. James, Eur. J. Cardiol., 9, 345 (1979).
28. J. Ferlinz, J. L. Easthope and W. S. Aronow, Circulation, 59, 313 (1979).
29. R. Mannhold, R. Steiner, W. Haas and R. Kaufmann, Naunyn Schmiedebergs, Arch. Pharmacol., 302, 217 (1978).
30. H. Ramuz, Arzneim. Forsch., 28II, 2048 (1978).
31. T. Yajima, T. Nakahara and K. Nakamura, Jpn. J. Pharmacol., (1978).
32. R. Gmeiner, C. K. Ng, H. Simma and M. Gstottner, Eur. J. Cardiol., 9, 77 (1979).
33. R. Gmeiner, C. K. Ng and M. Gstottner, Eur. J. Clin. Pharmacol., 16, 155 (1979).
34. M. Eichelbaum, M. Ende, G. Remberg, M. Schomerus and H. J. Dengler, Drug Metab. Dispos., 7, 145 (1979).
35. G. Cocco, C. Strozzi, D. Chu, R. Amrein and E. Castagnoli, Eur. J. Cardiol., 10, 59, 1979.
36. C. dePonti, F. Mauri, G. R. Ciliberto and B. Caru, Eur. J. Cardiol., 10, 47, 1979.
37. Drug Ther. Bull., 17, 22 (1979).
38. J. A. Owen, Jr., Hosp. Formul., 14, 682 (1979).
39. Symposium on Nifedipine, Am. J. Cardiol., 44, 779 (1979).
40. E. Rowland, T. Evans and D. Krikler, Br. Heart J., 42, 124 (1979).
41. L. Padeletti, F. Franchi, A. Brat, R. P. Dabizzi and A. Michelucci, Int. J. Clin. Pharmacol. Biopharm., 17, 290 (1979).
42. M. Lievre, J. Descotes, M. Ollagnier and G. Faucon, J. Pharmacol. (Paris), 10, 159, 1979.
43. M. Raschack, Arzneim. Forsch., 26, 1330 (1976).
44. A. P. Selwyn, E. Welman, K. Fox, P. Horlock, T. Pratt and M. Klein, Circ. Res., 44, 16 (1979).
45. A. Narimatsu, K. Satoh and N. Taira, Clin. Exper. Pharmacol. Physiol., 5, 107 (1978).
46. Drugs of the Future, IV, 655 (1979).
47. M. Hiwatari and N. Taira, Arzneim. Forsch., 29 II, 1373 (1979).
48. J. Am. Med. Assn., 241, 1275 (1979).
49. Drugs Future, IV, 580 (1979).
50. T. Takenaka and J. Handa, Internat. J. Clin. Pharmacol. Biopharma., 17, 1 (1979).
51. T. Seki and T. Takenaka, Internat. J. Clin. Pharmacol. Biopharma., 15, 267 (1977).
52. P. N. Whitehead, H. Chesterman, A. E. Street, D. E. Prentice, R. Heywood and T. Sado, Toxicol. Lett., 4, 57 (1979).
53. N. Sakamoto, M. Terai, T. Takenaka and H. Maeno, Biochem. Pharmacol., 27, 1269 (1978).
54. M. Iwanami, T. Shibanuma, M. Fujimoto, R. Kawai, K. Tamazawa, T. Takenaka, K. Takahashi and M. Murakami, Chem. Pharmacol. Bull., 27, 1426 (1979).
55. R. Weishaar, K. Ashikawa and R. J. Bing, Am. J. Cardiol., 43, 1137 (1979).
56. I. Nakayama, Internat. J. Clin. Pharmacol. Biopharm., 17, 410 (1979).

57. Y. Oyama, Y. Imai, H. Nakaya, K. Kanda and T. Satoh, Jpn. Circ. J., 42, 1257 (1978).
58. M. Kinoshita, Arzneim. Forsch., 29, 676 (1979).
59. A. Fleckenstein, Annu. Rev. Pharmacol. Toxicol., 17, 149 (1977).
60. B. Pourrias and F. Friedrich, Eur. J. Pharmacol., 49, 203 (1978).
61. S. Vogel, R. Crampton and N. Sperelakis, J. Pharmacol. Exp. Ther., 210, 378 (1979).
62. B. E. Strauer and A. Scherpe, Am. Heart J., 95, 210 (1978).
63. A. Malm, M. Arborelius, B. Lilja, R. L. Gil and S. Bornmyr, Cardiovasc. Res., 13, 281 (1979).
64. S. P. Michaelson, W. P. Batsford and B. L. Zaret, Cardiovasc. Res., 13, 407 (1979).
65. J. P Derrida, R. Sal and P. Chiche, Am. Heart J., 96, 833 (1978).
66. W. S. Aronow, Modern Concepts Cardiovascular Dis., 48, 31 (1979).
67. M. Sakanashi, E. Tomomatsu, R. Fukai, Y. Oyama, S. Ueda, F. Takenaka and M. Higuchi, Arzneim. Forsch., 29 II, 1530 (1979).
68. N. Taira, K. Satoh, T. Yanogisawa, Y. Imai and M. Hiwatari, Clin. Exp. Pharmacol. Physiol., 6, 301 (1979).
69. E. Grund, E. R. Muller-Ruchholtz, E. R. Lapp, H. M. Losch and W. Lochner, Arzneim. Forsch., 28 II, 1624 (1978).
70. K. R. Karsch, K. P. Rentrop, H. Blanke and H. Kreuzer, Eur. J. Clin. Pharmacol., 13, 241 (1978).
71. S. Guerchicoff, A. Vayquez, H. Kunik, S. Drajer and F. Diaz, Eur. J. Clin. Pharmacol., 13, 247 (1978).
72. V. J. Scholtholt, V. B. Fiedler and M. Keil, Arzneim. Forsch. 28 II, 1619 (1978).
73. N. Grewe and M. Stauch, Deutsch. Med. Wochenschr., 102, 1758 (1977).
74. G. Blazek, H. Heeger and F. Kubiçek, Dtsch. Med. Wochenschr., 102, 81 (1977).
75. E. H. Herman, T. Balazs, R. Young, F. L. Earl, S. Krop and V. J. Ferrans, Toxicol. Appl. Pharmacol., 47, 493 (1979).
76. S. Degre, A. Lenaers, R. Messin, P. Vandermoten, P. Salhadin, M. Limage and H. Denolin, Cardiology, 64, 35 (1979).
77. D. Wellens and A. Reyntjens, Acta Cardiol., 33, R31 (1978).
78. L. Nordstrom and F. L. Gobel, Chest, 74, 50 (1978).
79. A. Verheyen, R. Xhonneux, M. Borgers and R. S. Reneman, J. Mol. Cell. Cardiol., 8, 53 (1976).
80. G. Tornling, G. Unge, L. Skoog, A. Ljungqvist, S. Carlsson and J. Adolfsson, Cardiovascular Res., 12, 692 (1978).
81. D. R. Harder, L. Belardinelli, N. Sperelakis, R. Rubio and R. M. Berne, Circ. Res., 44, 176 (1979).
82. F. A. Fuhrman, G. J. Fuhrman, Y. H. Kim, L. A. Pavelka and H. S. Mosher, Science, 207, 193 (1980).
83. W. Schutz, G. Raberger and O. Kraupp, Arzneim. Forsch., 28 II, 207 (1978).
84. Drugs Future, III, 448 (1978).
85. R. A. Olsson, E. M. Khouri, J. L. Bedynek, Jr., and J. McLean, Circ. Res., 45, 468 (1979).
86. S. J. Mustafa, Biochem. Pharmacol., 28, 2617 (1979).
87. R. Sirbulescu and J. Scholtholt, Arzneim. Forsch., 29 I, 469 (1979).
88. F. Aparti, M. Finessa and L. Granata, Pharmacol. Res. Commun., 10, 469 (1978).
89. Drugs Future, IV, 15 (1979).
90. M. L. Ogletree and A. M. Lefer, Circ. Res., 42, 218 (1978).
91. J. Nowak, Acta Physiol. Scand., Supp. 467, 7 (1979).
92. M. L. Ogletree, J. B. Smith and A. M. Lefer, Am. J. Physiol., 235, H400 (1978).
93. K. Schror and S. Moncada, Prostaglandins, 17, 367 (1979).
94. M. L. Ogletree, A. M. Lefer, J. B. Smith and K. C. Nicolaou, Eur. J. Pharmacol., 56, 95 (1979).
95. A. M. Lefer, M. L. Ogletree, J. B. Smith, M. J. Silver, K. C. Nicolaou, W. E. Barnette and G. P. Gasic, Science, 200, 52 (1978).
96. K. Schror, U. Metz and R. Krebs, Naunyn-Schmiedebergs Arch. Pharmacol., 307, 213 (1979).
97. W. Forster, P. Mentz, K. Ponicke and U. Zehl, Acta Biol. Med. Ger., 37, 755 (1978).
98. R. Charlier in "Antianginal Drugs", Handbook of Experimental Pharmacol. Vol. 31, Springer Verlag, Berlin-Heidelberg-New York, 1971.
99. M. A. Mir and E. M. Kafetzakis, Am. Heart J., 96, 350 (1978).
100. L. J. Day and E. Sowton, Practitioner, 220, 965 (1978).
101. J. D. Horowitz and M. L. Mashford, Med. J. Aust., 1, 232 (1979).
102. P. Bouche, M. G. Bousser, M. A. Peytour and H. P. Cathala, Neurology, 29, 739 (1979).
103. P. Cote, M. G. Bourassa, J. Delaye, A. Janin, R. Froment and P. David, Circulation, 59, 1165 (1979).
104. R. H. Charlier, J. C. Richard and J. A. Bauthier, Arzneim. Forsch., 27 II, 1445 (1977).
105. Drugs Future, III, 349 (1978).
106. D. Cosnier, P. Duchene-Marullaz, G. Rispat and G. Streichenberger, Arch. Int. Pharmacodyn. Ther., 225, 133 (1977).

107. P. Mouille, G. Cheymol and J. Duteil, Arzneim. Forsch., 27 II, 1954 (1977).
108. K. Hashimoto, Y. Nakagawa, H. Nabata and S. Imai, Arch. Int. Pharmacodyn. Ther., 231, 212 (1978).
109. V. A. Fleckenstein, G. Fleckenstein-Grun and Y. K. Byon, Arzneim. Forsch., 27 I, 562 (1977).
110. R. J. Marshall and J. R. Parratt, Br. J. Pharmacol., 59, 311 (1977).
111. R. M. Gunnar, H. S. Loeb, P. J. Scanlon, J. F. Moran, S. A. Johnson and R. Pifarre, Prog. Cardiovasc. Dis., 22, 1 (1979).
112. W. Frishman and R. Silverman, Am. Heart J., 97, 797 (1979).
113. D. B. Evans, R. Fox and F. P. Hauck, Annu. Rep. Med. Chem., 14 81 (1979).
114. H. Morishita, Arch. Int. Pharmacodyn. Ther., 239, 195 (1979).
115. A. Berdeaux, M. Garnier, J. R. Boissier and J. F. Giudicelli, Eur. J. Pharmacol., 53, 261 (1979).
116. Y. Nose, M. Nakamura, H. Tomoike, O. Nakagaki, T. Fukuyama, A. Takeshita, A. Kuroiwa and M. Fujii, Arzneim. Forsch., 28, 2218 (1978).
117. B. E. Sobel, N. Engl. J. Med., 300, 191 (1979).
118. U. Thadani, C. Davidson, W. Singleton and S. H. Taylor, N. Engl. J. Med., 300, 750 (1979).
119. M. M. LeWinter, Med. Times, 106, 44 (Aug. 1978).
120. S. Goldstein, Arch. Int. Med., 139, 1089 (1979).
121. P. R. Maroko, C. B. Carpenter, M. Chiariello, M. C. Fishbein, P. Radvany, J. D. Knostman and S. L. Hale, J. Clin. Invest., 61, 661 (1978).
122. A. M. Lefer and E. W. Polansky, Cardiology, 64, 265 (1979).
123. R. A. Kloner, E. Braunwald and P. R. Maroko, Circulation, 58, 220 (1978).
124. J. M. Sullivan, N. Engl. J. Med., 301, 836 (1979).
125. W. Kobinger, C. Lillie and L. Pichler, Eur. J. Pharmacol., 58, 141 (1979).
126. T. Godfraind and X. Sturbois, Arch. Int. Pharmacodyn. Ther., 237, 288 (1979).
127. T. Godfraind, Arzneim. Forsch., 29, 1445 (1979).
128. A. Marzo and P. Ghirardi, Arzneim. Forsch., 29, 1449 (1979).
129. G. Botti and V. Bonatti, Arzneim. Forsch., 29, 1491 (1979).
130. G. F. Melloni, G. M. Minoja, G. F. Lureti, L. Merlo, F. Pomparana and B. Brusoni, Arzneim. Forsch., 29, 1477 (1979).
131. A. Cadel, A. Palumbo, G. Zerilli, R. Pria, R. Fanciulli, S. Conversano and R. Galbiati, Arzneim. Forsch., 29 1485 (1979).
132. U. Thadani, M. A. Chiong and J. O. Parker, Cardiology, 64, 333 (1979).
133. A. J. Liedtke and S. H. Nellis, J. Clin. Invest., 64, 440 (1979).
134. L. H. Opie, Am. Heart J., 97, 375 (1979).
135. L. Atkinson, G. Bergman, N. Jackson, D. E. Jewitt and J. M. Metcalfe, Br. J. Pharmacol., 66, 444P (1979).
136. K. J. Blackburn, R. A. Burges, D. G. Gardiner, A. J. Higgins, M. Morville and M. G. Page, Br. J. Pharmacol., 66, 443P (1979).
137. C. Bergman, L. Atkinson, J. M. Metcalfe, N. Jackson and D. E. Jewitt, Br. Heart J., 42, 240 (1979).
138. J. D. Folts, A. L Shug, J. R. Koke and N. Bittar, Am. J. Cardiol., 41, 1209 (1978).

Chapter 11. Diuretics

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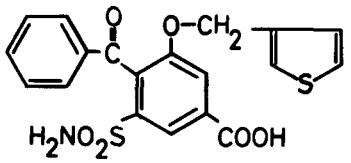
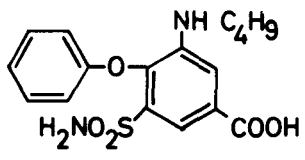
During recent years, diuretic development progressed in two directions. On one hand, the potency of the high ceiling sulfonamide diuretics was increased and compounds were found which are active in animals in the microgram range. On the other hand, compounds were introduced into the market which are far less potent than hydrochlorothiazide (HCT) but show a different spectrum of efficacy. The structures of the sulfonamides and phenoxyacetic acids guided several promising developments. Other structural types have increased in number, but only etozoline (13) was introduced into the market. No real progress was achieved in the field of potassium-sparing compounds. At this time, the combination of strong salidiuretic activity and potassium-sparing properties has not been found in one molecule. Fixed combinations of low ceiling diuretics other than HCT and triamterene are under development, but in most cases, an advantage over HCT/triamterene combinations has yet to be proven.

Sulfonamides - As in the past, strong efforts have been made to find new diuretics in the sulfonamide series, and much work has been published on efforts to improve the activity of furosemide, the leading high ceiling diuretic. Analogs more potent than furosemide have been obtained by the Leo group. The most potent compound (1) reported to date shows salidiuretic activity in dogs after oral dosing of only 1 μg/kg and is, accordingly, 5-10 times more potent than bumetanide (2).[1]

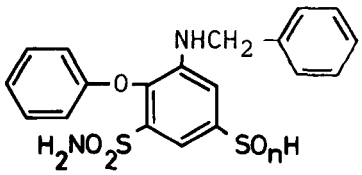


1



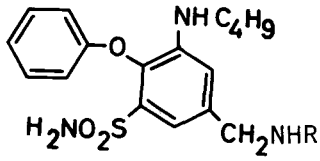
2

A review on the results of the Leo group was recently published.[2] A remarkable result is the observation that the carboxylic acid group can be replaced by a sulfonic or a sulfinic acid moiety, or an aminomethyl group,[2] leading to compounds 3 and 4. Structure activity relationship (SAR) studies demonstrate high salidiuretic activity even after these drastic changes.



n = 2 or 3

3

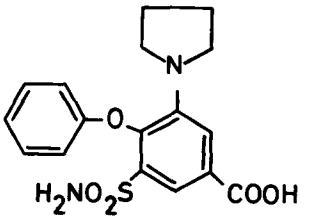


R = CH2 ;

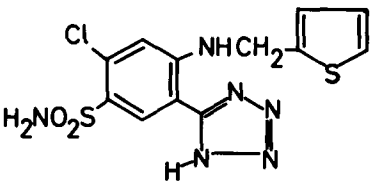
4

ISBN 0-12-040515-6

Numerous 3,4-disubstituted 5-sulfamoyl benzoic acids were synthesized by the Hoechst group utilizing a new method to introduce amine residues in the presence of other functional groups on the aromatic nucleus.[3,4] By far the most interesting compound is piretanide, 5,[4] now under extensive clinical investigation. It is a natriuretic acting largely by inhibiting sodium chloride transport in the ascending portion of the loop of Henle, with an additional component of activity on the proximal tubule. Sodium excretion increased by a factor of almost 10, and there was a concomitant 2 to 3-fold increase in potassium excretion. The compound increased calcium excretion and enhanced both acid and base excretion so that urinary pH was unchanged. No phosphaturia or side effects were seen.[5] Although body weight and plasma potassium decreased significantly, muscle water and intracellular potassium concentration did not change.[6] Comparative studies showed that a single 6 mg dose of piretanide is similar in potency to 40 mg furosemide in terms of diuresis, natriuresis and kaliuresis, but is less potent than 1 mg bumetanide. All three diuretics caused a decrease in urate excretion and a rise in serum uric acid levels. Interestingly, urate excretion was increased in the first 6 hours following drug administration and was decreased in subsequent hours. Piretanide is well absorbed after oral doses.[7]



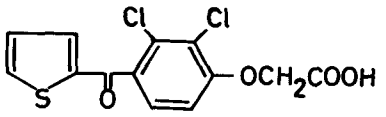
5



6

Clinical trials show that azosemide (6), a compound developed by Boehringer (Mannheim), is 5 times more potent on a weight basis when given intravenously than furosemide. However, both are equal in potency following oral administration.[8] Azosemide inhibits solute reabsorption throughout the thick ascending limb of the loop of Henle[9] and, in addition, affects solute transport proximal to the diluting segment, similar to bumetanide but differing from furosemide and ethacrynic acid.[10] In normal volunteers, azosemide and furosemide were similar in regard to volume, sodium, or chloride excretion when analyzed at 4, 8 or 12 hours, although azosemide tended to have a slower onset of effect.[11] Blood pressure did not change significantly at any time in either standing or supine position following 9-day treatment in healthy volunteers. A predictable rise in sodium and potassium excretion was demonstrated after the first dose. However, on the ninth day of treatment, excretion did not differ from control levels indicating that compensation had occurred.[10]

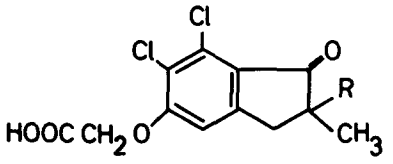
Phenoxyacetic Acids - Ticrynafen, (7) a diuretic with uricosuric and antihypertensive properties, has been reviewed[12,13] in Cragoe's excellent book on Diuretic Agents. A two-day symposium on clinical trials was held in Dallas.[14,15] In various clinical studies, the antihypertensive activity of ticrynafen was compared with that of HCT[16] and chlorthalidone, ethacrynic acid[17] or cyclopenthiazide.[18] Ticrynafen



7

(250 mg) gave comparable natriuretic activity to 50 mg ethacrynic acid or 25 mg chlorthalidone.[17] Ticrynafen resembles the thiazides in diuretic, antihypertensive and side effects, but lowers serum uric acid levels rather than increasing them.[16] The drug could play an important therapeutic role in the management of hyperuricemia when there is a concomitant need for diuretic or hypotensive treatment.[19] The antihypertensive activity of 7 and propranolol given as a combination are additive.[20] Like other uricosuric agents, ticrynafen can precipitate acute gout but may be less likely to damage the urinary tract than other uricosuric drugs, because the uricosuria is accompanied by diuresis.[21,22] In January, 1980, ticrynafen was removed from the U.S. market because of a suspicious incidence of liver disease and some deaths.

Indanyloxyacetic Acids - Among numerous compounds synthesized and tested by the Merck group, indacrinone (8) (MK 196), a salidiuretic with uricosuric and antihypertensive activities, exhibited optimum activity.[23-26] The compound is undergoing extensive clinical trials.[23]

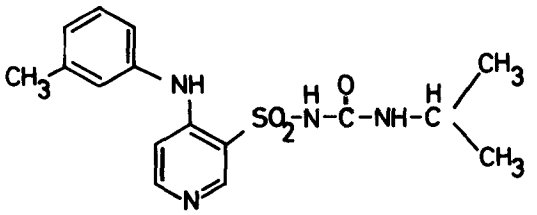


R= C_6H_5 8

R=  9

Besides indacrinone, the cyclopentyl derivative MK 473 (9) was studied in greater detail. Similar to indacrinone, MK 473 is diuretic, saluretic and uricosuric, and possesses substantial antihypertensive activity.[24] In man, it is well absorbed but extensively metabolized to a number of diastereomeric hydroxylated cyclopentyl derivatives.[27]

Diuretics of Various Structures - A series of 1-alkyl-3-(4-phenylamino-3-pyridyl)-sulfonylureas was reported and SAR discussed.[28] A member of this series, torasemide (10) proved to be a high ceiling diuretic in rat, dog and man. It appears to have greater diuretic capacity than available agents, shows no significant side effects or toxicity, and appears to have good long-term tolerance.



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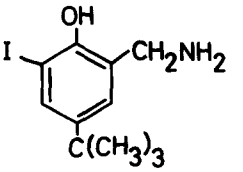
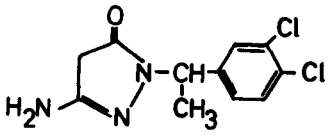


11

Additional diuretic and saluretic data in rats, dogs and chimpanzees were reported for MK 447 (11).[29] At doses ranging from 0.1-10 mg/kg, p.o., MK 447 was more effective than furosemide at the same or higher doses for increasing the excretion of Na^+, K^+ and Cl^- in rats and dogs. At single oral doses, MK 447 had antihypertensive activity in spontaneously hypertensive rats and renal hypertensive dogs.[30] The compound is rapidly and completely absorbed in

both rats and dogs. The half-life is approximately 7.5 hr in the dog and 1.0 hr in the rat.[31] In man, a dose of 100 mg was approximately equipotent to 80 mg of furosemide, although the duration of action was longer.[32]

Muzolimine (12) was discovered by the Bayer group years ago, and numerous publications have appeared in 1977.[33-35] In a recent study in healthy male volunteers, there was only a short rise in potassium excretion, whereas significant sodium and water diuresis were not accompanied by significant increases in renin-activity, angiotensin-II or aldosterone concentrations.[36] Pharmacokinetic results in dogs, healthy volunteers and in patients with renal insufficiency show that the compound is readily absorbed after oral administration. Elimination of unchanged drug from plasma was biphasic. The drug is assumed to be eliminated mainly in bile, probably after biotransformation; elimination in the urine has been confirmed to be a minor pathway.[34] Clearance investigations in healthy children of various ages confirmed findings in adults that muzolimine is an effective diuretic with a dose-related response.[37]



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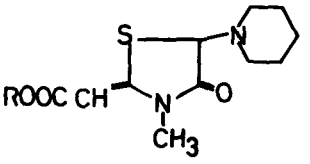

13 R=C_2H_5 Etozoline

14 R=H Ozolinone

Etozoline (13) was reported a number of years ago[38] and reviewed in Cragoe's book.[39] In 1979, it was introduced into the market in Germany as a diuretic with antihypertensive properties without causing potassium depletion. During a five-day workshop, the mode of action, toxicology, pharmacokinetic and pharmacodynamic properties of different diuretics, including etozoline were discussed.[40] Etozoline (800 mg, p.o.) was compared to 80 mg, p.o., furosemide in patients with left and/or right ventricular failure. Both drugs reduced body weight to the same extent. Heart rate and arterial blood pressure declined significantly during the trial. Etozoline was classified as a thiazide-like diuretic for its 24 hr profile but has an effect like the loop diuretics.[41]

Ozolinone (14), the main metabolite of etozoline, is more potent and less toxic than the parent compound.[42] Micropuncture studies in rats showed that ozolinone markedly increases urine volume, urinary sodium, and to a minor extent, urinary potassium. An impressive reduction of fluid, sodium and potassium reabsorption occurred in the loop of Henle. No further inhibition of fluid and sodium reabsorption could be detected in the distal convoluted tubules using the free flow recollection technique. Concerning the tubular site of renal action, there is a striking similarity between ozolinone and furosemide. The major difference between the two drugs concerns the end-proximal tubular chloride concentration, which was decreased by furosemide but was not affected by ozolinone.[43]

In anesthetized dogs, ozolinone was somewhat less potent than furosemide regarding effective doses and maximal changes of tubular sodium excretion.[44] Ozolinone has an asymmetric center in the 5-position. Examination of both antipodes showed that (-) oxolinone

has twice the diuretic activity of the racemate and is far more active than the (+) isomer in rats and dogs after oral dosing. LD_{50} values in rats for the racemate are nearly equal to those of (-) ozolinone.[45] The (+) antipode of ozolinone, which shows no significant diuretic activity, antagonizes furosemide activity by diminishing its secretion in the proximal tubule.[46]

In conclusion, research in the field of diuretics has led to the discovery of several new potent compounds, which demonstrates that diuretic activity is found among widely divergent chemical structures. The main effort during the past two years, however, was confined to increased clinical evaluation of compounds known for many years. Diuretics with uricosuric properties, suggesting use in the treatment of gout, are receiving increasing attention. Efforts to improve the profile of existing diuretics can be foreseen in the coming years.

References

1. O. B. Tvaermose Nielsen, A. Bruun, C. Brething and P. W. Feit, J. Med. Chem. 18, 41 (1975).
2. O. B. Tvaermose Nielsen and P. W. Feit in "Diuretic Agents" edited by E. J. Cragoe, Jr., ACS-Symposium Series 83, American Chemical Society, Washington DC, 1978, pages 12-23.
3. D. Bormann and W. Merkel Ger. Offen No 2453548, published 18.3.1976, granted to Hoechst AG.
4. W. Merkel, D. Bormann, D. Mania, R. Muschaweck and M. Hropot, Eur. J. Med. Chem. 11, 399 (1976).
5. P. Teredasi and J. B. Puschett, Clin. Pharm. Ther. 25, 331 (1979).
6. A. Alvestrand and J. Bergstrom, Curr. Ther. Res. 25, 786 (1979).
7. C.J.C. Roberts, M. Homeida, F. Roberts and W. Bogie, Br. J. Clin. Pharmacol. 6, 129 (1978).
8. F. Krueck, W. Bablok, E. Bensenfelder, G. Betzien and B. Kaufmann, Eur. J. Clin. Pharmacol. 14, 153 (1978).
9. D. C. Brater, Clin. Pharm. Ther. 25, 428 (1979).
10. D. C. Brater, S. Anderson and C. Muckleroy, Clin. Pharm. Ther. 26, 437 (1979).
11. D. C. Brater, S. A. Anderson and S. Strowig, Clin. Pharmacol. Ther. 25, 435 (1979).
12. P. Bessin, J. Bonnet, M. F. Malin, C. Jacquemin, N. DeBreze, J. Pelas, L. Desgroux, B. Agier and M. Dutarte in "Diuretic Agents" (see Ref. 2), pages 56-83.
13. A. R. Maass, J. B. Snow, R. Erickson and R. M. Stote in "Diuretic Agents" (Ref., 2) pages 84-92.
14. A New Class of Diuretics with Uricosuric Activity, Symposium, 16./17. November 1978 in Dallas, Texas (USA).
15. The Medical Letter 21, No. 15 (1979).
16. J. Furrer, W. Vetter and W. Siegenthaler, Schweiz. Med. Wschr. 108, 1983 (1978).
17. R. M. Stote, J. W. Dubb, R. G. Familiar and F. Alexander, Clin. Pharmacol. Ther. 23, 456 (1978).
18. P. Bolli, F. O. Simpson and H. J. Waal-Manning, The Lancet 1978, 595.
19. C. T. Gibson, H. A. Simmonds and R. J. Gleadle, J. Clin. Chem. Clin. Biochem. 17, 408 (1979).
20. R. M. Pearson, C. J. Bulpitt and C. W. H. Harvard, The Lancet 1979, 697.
21. A. H. B. Gillies and T. O. Morgan, Br. J. Clin. Pharmacol. 6, 357 (1978).
22. A. Friedman and T. H. Steele, J. Lab. Clin. Med. 1978, 447.
23. O. W. Woltersdorf, Jr., S. J. de Solms and E. J. Cragoe, Jr. in "Diuretic Agents" (see Ref. 2) pages 190-230.
24. O. W. Woltersdorf, Jr., S. J. de Solms, E. M. Schultz and E. J. Cragoe, Jr., J. Med. Chem. 20, 1400 (1977).
25. S. J. De Solms, O. W. Woltersdorf, Jr. and E. J. Cragoe, Jr., J. Med. Chem. 21, 437 (1978).
26. R. L. Smith, O. W. Woltersdorf, Jr. and E. J. Cragoe, Jr. in Annual Reports in Medicinal Chemistry 13, 62 (1978) and also 11, 72 (1976).
27. A. G. Zacchei, I. J. Wishousky, B. H. Arison and G. Hitzenberger, Drug Metab. Disposition 6, 303 (1978).
28. J. Delarge and C. L. Lapière, Ann. pharm. francaises 36, 369 (1978).
29. R. L. Smith, E. M. Schultz, G. E. Stokker and E. J. Cragoe, Jr. in "Diuretic Agents", pages 93-124 (see Ref. 2).
30. A. Scriabine, L. S. Watson, H. F. Russo, C. T. Ludden, C. S. Sweet, G. M. Fanelli, Jr., N. R. Bohidar and C. A. Stone, J. Pharmacol. Exp. Ther. 208, 148 (1979).
31. D. T. Lowenthal, G. Onesti, M. B. Affrime, J. J. Schrogie, K. E. Kim, P. Busby and C. D. Swartz, J. Clin. Pharmacol. 18, 414 (1978).

32. D. J. Tocco, G. E. Stokker, R. L. Smith, R. W. Walker, B. H. Arison and W. J. A. Vandenheuvel, Pharmacologist 20, 214 (1978).
33. H. Horstmann, E. Möller, E. Wehinger and K. Meng in "Diuretic Agents", pages 125-139 (see ref. 2).
34. W. Ritter, Curr. Med. Res. Opin. 4, 564 (1977).
35. E. Schnurr, H. Küppers, K. Wiesen and B. Grabensee, Pharmatherapeutica 1, 415 (1977).
36. E. Schnurr, H. Küppers, Int. J. of Clin. Pharm. and Biopharm. 17, 453 (1979).
37. D. Gekle, Pharmatherapeutica 2, 120 (1978).
38. G. Satzinger, Justus Liebigs Ann. Chem. 665, 150 (1963).
39. G. Satzinger, M. Herrmann, K. O. Vollmer, A. Merzweiler, H. Gohmar, O. Heidenreich and J. Greven in "Diuretics Agents", pages 155-189 (see Ref. 2).
40. Abstracts of the lectures given in Lissabon May 23rd to 27th 1979, see Med. Welt 30, 1013 (1979).
41. G. Biamino, H. Kopp, W. Rudroff and B. Schneider, Med. Klin. (München) 74, 624 (1979).
42. G. Satzinger, M. Herrmann and K. Vollmer, Ger. Pat. No 2414345, published 5.4.1979, granted to Gödecke AG.
43. J. Greven, H. Klein and O. Heidenreich, Naunyn-Schmied. Arch. Pharmacol., 304, 289 (1978).
44. J. Greven and O. Heidenreich, Naunyn-Schmied. Arch. Pharmacol. 304, 283 (1978).
45. W. D. Herrmann, M. Herrmann, G. Satzinger and W. Steinbrecher, Ger. Offen No 2658858, published 29.6.1978, granted to Gödecke AG.
46. J. Greven and O. Heidenreich, Med. Welt 30, 1014 (1979).

Section III - Chemotherapeutic Agents

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Ann Arbor, MI 48105

Chapter 12. Antibacterial Agents

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Introduction - This chapter will be limited to antibacterial agents with subjects related to biosynthesis being excluded. A number of antibacterial topics were presented at the joint meetings of the 11th International Congress of Chemotherapy/19th Interscience Conference on Antimicrobial Agents and Chemotherapy for which abstracts were published.[1] Two books of general interest are concerned with the origin, nature and properties of antibiotics.[2,3] In addition, two excellent reviews of β-lactams were published.[4,5]

Cephalosporins and Cephamycins - Interest continues to center on antipseudomonal cephalosporins with broad activity and resistance to β-lactamases, as well as long-acting preparations. No significant oral agents were reported. Ceftizoxime (FK 749, 1) is undergoing extensive laboratory and clinical trials.[6] It is active *in vitro* and *in vivo* against gram-positive and gram-negative organisms including many opportunistic pathogens such as *Enterobacter, Citrobacter, Serratia, Proteus, Pseudomonas* and *Haemophilus influenzae*.[7-9] It is one of the most potent β-lactams against gram-negative bacilli, being highly resistant with low binding affinity to β-lactamases including those in R-factor bearing *E. coli* strains.[10-12] In laboratory animals, relatively high serum and urine levels were achieved with good tissue distribution, although biliary excretion was low.[13] In man, the compound is safe given i.v. and has a good pharmacokinetic profile.[14] Cefotaxime (HR-756, 2) continues to undergo intensive clinical trial.[15-23] It has a short half-life and is converted to a less active desacetyl metabolite.[24,25] GR 20263 (3) is similar to 1 and 2 in its bacteriologic profile with superior antipseudomonal and poorer gram-positive activities.[26,27] It is β-lactamase stable, has low toxicity and good animal pharmacokinetic properties. Cefoperazone (T-1551) also is under detailed clinical evaluation,[28-32] as are the long-acting ceforanide (BL S786)[33-35] and cefonicid (SK&F 75073).[34-38] SK&F 80303 is reported to have the longest half-life in animals.[39] SM-1652 (4) a parenteral antipseudomonal, β-lactamase-stable compound displays good protection and pharmacokinetics in animals, however, high inoculum reduces its activity.[40,41] Cefathiamidine (5) was efficacious in man in gram-positive infections, including those caused by *Enterococcus*.[42] AL-226 (6) has broad spectrum parenteral activity.[43] Cephalexin analogs (7,8) unexpectedly lacked oral activity.[44] The acetoxymethyl ester of cefamandole was shown to be absorbed orally in mice when administered in 50% propylene glycol.[45] An extensive series of naphthalene-substituted cephalosporins had weak activity.[46] Synthetic studies were also directed towards nuclear analogs and conversion of penicillins to oxacephalosporins as exemplified by the synthesis of 6059-S (LY-127935) and other related 1-oxacephems.[47-51] In

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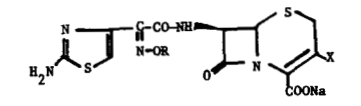

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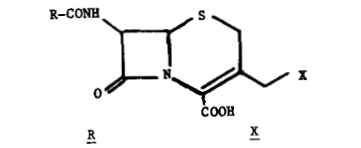
3	$-C(CH_3)_2CO_2H$	$-CH_2-$



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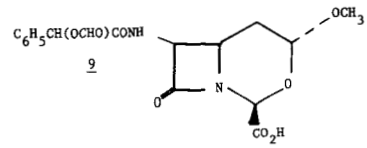
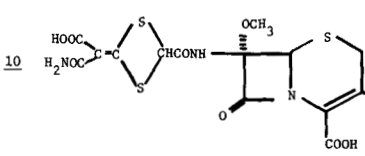
6		-H
7		
8		

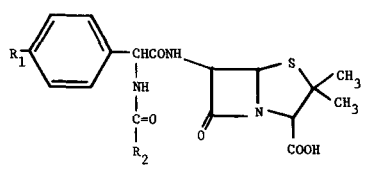




addition, syntheses of 3-oxa-analogs, including 9 with oral activity in mice, have been reported.[52,53] YM-09330 (10) is a broad spectrum β-lactamase stable cephamycin with good gram-negative activity. However, its gram-positive activity is weaker, and it displays significant animal species variation in its pharmacokinetics.[54-56] Modification of cefmetazole (CS-1170) at the 7β-acyl, the 7α-methoxy or the 3-position failed to enhance activity.[57]

Penicillins and Other β-Lactams - The search for penicillins with antipseudomonal activity continues. CI-867 (11) is an injectable, safe penicillin with broad-spectrum activity. It shows good protection in mice and a longer half-life than piperacillin.[58-60] The activity of TA-058 (12) against *P. aeruginosa* was found to be weaker than that of carbenicillin.[61] In contrast, BL-P1908 (13) and TEI-1194 (14) and TEI-2012 (15) showed better *in vitro* antipseudomonal activity than carbenicillin and ticarcillin and also higher serum levels in mice after i.m. injection.[62,63] The stable N-formimidoyl derivative (MK0787, 16) of thienamycin[64] was found to retain the spectrum of thienamycin with increased potency against *P. aeruginosa*.[65-67] The total synthesis of nocardicins A and D and that of their analogs was reported.[68,69] Three novel β-lactams (17, 18, 19) isolated from *S. clavigerus* contain the clavulanic acid nucleus.[70] The preparation of a simple penem nucleus (20) of clavulanic acid,[71] 6-APA[72] or total synthesis[73] has been described.

β-Lactamase Inhibitors - Olivanic acids are thienamycin-like β-lactams produced by *S. olivaceus*.[74-76] Structures for 7 members of this class were established: MM 4550 (21), MM 13902 (22), MM 7880 (23), MM 22380 (24), MM 22382 (25), MM 22381 (26) and MM 22383 (27). They have weak antibacterial activity and are irreversible inhibitors of various types of β-lactamases. Some olivanic acids also were obtained by synthesis.[77] The novel semisynthetic penicillinase inactivator, 6-α-chloropenicillanic acid sulphone (28) is similar in activity to clavulanic acid and CP-45,899.[78] Chemical and biological studies on clavulanic acid continue. A new total synthesis of (±) clavulanic acid[79] and some analogs were reported.[80,81] A fixed oral formulation (augmentin, BRL 25,000) of amoxicillin and clavulanic acid was reported to be successful in the treatment of urinary tract infections caused by β-lactamase-producing bacteria.[82-85] The pivaloyloxmethyl ester (CP-47,904) of CP-45,899 showed good oral absorption.[86-87] 6-β-Bromopenicillanic acid was found to inactivate β-lactamase I from *B. cereus* by



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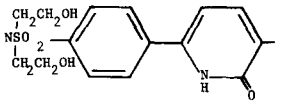
11	OH	
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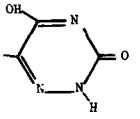
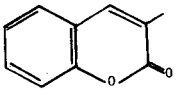
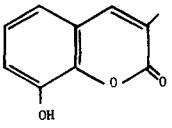
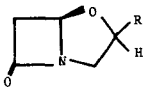
13	OH	
14	H	
15	H	



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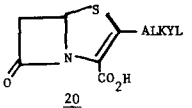
18 R=CH_2OCOH
19 R=CO_2H



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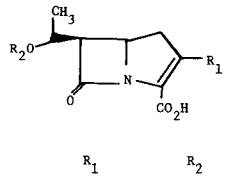

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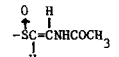
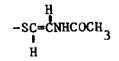
21		$-SO_3Na$
22		$-SO_3Na$
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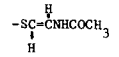
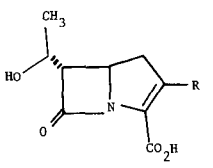
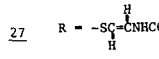
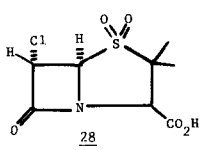
25		-H



26 R = $-SCH_2CH_2NHCOCH_3$

27 R = 

16 R=$-SCH_2CH_2NH-CH=NH$

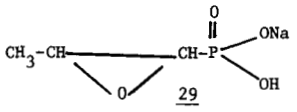


28

binding to a specific serine residue.[88-90] Cefonicide (SK&F 75073) and closely related cephalosporins were found to be stable to β-lactamases[91] and to be an inhibitor of type I β-lactamase from *E. cloacae*.[92] Controversy exists as to the validity of the combination approach for the treatment of infections caused by β-lactamase producing gram-negative organisms.[93]

Other Cell Wall Inhibitors - The therapeutic success of the β-lactams has prompted the search for other agents with a similar mechanism of action.[94,95] Many compounds currently being explored are those containing phosphonic acid, as exemplified by the earlier-reported fosfomycin (29): FR 800098 (30) and FR 33289 (31), products of *S. rubellomurinus* subsp. *indigoferus*, FR 32863 (32) and FR 31564 (33) produced by *S. lavendulae*.[96,97] Of these, 33 was found to be the most potent against a broad spectrum of gram-negative organisms, including *P. aeruginosa*. Like 29, it appears to be incorporated into the bacterial cells by active transport. It was found to be superior to 29 and more potent in mice than would be expected from *in vitro* data. On the basis of its favorable pharmacokinetic properties,[98] it is being developed in both oral and parenteral forms. Ensanchomycin (MSD-820A) and prenomycin are active *in vitro* and *in vivo* against gram-negative and gram-positive bacteria.[99] The rationale behind the discovery of the earlier reported alafosfalin (alaphosphin, Ro-03-7008, 34) and its biological data were reported in detail.[100-104] In rats, baboons and man, it displayed good pharmacokinetics,[105] and clinical trials are in progress.

A specific screening procedure to search for inhibitors of cell wall synthesis based on lack of mycoplasma activity and inhibition of incorporation of diaminopimelic acid was used to screen 10,000 soil isolates. This led to the isolation of azureomycins A and B produced by *Pseudonocardia azurea*.[106,107] These water-soluble basic compounds have predominantly gram-positive activity and inhibit peptidoglycan synthesis with lysis.[108] A cell wall active complex, polypeptide A 38533, produced by a Streptomycete, possesses *in vitro* and *in vivo* activity mainly against *P. aeruginosa*,[109,110] but development of



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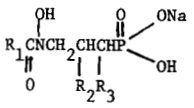
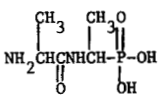
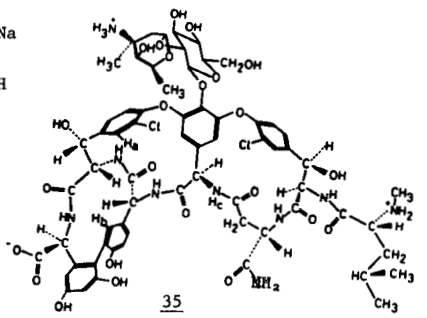


30 R_1=CH_3, R_2=R_3=H
31 R_1=CH_3, R_2=OH, R_3=H
32 R_1=H, R_2, R_3=OLEFIN
33 R_1=R_2=R_3=H



34



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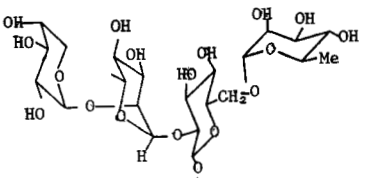

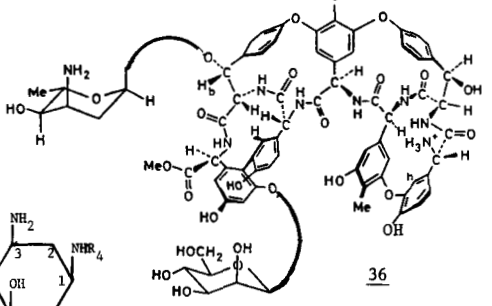

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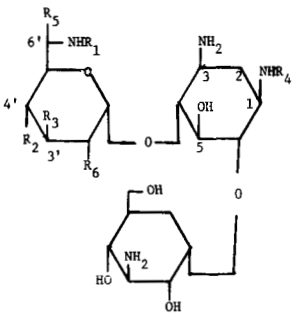
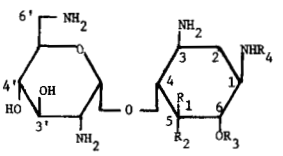
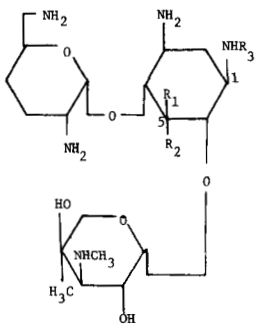


37 R_1=R_5=H, R_2=R_3=R_6=OH, R_4=$COCH(OH)CH_2CH_2NH_2$
38 R_1=CH_3, R_2=R_5=H, R_3=R_6=OH, R_4=$COCH(OH)CH_2CH_2NH_2$
39 R_1=R_2=R_3=R_4=H, R_5=CH_3, R_6=NH_2
52 R_1=R_5=H, R_2=R_3=R_6=OH, R_4=$CO_2CH_2CH_2NH_2$



40 R_1=OH, R_2=R_3=R_4=H
41 R_1=R_3=R_4=H, R_2=Cl
42 R_1=R_4=H, R_2=OH R_3=3-AMINOGLUCOSE
53 R_1=OH, R_2=H, R_3=3-AMINOGLUCOSE, R_4=$CH(CH_2OH)_2$



43 R_1=R_2=R_3=H
44 R_1=R_3=H, R_2=OH
45 R_1=R_3=H, R_2=NH_2
46 R_1=R_3=H, R_2=N_3
47 R_1=NH_2, R_2=R_3=H
48 R_1=R_3=H, R_2=F
49 R_1=F, R_2=R_3=H
50 R_1=H, R_2=OH, R_3=Et
51 R_1=H, R_2=OH, R_3=HAPA

permeability resistant mutants was observed.[111] The structures of the cell wall active aromatic glycopeptides vancomycin (35)[112,113] and ristocetin A (ristomycin A, 36)[114-116] were determined based on X-ray, chemical and spectrodata.

Structural studies on avoparcin[117] and actinodin[118] (same class) were published.

Aminoglycosides - To circumvent bacterial enzymatic inactivation, numerous semisynthetic analogs were prepared. Amikacin (37), susceptible to 4'-O-adenylation and 6'-N-acetylation was converted to its 4'-deoxy-6'-N-methyl derivative, BBK-311 (38), which is refractory to all known inactivating enzymes with somewhat lower potency.[119,120] However, removal of only the hydroxyls at the 3'- and/or 4'-position enhances the activity.[119-121] The 6'-C-methyl derivative (39) of 3', 4'-dideoxykanamycin B was resistant to 6'-N-acetylation with retained activity.[122]

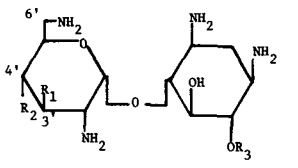
Several modifications at the 5- and 6-(cyclitol) positions have been reported. Substitution of an amino group for hydroxyl at these positions in neamine (40) resulted in less active products.[123] Similarly, 5-epichloro-5-deoxyneamine (41)[124] and 5-epikanamycin B (42)[125] were less active than their parents. A series of 3',4',5-trideoxypseudotrisaccharides were found to be less active than gentamycin.[124] Neamine intermediates lacking their chemically more reactive O-6 hydroxyl groups were used to selectively prepare active 6-deoxyribostomycin analogs *via* glycosylation on their 5-positions.[126] Modification at the 5-position

did not lead to more active derivatives in the kanamycin series but did in the gentamicin (sisomicin) series. Using a mutasynthetic approach, a series of sisomicins (43-49) modified at the 5-position was prepared.[127] In many cases, bioconversion was poor, and a synthetic approach was necessary to prepare quantities of interesting candidates. Most of the products were as active as sisomicin and displayed resistance to enzymatic inactivation. Sch 22703 (5-epinetilmicin, 50) was apparently less nephrotoxic and more active than sisomicin, especially against 2"-O-adenylating and 2'-N-acetylating organisms. Win 42122-2 (2-hydroxygentamicin) was reported to be almost as active as gentamicin but less toxic.[128]

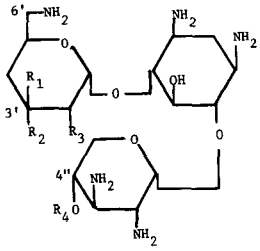
Interest continues in developing procedures for selective acylation of aminoglycosides, useful for the preparation of amikacin and other 1-N-amides.[129-131] Such procedures were used to prepare the 3-amino-2-hydroxypropionyl (HAPA) derivatives of sisomicin, gentamicin B and their 5-epi isomers.[132-134] The best of them, 1-N-HAPA-5-episisomicin (51) was active against all inactivating strains tested. Similarly, a series of 1-N-mono-, di- and tri-peptide derivatives of sisomicin were prepared, of which the 1-N-seryl and the 1-N-glycyl-glycyl showed enhanced activity and reduced toxicity.[135] Many 1-N-urethanes and ureas of sisomicin, gentamicins B and C_{1a} and kanamycin A were active.[136] The 1-N-(2-aminoethoxy)-carbonyl analog of kanamycin A (52) was more active than 37. Reduction of the 1-N-amide side chain of butirosin resulted in a modest loss of activity.[137] A new kanamycin B derivative, UK-31,214 (53), displayed enhanced activity especially against *Pseudomonas* and kanamycin-resistant strains.[138]

The 3'- and 4'-thiodeoxyneamines (54, 55) and 4'-thiodeoxykanamycin B (56) were less active than their parent compounds except for some enhancement against *P. aeruginosa* and phosphorylating *E. coli*.[139]

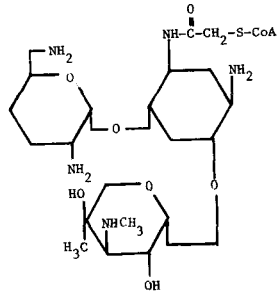
Removal of the 4"-O-methyl group (57) from seldomycin factor 5 (SK-5) and 3'-deoxy SF-5 (58) had little effect on activity whereas addition of a methyl group to the 4"-position of gentamicin C_{1a} reduced activity.[140] The 3'-axial SF-5 derivative (59) had comparable activity to SF-5, but the aziridine (60) was inactive.[141] All new aminoglycoside structures isolated from fermentation were various deoxy and epimeric analogs of fortimicin.[142-152] Like fortimicin, these lacked appreciable antipseudomonal activity and were susceptible to 3-N-acetylating enzymes.



54 R_1=SH, R_2=OH, R_3=H
55 R_1=OH, R_2=SH, R_3=H
56 R_1=OH, R_2=SH, R_3=3-AMINOGLUCOSE



57 R_1=OH, R_2=R_4=H, R_3=NH_2
58 R_1=R_2=R_4=H, R_3=NH_2
59 R_1=R_3=H, R_2=NH_2, R_4=Me
60 R_1=H, R_2, R_3=N⟋, R_4=Me



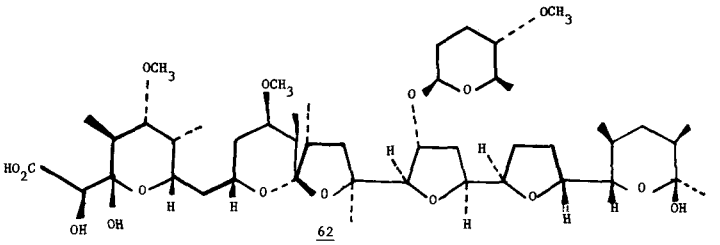
61

In a novel approach to overcome plasmid-mediated enzymatic inactivation, a gentamicin-derivative (61) with covalently attached coenzyme A (CoA) was stereospecifically prepared.[153] Using the specificity of an isolated preparation of gentamicin acetyl transferase (AAC(3)-I), gentamicin was selectively chloroacetylated on its N-3-position with chloroacetyl-CoA and then in a second step selectively alkylated on the chloroacetyl group with

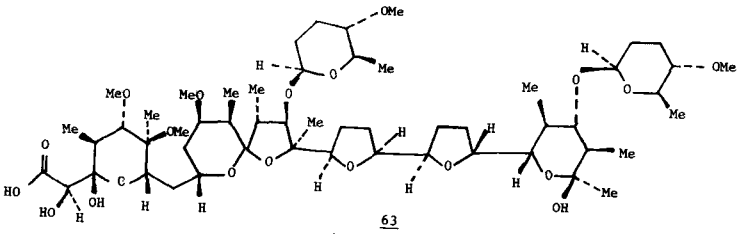
free CoA. The product was an inhibitor of isolated AAC(3)-I but had no effect in an intact organism implying a lack of uptake. Two syntheses of spectinomycin[154,155] and a quick method for presumptive early identification of aminoglycosides[156] were published.

Macrolides - A6888, a new complex related to the cirramycins, is produced by *S. flocculus* and active against gram-positive bacteria.[157] Diacetylation of midecamycin at the 9- and 3"-positions enhanced its pharmacokinetic properties in animals.[158] The structure of Sch 23831, a weakly active by-product of the rosaramycin fermentation, was reported.[159] A proposed anagolamycin-like structure of staphcoccomycin, produced by *Streptomyces* sp., was published.[160] The structures of the megalomycins were revised by ^{13}C NMR (CMR) and X-ray crystallography.[161] The total syntheses of erythronolide,[162] methylnolide[163] and A26771B methylester[164,165] were reported. Primycin, the largest nonpolyene macrolide, has been crystallized and studied.[166]

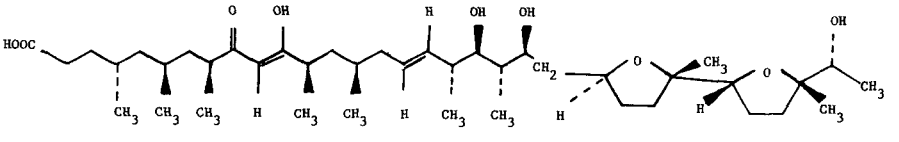
Ionophores - A set of empirical rules to aid in the structure determination of polyether antibiotics based on their CMR spectra was published.[167] It was used to confirm the structure of antibiotic 6016 (62),[168] a novel monovalent, monoglycosyl, Mg-selective ionophore[169] and to revise the structures of alborixin,[170] mutalomycin and noboritomycins A and B.[171] These revisions, which involved reassignment of configurations at one carbon in each structure, corrected original misinterpretations of X-ray data. Similar CMR data was used to determine the structure of the monovalent diglycoside K-41B (63),[172] whereas X-ray determination revealed the structures of the calcium specific polyether ionomycin (64)[173] and the two new monovalent monoglycosides, CP-47,433 (65) and CP-47,434 (66), produced by an *Actinomadura* sp.[174] The details on the isolation and characterization of X-14547A were published.[175] The total syntheses of monensin[176] and A-23187[177] were reported, as were successful attempts to mimic ionophoric activity with totally synthetic open chain polyethers.[178] Microbial modification of A-23187 methyl ester yielded less active hydroxylated analogs.[179]



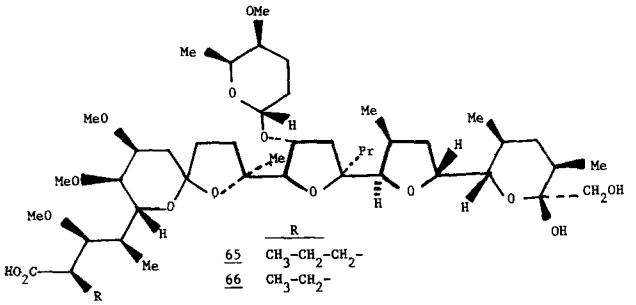
62



63



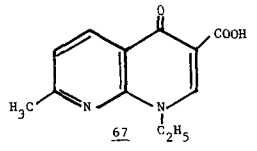
64



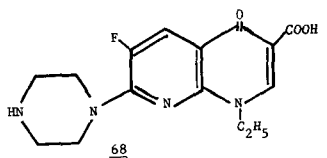
	R
65	$CH_3-CH_2-CH_2-$
66	CH_3-CH_2-

Synthetic Agents - Several new naphthyridine derivatives related to nalidixic acid[180] (67) were reported.[181-183] The most promising of these, AT 2266 (68), unlike 67 has excellent broad-spectrum activity against a series of organisms, including *S. marcescens*, *P. aeruginosa* and *S. aureus*. It was generally more effective orally in mice than pipemidic acid, oxolinic acid, cephalexin or ampicillin. Low serum protein binding and good plasma, tissue and urine concentrations make this compound a candidate for further clinical study. DJ-6782 (69) has similar characteristics to 68, but it has less activity against *P. aeruginosa*. It is well absorbed orally in man with most of the drug excreted as glucuronide.[184,185] Rosoxacin, a pyridyl-quinoline derivative with broad gram-negative activity, including gentamicin-resistant *Pseudomonas*, is currently undergoing clinical trial.[186]

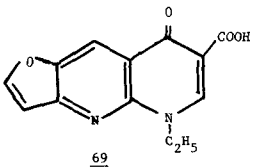
Tetroxoprim, a new benzylpyrimidine is structurally similar to, but less active than, trimethoprim.[187] The treatment of anerobic infections, especially those caused by *B. fragilis*, with nitroimidazoles is actively being pursued. Two newer analogs, secnidazole (1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole) and ornidazole (α-chloromethyl-2-methyl-5-nitroimidazole) are of current interest.[188-190] Sodium fusidate, an older antibiotic, was reported to possess antianaerobic activity, including activity against *B. fragilis*.[191]



67



68



69

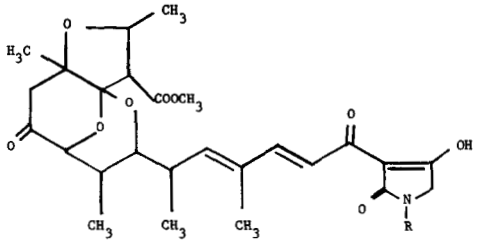
Miscellaneous Antibiotics - Other primarily antibacterial antibiotics reported are listed in Table 1. Structural work also was completed for saframycins B and C, (78,79)[203] rubradirin (80) and rubradirin B (81).[204] A revised structure for flambamycin (82),[205,206] some refinements in the everninomicin D structure,[207] and tentative structures for curamycin A 83 [208] and avilamycins A (84) and C (85),[209] were determined. All are members of the recently reviewed orthosomycins.[210] A series of glycosides of the known antibiotic pleuromutilin was prepared after the discovery of its β-xyloside, A40104A, in the broth of *Clitopilus pseudo-pinsitus*.[211,212] The β-thioxyloside (LY 92206), its dihyro derivative (LY 92207) and dihydro-A40104A (LY 235973), have primarily gram-positive activity[213] with potential use as feed additives.[214]

Table 1 New Antibiotic Entities

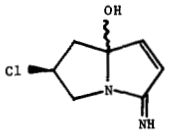
Antibiotic	Producing Organism	Activity*	Structure	Reference
Bu 2313 A,B	Actinomycete	G+,G-,AA	70,71	192,193
Clazamycins A,B	*Streptomyces* sp.	G+,G-	72,73	194
G1549 (BN-227, BN-227-F)	*Pseudomonas* sp.	G+,G-	74	195,196
Gunacin	*Ustilago* sp.	G+,G-,AF	75	197
Heneicomycin (3-Deoxyaurodox)	*Streptomyces filipinensis*	G+,G-	-	198
2-Hydroxy-5-iminoaza-cyclopent-3-ene	*Streptoverticillium parvisoporgenes*	G+,G-	-	199
Nanaomycin E (Epoxy-nanaomycin A)	*Streptomyces* sp.	G+	-	200
Neosidomycin	*Streptomyces hygroscopicus*	G-	76	201
Permetin A	*B. circulans*	G+,G-,AA	77	202

*G+,G-(gram-positive, -negative); AF (antifungal); AA (anaerobic)

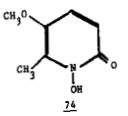
Replacement of the 3-hydroxyl group of chloramphenicol (CM) and thiamphenicol with fluorine led to derivatives (Sch 24893, 86; Sch 25298, 87 and Sch 25392, 88) with activity against CM resistant (acetylating) organisms.[215-218] N-9174, an inhibitor of CM acetyl transferase, was isolated from a *Streptomyces* sp. and found to enhance the activity of CM against a CM-acetylating *Streptococcus*.[219] A synthetic tetracycline analog (89) with a hydroxyl at the 8-position had low activity.[220] Of several imine derivatives of chelocardin, only the aniline condensation product retained activity.[221] None of 59 semisynthetic bicyclomycin derivatives were as active as bicyclomycin.[222] Reverse phase HPLC was successfully used to resolve complex mixtures of the peptides cerexins and tridecaptins into several components.[223,224] The total synthesis of d,l terramycin[225] and syntheses of tuberactinomycin[226] and of some capreomycin analogs were reported.[227]



70 R = CH_3
71 R = H



72 α
73 β



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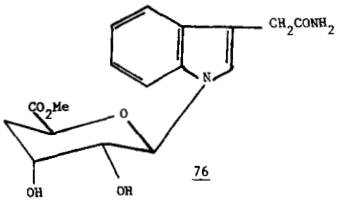

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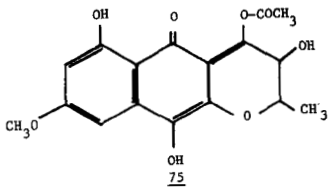

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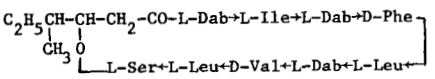

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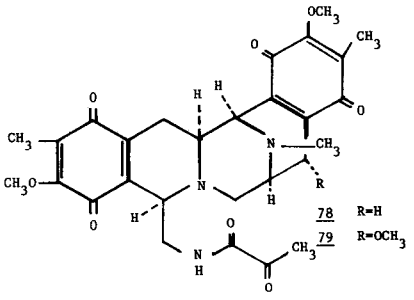
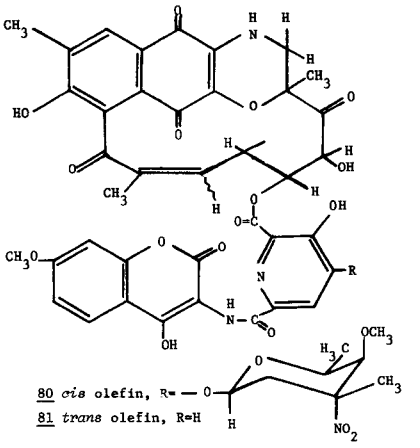
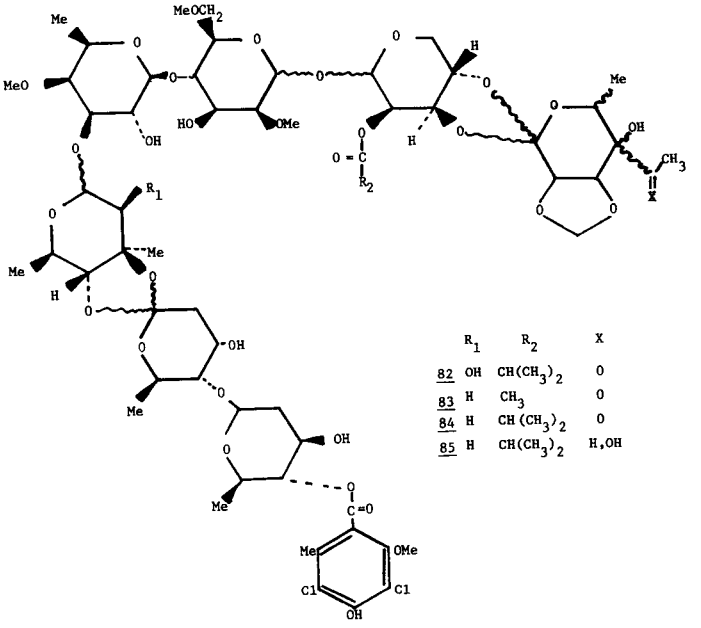


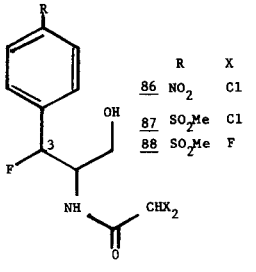
78 R=H
79 R=OCH_3



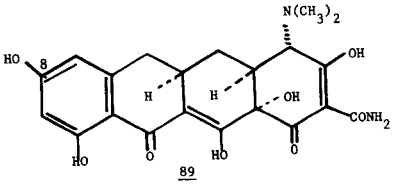
80 *cis* olefin, R= — O —
81 *trans* olefin, R=H



	R_1	R_2	X
82	OH	$CH(CH_3)_2$	O
83	H	CH_3	O
84	H	$CH(CH_3)_2$	O
85	H	$CH(CH_3)_2$	H,OH



	R	X
86	NO_2	Cl
87	SO_2Me	Cl
88	SO_2Me	F



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References

1. Abstracts, 11th International Congress of Chemotherapy and 19th Interscience Conference on Antimicrobial Agents and Chemotherapy (11th ICC/19th ICAAC), Boston, Mass., October 1-5, 1979.
2. J. S. Glasby, Encyclopedia of Antibiotics, John Wiley & Sons, New York (1979).
3. T. Korzybski, Z. Kowszyk-Gindifer and W. Kuryłowicz, Antibiotics - Origin, Nature and Properties, Vols. I, II, III, American Society for Microbiology, Washington, D.C., (1978).
4. J. R. E. Hoover and G. L. Dunn, The β-Lactam Antibitoics, in Burger's Medicinal Chemistry, 4th Ed. Part II, M. E. Wolff, Ed., John Wiley & Sons, New York (1979).
5. C. H. O'Callaghan, J. Antimicrob. Chemother. 5, 635 (1979).
6. R. Heymès, R. Bucourt, A. Lutz, L. Pénase and J. Perronnet, Drugs Exptl. Clin. Res. 5, 23 (1979).
7. T. Takaya, T. Kamimura, H. Kojo, Y. Mine, M. Nishida, S. Goto and S. Kuwahara, 11th ICC/19th ICAAC, 556 (1979).
8. T. Kamimura, Y. Matsumoto, N. Okada, Y. Mine, M. Nishida, S. Goto and S. Kuwahara, Antimicrob. Ag. Chemother. 16, 540 (1979).
9. M. Nishida, T, Kamimura, N. Okada, Y. Matsumoto, Y. Mine and T. Murakowa, J. Antibiot. 32, 1319 (1979).
10. H. Kojo, M. Nishida, S. Goto and S. Kuwahara, Antimicrob. Ag. Chemother. 16, 549 (1979).
11. T. Yokota, R. Sekiguchi and E. Azuma, 11th ICC/19th ICAAC, 558 (1979).
12. H. C. Neu and K. P. Fu, ibid, 561 (1979).
13. T. Murakawa, H. Sakamoto, S. Fukada, T. Hirose, S. Nakamoto and M. Nishida, ibid. 557 (1979).
14. M. Nakashima, H. Hashimoto, K. Suzuki and K. Nishijima, ibid. 555 (1979).
15. N. Clumeck, R. Vanhoof, Y. Vanlaethem and J. P. Butzler, ibid. 16 (1979).
16. G. G. Grassi, R. Dionigi, A. Ferrara and E. Pozzi, ibid. 15 (1979).
17. M. W. McKendrick, A. M. Geddes and R. Wise, ibid. 17 (1979).
18. S. W. B. Newsom, J. Matthews, S. J. Connellan and V. R. Pearce, ibid. 18 (1979).
19. H. F. Helwig, ibid. 20 (1979).
20. D. H. Wittmann and H. H. Schassan, ibid. 12 (1979).
21. I. Ho, P. Aswapokee, K. P. Fu, C. Matthijssen and H. C. Neu, ibid. 13 (1979).
22. D. M. D. Rimmer, ibid. 871 (1979).
23. R. Lüthy, R. Münch, J. Blaser, H. Bhend and W. Siegenthaler, Antimicrob. Ag. Chemother. 16, 127 (1979).
24. R. Wise, J. M. Andrews, D. Hammond, P. J. Wills, A. M. Geddes and M. McKendrick, 11th ICC/19th ICAAC, 14 (1979).
25. L. O. White, H. A. Holt, D. S. Reeves, M. J. Bywater and R. P. Bax, ibid. 881 (1979).
26. P. B. Harper, S. M. Kirby and C. H. O'Callaghan, ibid. 559 (1979).
27. P. Acred, D. M. Ryan and P. W. Muggleton, ibid. 560 (1979).
28. S. Matsuda, M. Tanno, T. Kashiwagura and H. Furuya, ibid. 402 (1979).
29. Y. Ueda, M. Ohmori, A. Saito and K. Shiba, ibid. 401 (1979).
30. B. Sambe, K. Jyo and M. Inafuku, ibid. 405 (1979).
31. J. Sasaki, J. Goto, T. Konnai, S. Miyachi, Y. Yamada, T. Imoto, H. Kobune and K. Takeyasu, ibid. 406 (1979).
32. O. Sekine, Y. Usuda, T. Shimizu, N. Aoki, Y. Hirasawa and T. Aoki, ibid. 398 (1979).
33. R. D. Smyth, M. Feffer, D. R. Van Harken, R. A. Trompeter and G. H. Hottendorf, ibid. 517 (1979).
34. D. Rawson, D. Jones and C. Perlino, ibid. 518 (1979).
35. D. M. Musher, V. Fainstein and E. J. Young, ibid. 519 (1979).
36. S. F. Grappel, L. Phillips, P. Actor and J. A. Weisbach, ibid. 729 (1979).
37. I. Zajac, R. Bartus, P. Actor and J. A. Weisbach, ibid.730 (1979).
38. D. H. Pitkin, P. Actor, F. Alexander, J. Dubb, R. Stote and J. A. Weisbach, ibid. 731 (1979).
39. J. V. Uri, P. Actor, I. Zajac, D. H. Pitkin, L. Phillips, J. R. Guarini, H. F. Bartus, T. J. Polansky, G. L. Dunn, J. R. E. Hoover and J. A. Weisbach, J. Antibiot. 32, 1161 (1979).
40. T. Komatsu, T. Okuda, H. Noguchi, M. Fukazawa, K. Yano, M. Kato and S. Mitsuhashi, 11th ICC/19th ICAAC, 565 (1979).
41. H. Matsui, K. Yano, H. Nakatani and K. Mashimo, ibid. 562 (1979).
42. T. Y. Tai, F. Wang, C. L. Chang, M. F. Chang, C. T. Chang, Y. Y. Chang and Y. K. Liu, Chinese Med. J. 92, 26 (1979).
43. A. A. Dominguez-Gil, M. Cepeda, J. L. Vila and J. J. Garcia, J. Antibiot. 32, 482 (1979).
44. M. L. Edwards and R. C. Erickson, J. Med. Chem. 22, 1416 (1979).
45. W. E. Wright, W. J. Wheller, V. D. Line, J. A. Frogge and D. R. Finley, J. Antibiot. 32, 1155 (1979).
46. E. Quaresima, M. O. Tinti, P. Foresta, P. De Witt and M. Ramacci, ibid. 32, 1311 (1979).
47. M. Narisada, T. Yoshida, H. Onoue, M. Ohtani, T. Okada, T. Tsuji, I. Kikkawa, N. Haga, H. Satoh, H. Itani and W. Nagata, J. Med. Chem. 22, 757 (1979).

48. S. Uyeo, I. Kikkawa, Y. Hamashima, H. Ona, Y. Nishitani, K. Okada, T. Kubota, K. Ishikura, Y. Ide, K. Nakano and W. Nagata, J. Am. Chem. Soc. 101, 4403 (1979).
49. Y. Hamashima, T. Kubota, K. Ishikura and W. Nagata, Tetrahedron Lett. 4943 (1979).
50. M. Yoshioka, I. Kikkawa, T. Tsuji, Y. Nishitani, S. Mori, K. Okada, M. Murakami, F. Matsubara, M. Yamaguchi and W. Nagata, ibid. 4287 (1979).
51. C. L. Branch and M. J. Pearson, J.C.S. Perkin I, 2268 (1979).
52. M. S. Manhas, S. G. Amin, B. Ram, V. V. Rao and A. K. Bose, MEDI 47, ACS/CSJ Chemical Congress, Honolulu, Hawaii, April 1-6, 1979.
53. J. G. Gleason, T. F. Buckley, K. G. Holden, D. B. Bryan and P. Siler, J. Am. Chem. Soc. 101, 4730 (1979).
54. K. Yano, K. Suzaki, M. Saito, M. Toda, T. Saito and S. Mitsuhashi, 11th ICC/19th ICAAC, 564 (1979).
55. A. Tachibana, M. Komiya, Y. Kikuchi, K. Yano and K. Mashimo, ibid. 563 (1979).
56. M. Iwanami, T. Maeda, M. Fujimoto, Y. Nagano, N. Nagano, A. Yamazaki, T. Shibanuma, K. Murase, K. Tamazawa, M. Aruga and R. Ishikawa, MEDI 44, ACS/CSJ (1979).
57. H. Nakao, H. Yanagisawa, S. Ishihara, E. Nakajama, A. Ando, J. Nakazawa, B. Shimizu, M. Kaneko, M. Nagano and S. Sugawara, J. Antibiot. 32, 320 (1979).
58. J. Cardenas, R. Delbusto, K. Burch, T. Madhavan, E. Fisher and E. L. Quinn, 11th ICC/19th ICAAC, 242 (1979).
59. C. L. Heifetz and J. A. Sesnie, ibid. 244 (1979).
60. J. S. Kaltenbronn, T. Haskell, L. Doub, J. Knoble, D. DeJohn, U. Krolls, N. Jenesel, G. G. Huang, K. L. Heifetz and M. W. Fisher, J. Antibiot. 32, 621 (1979).
61. T. Nishino, N. Ishii, T. Tanino, S. Ohshima and T. Yamaguchi, 11th ICC/19th ICAAC, 241 (1979).
62. S. R. Baker, C. T. Holdrege and D. N. McGregor, J. Antibiot. 32, 468 (1979).
63. Y. Suzuki, H. Ohmori, A. Azuma, Y. Hashimoto, Y. Ichikawa and T. Noguchi, ibid. 32, 711 (1979).
64. J. S. Kahan, F. M. Kahan, R. Goegelman, S. A. Currie, M. Jackson, E. O. Stapley, T. W. Miller, A. K. Miller, D. Hendlin, S. Mochales, S. Hernandez, H. B. Woodruff and J. Birnbaum, ibid. 32, 1 (1979).
65. W. J. Leanza, K. J. Wildonger, T. W. Miller and B. G. Christensen, J. Med. Chem. 22, 1435 (1979).
66. H. Kropp, J. G. Sundelof, J. S. Kahan, F. M. Kahan and J. Birnbaum, 11th ICC/19th ICAAC, 231 (1979).
67. K. J. Wildonger, W. J. Leanza, T. W. Miller and G. B. Christensen, ibid. 232 (1979).
68. T. Kamiya, M. Hashimoto, O. Nakaguchi and T. Oku, Tetrahedron 35, 323 (1979).
69. T. Kamiya, T. Oku, O. Nakaguchi, H. Takeno and M. Hashimoto, Tetrahedron Lett. 5119 (1979).
70. D. Brown, J. R. Evans and R. A. Fletton, J.C.S. Chem. Comm. 282 (1979).
71. P. C. Cherry, C. E. Newall and N. S. Watson, ibid. 663 (1979).
72. C. M. D. Beels, M. S. Abu-Rabie, P. Murray-Rust and J. Murray-Rust, ibid. 665 (1979).
73. M. Lang, K. Prasad, W. Holick, J. Gosteli, I. Ernest and R. B. Woodward, J. Am. Chem. Soc. 101, 6296 (1979).
74. D. Butterworth, M. Cole, G. Hanscomb and G. N. Rolinson, J. Antibiot. 32, 287 (1979).
75. J. D. Hood, S. J. Box and M. S. Verrall, ibid. 32, 295 (1979).
76. A. G. Brown, D. F. Corbett, A. J. Eglington and T. T. Howarth, ibid. 32, 961 (1979).
77. A. J. G. Baxter, K. H. Dickinson, P. M. Roberts, T. C. Smale and R. Southgate, J.C.S. Chem. Comm. 236 (1979).
78. S. J. Cartwright and A. F. W. Coulson, Nature 278, 360 (1979).
79. P. H. Bentley, G. Brooks, M. L. Gilpin and E. Hunt, Tetrahedron Lett. 1889 (1979).
80. P. Lombardi, G. Franceschi and F. Arcamone, ibid. 3777 (1979).
81. E. Hunt, J.C.S. Chem. Comm. 686 (1979).
82. J. Cosmidis, A. Anifantis, C. H. Stathakis, K. Mantopoulos and G. K. Daikos, 11th ICC/19th ICAAC, 300 (1979).
83. D. A. Leigh and K. Bradnock, ibid. 301 (1979).
84. K. Comber, R. Horton, L. Minzen, A. White and R. Sutherland, ibid. 306 (1979).
85. F. W. Goldstein, M. D. Kitzis, C. Malhuret, P. Bourquelot and J. F. Acar, ibid. 309 (1979).
86. J. A. Retsema, A. E. Girard, A. R. English and J. E. Lynch, ibid. 311 (1979).
87. G. Foulds, W. E. Barth, J. R. Bianchine, A. R. English, D. Girard, S. L. Hayes, M. M. O'Brien and P. Somani, ibid. 312 (1979).
88. V. Knott-Hunziker, B. S. Orlek, P. G. Sammes and S. G. Waley, Biochem J. 177, 365 (1979).
89. B. S. Orlek and P. G. Sammes, J.C.S. Chem. Comm. 962 (1979).
90. V. Knott-Hunziker, S. G. Waley, B. S. Orlek and P. G. Sammes, Febs Letters 99, 59 (1979).
91. R. J. Mehta, D. J. Newman, B. A. Bowie, C. H. Nash and P. Actor, 11th ICC/19th ICAAC, 728 (1979).
92. D. J. Newman, R. J. Mehta, B. A. Bowie, C. H. Nash and P. Actor, ibid. 727 (1979).
93. M. H. Richmond, Rev. Inf. Dis. 1, 30 (1979).

94. D. J. Tipper, ibid. 1, 39 (1979).
95. K. Kitano and A. Tomasz, Antimicrob. Ag. Chemother. 16, 838 (1979).
96. T. Kamiya, K. Hemmi, H. Takeno and M. Hashimoto, 11th ICC/19th ICAAC, 235 (1979).
97. M. Okuhara, Y. Kuroda, J. Hosoda, T. Goto, M. Okamoto, H. Terano, E. Iguchi, M. Kohsaka, H. Aoki and H. Imanaka, ibid.236 (1979).
98. H. Kojo, T. Kamimura, S. Nonoyama, Y. Mine, M. Nishida, S. Goto and S. Kuwahara, ibid. 234 (1979).
99. E. O. Stapely, S. B. Zimmerman, M. Jackson, R. T. Goegelman, A. K. Miller, B. A. Pelak, T. W. Miller, S. A. Currie, D. Hendlin, S. Hernandez and J. M. Mata, ibid. 233 (1979).
100. J. G. Allen, F. R. Atherton, M. J. Hall, C. H. Hassall, S. W. Holmes, R. W. Lambert, I. Lennox-Smith, W. J. Lloyd, L. J. Nisbet and P. S. Ringrose, Drugs Exptl. Clin. Res. 5, 187 (1979).
101. F. R. Atherton, M. J. Hall, C. H. Hassall, R. W. Lambert and P. S. Ringrose, Antimicrob. Ag. Chemother. 15, 677 (1979).
102. J. G. Allen, F. R. Atherton, M. J. Hall, C. H. Hassall, S. W. Holmes, R. W. Lambert, L. J. Nisbet and P. S. Ringrose, ibid. 15, 684 (1979).
103. F. R. Atherton, M. J. Hall, C. H. Hassall, R. W. Lambert, W. J. Lloyd and P. S. Ringrose, ibid. 15, 696 (1979).
104. H. B. Maruyama, M. Arisawa and T. Sawada, ibid. 16, 444 (1979).
105. J. G. Allen, L. Havas, E. Leicht, I. Lenox-Smith and L. J. Nisbet, ibid. 16, 306 (1979).
106. S. Ōmura, H. Tanaka, R. Ōiwa, T. Nagai, Y. Koyama and Y. Takahashi, J. Antibiot. 32, 978 (1979).
107. S. Ōmura, H. Tanaka, Y. Tanaka, P. Spiri-Nakagawa, R. Ōiwa, Y. Takahashi, K. Matsuyama and Y. Iwai, ibid. 32, 985 (1979).
108. P. Spiri-Nakagawa, Y. Tanaka, R. Ōiwa, H. Tanaka and S.Ōmura, ibid. 32, 995 (1979).
109. R. D. Johnson, L. D. Boeck, H. R. Papiska, Y. H. B. Chao and R. Nagarajan, 11th ICC/19th ICAAC, 1032 (1979).
110. F. T. Counter, P. W. Ensminger and L. F. Ellis, ibid. 1033 (1979).
111. J. N. Hobbs and N. E. Allen, ibid. 1034 (1979).
112. G. Sheldrick, P. G. Jones, O. Kennard, D. H. Williams and G. A. Smith, Nature 271, 223 (1978).
113. D. H. Williams, V. Rajananda, G. Bojesen and M. P. Williamson, J.C.S. Chem. Comm. 906 (1979).
114. C. M. Harris, J. J. Kibby, J. R. Fehlner, A. B. Raabe, T. A. Barber and T. M. Harris, J. Am. Chem. Soc. 101, 437 (1979).
115. D. H. Williams, V. Rajananada and J. R. Kalman, J. Chem. Soc. Perkin I, 787 (1979).
116. F. Sztaricskai, C. M. Harris and T. M. Harris, J. Antibiot. 32, 446 (1979).
117. W. J. McGahren, J. H. Martin, G. O. Morton, R. T. Hargreaves, R. A. Leese, F. M. Lovell, G. A. Ellestad, J. Am. Chem. Soc. 101, 2237 (1979).
118. F. Sztaricskai, C. M. Harris and T. M. Harris, Tetrahedron Lett. 2861 (1979).
119. T. Naito, S. Nakagawa, S. Toda, K. Fujisawa and H. Kawaguchi, J. Antibiot. 32, 659 (1979).
120. P. Kresel, T. Pursiano, K. Price, Misiek and F. Leitner, 11th ICC/19th ICAAC, 770 (1979).
121. T. Tsuchiya, T. Jikihara, T. Miyake, S. Umezawa, M. Hamada and H. Umezawa, J. Antibiot. 32, 1351 (1979).
122. H. Umezawa, D. Ikeda, T. Miyasaka and S. Kondo, ibid. 32, 1360 (1979).
123. T. Suami, CARB 4, ACS/CSJ, (1979).
124. J. P. H. Verheyden, D. B. Repke, T. C. Tompkins and J. G. Moffatt, CARB 37, ACS/CSJ (1979).
125. T. Suami and K. Nakamura, Bull. Chem. Soc. Japan 52, 955 (1979).
126. B. J. Magerlein, CARB 32, ACS/CSJ (1979).
127. P. J. Daniels and D. F. Rane, Microbiology-1979, p. 314.
128. P. Came, J. O'Connor, R. Dobson, R. Wagner and R. Fabian, Antimicrob. Ag. Chemoth. 16, 813 (1979).
129. T. L. Nagabhushan, A. B. Cooper, W. N. Turner, H. Tsai, S. McCombie, A. K. Mallams, D. Rane, J. J. Wright, P. Reichert, D. L. Boxler and J. Weinstein, J. Am. Chem. Soc. 100, 5253 (1978).
130. T. Tsuchiya, Y. Takagi and S. Umezawa, Tetrahedron Lett. 4951 (1979).
131. M. J. Cron, J. G. Keil, J. S. Lin, M. V. Ruggeri and D. Walker, J.C.S. Chem. Comm. 266 (1979).
132. R. Hare, T. Schafer, P. Chiu, F. Sabatelli, E. Moss, Jr. and G. H. Miller, 11th ICC/19th ICAAC, 765 (1979).
133. D. Rane and P. Daniels, ibid. 768 (1979).
134. T. Nagabhushan and A. Cooper, ibid. 769 (1979).
135. A. Afonoso and F. Hon, ibid. 766 (1979).
136. A. Mallams, P. Reichert and J. Morton, ibid. 767 (1979).
137. T. Hayashi, H. Saeki, N. Takeda and E. Ohki, J. Antibiot. 32, 1280 (1979).

138. K. Richardson, K. Brammer, S. Jevons, R. Plews and J. Wright, ibid. 32, 973 (1979).
139. T. W. Ku, R. D. Sitrin, D. J. Cooper, J.R.E. Hoover and J. A. Weisbach, CARB 36, ACS/CSJ (1979).
140. J. McAlpine, R. Carney, R. DeVault, A. Sinclair, R. Egan, M. Cirovic, R. Stanaszek and S. Mueller, ibid. 34 (1979).
141. H. Matsushima, Y. Mori and K. Kitaura, Bull. Chem. Soc. Japan 51, 3553 (1978).
142. T. Iida, M. Sato, I. Matsubara, Y. Mori and K. Shirahata, J. Antibiot. 32, 1273 (1979).
143. M. Sugimoto, S. Ishii, R. Okachi and T. Nara, ibid. 32, 868 (1979).
144. T. Deushi, M. Nakayama, I. Watanabe, T. Mori, H. Naganawa and H. Umezawa, ibid. 32, 187 (1979).
145. T. Deushi, A. Iwasaki, K. Kamiya, T. Kunieda, T. Mizoguchi, M. Nakayama, H. Itoh, T. Mori and T. Oda, ibid. 32, 173 (1979).
146. Y. Okami, K. Hotta, M. Yoshida, D. Ikeda, S. Kondo, and H. Umezawa, ibid. 32, 964 (1979).
147. T. Deushi, A. Iwasaki, K. Kamiya, T. Mizoguchi, M. Nakayama, H. Itoh and T. Mori, ibid. 32, 1061 (1979).
148. S. Inouye, K. Ohba, T. Shomura, M. Kojima, T. Tsurouoka, J. Yoshida, N. Katō, M. Itō, S. Omoto, N. Ezaki, T. Itō, T. Nida and K. Watanabe, ibid. 32 1354 (1979).
149. R. S. Egan, R. S. Stanaszek, M. Cirovic, S. L. Mueller, A. W. Goldstein, P. Collum, J. R. Martin, E. E. Fager, G. G. Post, P. Kurath, J. B. McAlpine, R. E. Carney, D. Grampovnik and J. H. Seely, CARB 21, ACS/CSJ (1979).
150. K. Takahashi, T. Iida, S. Takazawa, G. Shimura and K. Shirahata, ibid. 22 (1979).
151. M. Sato and Y. Mori, J. Antibiot. 32, 371 (1979).
152. T. Yamaguchi, Y. Kyotani, I. Watanabe, S. Sato, Y. Takahashi, M. Nagakura and T. Mori, ibid. 32, 1137 (1979).
153. J. W. Williams and D. Northrop, ibid. 32, 1147 (1979).
154. S. Hanessian and R. Roy, J. Am. Chem. Soc. 101, 5839 (1979).
155. D. R. White, R. D. Birkenmeyer, R. C. Thomas, S. A. Mizsak and V. H. Wiley, Tetrahedron Lett. 2737 (1979).
156. J. V. Uri, P. Actor and J. A. Weisbach, Experientia 35, 1034 (1979).
157. S. M. Nash, L. D. Boeck, P. W. Ensminger, M. M. Hoehn and K. F. Koch, 11th ICC/19th ICACC, 1026 (1979).
158. K. Umemura, T. Shomura, S. Someya, S. Murata and Y. Kazuno, ibid. 757 (1979).
159. M. S. Puar, R. Brambilla, P. Bartner, D. Schumacher and R. S. Jaret, Tetrahedron Lett. 2767 (1979).
160. I. R. Shimi, S. Shoukry and F. T. Ali, J. Antibiot. 32, 1248 (1979).
161. P. Bartner, D. L. Boxler, R. Brambilla, A. K. Mallams, J. B. Morton, P. Reichert, F. D. Sancilio, H. Surprenant, G. Tomalesky, G. Lukacs, A. Olesker, T. T. Thang, L. Valente and S. Ōmura, J.C.S. Perkin I, 1600 (1979).
162. E. J. Corey, P. B. Hopkins, S. Kim, S. Yoo, K. P. Nambiar and J. R. Falck, J. Am. Chem. Soc. 101, 7131 (1979).
163. P. A. Grieco, Y. Ohfune, Y. Yokoyama and W. Owens, ibid. 101, 4749 (1979).
164. T. A. Hase and E. L. Nylund, Tetrahedron Lett. 2633 (1979).
165. K. Tatsuta, T. Yamauchi, A. Nakagawa and M. Kinoshita, CARB 72, ACS/CSJ (1979).
166. J. V. Uri and P. Actor, J. Antibiot. 32, 1207 (1979).
167. H. Seto, K. Mizoue, H. Nakayama, K. Furihata, N. Ōtake, H. Yonehara, ibid. 32, 239 (1979).
168. H. Seto, H. Nakayama, T. Ogita, K. Furihata, K. Mizoue and N. Ōtake, ibid. 32, 244 (1979).
169. N. Ōtake and M. Mitani, Agric. Biol. Chem. 43, 1543 (1979).
170. H. Seto, K. Mizoue, N. Ōtake, P. Gachon, A. Kergomard and J. W. Westley, J. Antibiot. 32, 970 (1979).
171. T. Fehr, C. Keller-Juslén, H. King, H. Loosli, M. Kuhn and A. von Wartburg, ibid. 32, 535 (1979).
172. N. Tsuji, K. Nagashima, Y. Terui, and K. Tori, ibid. 32, 169 (1979).
173. B. K. Toeplitz, A. J. Cohen, P. T. Funke, W. L. Parker and J. Z. Gougoutas, J. Am. Chem. Soc. 101, 3344 (1979).
174. J. Tone, R. Shibakawa, H. Maeda, K. Inoue, Y. Yamauchi, K. Tsukuda, M. Yamada, W. Cullen, L. Chappel, C. Moppett, J. Oscarson, C. LaPlante, L. Huang and W. Celmer, 11th ICC/19th ICAAC, 1030 (1979).
175. J. Westley, R. Evans, Jr., L. Sello, N. Troupe, C. Liu and J. Blount, J. Antibiot. 32, 100 (1979).
176. T. Fukuyama, K. Akasaka, D. Karanewsky, C. Wang, G. Schmid and Y. Kishi, J. Am. Chem. Soc. 101, 262 (1979).
177. D. A. Evans, C. E. Sacks, W. H. Kleschick and T. R. Tabor, ibid. 101, 6789 (1979).
178. W. Wierenga, B. R. Evans and J. Wolterson, ibid. 101 1334 (1979).
179. B. Abbott, D. Fukuda, D. Dorman, J. Occolowitz, M. Debono and L. Farhner, Antimicrob. Ag. Chemother. 16, 808 (1979).

180. P. M. Gilis and A. Haemers, Drugs Exptl. Clin. Res. 5, 287 (1979).
181. J. Matsumoto, T. Miyamoto, A. Minamida, Y. Nishimura, H. Egawa and H. Nishimura, 11th ICC/19th ICAAC, 1022 (1979).
182. M. Shimizu, Y. Takase, S. Nakamura, H. Katae, S. Inoue, A. Minami, K. Nakata and Y. Sakaguchi, ibid. 1021 (1979).
183. S. Nakamura, Y. Takase, N. Kurobe, S. Kashimoto and M. Shimizu, ibid. 1023 (1979).
184. Y. Osada, T. Une, H. Ogawa and K. Satoh, ibid. 1024 (1979).
185. H. Sano, N. Takasugi, K. Abe, M. Tsumura, H. Nomura, H. Ogawa and Y. Osada, ibid. 1025 (1979).
186. J. R. O'Connor, R. A. Dobson, P. E. Came and R. B. Wagner, ibid. 511 (1979).
187. K. Hoxer, ibid. 352 (1979).
188. J. Symonds, J. Antimicrob. Chemother. 5, 484 (1979).
189. J. A. Garcia-Rodriguez, J. Prieto, J. E. Garcia-Sanchez, N. Rodrigo and A. M. Martin, 11th ICC/19th ICAAC, 817 (1979).
190. E. Rodriguez-Noriega, M. A. Izaguirre-Casillas, S. Esparaza-Ahumada and J. J. Rodrigez-Chagollan, ibid. 816 (1979).
191. G. E. Steinkraus and L. R. McCarthy, Antimicrob. Ag. Chemother. 16, 120 (1979).
192. H. Tsukiura, K. Tomita, M. Hanada, S. Kobaru, M. Tsunakawa, K. Fujisawa and H. Kawaguchi, 11th ICC/19th ICAAC 1028 (1979).
193. M. Tsunakawa, S. Toda, T. Okita, M. Hanada, S. Nakagawa, H. Tsukiura, T. Naito and H. Kawaguchi, ibid. 1027 (1979).
194. Y. Horiuchi, S. Kondo, T. Ikeda, K. Miura, M. Hamada, T. Takeuchi and H. Umezawa, J. Antibiot. 32, 762 (1979).
195. W. Barker, C. Callaghan, L. Hill, D. Noble, P. Acred, P. Harper, M. Sowa and R. Fletton, ibid. 32, 1096 (1979).
196. J. Itoh, S. Miyadoh, S. Takahasi, S. Amano, N. Ezaki and Y. Yamada, ibid. 32, 1089 (1979).
197. R. Werner, K. Appel and W. Merk, ibid. 32, 1104 (1979).
198. S. B. Zimmerman, J. H. Chalmers, Jr., R. S. Dewey, E. O. Stapley and S. Hernandez, ibid. 32, 665 (1979).
199. A. Okuyama, S. Kondo, T. Ikeda, K. Miura, M. Hamada and H. Umezawa, ibid. 32, 768 (1979).
200. M. Kasai, K. Shirahata, S. Ishii, K. Mineura, H. Marumo, H. Tanaka and S. Ōmura, ibid. 32, 442 (1979).
201. R. Furuta, S. Naruto, A. Tamura and K. Yokogawa, Tetrahedron Lett. 1701 (1979).
202. Y. Takeuchi, A. Murai, Y. Takahara and M. Kainosho, J. Antibiot. 32, 121 (1979).
203. T. Arai, K. Takahashi, A. Kubo, S. Nakahara, S. Sato, K. Aiba and C. Tamura, Tetrahedron Lett. 2355 (1979).
204. H. Hoeksema, S. Mizasak and L. Baczynskyj, J. Antibiot. 32, 773 (1979).
205. W. D. Ollis, C. Smith and D. Wright, Tetrahedron 35, 105 (1979).
206. L. Valente, L. E. S. Barata, A. Olesker, R. Rabanal, G. Lukacs and T. T. Thang, Tetrahedron Lett. 1149 (1979).
207. A. K. Ganguly, O. Z. Sarre, A. T. McPhail and R. W. Miller, J.C.S. Chem. Comm. 22 (1979).
208. A. K. Ganguly, A. K. Bose and N. F. Cappuccino, J. Antibiot. 32, 1213 (1979).
209. W. Keller-Schierlein, W. Heilman, W. D. Ollis and C. Smith, Helv. Chim. Acta 62, 7 (1979).
210. D. E. Wright, Tetrahedron 35, 1207 (1979).
211. K. H. Michel, D. E. Dorman and J. L. Occolowitz, 11th ICC/19th ICAAC, 1036 (1979).
212. A. Schable, S. M. Nash and R. Nagarajan, ibid. 1037 (1979).
213. P. W. Ensminger and F. T. Counter, ibid. 1038 (1979).
214. E. E. Ose, ibid. 1039 (1979).
215. T. L. Nagabhushan, D. Kandasamy, H. Tsai, W. N. Turner and G. H. Miller, ibid. 512 (1979).
216. T. W. Achafer, E. L. Moss, Jr., T. L. Nagabhushan and G. H. Miller, ibid. 513 (1979).
217. K. Kung, K. P. Fu and H. C. Neu, ibid. 514 (1979).
218. V. P. Syripoulou, A. L. Harding, D. A. Goldmann and A. L. Smith, ibid. 515 (1979).
219. S. Yiyamura, K. Koizumi and Y. Nakagawa, J. Antibiot. 32, 1217 (1979).
220. B. Glatz, G. Helmchen, H. Muxfeldt, H. Procher, R. Prewo, J. Senn, J. J. Stezowski, R. J. Stojda and D. R. White, J. Am. Chem. Soc. 101, 2171 (1979).
221. D. L. Garmaise, D. T. W. Chu, E. Bernstein, M. Inaba and J. M. Stamm, J. Med. Chem. 22, 559 (1979).
222. B. W. Müller, O. Zak, W. Kump, W. Tosch and O. Wacker, J. Antibiot. 32, 689 (1979).
223. J. Shoji, T. Kato, S. Terabe and R. Konaka, ibid. 32, 313 (1979).
224. T. Kato, R. Sakazaki, H. Hinoo and J. Shoji, ibid. 32, 305 (1979).
225. H. Muxfeldt, G. Haas, G. Hardtmann, F. Kathawala, J. B. Mooberry and E. Vedejs, J. Am. Chem. Soc. 101, 689 (1979).
226. T. Shiba, T. Ando and T. Teshima, J. Antibiot. 32, 1078 (1979).
227. S. Nomoto and T. Shiba, Bull, Chem. Soc. Japan 52, 1709 (1979).

Chapter 13. Antiparasitic Agents

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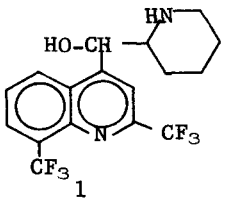
Protozoal Disease

General - In view of the increasing role of the W.H.O. in research on tropical diseases, an article on their new policies in research for new tools to control six major tropical diseases is of interest.[1] A fascinating summary has appeared on the philosophy of a pharmaceutical company towards involvement with tropical diseases.[2]

Malaria - A review on new experimental antimalarial drugs[3] and a volume on "New Trends in Malaria Chemotherapy" have appeared.[4] An overview of the literature indicates an encouraging trend towards the application of more basic science and thus a more rational approach to drug development in this area. An increase is apparent in papers dealing with the basic biochemistry of the parasite,[5-8] the mode of drug action[9-11] and in the application of contemporary quantitative structure activity relationships (QSAR) technology[12-14] towards the design of better agents. Drug delivery systems have also received attention,[15-17] although it is still not clear that major improvements could be expected in malaria chemotherapy in this fashion. The need is well defined as the scourge of chloroquine resistance spreads slowly into areas such as Africa which have as yet remained untouched.[18-22] Moreover, it has been concluded recently that malaria and beriberi were the unresolved military medical problems contributing to the fall of Cambodia.[23]

New therapeutic entities are sorely wanting and the recent literature has dealt mainly with further study of those agents revealed previously. Additional information is available on mefloquine (1), perhaps the most important new entity to appear in recent years. Studies on its clinical efficacy[24] pharmacokinetics,[25-28] as well as on a pyridine methanol analog have appeared.[29,30] The therapeutic superiority of mefloquine may be explained by the fact that it, like amodiaquine, is undiminished in accumulation by erythrocytes infected with chloroquine-resistant P. berghei.[31] The novel acridinedione, 2, reported last year has now acquired a name (floxacrine), and a detailed evaluation of its biological activity has appeared.[32] Use of this compound in combination with antimalarial agents such as chloroquine, quinine, pyrimethamine, etc., has been claimed to result in synergistically increased activity against the normal and chloroquine-resistant erythrocytic forms of P. berghei.[33]



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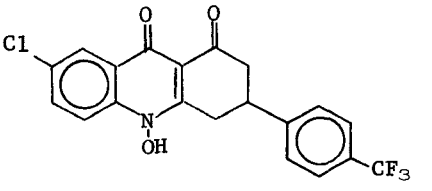

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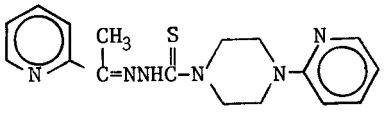
ISBN 0-12-040515-6

Complete primate data has been published on a number of the very potent 2,4-diaminoquinazoline antifol antimalarials.[34-36] The need for an improved tissue schizontocide continues to stimulate substantial effort. Synthetic efforts have been unrewarding thus far,[37-40] as have efforts to understand the toxicity of primaquine, the best currently available drug.[41-42] An effort to overcome drug toxicity by utilizing a lower dose via a slow release preparation was also unsuccessful.[43]

The enantiomers of chloroquine have been separated, and it was shown that the (+) isomer was some three times more active against P. vinckei than the (-) isomer. No correlation was observed between the antimalarial activity and the DNA binding of the enantiomers.[44] One novel structure reported recently to have substantial curative activity in the P. berghei mouse screen is the pyridine thiosemicarbazone 3.[45] An α-aminocresol derivative (4) has been shown to be more active[46] in the primate model than in the mouse model, with efficacy against both normal and resistant parasites very similar to that of mefloquine.



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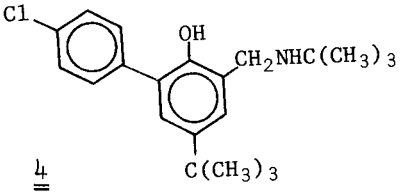

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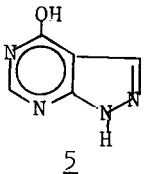
Leishmaniasis - Little has appeared which would suggest that major therapeutic advances are forthcoming for this disease entity, or even that new approaches are available worthy of further study. As Marsden puts it, the need is for a cheap, non-toxic, orally administered drug and no prospect is in sight.[47] He compares our current knowledge of the disease to that of malaria at the end of World War II. Once again, expanded interest in the biochemistry of the parasite is evident.[48-53] Hopefully, with time this may be translated into useful drug discovery results.

The antileishmanial effect of allopurinol (5) on the parasite



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in vitro has been demonstrated at concentrations comparable to those achieved in human plasma. Work has continued in an effort to understand the actions of this drug and the unique aspects of its metabolism in the parasite.[54-57] It is suspected that activation within the cells to an active form is required, but as yet it is not clear how a useful drug may result from these observations. To our knowledge, no in vivo test system can demonstrate antiparasitic activity for this compound.

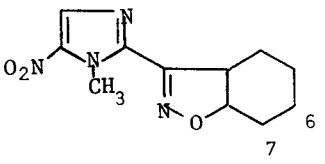
Nifurtimox was evaluated in 26 patients with mucocutaneous disease. Only limited response was obtained when the drug was given in a daily oral divided dose of 10 mg/kg for 30 days, and the drug was not recommended for routine use in the disease.[58]

Three other observations are worthy of follow-up, however. Oral administration of 150 mg/kg/day for 30 days of amphotericin B inhibited the

development of symptoms in mice and hamsters.[59] Rifampicin given at 600 mg daily for periods from 2 weeks to 2 months to 10 patients with cutaneous disease afforded disappearance of the lesions, but no parasitic evaluation was presented.[60] In 12 adult patients with Leishmania tropica, levamisole treatment at 150 mg/day for 2 days for 3-7 weeks was said to be effective.[61]

Trypanosomiasis - A review on the human (T. cruzi, T. gambiense, T. rhodesiense) and animal (T. brucei, T. vivax, T. congolense, T. evansi, T. equinum, T. equiperdum) trypanosomiases as world public health problems has appeared,[62] as well as one on the present status of chemotherapy and chemoprophylaxis of human trypanosomiasis in the Western hemisphere.[63]

Nitroheterocycles continue to be a fertile field for investigation. The nitroimidazole 6 was active against T. cruzi, and a study of its metabolites in the dog led to the 6,7-cis dihydroxy compound which had greater trypanocidal activity in vivo than 6.[64]



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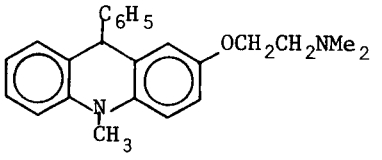

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An interesting non nitroheterocycle, acridine 7, is reported in a recent patent to be a trypanosomicide, but details are not available.[65] Two babesicides, amicarbalide (Diampion; 3,3'-diamidinocarbanilide diisethionate) and imidocarb (Imezol; 3,3'-bis(2-imidazolin-2-yl) carbanilide, dihydrochloride) were reported to cure T. brucei infections in mice when given ip at 10 mg/kg for 3 days starting 24 hours post infection.[66]

Rats infected with T. brucei were cured with just sublethal doses of salicylhydroxamic acid plus glycerol. It was thought that this was a result of the inhibition of L-glycerol-3-phosphate oxidase, which was also thought to be one of the principal sites of action of suramin. However, treatment with glycerol did not affect the mobility of the trypanosomes nor the survival of infected rats after treatment with suramin.[67]

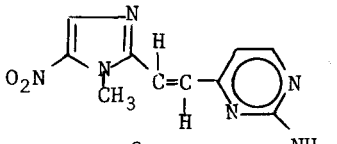
Antitrypanosomal activity was reported for several benzyltriphenylphosphonium salts against T. rhodesiense infections in mice.[68]

Both African trypanosomes and some tumor cells undergo a high rate of aerobic glycolysis as a result of inefficient or nonfunctional mitochondrial systems and in each the key "pacemaking" glycolytic enzymes are hexokinase, phosphofructokinase and pyruvic kinase. Using this as a rationale, 49 compounds known to have antitumor properties were screened against T. rhodesiense infections in mice. Six were found active with the most interesting being 5-(3,3-dimethyl-1,1-triazeno)imidazole-4-carboxamide.[69]

Studies to reexamine the synthesis of purines and pyrimidines in T. cruzi indicate that no form of the parasite can synthesize substantial quantities of purine de novo and that therefore dependence is on salvage. Moreover, it seems that amastigotes and trypomastigotes will depend on de novo synthesis for their pyrimidines and thus, drugs that can block this are likely to be trypanocidal.[70]

Benznidazole (N-benzyl-2-nitro-1-imidazoleacetamide) has been reported to be a very efficient agent against Chagas disease, and its pharmacokinetics have been reviewed.[71]

Trichomoniasis - Discussions as to the potential toxicity of metronidazole continue[72,73] as the drug continues to be used as very likely the best available agent for this infection. Two separate publications have indicated that there is no evidence for cancer due to its use,[74] and that it is safe for short-term treatment.[75] Moreover, it was reported that the mutagenic effects of the drug can be dissociated from its antiparasitic properties in experimental models by pretreatment of 2(3)-t-butyl-4-hydroxyanisole or administration of erythromycin.[76] An interesting anaerobic metabolite of metronidazole, N-(2-hydroxyethyl)oxamic acid has been reported.[77] Other than nitroheterocycles, little of interest against trichomonal infections has appeared, and as a matter of fact, not much of anything appears ready to compete with metronidazole as the drug of choice. Toxicological and teratological studies appeared on azanidazole (8), a new systemic trichomonacide.[78]



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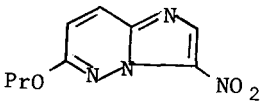

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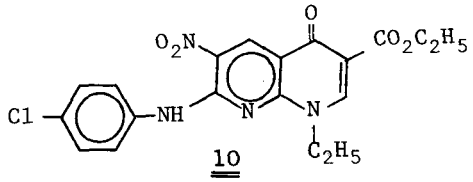
Another nitroheterocyle, of more unusual structure, is the imidazo[1,2-b]pyridazine 9, which is apparently being readied for clinical trial.[79] Nitro-1,8-naphthyridines such as 10 were reported to have nearly the same activity as metronidazole against T. vaginalis infections.[80]



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In vitro studies among a wide range of structural types revealed activity with saponins, such as leontoside,[81] and 3-hydroxymethylene-pyrazoltetradecanoic acid picrate.[82] Tricandil (mepartricin), a polyene antibiotic has been introduced into Brazil by Searle for the treatment of trichomoniasis and candidiasis.[83]

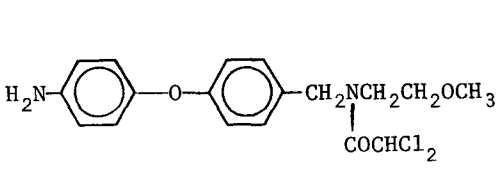
Amoebiasis - The literature on this disease area remains sparse. A belated summary on all aspects of amoebiasis research up to 1977 reported at the Seventh Seminar on Amoebiasis at Mexico City in November of 1977 has been published.[84]

A progress report on all intestinal protozoa has also appeared.[85] The need for a cheap, non-toxic agent in rural environments has led to a recommendation for a diiodohydroxyquinoline-oxytetracycline combination[86] - the tetracycline acting presumably on the gut flora and thus indirectly on the infectious organism.[87]

The classic drug in this area, emetine, has been shown to be amoebicidal by inhibiting protein synthesis.[88] It has been the subject of two stereoselective total syntheses.[89,90] Application of this technology may allow the syntheses of less complex structural analogs.

The imidazo[1,2-b]pyridazine mentioned previously as a trichomonacide is also reported to be a potent agent against cecal E. histolytica in rats and hepatic E. histolytica in hamsters.

A recent patent claims still another dichloroacetamide (11) as a potent amoebacide in vitro.[91] The amidines represented by 12 continue under investigation and 12 is reported to have no teratogenic effect, to be as potent as metronidazole against cecal amoebiasis (albeit with a lower safety margin) and to be more potent than metronidazole against the hepatic infection.[92]



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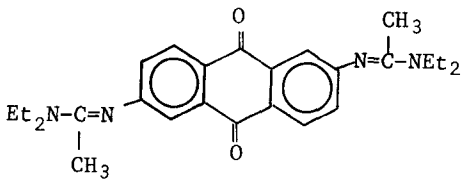

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Giardiasis - A thorough review of the infection of the human gastrointestinal tract by Giardia lamblia, known both as giardiasis or lambliasis, has appeared.[93] The use of nitroimidazoles as therapy is stressed. In a comparison of metronidazole and quinacrine in 160 cases of infants and children, the low failure rate, minimal side effects and more tolerable flavor favored metronidazole given at 15-25 mg/kg/day for 5 days.[94] A newer nitroimidazole, ornidazole (Tiberal) was studied in children and found to be effective in a single dose. It was preferred to metronidazole.[95] Another nitroimidazole, tinidazole, was also used successfully in children in a single dose treatment.[96]

Toxoplasmosis - Several reviews have appeared dealing with the disease and its treatment.[97-100] Therapeutic emphasis continues to center around combinations of pyrimethamine and a long-acting sulfonamide.

Coccidiosis - Commercial potential for the control of this resistance-prone parasite continues to spark research interest. For example, of the approximately 130 million cattle in the United States, about 77 million are susceptible to coccidiosis each year. Of these, some 3,850,000 are treated and about 180,000 die.[101]

Ionophorous antibiotics recently have been found effective in poultry and mammals. Lasalocid added to feed in a dose to produce the equivalent of about 3.0 mg/kg has been found effective in controlling clinical coccidiosis in calves.[101] A review of a variety of drug types has appeared.[102] Studies are ongoing with arprinocid [9-(2-chloro-6-fluorobenzyl)adenine]. Mode of action studies have led to the tentative conclusion that inhibition of hypoxanthine transport may play a critical role in its action on the parasite.[103,104] A recent study has concluded that its anticoccidial activity is due to a metabolite in the chick.[105] Modification of the 6-azauracil structure resulted in 1-(3',5'-dichlorophenyl)-6-azauracil. Further modifications have apparently resulted in the separation of potency from the undesirable persistence of these compounds and also prevent emergence of resistant strains.[106]

N,N'-bis(3,4-ditrifluoromethylphenyl)methylmalonamide has been shown to be effective vs. amprolium, zoalene, aklomide or nicarbazine resistant strains of E. tenella.[107]

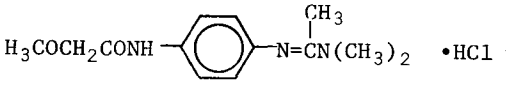
Helminth Diseases

General - Recent articles and reviews consider a wide range of subjects including the problem of resistance of animal helminths to current chemotherapy,[108] parasitic zoonoses[109] and the complex interrelationships of the variety of disciplines contributing to human health.[110] Two provocative articles outlining broad biochemical strategies toward parasite chemotherapy appeared recently.[111,112]

Filaria - Basic biochemical studies with this parasitic group seem to be increasing, with work being reported on comparative metabolism of different genera of adult worms,[113] and effects of larval forms in infected mosquitoes.[114-116] Methods of cryopreservation and *in vitro* cultivation may allow improved studies with the human parasite, *Onchocerca volvulus*.[117] Clinical trials with a variety of antiparasitic drugs, such as metronidazole,[118] nifurtimox,[119] mebendazole,[120,122] amodiaquine,[121] and levamisole[121,122] gave marginally promising results at best with the possible exception of mebendazole against *Dipetalonema perstans*.[122] An interesting approach uses electron microscopy of skin snips to evaluate the effect of metrifonate on onchocerciasis patients.[123] Recent reviews of both the clinical[124] and laboratory[125] work on filariasis chemotherapy are available.

Other Nematodes - An exciting new group of compounds, known collectively as avermectins was isolated from fermentation of *Streptomyces avermitilis*.[126] This group consists of at least 8 disaccharides of 16-membered pentacyclic lactones. The compound designated avermectin B1a has demonstrated a broad spectrum of activity at single oral doses of 0.1 mg/kg or less,[127] including a 95% reduction of *Haemonchus contortis*, *Ostertagia circumcincta* and *Cooperia oncophora* in sheep, *H. placei*, *O. ostertagi* and *C. punctata* in cattle at 0.1 mg/kg, and *Ancylostoma caninum* in dogs. It was also effective against *Capillaria obsignata*, but not *Heterakis gallinarum*, in poultry.[128] Activity was also seen against the pre-cardiac stage of *Dirofilaria immitis* in ferrets.[129]

Amidantel, a new phenylenediamine derivative (13),[130] has shown an interesting anthelmintic spectrum including, *Hymenolepis diminuta* in rats,[130] and *Ancylostoma caninum* in dogs.[131] It was also effective against both the microfilaria and adult *Dipetalonema witei*, but only the microfilaria of *Litomosoides carinii* in *Mastomys natalensis*.[130] Still another new phenylenediamine structure, febantel (14), has been shown



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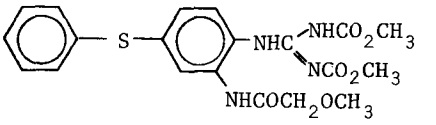

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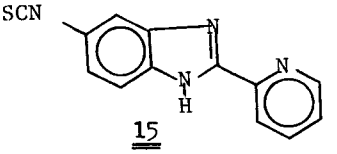
highly effective against various nematodes and cestodes in rodents, dogs, sheep and cattle.[132] A new benzimidazole derivative, (15), given to



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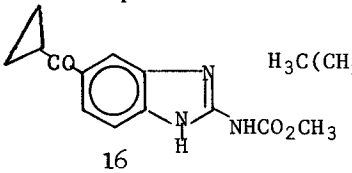
sheep naturally infected with intestinal nematodes at 50 and 200 mg/kg gave complete reduction in fecal egg count, despite a lack of activity against Nematospiroides dubius in mice.[133] Results from a double-blind clinical study comparing ciclobendazole (16) and mebendazole, indicate that the two drugs are equally effective for treating ascaris and hookworm infections and that mebendazole is significantly better against trichuriasis. Both drugs were well tolerated.[134] A variety of other structures including 17 [135] and 18 [136] have appeared in the recent patent literature claiming nematocidal activity.



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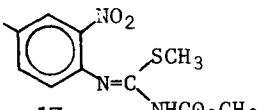

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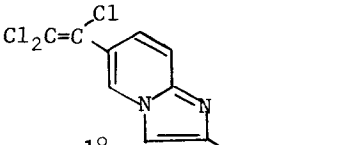

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Schistosomiasis - The first clinical reports using praziquantel appear promising. The pharmacokinetic behavior was dominated by rapid metabolism.[137] No clinically relevant changes were found for any of the laboratory parameters measured in healthy volunteers given total doses as high as 75 mg/kg. In initial multicenter clinical trials,[138-142] the drug was found effective against Schistosoma haematobium, S. mansoni, and S. japonicum infections in Africa, South America, and Asia at total doses of 60 mg/kg or less. Further studies on the carcinogenic potential of hycanthone in rodents were conducted.[143,144] The absence of mutagenic activity for praziquantel was confirmed in several systems, including mammalian cells.[145] An interesting mechanized in vitro screening approach was developed using several known schistosomicides.[146]

References

1. E. W. J. deMaar, Trans. Roy. Soc. Trop. Med. Hyg., 73, 147 (1979).
2. J. R. Vane, Trans. Roy. Soc. Trop. Med. Hyg., 73, 140 (1979).
3. R. S. Rozman and C. J. Canfield, in Adv. Pharmacol. Chemother., 16, 1 (1979), Eds. S. Garattini, A. Goldin, F. Hawking and I. J. Kopin, Academic Press.
4. Chemotherapy, 10 in Advances in Pharmacol. Therapeutics, Ed. M. Adolphe, Pergamon Press, 1978 (Proceedings of the 7th Int. Cong. Pharmacol., Paris, 1978).
5. C. A. Homewood in "Rodent Malaria", Eds. R. Killick Kendrick and W. Peters, Academic Press, 1978, pp. 170-200.
6. W. E. Gutteridge, D. Dave, and W. H. Richards, Biochim. Biophys. Acta, 582 390 (1979).
7. P. G. Shakespeare, P. I. Trigg, S. I. Kyd and L. Tappenden, Ann. Trop. Med. Parasitol. 73, 407 (1979).
8. A. Gero and W. J. O'Sullivan, Clin. Exp. Pharmacol. Physiol., 6, 454 (1979).
9. R. L. Jones, M. W. Davidson and W. D. Wilson, Biochim. Biophys. Acta, 561, 77 (1979).
10. J. Bolte, C. Demuynck, J. Lhomme, M. C. Fournie-Zaluski, and B. P. Roques, Biochem., 1979, 4928.
11. W. A. Ritschel, G. V. Hammer, and G. A. Thompson, Inter. J. Clin. Pharmacol. Biopharm., 16, 395 (1978).
12. E. A. Coats, C. S. Genther and C. S. Smith, Eur. J. Med. Chem. 14, 261 (1979).

12a. K. H. Kim, C. Hansch, J. Y. Fukunaga, E. E. Steller, P. Y. C. Jow, P. N. Craig and J. Page, J. Med. Chem., 22, 366 (1979).

13. C. C. Smith, C. S. Genther and E. A. Coats, Eur. J. Med. Chem., 14, 271 (1979).
14. S. W. Dietrich, R. Nelson Smith, J. Y. Fukunaga, M. Olney and C. Hansch, Arch. Biochem. Biophys., 194, 600 (1979).

15. P. Puson, R. F. Steiger, A. Trouet, J. Gillet and F. Herman, Trans. Roy. Soc. Trop. Med. Hyg., 73, 347 (1979).
16. D. L. Wise, J. D. Grisser and G. J. McCormick, J. Pharm. Pharmacol., 31, 201 (1979).
17. R. S. Chawla, I. W. Kellaway, C. Marriott and J. Stevens, J. Pharm. Pharmacol., 30 Suppl, 37P (1978).
18. P. N-Dinh and W. Trager, Science, 200, 1397 (1978).
19. B. H. Kean, J. Am. Med. Assoc., 241, 395 (1979).
20. M. Simpson and P. Williams, Med. J. Aust., 1978, 41.
21. S. Fogh, S. Jepsen and P. Effersoe, Trans. Roy. Soc. Trop. Med. Hyg., 73, 228 (1979).
22. R. A. Eke, Am. J. Trop. Med. Hyg., 28, 1074 (1979).
23. W. D. Everett, Milit. Med., 144, 158 (1979).
24. E. B. Doberstyn, P. Phintagothin, S. Noeypatimanondh and C. Teerakiart-kamjorn, Bull. W.H.O., 57, 275 (1979).
25. R. E. Desjardins, C. L. Pamplin, J. Von Bredow, K. G. Barry and C. J. Canfield, Clin. Pharmacol. Therap., 26, 372 (1979).
26. H. Chung, V. Jimmerson, D. Bounds, R. Keller and R. Rozman, Toxicol. Appl. Pharmacol., 48, No. 1, Pt. 2, A10 (1979).
27. R. S. Rozman, N. A. Molek and R. Koby, Drug Metab. Dispos., 6, 654 (1978).
28. D. W. Mendenhall, T. Higuchi and L. A. Sternson, J. Pharm. Sci., 68, 746 (1979).
29. J. R. Hodgson, J. L. Minor, C. C. Lee and H. Chung, Toxicol. Appl. Pharmacol., 48 (1) Pt. 2, A8 (1979).
30. O. J. Bouwsma, J. T. Stewart, and J. J. Vallner, J. Pharm. Sci., 68, 45 (1979).
31. C. D. Fitch, R. L. Chan and R. Chevli, Antimicrob. Agents Chemother., 15 258 (1979).
32. L. H. Schmidt, Antimicrob. Agents Chemother., 16, 475 (1979)., W. Raether and E. Fink, Ann. Trop. Med. Parasitol., 73, 505 (1979).
33. German Patent DT2748333, Hoechst Ag, May 3, 1979.
34. L. H. Schmidt and R. N. Rossan, Am. J. Trop. Med. Hyg. 28, 781 (1979).
35. L. H. Schmidt, ibid., 793.
36. L. H. Schmidt, ibid., 808.
37. M. S. Khan, M. P. LaMontagne, J. Med. Chem., 22, 1005 (1979).
38. J. P. Scovill, D. L. Klayman, T. S. Woods, and T. R. Sweeney, J. Med. Chem., 22, 1164 (1979).
39. F. I. Carroll, B. D. Berrang and C. P. Linn, J. Med. Chem., 22, 1363 (1979).
40. F. I. Carroll, C. P. Linn, and C. E. Twine, Jr., J. Med. Chem., 22, 694 (1979).
41. E. R. Seidel and R. L. Mundy, Pharmacologist, 21, 236 (1979).
42. J. Greaves, D. A. Price Evans, H. M. Gilles, K. A. Fletcher, D. Bunnag and T. Harinasuta, Trans. Roy. Soc. Trop. Med. Hyg., 73, 328 (1979).
43. B. M. Judge and R. E. Howells, Trans. Roy. Soc. Trop. Med. Hyg., 73, 327 (1979).
44. E. Fink, G. Minet and P. Nickel, Arzneim. Forsch., 29, 163 (1979).
45. D. L. Klayman, J. P. Scovill, J. F. Bartosevich and C. J. Mason, J. Med. Chem., 22, 1367 (1979).
46. L. H. Schmidt and R. Crosby, Antimicrob. Agents Chemother., 14, 672 (1978).
47. P. D. Marsden, New Eng. J. Med., 300, 350 (1979).
48. J. J. Marr, R. L. Berens, and D. J. Nelson, Biochim. Biophys. Acta 544, 360 (1978).
49. E. Martin and A. J. Mukkada, J. Protozool., 26, 138 (1979).
50. G. W. Koszalka and T. A. Krenitsky, J. Biol. Chem., 254, 8185 (1979).
51. S. S. Law and A. J. Mukkada, J. Protozool., 26, 295 (1979).
52. U. Bachrach, S. Brem, S. B. Wertman, L. F. Schnur and C. L. Greenblatt, Exp. Parasitol., 48, 464 (1979).
53. R. P. Brazil and J. D. McCarthy, Trans. Roy. Soc. Trop. Med. Hyg., 73, 323 (1979).
54. E. Konigk, Tropenmed. Parasit., 29, 435 (1978).
55. D. J. Nelson, C. J. Bugge, G. B. Elion, R. L. Berens and J. J. Marr, J. Biol. Chem., 254, 3959 (1979).
56. T. Spector, T. E. Jones and G. B. Elion, J. Biol. Chem., 254, 8422 (1979).
57. J. J. Marr, R. L. Berens and D. J. Nelson, Clin. Res., 27, 350A (1979).
58. P. D. Marsden, C. C. Cuba, A. C. Barreto, R. N. Sampaio and R. A. H. Rocha, Trans. Roy. Soc. Trop. Med. Hyg., 73, 391 (1979).
59. L. K. Lyubimova, L. I. Fatelva and L. A. Sergeeva, Antibiotiki (Moscow) 24, 281 (1979). Chem. Abstr. 91, 32862 (1979).
60. I. O. Iskandar, J. Int. Med. Res., 6, 280 (1978).
61. P. G. Butler, J. Trop. Med. Hyg., 81, 221 (1978).
62. W. E. Ormerod, Pharmacol. Therapeutics, 6, 1 (1979).
63. Z. Brener, Pharmacol. Therapeutics, 7, 71 (1979).
64. W. J. A. Vandenheuvel, B. H. Arison, T. W. Miller, P. Kulsa, P. Eskola, H. Mrozik, A. K. Miller, H. Skeggs, S. B. Zimmerman and B. M. Miller, J. Pharm. Sci., 68, 1156 (1979).
65. U.S. Patent 4,150,134 to Sterling Drug, April 17, 1979.
66. H. C. Nathan, K. V. M. Soto, R. Moriera, D. Resigno, D. Stumpf and C. J. Bacchi, J. Protozool., 26, (3,I) Abstr. #86 (1979).
67. C. Van DerMeer, J. A. M. Verslujs-Broers and F. R. Opperdoes, Exp. Parasit., 48, 126 (1979).
68. K. E. Kinnamon, E. A. Steck and D. S. Rane, J. Med. Chem., 22, 452 (1979).

69. K. E. Kinnamon, E. A. Steck and D. S. Rane, Antimicrob. Agents Chemother., 15, 157 (1979).
70. W. E. Gutteridge and M. Gaborak, Int. J. Biochem., 10, 415 (1979).
71. J. Riaflaub, W. H. Zeiger, Arzneimit. Forsch., 29, 1611 (1979).
72. B. Hartley-Asp, Lancet 1, (8110) 275 (1979).
73. C. M. Voogd, J. J. van der Stel and J. J. J. A. A. Jacobs, Mut. Res., 66, 207 (1979).
74. C. M. Beard, K. L. Noller, W. M. O'Fallon, L. T. Kurland, and M. B. Dockerty, N. Engl. J. Med., 301, 519 (1979).
75. A. B. Hartley, Lancet, 8110, 275 (1979).
76. D. A. Bruckner, E. Bueding and M. Voge, J. Parasitol., 65, 474 (1979).
77. R. L. Koch and P. Goldman, J. Pharmacol. Exp. Ther., 208, 406 (1979).
78. R. Tammiso, G. Olivari, C. Coccoli, G. Garzia and G. Vittadini, Arzneim-Forsch, 28, (II), 2251 (1978).
79. P. F. Fabio, A. E. Lanzilotti and S. A. Lang, Jr., J. Labelled Cpds. Radiopharmaceut., 15, 407 (1978).
80. N. Suzuki, M. Kato and R. Dohmori, Yakugaku Zasshi (J. Pharm. Sci. Jap.) 99, 155 (1979).
81. Kh. Abdullaev, Farmakol. Prir. Veschistv, 1978, 103 (C.A. 91, 49295 (1979)).
82. Kh. Abdullaev, ibid., 139, C. A. 91, 151104 (1979).
83. F. D. C. Reports "The Pink Sheet", May 21, 1979; U.S. Patent 3,780,173 to S. P. A., December 18, 1973.
84. Arch. Invest. Med., 9, Suppl. 1, 85 (1978).
85. R. Knight and S. G. Wright, Gut., 19, 940 (1978).
86. D. K. Masters and A. D. Hopkins, J. Trop. Med. Hyg., 82, 99 (1979).
87. M. A. Roemer, Internist, 19, 680 (1978).
88. N. Entner, J. Protozool., 26, 324 (1979).
89. T. Kametani, Y. Suzuki, M. Terasawa and M. Imara, J. Chem. Soc. Perkin. Trans. I., 1979, 1211.
90. T. Fujii, and S. Yoshifuji, Chem. Pharm. Bull., 27, 1486 (1979).
91. German Patent No. 2,842,752, to Carlo Erba, April 5, 1979.
92. E. J. Burden, S. G. Carvajal, P. F. Fabio, T. L. Fields, Yans-I Lin, K. C. Murdock, S. A. Lans, Jr., Experientia 35, 33 (1979).
93. H. J. Spech, Deut. Med. Wochenschr, 103, 2008 (1978).
94. S. Kavousi, Am. J. Trop. Med. Hyg., 28, 19 (1979).
95. N. Iyngkaran, E. L. Lee, and M. L. Robinson, Scand. J. Infect., Dis., 10, 243 (1978).
96. G. C. Levi, V. Amato Neto, H. N. V. Stefani, N. A. Romero and N. L. Neto, Rev. Inst. Med. Trop. Sao Paulo, 21, 26 (1979).
97. V. Maternova and E. A. Shevkunova, Klinich Med., 57, 21 (1979).
98. I. T. El Dasouqi, J. Egypt Soc. Parasitol., 9, 89 (1979).
99. R. J. Scott, Trop. Dis. Bull., 75, 809 (1978).
100. J. van der Veen, Ned. Tijdschr. Geneesk., 123, 564 (1979).
101. P. R. Fitzgerald and M. E. Mansfield, J. Parasitol., 65, 824 (1979).
102. K. Imai and T. Matsuno, Farumashia, 14, 676 (1978) [C. A., 90, 80441 (1979)].
103. C. C. Wang, P. M. Simashkevich and R. L. Stotish, Biochem. Pharmacol., 28, 2241 (1979).
104. C. C. Wang, R. L. Tolman, P. M. Simashkevich and R. L. Stotish, Biochem. Pharmacol., 28, 2249 (1979).
105. V. S. Latter and R. G. Wilson, Parasitol., 79, 169 (1979).
106. M. W. Miller, B. L. Mylari, H. L. Howes, Jr., J. E. Lynch, M. J. Lynch and R. C. Koch, J. Med. Chem., 22, 1483 (1979).
107. F. Panitz, Parasitol., 78, 33 (1979).
108. J. D. Kelly and C. A. Hall, Adv. Pharmacol. Chemother., 16, 89 (1979).
109. Tech. Rept. Ser. 637, World Health Organization, Geneva, 1979.
110. W. S. Bailey, Am. J. Trop. Med. Hyg., 27, 441 (1978).
111. T. E. Mansour, Science, 205, 462 (1979).
112. S. S. Cohen, Science, 205, 964 (1979).
113. K. R. Middleton and H. J. Saz, J. Parasitol., 65, 1 (1979).
114. J. J. Jaffe and L. R. Chrin, J. Parasitol., 65, 226 (1979) ibid., 550 (1979).
115. M. G. Simpson and B. R. Laurence, J. Parasitol., 65, 732 (1979).
116. J. J. Jaffe and L. R. Chrin, Biochem. Pharmacol., 28, 1831 (1979).
117. E. L. Schiller, V. M. Turner, H. F. Marroquin and R. D'Antonio, Am. J. Trop. Med. Hyg., 28, 997 (1979).
118. V. P. Sharma, H. S. Rathore, and M. M. Sharma, Am. J. Trop. Med. Hyg., 28, 658 (1979).
119. H. Fuglsang and J. Anderson, Tropenmed. Parasit., 29, 355 (1978).
120. J. M. Golsmid and S. Rogers, Central Afr. J. Med., 25, 3 (1979).
121. J. E. McMahon, Ann. Trop. Med. Parasit., 73, 465 (1979).
122. M. U. V. L. Narashinham, S. P. Roychowdhury, M. Das and C. K. Rao, S. E. Asian, J. Trop. Med. Pub. Health, 9, 571 (1978).
123. G. D. Burchard, E. J. Albiez and M. Bierther, Tropenmed. Parasit., 30, 97 (1979).
124. F. Hawking, Advan. Pharmacol. Chemother., 16, 129 (1979).
125. D. A. Denham, J. Helminth., 53, 175 (1979).

126. T. W. Miller, L. Chaiet, D. J. Cole, L. J. Cole, J. E. Flor, R. T. Goegelman, V. P. Gullo, H. Joshua, A. J. Kempf, W. R. Krellwitz, R. L. Monaghan, R. E. Ormond, K. E. Wilson, G. Albers-Schonberg and I. Putter, Antimicrobial Agents and Chemother., 15, 638 (1979).
127. R. W. Burg, B. M. Miller, E. E. Baker, J. Birnbaum, S. A. Currie, R. Hartman, Y. Kong, R. L. Monaghan, G. Olsen, I. Putter, J. B. Tunac, H. Wallick, E. O. Stapley, R. Oiwa, and S. Omura, Antimicrobial Agents and Chemother., 15, 361 (1979).
128. J. R. Egerton, D. A. Ostlind, L. S. Blair, C. H. Eary, D. Suhayda, S. Cifelli, R. F. Riek and W. C. Campbell, Antimicrobial Agents and Chemother., 15, 372 (1979).
129. L. S. Blair and W. C. Campbell, J. Parasitol., 64, 1032 (1978).
130. H. Wollweber, E. Niemers, W. Flucke, P. Andrews. H. Schultz and H. Thomas, Arzneim. Forsch., 29, 31 (1979).
131. H. Thomas, Tropenmed. Parasit., 30, 404 (1979).
132. H. Wollweber, H. Kolling, A. Widdig, H. Thomas, H. Schultz and P. Murmann, Arzneim. Forsch., 28, 2193 (1978).
133. R. D. Haugwitz, B. V. Maurer, G. A. Jacobs, V. L. Narayanan, L. Cruthers and J. Szanto, J. Med. Chem., 22, 1113 (1979).
134. R. Guggenmoos, K. M. Akhtaruzzaman, F. Rosenkaimer, W. Gaus, U. Bienzle and M. Dietrich, Tropenmed. Parasit., 29, 423 (1978).
135. U.S. Patent 4021570 to Schering Corp., May 3, 1977; D. Loebenburg, M. M. Nafissi-Vorchei, B. Antonacci and J. A. Waitz, J. Parasitol., 65, 823 (1979).
136. U.S. Patent 4154835 to Merck & Co., May 15, 1979.
137. G. Leopold, W. Ungethum, E. Groll, H. W. Diekmann, H. Nowak and D. H. G. Wegner, Eur. J. Clin. Pharm., 14, 281 (1978).
138. A. Davis and D. H. G. Wegner, Bull. W. H. O., 57, 767 (1979); A. Davis, J. E. Biles and A. M. Ulrich, ibid., 773.
139. N. Katz, R. S. Rocha and A. Chavas, ibid., 781.
140. T. Ishizaki, E. Kamo and K. Boehme, ibid., 787.
141. A. T. Santos, B. L. Blas, J. S. Nosenas, G. P. Portillo, O. M. Ortega, M. Hayashi and K. Boehme, ibid., 793.
142. J. E. McMahon and N. Kolstrap, Brit. Med. J., 2, 1396 (1979).
143. O. Bulay, H. Urman, K. Patil, D. B. Clayson and P. Shubik, Int. J. Cancer, 23, 97 (1979).
144. H. Tsuda, D. S. R. Sarma, S. Rajalakshmi, J. Zubroff, E. Farber, R. P. Batzinger, Y. Cha and E. Bueding, Cancer Res., 39, 4491 (1979).
145. H. Bartsch, T. Kuroki, C. Malaveille, N. Loprieno, R. Barale, A. Abbondandolo, S. Bonatti, G. Rainaldi, E. Vogel and A. Davis, Mutation Res., 58, 133 (1978).
146. M. C. Brown, D. F. Norman, D. R. Bell and C. J. Chavasse, Med. Biol. Eng. Comput., 16, 408 (1978).

Chapter 14. Antineoplastic Agents

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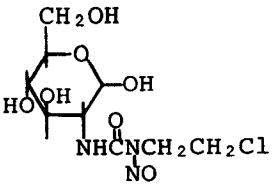
Introduction - A literature survey on the topic of antineoplastic agents resulted in the citation of over 2100 articles or reviews published in 1979. Of primary interest to medicinal chemists are agents under active clinical investigation and development in the United States and in other countries, and the National Cancer Institute maintains a listing[1] of these investigational drugs in various stages of development. Structures of these agents, which serve as inspiration for the design of related agents of improved value, are available for distribution.[2] Reviews or proceedings of symposia have been published on VM-26[3], VP-16-213[4], cis-Pt[5,6], mitomycin C[3], dibromodulcitol[7], methotrexate[8], pepleomycin[9], and interferon.[10] Of particular interest is a review on new developments on the mechanism of action of anticancer drugs.[11]

Alkylating Agents - Comparison of the mutagenicity and antitumor effect of nitrosoureas and nitrosoamides suggested a possible dissociation of these biological effects.[12] L-Chlorozotocin (1) was not significantly different in toxicity to mice or antitumor activity against L1210 leukemia (LL) from the usual D-enantiomorph.[13] The reduced bone marrow toxicity of chlorozotocin to mice in comparison to CCNU was found to correlate with an advantageous ratio of DNA alkylation in LL cells vs. bone marrow.[14] Of six mono- and dissaccharide analogs of chlorozotocin and GANU (2), all but a mannopyranosyl derivative were curative against LL.[15] 2-Chloroethylureidocyclopentanetetrols and -cyclohexanetetrols also cured LL.[16] Methyl 6-[[[2-chloroethyl)nitrosoamino]-carbonyl]amino]-6-deoxy-α-D-glucopyranoside (MCNU) exhibited a marked effect against mouse tumors by IP, IV, or oral administration; bone marrow toxicity was less than that of CCNU but more than that of chlorozotocin.[17] ACNU (3) was an active agent against experimental brain tumors,[18] and four steroidal nitrosoureas were active against a rat mammary tumor.[19]



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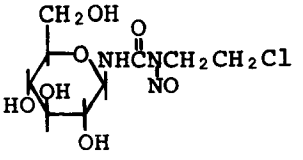

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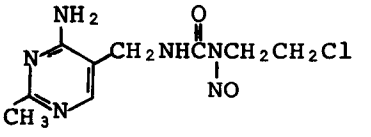

(3)

Studies on the metabolism of (+)- and (-)-cyclophosphamide indicated that the enantiomers and the racemate will not differ appreciably in their therapeutic effect, although stereoselective uptake of the 4 stereoisomers of the intermediate 4-hydroxycyclophosphamide (4) could result in different intracellular levels of the ultimate cytotoxic metabolite phosphoramide mustard.[20,21] Alternatively, the 4 stereoisomers could differ in their spontaneous, extracellular production of phosphoramide mustard. A

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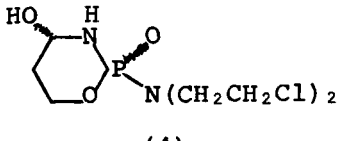
diastereomer of 5-bromocyclophosphamide was weakly active in vivo in comparison with cyclophosphamide.[22] 2',3'-Bis(2-chloroethyl)aminophosphoryl-3'-amino-3'-deoxyadenosine (5) was active in vitro,[23] presumably as a result of partial conversion to the phosphoramide mustard analog by hydrolysis at the 2'-position; evaluation in vivo would be of interest as an indication of either phosphoramide mustard, pro-drug activity or mixed-function oxidase hydroxylation of C-3 to generate an analog of 4-hydroxy-cyclophosphamide. Evaluation of 31 aziridinylbenzoquinones against a series of murine tumors resulted in the demonstration of superior activity for 2,5-bis(1-aziridinyl)-3,6-dioxo-1,4-cyclohexadiene-1,4-dicarbamate (6).[24] Structure activity studies on aziridinylbenzoquinones showed that greater hydrophilicity correlated with better activity against LL.[25] Antitumor data on a new Russian analog of TEPA, diiodobenzo-TEPA, were reported.[26] Procarbazine was shown to be a hypoxic cell sensitizer, suggeseing possible development of a new class of sensitizers.[27] Dianhydrogalactitol was the most active of 177 agents tested against a mouse ependymoblastoma and, in combination with radiation, resulted in longer median survival in patients with supratentorial astrocytomas than those treated with radiation alone.[28] Some N-glycosylhalomethyl-triazoles and -pyrazoles were active against murine tumors.[29,30]



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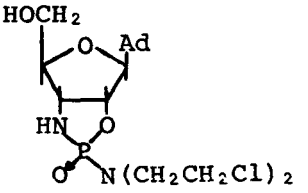

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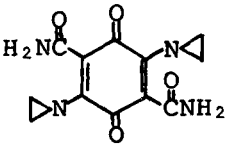
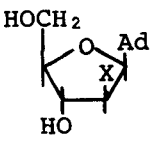


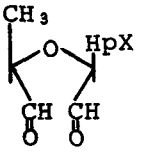
(6)

Purine, Pyrimidine and Folate Antagonists - 9-(3,5-Dideoxy-β-D-glycero-pent-4-enofuranosyl)adenine inhibited LL in vitro but other ribose-modified analogs did not.[31] 2'-Azido-2'-deoxy-araA (arazide) (7) was equivalent to araA for inhibition of LL cells in vitro, whereas 2'-amino-2'-deoxy-araA (aramine) (7) was less active. Arazide has an advantage over araA in being 25-fold more water soluble.[32] 6-Dimethyladenosine was cytotoxic to trophoblastic cells while non-trophoblastic cells were resistant.[33] 3-Deazaadenosine caused a reduction in biochemical transmethylations in vivo, indicating that this enzyme is a prime target for regulation of transmethylation.[34] The dialdehyde derivative (8) of 5'-deoxyinosine was a more potent antitumor agent than the corresponding derivative of inosine.[35] Cytotoxicity of 2'-O-acyl derivatives of 6-thioinosine cyclic 3',5'-phosphate to mouse lymphoma cells deficient in hypoxanthine-guanine phosphoribosyltransferase increased with lipophilicity.[36] 3'-Branched homologs of 2'-deoxythioguanosine were inhibitory to human lymphoblastoid cells and were more effective than the parent.[37] The in vivo antitumor activity of the 3-carboxamido derivative of 4-(amino-1-β-D-ribofuranosyl)pyrazolo[3,4-d]pyrimidine was greater than that of the parent.[38] 1-,2- (9), and 7-Methyl-formycins were cytotoxic to LL cells in vitro and were capable of forming nucleotides if deamination was prevented by their structure or by a deaminase inhibitor.[39]



Arazide: X = N_3
Aramine: X = NH_2

(7)



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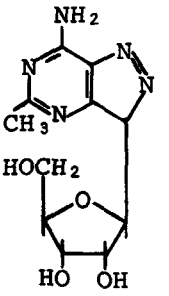
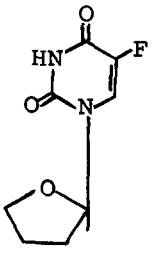


(9)

Two human urinary metabolites of Ftorafur (FT-207) (10), trans-3'-hydroxy- and cis-4'-hydroxy-ftorafur, showed no significant activity against L1210 leukemia in vivo.[40,41] 3-Oxa-FU but not 3-oxathymine was inhibitory to S. faecium and E. coli at low concentrations and to LL cells at higher concentrations.[42] Modification of the N^4-position of ara-C with long-chain saturated fatty acids resulted in an improvement in chemotherapeutic effect.[43] 1-β-D-Arabinofuranosyl-2-amino-1,4(2H)-4-iminopyrimidine and its 3'-phosphate were effective, deaminase-resistant depot forms of ara-C; both agents produced the corresponding 2,2'-anhydro derivative upon hydrolysis.[44,45] 3',5'-Di-O-acyl and 3'-O-acyl-5'-phosphates of anhydro-ara-C showed high activity against LL.[46] 5'-(Cortisol- and cortisone-21-phosphoryl)-ara-C were superior to ara-C against LL.[47] The carbocyclic analog of ara-C was also active against LL.[48] 5-Aza-ara-C (11) was somewhat superior to both ara-C and 5-azacytidine against LL and was also less toxic.[49] Ara-thymine and corresponding 5-bromo and 5-iodo analogs inhibited the growth of sarcoma 180 and LL in culture.[50] Hydroxynitrodihydrothymine inhibited growth of tumor cells,[51] and psi-isocytidine, an antitumor C-nucleoside, was incorporated into RNA and DNA.[52] 5-Formimidoyl-barbituric acid was highly active against several mouse tumors.[53] A methotrexate analog with an additional nitrogen atom between the phenyl ring and the carbonyl group of the side chain showed significant activity in comparison to methotrexate against LL.[54] Among analogs of methotrexate with modifications in the ring and in the side chain, several were equivalent to methotrexate against LL.[55] 2,4-Diamino-5-methyl-6-(3,4,5-trimethoxyanilinomethyl)- and 2,4-diamino-5-chloro-6-(3,4-dichloroanilinomethyl)quinazoline (12) were active against a spectrum of mouse leukemias and solid tumors.[56] Carboxypeptidase G_1 enhanced the in vivo antitumor activity of triazinate (Baker's antifol) and 2,4-diamino-5-(3',4'-dichlorophenyl)-6-methylpyrimidine (DDMP).[57]



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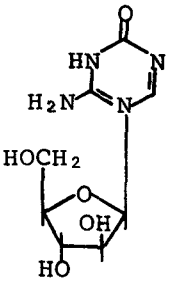

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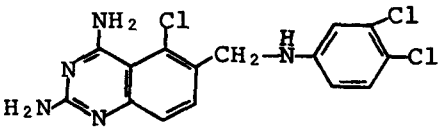
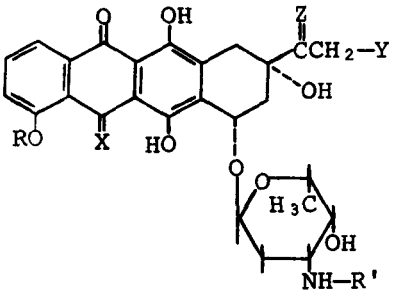
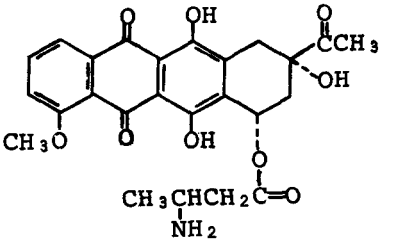


(12)

Anthracyclines - A thorough review of anthracyclines was published,[58] and a general mechanism of their activation was observed.[59] 5-Iminodaunorubicin (13) retained antitumor activity and was less cardiotoxic than the parent.[60] 4'-O-Methyldaunorubicin and -adriamycin and their 4'-epi analogs were active against mouse leukemia.[61] Some amino acid derivatives were superior to daunorubicin against murine tumors.[62] Replacement of the sugar moiety of daunorubicin with a β-alaninoyl group gave an analog (14) that was still active against P388 leukemia,[63] as was also true of the 9-formyl analog.[64] Some N-acyl derivatives of daunorubicin were active *in vitro* without being hydrolyzed to the parent.[65] The free and acetyl-protected L-lyxose analogs of daunorubicin were effective against P388 leukemia.[66] N,N-Dibenzyldaunorubicin showed increased activity *vs.* P388 leukemia, almost complete loss of mutagenicity, and a 10-fold reduction in cardiotoxicity.[67] DNA complexes of daunorubicin and adriamycin were cytotoxic to LL *in vitro*[68] and showed equivalent activity with less cardiotoxicity in the clinic.[69] Phase I studies were reported for AD-32[70] (15) and 4'-epi-adriamycin.[71] Various studies were reported for quelomycin (triferric adriamycin),[72,73] rubidazone (16),[74,75] detorubicin (semisynthetic derivative of daunorubicin),[76,77] aclacinomycin A,[78,79,80] and carminomycin (17).[81] 7(R)-O-Methylnogarol, a derivative of nogalamycin, and daunorubicin were both inactive against adriamycin-resistant P388 leukemia cells.[82]





(14)

(13): R = CH_3, X = NH,
Y = R' = H, Z = O

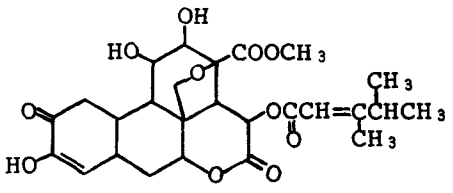
(15): R = CH_3, X = Z = O, $\overset{O}{\|}CCF_3$
Y = O–valeryl, R' =

(16): R = CH_3, X = O, $\overset{O}{\|}$
Y = R' = H, Z = $NNHCPh$

(17): R = Y = R' = H,
X = Z = O

Natural Products and Semi-Synthetics - 1,4-Oxazinone derivatives of the phenoxazinone chromophore in actinomycin D were more active than the parent against P388 leukemia and less toxic.[83] The antitumor antibiotic AT-125 was active against mouse leukemias and an ovarian tumor,[84] and toxicity to mice could be reduced by testosterone.[85] Auromomycin was active against several mouse tumors and was converted to macromomycin on Amberlite^R XAD. Macromomycin with ara-C or cyclophosphamide was synergistic against LL and was not inactivated by serum although neocarcinostatin was.[86] The structure of nocamycin was found to be related to tirandamycin.[87] Prumycin was active against several mouse tumors,[88] and cytidine was twice as effective in reducing toxicity of showdomycin for

murine bone marrow cells in vitro as it was for LL.[89] Tunicamycin demonstrated selective cytotoxicity for transformed cells.[90] An acetoxy group at position 15 appears to be necessary for good activity of anguidine analogs.[91] Inhibition of DNA synthesis by bruceantin (18), brucein D, brucein E, bruceoside A, and brusatol correlated more directly with activity against P388 leukemia than inhibition of RNA and protein synthesis.[92] Studies on the antitumor activity and metabolism of indicine N-oxide (19) suggest that conversion to indicine is not essential for its activity.[93] An extensive series of studies on pepleomycin (NK 631), a bleomycin derivative with less pulmonary toxicity, was reported.[94] A tetrahydroprotoberberine alkaloid with a trans-quinolizidine conformation was active against P388 leukemia.[95] Two glycosides of 25-spirost-5-en-3-β-ol were active against murine leukemia in vitro.[96] Structure-activity studies on tenulin, a sesquiterpene lactone, indicated that the cyclopentenone and the hemiketal units were necessary for high in vivo activity.[97] Isohelenol, a new antileukemic sesquiterpene lactone, was isolated and its structure determined.[98] In vitro cytotoxicity studies on norditerpenoid dilactones revealed several features essential for activity.[99] The importance of 9-hydroxy substitution for improving anticancer activity of ellipticine was confirmed.[100] The coumarins micromelin and scopoletin showed antitumor activity,[101] and L-glutamic acid γ-(2,5-dihydroxyanilide) was active against B16 melanoma cells.[102] Examination of several hundred natural and synthetic flavonoids led to the conclusion that pursuit of these compounds as antitumor agents was not warranted.[103] Evaluation of vindesine and some N-substituted analogs against 3 tumors indicated that vindesine was superior.[104] Proceedings of a workshop on vindesine appeared,[105] and chemical synthesis of vinblastine was reported.[106] A common leukemia-active constituent of extracts of Wikstroemia was the lignan wikstromol.[107] Studies on L-dopa analogs confirmed that a catechol moiety was essential for activity; 3,4-dihydroxybenzylamine and N-acetyldopamine showed low neurotoxicity but were the most active.[108]



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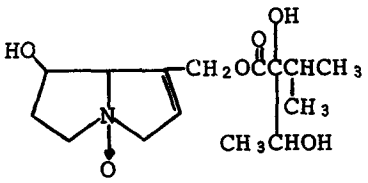
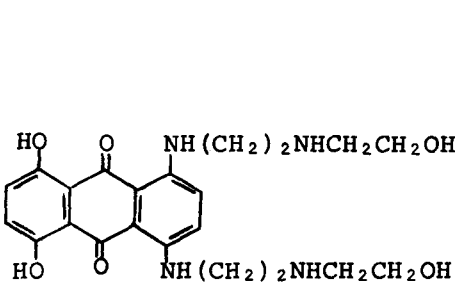


(19)

Miscellaneous Synthetic Agents - Of a series of alkylaminoanthraquinones, two members were curative against several tumors.[109,110] The 2-(2-hydroxyethyl)aminoethyl moiety is an important but not sufficient factor for high activity in this series.[111] In contrast to adriamycin, the 5,8-dihydroxy derivative (20) was active against I.V.-implanted LL and moderately active against adriamycin-resistant-P388 leukemia.[112] In vitro mutagenicity and in vivo antitumor activity of 9-anilinoacridines could be separated by structural modifications.[113] 3,4-Dihydroxybenzohydroxamic acid was found to be an active antitumor agent,[114,115] and the major factor influencing antitumor selectivity of 174 bisquaternary ammonium heterocycles was their ability to distinguish alternating adenine-thymine sequences in DNA.[116] Some boron betaine analogs were active against a spectrum of experimental tumors.[117] Macromolecular dextrans with substituents terminated by sulfhydryl groups or aromatic amines inhibited growth of tumors in a syngeneic mouse model but were inactive in vitro.[118] Clinical use of diphenylhydantoin against astrocytic tumors was suggested by

the observation of its activity against 2 rat gliomas; in addition, an in vitro test predicted the response in vivo.[119] Re-evaluation of DON and azotomycin indicated high activity against a spectrum of mouse tumors and human tumor xenografts, suggesting a second look at these drugs, as well as azaserine, in the clinic.[120,121] N-Diazoacetylglycine derivatives were inactive against primary Lewis lung tumor but were effective against formation of lung metastases.[122] Eight fluorenone-azomethines without alkylating functions were effective against LL.[123] Two 7H-pyridocarbazole dimers (21), DNA bifunctional intercalators, gave some cures of LL.[124] Studies on pyridones indicated that carbamate or acyloxy groups in position-3 were the most active.[125] Phenyl-substituted derivatives of 1,2-dimethyl-3,4-bis(hydroxymethyl)-5-phenylpyrrole bis(N-methylcarbamate) were active against P388 leukemia.[126] Electron-donating and -attracting groups in the 6-position of 1-methylquinolinium-2-dithioacetic acid zwitterions gave compounds with antileukemic activity.[127] A study of ureidothiazole and ureidothiadiazole derivatives related to ethyl 4-(2-thiazolylamino)carbonylaminobenzoate revealed that active agents contain either an N-C(S-)=N- or N-C-(S-)=N-N= unit.[128]



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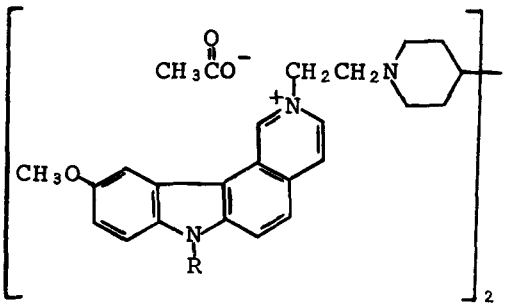

(21)

Metal Complexes - Probenecid reduced the renal toxicity of cis-Pt in rats and raised its LD_{50} in mice but did not affect its response against LL.[129] A series of alicyclic amine complexes of $PtCl_2$ showed activity against various animal tumors,[130] and two platinum complexes of ICRF-159 were active against LL.[131] Tumor-active tetra-μ-carboxylatodirhodium(II) complexes inhibited DNA and protein synthesis, but not RNA synthesis, in LL in vitro.[132] Titanocene dichloride was observed to be the first metallocene with cancerostatic activity,[133] and 2-formylpyridine thiosemicarbazone zinc sulfate complex was more active as an antitumor agent than the uncomplexed drug.[134]

Enzymes - A tumor-active L-asparaginase was isolated from Pseudomonas geniculata and may offer advantages over the currently-used, E. Coli enzyme, which sometimes causes immunological problems.[135] A combination of this tumor-active enzyme with AT-125 (NSC 163501) inhibited growth of Ehrlich ascites carcinoma much more than either agent alone.[136] Polymers produced by binding L-asparaginase to dextrans were found to persist well in blood, and it is anticipated that the dextran complex may demonstrate increased antitumor activity.[137] Succinylated glutaminase-asparaginase, a preparation with the same enzyme activity but increased plasma half life, demonstrated clinical activity against leukemia and solid lymphoid tumors in children.[138] In vitro studies with L-threonine deaminase from sheep liver demonstrated cytotoxicity to two murine leukemia cell lines but not to L cells or normal human fibroblasts.[139]

Drug Delivery - Methotrexate bound to bovine serum albumin was more effective than free methotrexate in reducing metastases of Lewis lung carcinoma,[140] and polymer-linked methotrexate had similar or only slightly-reduced activity in comparison with methotrexate against L5178Y cells in vitro.[141] The potential utility of antitumor drugs in liposomes continues under exploration with methotrexate[142,143] and ara-C.[144] Results after intratracheal administration suggest that liposome-encapsulated ara-C may be able to produce a selective effect in the lung without adverse effects in other tissues.[145] Studies on infusions of microencapsulated mitomycin C into dog kidney and tissue distribution in mice of 5-FU entrapped in albumin microspheres were reported.[146,147] Trial of 5-FU and urokinase embedded in silastic against brain tumors was also reported.[148] Sustained release of BCNU from an episcleral-implanted silicone device delayed growth of an epithelioma in rabbit eyes.[149]

References

1. DCT Bulletin, Ed. J. Henney, DHEW, NIH, NCI, DCT, Bethesda, Maryland 20205, Dec. 1979.
2. "Chemical Structures of Interest to DCT", V. Narayanan, NCI, NIH, Blair Bldg, Room 4A17, 8300 Colesville Rd, Silver Spring, Maryland 20205.
3. M. Gutierrez and S. Crooke, Cancer Treat. Rev., 6, 153 (1979).
4. B. Issell and S. Crooke, Cancer Treat. Rev., 6, 107 (1979).
5. C. Williams and J. Whitehouse, Brit. Med. J., 23, 1689 (1979).
6. A. Prestayko, J. D'Aoust, B. Issell and S. Crooke, Cancer Treat. Rev., 6, 17 (1979).
7. N. Michler, R. Earhart, B. Carr and D. Tormey, Cancer Treat. Rev., 6, 191 (1979).
8. Symposium, Clin. Exp. Pharmacol. Physiol., 1 (1979).
9. Symposia, Jpn. J. Antibiot., 32, 106, 259, 387 (1979).
10. T. Priestman, Cancer Treat. Rev., 6, 223 (1979).
11. R. Donehower, C. Meyers and B. Chabner, Life Sci., 25, 1 (1979).
12. R. Brundrett, M. Colvin, E. White, J. McKee, P. Hartman and D. Brown, Cancer Res., 39, 1328 (1979).
13. T. Johnston, G. McCaleb, T. Anderson and D. Murinson, J. Med. Chem., 22, 597 (1979).
14. L. Panasci, D. Green and P. Schein, J. Clin. Invest., 64, 1103 (1979).
15. T. Suami, T. Machinami and T. Hisamatsu, J. Med. Chem., 22, 247 (1979).
16. T. Suami, K. Tadano and W. Bradner, J. Med. Chem., 22, 314 (1979).
17. S. Sekido, K. Ninomiya and M. Iwasaki, Cancer Treat. Rep., 63, 961 (1979).
18. H. Hasegawa, W. Shapiro, J. Posner and G. Basler, Cancer Res., 39, 2687 (1979).
19. H. Lam, A. Begleiter, G. Goldenberg and C. Wong, J. Med. Chem., 22, 200 (1979).
20. F. Tsui, J. Brandt and G. Zon, Biochem. Pharmacol., 28, 367 (1979).
21. M. Jarman, R. Milsted, J. Smyth, R. Kinas, K. Pankiewicz and W. Stec, Cancer Res., 39, 2762 (1979).
22. S. Ludeman, G. Zon and W. Egan, J. Med. Chem., 22, 151 (1979).
23. A. Okruzek and J. Verkade, J. Med. Chem., 22, 882 (1979).
24. J. Driscoll, L. Dudeck, G. Congleton and R. Geran, J. Pharm. Sci., 68, 185 (1979).
25. M. Yoshimoto, H. Miyazawa, H. Nakao, K. Shinkai and M. Arakawa, J. Med. Chem., 22, 491 (1979).
26. F. Trinus, P. Sologub, L. Protsenko and M. Tarnavskaya, Vopr. Onkol., 25, 53 (1979).
27. P. Roberts, Brit. J. Cancer, 39, 755 (1979).
28. R. Eagan, D. Childs, D. Layton, E. Laws, H. Bisel, M. Holbrook and T. Fleming, J. Am. Med. Assoc., 241, 2046 (1979).
29. F. de las Heras, R. Alonso and G. Alonso, J. Med. Chem., 22, 296 (1979).
30. M. Garcia-Lopez, R. Herranz and G. Alonso, J. Med. Chem., 22, 807 (1979).
31. V. Srivastava and L. Lerner, J. Med. Chem., 22, 24 (1979).
32. S. Lee, F. Unger, R. Christian and A. Sartorelli, Biochem. Pharmacol., 28, 1267 (1979).
33. R. Trewyn and S. Kerr, Biochem. Pharmacol., 28, 607 (1979).
34. P. Chiang and G. Cantoni, Biochem. Pharmacol., 28, 1897 (1979).
35. J. Corey and S. Parker, Biochem. Pharmacol., 28, 867 (1979).
36. R. Meyer, T. Stone and B. Ullman, J. Med. Chem., 22, 811 (1979).
37. E. Acton, R. Goerner, H. Uh, K. Ryan, D. Henry, C. Cass and G. LePage, J. Med. Chem., 22, 518, (1979).
38. L. Wotring and L. Townsend, Cancer Res., 39, 3018 (1979).
39. G. Crabtree, R. Agarwal, R. Parks, A. Lewis, L. Wotring and L. Townsend, Biochem. Pharmacol., 28, 1491 (1979).
40. J. Benvenuto, J. Liehr, T. Winkler, D. Farquhar, R. Caprioli and T. Loo, Cancer Res., 39, 3199 (1979).
41. A. Lin, R. Benjamin, P. Rao and T. Loo, J. Med. Chem., 22, 1096 (1979).
42. M. Bobek, S. Kuhar and A. Bloch, J. Med. Chem., 22, 592 (1979).
43. T. Tsuro, H. Iida, S. Tsukagoshi and Y. Sakurai, Cancer Res., 39, 1063 (1979).

44. A. Mian, R. Long, L. Allen, R. Sidwell, R. Robins and T. Khwaja, J. Med. Chem., 22, 514 (1979).
45. T. Khwaja, L. Kigwana and A. Mian, Cancer Res., 39, 3129 (1979).
46. K. Kondo, T. Nagura, Y. Arai and I. Inoue, J. Med. Chem., 22, 639 (1979).
47. C. Hong, A. Nechaev and C. West, Biochem. Biophys. Res. Commun., 88, 1223 (1979).
48. Y. Shealy and C. O'Dell, J. Pharm. Sci., 68, 668 (1979).
49. J. Beisler, M. Abbasi, and J. Driscoll, J. Med. Chem., 22, 1230 (1979).
50. W. Prusoff, R. Schinazi and M. Chen, J. Med. Chem., 22, 1273 (1979).
51. I. Mustea, I. Postescu, R. Comes and V. Cristea, Pharmazie, 34, 111 (1979).
52. M. Zedeck, Biochem. Pharmacol., 28, 1440 (1979).
53. A. Kreutzberger, Arzneim.-Forsch., 28, 1684 (1978).
54. J. Martinelli, M. Chaykovsky, R. Kisliuk, Y. Gaumont and M. Gittleman, J. Med. Chem., 22, 869 (1979).
55. J. Montgomery, J. Piper, R. Elliott, C. Temple, E. Roberts and Y. Shealy, J. Med. Chem., 22, 862 (1979).
56. J. Bertino, W. Sawiki, B. Moroson, A. Cashmore and E. Elslager, Biochem. Pharmacol., 28, 1983 (1979).
57. K. Kalghatgi, B. Moroson, C. Horvath and J. Bertino, Cancer Res., 39, 3441 (1979).
58. Symposium, Cancer Treat. Rep., 63, 807 (1979).
59. N. Bachur, Cancer Treat. Rep., 63, 817 (1979).
60. G. Tong, D. Henry and E. Acton, J. Med. Chem., 22, 36 (1979).
61. G. Cassinelli, D. Ruggieri and F. Arcamone, J. Med. Chem., 22, 121 (1979).
62. Y. Levin and B. Sela, FEBS Lett., 98, 119 (1979).
63. E. Acton, G. Tong, C. Mosher, T. Smith and D. Henry, J. Med. Chem., 22, 922 (1979).
64. T. Smith, A. Fujiwara and D. Henry, J. Med. Chem., 22, 40 (1979).
65. A. Aszalos, M. Macy, V. Sethi, V. Luc and C. Kalita, Biochem. Pharmacol., 28, 335 (1979).
66. E. Fuchs, D. Horton, W. Weckerle and E. Winter-Mihaly, J. Med. Chem., 22, 406 (1979).
67. G. Tong, H. Wu, T. Smith and D. Henry, J. Med. Chem., 22, 912 (1979).
68. R. Sorace and B. Sheid, Cancer Treat. Rep., 63, 43 (1979).
69. A. Trouet and G. Sokal, Cancer Treat. Rep., 63, 895 (1979).
70. R. Blum, M. Garnick, M. Israel, G. Canellos, I. Henderson and E. Frei, Cancer Treat. Rep., 63, 919 (1979).
71. V. Bonfante, G. Bonadonna, F. Villani, G. DiFronzo, A. Martini and A. Casazza, Cancer Treat. Rep., 63, 915 (1979).
72. H. Cortes-Funes, M. Gosalvez, A. Moyano, A. Ma Nas and C. Mendiola, Cancer Treat. Rep., 63, 925 (1979).
73. A. Brugarolas, J. Perez-Llanderal, M. Garcia-Miralles, A. Lacave, M. Garcia-Marco, J. Izquierdo, A. Rodriguez-Llorian and A. Ribas, Cancer Treat. Rep., 63, 909 (1979).
74. T. Ohnuma, F. Elias, J. Holland and E. Henderson, Eur. J. Cancer, 15, 363 (1979).
75. R. Benjamin, M. Keating, K. Swenerton, S. Legha and K. McCredie, Cancer Treat. Rep., 63, 925 (1979).
76. D. Deprez-DeCampeneere, R. Baurain and A. Trouet, Cancer Treat. Rep., 63, 861 (1979).
77. C. Jacquillat, M. Auclerc, M. Weil, J. Maral, L. Degos, G. Auclerc, G. Tobelem, G. Schaison and J. Bernard, Cancer Treat. Rep., 63, 889 (1979).
78. M. Ogawa, J. Inagaki, N. Horikoshi, K. Inoue, T. Chinen, H. Ueoka and E. Nagura, Cancer Treat. Rep., 63, 931 (1979).
79. M. Misumi, H. Yamaki, T. Akiyama and N. Tanaka, J. Antibiot. (Tokyo), 32, 48 (1979).
80. D. Dantchev, V. Slioussartchouk, M. Paintrand, M. Hayat, C. Bourut and E. Math, Cancer Treat. Rep., 63, 875 (1979).
81. L. Baker, D. Kessel, R. Comis, S. Reich, M. Defuria and S. Crooke, Cancer Treat. Rep., 63, 899 (1979).
82. M. Egorin, R. Clawson, J. Cohen, L. Ross and N. Bachur, J. Pharmacol. Exp. Ther., 210, 229 (1979).
83. S. Sengupta, D. Trites, M. Madhavarao and W. Beltz, J. Med. Chem., 22, 797 (1979).
84. D. Houchens, A. Ovejera, M. Sheridan, R. Johnson, A. Bogden and G. Neil, Cancer Treat. Rep., 63, 473 (1979).
85. G. Neil, A. Berger, R. McPartland, G. Grindey and A. Bloch, Cancer Res., 39, 852 (1979).
86. T. Yamashita, N. Naoi, T. Hidaka, K. Watanabe, Y. Kumada, T. Takeuchi and H. Umezawa, J. Antibiot. (Tokyo), 32, 330 (1979); T. Hidaka, Y. Yano, T. Yamashita and K. Watanabe, J. Antibiot. (Tokyo), 32, 340 (1979).
87. G. Horvath, M. Brazhnikova, N. Konstantinova, I. Tolstykh and N. Potapova, J. Antibiot. (Tokyo), 32, 555 (1979).
88. S. Okubo, N. Nakamura, K. Ito, H. Marumo, M. Tanaka and S. Omura, J. Antibiot. (Tokyo), 32, 347 (1979).
89. M. Rabinowitz, Y. Uehara and D. Vistica, Science, 206, 1085 (1979).
90. K. Olden, R. Pratt and K. Yamada, Int. J. Cancer, 24, 60 (1979).
91. C. Claridge, H. Schmitz and W. Bradner, Cancer Chemother. Pharmacol., 2, 181 (1979).
92. I. Hall, K. Lee, S. Eigebaly, Y. Imakura, Y. Sumida and R. Wu, J. Pharm. Sci., 68, 883 (1979).
93. G. Powis, M. Ames and J. Kovach, Res. Commun. Chem. Pathol. Pharmacol., 24, 559 (1979).
94. Symposium, J. Antibiot. (Tokyo), 32, 36, 106, 259, 387 (1979).

95. M. Cushman, F. Dekow and L. Jacobsen, J. Med. Chem., 22, 331 (1979).
96. P. Ravikumar, P. Hammesfahr and C. Sih, J. Pharm. Sci., 68, 900 (1979).
97. T. Waddell, A. Austin, J. Cochran, K. Gerhart, I. Hall and K. Lee, J. Pharm. Sci., 68, 715 (1979).
98. D. Sims, K. Lee and R. Wu, J. Nat. Prod., 42, 282 (1979).
99. Y. Hayashi, T. Matsumoto and T. Tashiro, Gann, 70, 365 (1979).
100. C. Paoletti, S. Cros, N. Xuong, P. Lecointe and A. Moisand, Chem. Biol. Interact., 25, 45 (1979).
101. J. Cassady, N. Ojima, C. Chang and J. McLaughlin, J. Nat. Prod., 42, 274 (1979).
102. A. Rosowsky, M. Wick and S. Kim, J. Med. Chem., 22, 1034 (1979).
103. J. Edwards, R. Raffauf and P. LeQuesne, J. Nat. Prod., 42, 85 (1979).
104. R. Conrad, G. Cullinan, K. Gerzon and G. Poore, J. Med. Chem., 22, 391 (1979).
105. Symposium, Cancer Chemother. Pharmacol., 2, 229 (1979).
106. P. Mangeney, R. Zo Andriamialisoa, N. Langlois, Y. Langlois and P. Potier, J. Am. Chem. Soc., 101, 2243 (1979).
107. S. Torrance, J. Hoffmann and J. Cole, J. Pharm. Sci., 68, 664 (1979).
108. M. Wick, Cancer Treat. Rep., 63, 991 (1979).
109. R. Johnson, R. Zee-Cheng, W. Lee, E. Acton, D. Henry and C. Cheng, Cancer Treat. Rep., 63, 425 (1979).
110. K. Murdock, R. Child, P. Fabio, R. Angier, R. Wallace, F. Durr and R. Citarella, J. Med. Chem., 22, 1024 (1979).
111. R. Zee-Cheng, E. Podrebarac, C. Menon and C. Cheng, J. Med. Chem., 22, 501 (1979).
112. R. Wallace, K. Murdock, R. Angier and F. Durr, Cancer Res., 39, 1570 (1979).
113. L. Ferguson and W. Denny, J. Med. Chem., 22, 251 (1979).
114. B. Van't Riet, G. Wampler and H. Elford, J. Med. Chem., 22, 589 (1979).
115. H. Elford, G. Wampler and B. Van't Riet, Cancer Res., 39, 844 (1979).
116. W. Denny, G. Atwell, B. Baguley and B. Cain, J. Med. Chem., 22, 134 (1979).
117. I. Hall, C. Starnes, B. Spielvogel, P. Wisian-Neilson, M. Das and L. Wojnowich, J. Pharm. Sci., 68, 685 (1979).
118. J. Pitha, K. Kociolek and C. Apffel, Cancer Res., 39, 170 (1979).
119. P. Kornblith, L. Hartnett, L. Anderson, E. Quindlen and B. Smith, Neurosurgery, 5, 259 (1979).
120. A. Ovejera, D. Houchens, R. Catane, M. Sheridan and F. Muggia, Cancer Res., 39, 3220 (1979).
121. R. Catane, D. Von Hoff, D. Glaubiger and F. Muggia, Cancer Treat. Rep., 63, 1033 (1979).
122. T. Giraldi, A. Guarino, C. Nisi and L. Baldini, Eur. J. Cancer, 15, 603 (1979).
123. W. Jungstand, W. Gutsche, K. Wohlrabe and W. Schulze, Arch. Geschwulstforsch., 49, 15 (1979).
124. B. Roques, D. Pelaprat, I. LeGuen, G. Porcher, C. Gosse and J. Le Pacq, Biochem. Pharmacol., 28, 1811 (1979).
125. D. Hwang and J. Driscoll, J. Pharm. Sci., 68, 816 (1979).
126. W. Anderson and M. Halat, J. Med. Chem., 22, 977 (1979).
127. W. Foye and J. Kauffman, J. Pharm. Sci., 68, 336 (1979).
128. R. Zee-Cheng and C. Cheng, J. Med. Chem., 22, 28 (1979).
129. D. Ross and G. Gale, Cancer Treat. Rep., 63, 781 (1979).
130. M. Tomaro, S. Venturini, C. Monti-Bragadin, G. Saincich, G. Mestroni and G. Zassinovich, Chem. Biol. Interact., 26, 179 (1979).
131. C. Bahner, T. Patterson, L. Rives and H. Harmon, J. Med. Chem., 22, 575 (1979).
132. R. Howard, A. Kimball and J. Bear, Cancer Res., 39, 2568 (1979).
133. H. Kopf and P. Kopf-Maier, Angew. Chem. (Engl.), 18, 477 (1979).
134. G. Atassi, P. Dumont and J. Harteel, Eur. J. Cancer, 15, 451 (1979).
135. G. Kitto, G. Smith, T. Thiet, M. Mason and L. Davidson, J. Bacteriol., 137, 204 (1979).
136. J. Holcenberg, Cancer Treat. Rep., 63, 1109 (1979).
137. J. Benbough, C. Wiblin, T. Rafter and J. Lee, Biochem. Pharmacol., 28, 833 (1979).
138. J. Holcenberg, L. Borella, B. Camitta and B. Ring, Cancer Res., 39, 3145 (1979).
139. D. Wellner and R. Greenfield, Cancer Treat. Rep., 63, 1089 (1979).
140. B. Chu and J. Whiteley, J. Nat. Cancer Inst., 62, 79 (179); 141. W. Fung, M.
141. W. Fung, M. Przybylski, H. Ringsdorf and D. Zaharko, J. Nat. Cancer Inst., 62, 1261 (1979).
142. J. Freise, P. Magerstedt and F. Schmidt, Z. Naturforsch. C, 34, 114 (1979).
143. J. Freise, F. Schmidt and P. Magerstedt, J. Cancer Res. Clin. Oncol., 94, 21 (1979).
144. Y. Rustum, C. Dave, E. Mayhew and D. Papahadjopoulos, Cancer Res., 39, 1390 (1979).
145. H. McCullough and R. Juliano, J. Nat. Cancer Inst., 63, 727 (1979).
146. T. Kato, R. Nemoto, I. Kumagai, T. Nishimoto and H. Mori, Nippon Gan. Chiryo Gakkai Shi., 14, 152 (1979).
147. K. Sugibayashi, Y. Morimoto, T. Nadai, Y. Kato, A. Hasegawa and T. Arita, Chem. Pharm. Bull. (Tokyo), 27, 204 (1979).
148. Y. Oda, Y. Tokuriki, E. Tsuda, H. Handa and J. Kieler, Acta Neurochir.[Suppl.] (Wien), 2, 489 (1979).
149. H. Liu, M. Refojo, H. Perry and D. Albert, Invest. Ophthalmol. Vis. Sci., 18, 1061 (1979).

Chapter 15. Antifungal Chemotherapy

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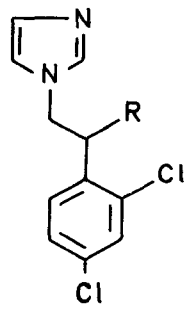
Introduction - During the last two years, comprehensive reviews have been published dealing with chemistry, mode of action and clinical use of various classes of antifungal agents.[1-4] A recent review is available in which drugs for systemic mycosis[5] and strategies in the treatment of these infections were discussed.[6] Antifungal agents and their use in the treatment of candidosis have been discussed by Odds in his outstanding book on Candida and candidosis.[7] The treatment of mycosis with imidazole derivatives was reviewed by Raab.[8] Miconazole 1a used in the treatment of superficial and life threatening systemic fungal infections was reviewed.[9,10] Treatment of candidosis with miconazole was the topic of a symposium[11] and of a review.[12] The potential of econazole 1b in dermatomycosis and systemic mycosis, including clinical evaluation, was the subject of two symposia[13,14] and a review.[15] Clinical efficacy of orally administered ketoconazole 10 was evaluated.[16,17] No new major antifungal drugs have been introduced into clinical practice.

New Antifungal Agents - Some new imidazole derivatives were described and studied in more detail. Tioconazole 1d was shown to be active against Candida spp., Torulopsis glabrata, Cryptococcus neoformans, dermatophytes and Aspergillus spp.[18] Isoconazole nitrate 1c showed broad-spectrum activity against gram positive bacteria, dermatophytes, yeasts, molds and Trichomonas. This activity was confirmed by topical cure of vaginal and skin mycoses in rats and guinea pigs, respectively.[19,20] Imidazoles of type 2 and 3, structurally related to miconazole, were described.[21,22] In vitro activity was hardly affected by chain length (X = 2,4-Cl_2, Y = 4-Cl and n = 1, 2) and no correlation was found with in vivo activity. Broad-spectrum



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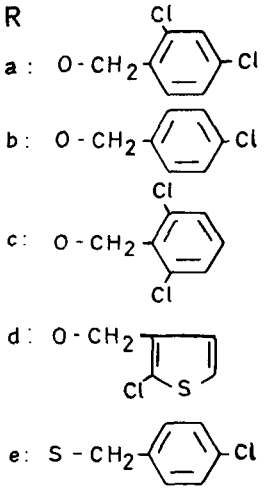

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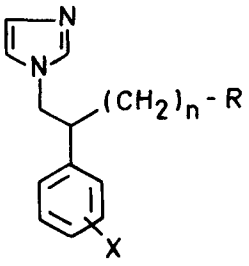
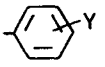
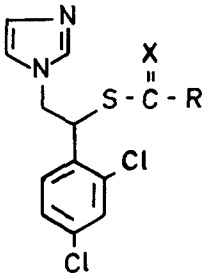


2 R = 

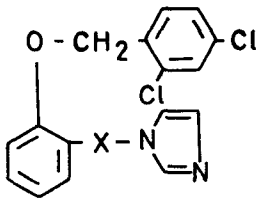
3 R = O 



4 a: X = O, R = 

b: X = S, R = OC_3H_7

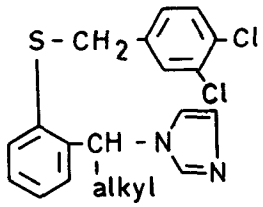
c: X = S, R = OC_4H_9



5 X = —

6 X = - CH - (alkyl)

7 X = - CH - $(CH_2)_n$ - (alkyl)



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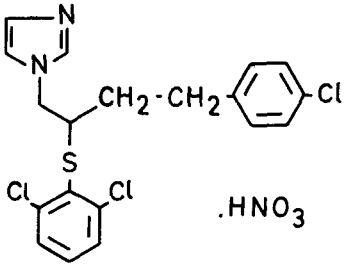
activity was found within a series of esters of type 4.[23] The synthesis of phenylethers of type 5, 6 and 7 and thiophenylethers 8 was reported.[24-27] Compound 6a (alkyl = nC_3H_7) was active in vitro against Trichophyton mentagrophytes (0.4 µg/ml), Trichophyton rubrum (0.8 µg/ml), Microsporum gypseum (1.6 µg/ml) and Candida albicans (25 µg/ml). In vivo, it was effective against vaginal candidosis in rats and trichophytosis in guinea pigs. In vivo, 6a and 8a (alkyl = nC3H7) were equipotent, and the latter compound has been selected for more detailed investigation.

Preliminary screening results showed comparable activity for miconazole and butoconazole 9 against Trichophyton spp., Microsporum spp., Epidermophyton floccosum, Candida spp. and Cr. neoformans and also against gram positive bacteria.[28] In vivo, 9 was particularly effective against vaginal candidosis in mice with low reinfection rates.

Ketoconazole 10, a new orally active, broad spectrum antimycotic was described.[29] It was active against crop candidosis in turkeys, vaginal candidosis in rats, systemic candidosis in chickens, skin- and systemic candidosis and dermatophytosis in guinea pigs and Coccidioides immitis in mice.[30,31] SN 105-843 11 showed good in vitro activity against Trichophyton, Epidermophyton, Aspergillus spp., Sporothrix schenckii and Candida spp.[32]



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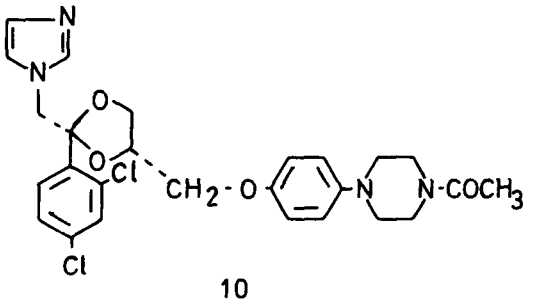

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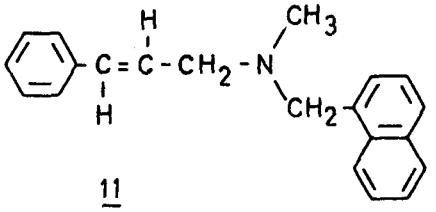

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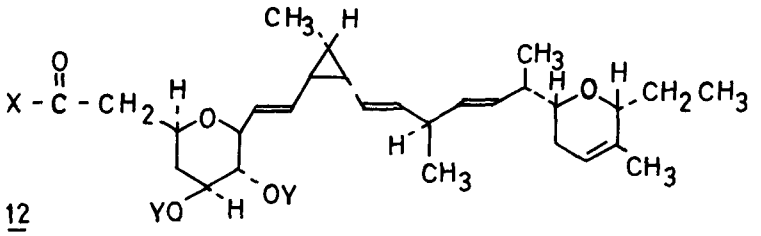
Synthesis and antifungal activity was described of esters and amides,[33] alcohol, ketone and oxime analogs[34] of ambruticin 12 (X = OH, Y = H).



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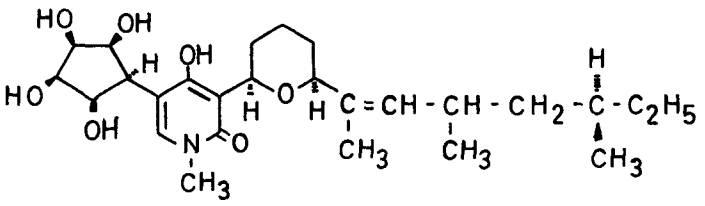
Mixtures of enzymes of fungal origin, "mycolases," containing various carbohydrases - including chitinase and lamirinase - were successfully used - alone or combined with amphotericin-B or nystatin - against systemic Aspergillus fumigatus infections in BALB/C mice.[35]

Frenolicin B, isolated from Streptomyces roseofulvus (Strain AM-3867) was active in vitro against yeast, dermatophytes and molds.[36] A 121, a non-polyene compound, produced by Streptomyces spp. was active against Rhizopus nigricans (25 µg/ml), Aspergillus niger (12 µg/ml, Histoplasma capsulatum (3 µg/ml) and dermatophytes.[37] Four new 2-pyridinethiol-1-oxide cephalosporins showed in vitro activity against Trichophyton mentagrophytes and C. albicans; none of them was active in systemic C. albicans infections in mice.[38]

Funicolusin 13 was active in vitro against T. mentagrophytes, T. rubrum, Microsporum ferrugineum, Microsporum canis, M. gypseum, E. floccosum, Blastomyces dermatitidis and Phialophora pedrosoi and in vivo against T. mentagrophytes infections in guinea pigs.[39]

Rapamycin (AY-22,989) 14 was reported to cure systemic C. albicans infections in mice and vaginal candidosis in rats after oral treatment.[40]



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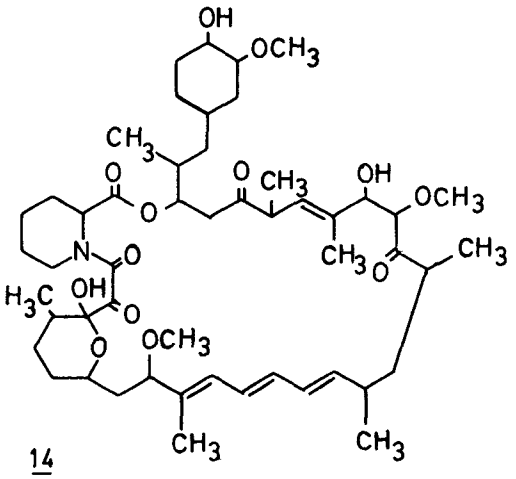

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Laboratory Methods - Methods have been developed for simultaneous determination of griseofulvin and its major metabolite[41] and for amphotericin-B, 5-fluorocytosine (5-FC) and econazole.[42] Econazole plasma levels have been determined by HPLC[43] and those of amphotericine-B by a modified agar diffusion assay.[44] A simple, reliable and inexpensive assay has been described for miconazole and ketoconazole.[45]

New test media for C. albicans include kidney homogenate[46] and Eagle's minimum essential medium (EMEM) supplemented with amino acids and fetal calf serum.[47] The latter medium, normally used for culturing mammalian cells favors mycelium formation of C. albicans. Since the predominating form in candidosis is the mycelial phase, it was proposed that in vitro testing of anti-fungals on mycelial phase cells would lead to a better prediction of their in vivo activity.[48] Furthermore, this system makes it possible to study the interaction between e.g. C. albicans and leukocytes with and without the addition of anti-fungal compounds.[49]

Ketoconazole was found to inhibit the growth of C. albicans and to suppress completely the formation of mycelia in EMEM at concentrations as low as 10 ng/ml. It was toxic to the co-cultivated leukocytes at 0.1 mg/ml only.[49] Ketoconazole inhibited filamentous forms of H. capsulatum, B. dermatitidis and Coccidioides immitis.[50] It was effective in murine coccidioidomycosis,[51] histoplasmosis and cryptococcosis,[52] and showed high therapeutic effect in rats, intrarenally infected with C. albicans, and in systemic murine candidosis.[53] Econazole diminished and delayed mortality in murine histoplasmosis.[54] In mice and guinea pigs, miconazole prevented establishment of C. albicans infections.[55] In rats, systemic candidosis was effectively controlled.[56]

In a comprehensive study,[57] ambruticin was highly effective against dimorphic and filamentous fungi. In an ICS agar assay it was superior to miconazole and amphotericin-B against C.immitis, H.capsulatium, B.dermatitidis and A.fumigatus, but inferior against Candida and Torulopsis spp., and less active than tolnaftate against dermatophytes.[58] Oral and topical activity in experimental trichophytosis has been described and serum levels and excretion have been discussed.[57] Oral ambrucitin was able to cure murine histoplasmosis[59] and coccidioidomycosis;[60] no resistance of C.immitis to the drug was induced on prolonged treatment.

Amphotericin-B methyl ester was slightly less active than amphotericin-B against a wide range of phatogenic fungi.[61] Candida endocarditis in rabbits could easily be prevented with 5-FC.[62]

Mechanisms of Action - After intrafungal deamination, 5-fluorocytosine (5-FC) inhibits both DNA and RNA synthesis,[63,64] whereas production of proteins, the composition of which may be abnormal, continues to some extent and a marked increase of the carbohydrate synthesis was observed. Thus exposure of C.albicans to 5-FC seems to result in so-called unbalanced growth.[62] The deamination of 5-FC to form the antimetabolite 5-fluorouracil is catalysed by cytosine deaminase. Absence or low activity of this enzyme has been observed in animal cells and is considered the basis of the drug's low toxicity in patients.[65] Polyene antibiotics promote leakage of cellular constituents, an effect dependent on drug binding to cell-membrane sterols.[8,66,67] Scanning electron microscopy on C.albicans treated with the polyene, nystatin, revealed an effect on the membranes followed by disruption of cells, effects antagonized by cholesterol and hydrocortisone.[68,69] Kotler-Brajtburg et al.[70] have studied 14 polyene antibiotics and 6 of their derivatives for permeabilizing and lethal effects. According to these studies, there is a clear correlation between the type of biologic action and the size of the polyene macrolide ring. The heptaenes induce considerable potassium leakage or a fungistatic effect at lower and cell death at higher concentrations. A triene, tetraenes, pentaenes and one hexaene induce little or no potassium leakage or fungistatic effect separable from cell death.[70] In addition to its antifungal effect, amphotericin-B appears to have immunoadjuvant properties. Experiments have shown that this polyene can enhance the number of antibody-producing cells in the spleen and lymph nodes of different mouse strains[75] and can augment delayed hypersensitivity reactions and cell-mediated immunity.[72,73]

The morphological effects of low dose levels of miconazole, clotrimazole[48] and ketoconazole[47] consisted of changes in cell volume, defective cell division and alterations of the plasma membrane. Electron microscopically, the primary structural changes caused by econazole were also seen in the plasmalemma of the fungus cell.[74] Studies of Yamaguchi and Iwata further proved that miconazole and clotrimazole affect the membrane function of C. albicans.[75] The authors related the fact that miconazole affected the membrane function more drastically than clotrimazole with the difference that clotrimazole tends to be fungistatic whereas miconazole is more fungicidal.[75] A significant difference was also seen between these drugs in induction of necrosis in outgrown C. albicans mycelium.[48]

The morphological effects described might be a reflection of the effects of N-substituted imidazole derivatives on ergosterol biosynthesis. In fact, the ergosterol synthesis was inhibited by miconazole in C. albicans[76,77] and Ustilago maydis,[78] by imazalil in Aspergillus nidulans,[79] Ustilago avenae[80] and in Penicillum expansum,[81] and by clotrimazole[82] and ketoconazole[83] in C. albicans. In all studies, an accumulation of sterols with a methyl group at C-14 was observed. It has been shown that such

sterols do not function effectively as membrane components.[84,85]

At higher doses than those interfering with ergosterol biosynthesis, miconazole also intervened with fatty acid and triglyceride synthesis.[76] In vitro, the action of clotrimazole and miconazole was partly antagonized by the addition of phospholipids with unsaturated acyl moieties or free fatty acids.[86] Yamaguchi and Iwata[87] reported that phospholipid liposomes are more sensitive to imidazoles when the esterified fatty acids are unsaturated. Sud et al observed that the presence of free fatty acids in liposome model membranes sensitized these membranes to the action of clotrimazole, miconazole and sulconazole (1e).[88] The effects of N-substituted imidazoles on sterol synthesis, fatty acids, triglycerides and phospholipids may be the basis of their damaging effects on membrane systems of fungal cells.

Ciclopirox (Hoe 296) the ethanolamine salt of 6-cyclohexyl-1-hydroxy-4-methyl-2-(1H)-pyridone also affected some membrane functions of C. albicans.[89] More than 97% of the total amount of ciclopirox taken up was bound to some cellular structures and organelles, including cell walls, cell membranes, mitochondria and microsomes.[90]

Drug Combinations - In adult female mice, significant mortality was seen on combined administration of cortisone acetate and amphotericin-B; microscopic examination revealed only dose-related renal lesions within 6 days.[91] In contrast to amphotericin-B, 5-FC is often synergistic with imidazoles.[92] Subinhibitory concentrations of antioxidants were synergistic with amphotericin-B against two strains of C. albicans.[93] Atromid-S (clofibrate) potentiated in vitro activity of miconazole on C. albicans.[94] Intravenous miconazole combined with intrathecal amphotericin-B was useful in a case of disseminated coccidioidomycosis.[95] However, in vitro, miconazole and clotrimazole appeared to decrease the effectiveness of amphotericin-B.[8,96] This has been related to their inhibition of ergosterol synthesis resulting in a decreased binding of amphotericin-B to the cells.[6] Amphotericin-B and rifampicin were synergistic in ocular fungal isolates[97] and were successfully used in the treatment of an invasive pulmonary aspergillosis.[98] Addition of small amounts of candicidin markedly enhanced the potency of 5-FC,[99] especially in vaginal candidosis.[100]

Therapeutic and Pharmacological Studies - Therapeutic failures of griseofulvin in T. rubrum infections have been correlated with in vitro resistances of the infecting fungus.[101] The bioavailability of this drug has been improved by a new formulation.[102] Encouraging results have been obtained in the treatment of chromomycosis with 5-FC.[103] Amphotericin-B gave excellent therapeutic responses against histoplasmosis in immunosuppressed patients, particularly in early diagnosis and on full therapy.[104] A strain of C. tropicalis with progressive resistance to amphotericin-B (>0.5 mg/ml) was unable to produce renal infection in steroid treated mice,[105] because of the reduced capacity for pseudo-mycelia production.

The elimination phase half-life of amphotericin-B was approximately 15 days with only 3% renally excreted.[106] Intravenously and intrathecally administered amphotericin-B methyl ester failed in meningoencephalitis, due to Coccidioides immitis.[107]

Clinical isolates of C. albicans showing "wild" and emergent resistance to 5-FC and to antifungal imidazole derivatives have been noted.[108] Oil solutions of clotrimazole, in contrast to oral tablet forms, yielded effective and sustained blood levels.[109] Clotrimazole cream gave good

results in the treatment of napkin dermatitis.[110] Both clotrimazole troches[111] and miconazole gel[112] were effective in chronic oral candidosis. Intravenous miconazole[113,114] resulted in dramatic clinical improvement in chronic mucocutaneous candidosis. Medicated tampons containing 100 mg miconazole, have been studied in 48 women with culture-proven vaginal candidiasis. With one case of reinfection excluded, the cure rate was 98%.[115]

The clinical values of miconazole in Malassezia furfur infection was reviewed.[116] The availability of i.v. miconazole and amphotericin-B - 5-FC combinations constitute real progress in the management of Candida meningitis.[117-119] The value of i.v. - and even oral miconazole administration in paracoccidioidomycosis was confirmed.[120-121] To prevent relapse, prolonged therapy courses may be necessary. Intravenous miconazole therapy was able to clear deep infections with Petriellidium boydii (formerly Allescheria boydii),[122] and miconazole gel was effective against tinea nigra, caused by Cladosporium werneckii.[123] Therapy failures in destructive arthritis due to S.schenckii, Cr.neoformans meningoencephalitis and disseminated A.fumigatus infections, respectively, were attributed to suboptimal body fluid levels of miconazole although all fungi were susceptible in vitro to about 1.5 µg/ml or less.[124] A patient with cryptococcal meningitis refractory to amphotericin-B was successfully treated with intraventricular miconazole.[125]

Miconazole and tioconazole were equipotent in vaginal candidosis, side effects occurring more frequently with tioconazole.[125] Oral econazole gave beneficial therapeutic results in chronic mucocutaneous candidosis and pulmonary aspergillosis in children with cellular immune deficiency.[126] An infusion treatment with econazole resulted in cure of pulmonary aspergillosis[127] and local treatment with 1 % econazole solution cured fungal nasosinusitis.[128] A clinical study was performed with vaginal suppositories of econazole. The suppositories were administered for three consecutive days followed by a seven-day medication-free interval. If the patient was still mycologically positive at the end of this period, a second course of treatment was started. The cure rate was 71.1 % after the 1st and 87.4 % after the second course of treatment.[129] A three-day treatment with econazole in a combination of vaginal ovules with cream gave good results in vaginal candidiasis.[130]

The preclinical and clinical profile of sulconazole has been reported.[131] In dermatophytosis SN 105-843 gave cure rates of 96 % within four weeks of topical treatment in 130 patients.[32]

Bioavailability studies in man revealed that ketoconazole was well-absorbed and that high and sustained plasma levels (about 5 mg/l) were found after a single oral dose of 200 mg.[132] Oral ketoconazole gave high cure rates in vaginal candidosis[133] and in superficial and systemic mycoses.[16] Dermatomycosis was treated successfully.[16,134,135] and a rapid relief of symptoms in chronic mucocutaneous candidosis patients was seen.[16] Oral ketoconazole therapy cleared dermatophytoses in patients with resistance or intolerance to griseofulvin.[16] In paracoccidioidomycosis[16,136] and in histoplasmosis[136] ketoconazole gave high cure rates and an encouraging response was seen in coccidioidomycosis.[137-139] Ketoconazole can offer an effective non-toxic alternative for amphotericin-B in the management of dermatophytoses and systemic mycoses.

References

1. P.F. D'Arcy and E.M. Scott, in "Progress in Drug Research", Vol. 22, E.Jucker, Ed., Birkhäuser Verlag, Basel und Stuttgart, 1978, p. 93.
2. E.R. Stiehm, T.J. Fischer and L.S. Young, Ann.Intern.Med., 89, 91 (1978).

3. A.J. Weinstein, in "Seminar in Infectious Disease", Weinstein and Fields, Eds., Stratton Intercontinental Medical Book Corporation, 1978.
4. D.C.E. Speller, The Practitioner, 222, 511 (1979).
5. D.A. Stevens, Ration.Drug Ther., 13, 51 (1979).
6. G. Medoff and G.S. Kobayashi, N.Eng.J.Med., 302, 145 (1980).
7. F.C. Odds in "Candida and Candidosis", Leicester University Press, Leicester, 1979, p.228
8. W. Raab in "Mykosebehandlung mit Imidazolderivaten", Springer Verlag, Berlin, 1978 (English edition in press).
9. R.C. Heel, R.N. Brogden, G.E. Pakes, T.M. Speight and G.S Avery, Drugs, 19, 7 (1980).
10. P.A.J. Janssen and W. Van Bever in "Pharmacological and biochemical properties of drug substances", M.E. Goldberg, Ed., publ. Amer.Pharm.Ass., Acad.Pharm.Sci., Washington, 1979, p. 333.
11. D. Gough "New Advances in the treatment of Candidal vaginitis", Roy.Soc.Med. Intern. Congress and Symposium Series, No. 7, Academic Press, London, 1979.
12. B. Chevrel, Med. Chir.Dig., 7, 177 (1978).
13. C.Diefenbach (Ed) in "Medical Mycology", Proceedings of the International Cilag-Chemie Symposium, Flims, Switzerland, January 24-26, 1977, published in Mykosen, Supplement 1, 1978.
14. H.Rieth, H.Becker and V.P.Nass, Symposium über Econazol-Nitrat, ein neues Breitspektrum Antimycoticum, Notabene Medici Verlag Pharmadolinguoa, Melsungen, 1978.
15. R.C. Heel, R.N. Brogden, T.M. Speight and G.S. Avery, Drugs, 16, 177 (1978).
16. First International Symposium on ketoconazole, Meddelin, Columbia, November 29-30, 1979, Rev.Infect.Dis., in press.
17. J. Symoens in "Proceedings of the International Symposium on Health Policy in Developing Countries", C.Wood, Ed., Beerse, April 1979, p., in press.
18. S. Jevons, G.E. Gymer, K.W. Brammer, D.A. Cox and M.R.G. Leening, Antimicrob.Ag.Chemother. 15, 597 (1979).
19. H.J. Kessler, Arzneim-Forsch., 29, 1344 (1979).
20. H.J. Kessler, D.Hande and C. Schöbel, ibid., 1352 (1979).
21. J.Heeres, J.H.Mostmans and J.Van Cutsem, J.Med.Chem., 20, 1511 (1977).
22. J.Heeres, L.J.J. Backx and J.Van Cutsem, ibid., 20, 1516 (1977).
23. K.A.M. Walker, A.C.Braemer, S.Hitt, R.E.Jones and T.R. Matthews, ibid., 21, 840 (1978).
24. P.Strehlke , Eur.J.Med.Chem., 14, 227 (1979).
25. P.Strehlke and H.J.Kessler, ibid., 14, 231 (1979).
26. P.Strehlke and H.J.Kessler, ibid., 14, 238 (1979).
27. P.Strehlke and H.J.Kessler, ibid., 14, 243 (1979).
28. K.A.M. Walker, A.C.Braemer, S.Hitt, R.E.Jones and T.R. Matthews, J.Med.Chem., 21, 840 (1978)
29. J.Heeres, L.J.J.Backx, J.H.Mostmans and J.Van Cutsem, ibid., 22, 1003 (1978).
30. D.Thienpont, J.Van Cutsem, F.Van Gerven, J.Heeres and P.A.J.Janssen, Experientia, 35, 606 (1979).
31. D.Borelli, J.Fuentes, E.Leiderman, A.Restrepo-M, J.L.Bran, R.Legendre, H.B.Levine and D.A. Stevens, Postgrad.Med.J., 55, 657 (1979).
32. A.Georgopoulos, D.Berney, G.Petrányi, J.Drews and H.Mieth, Abstract 11th International Congress of Chemotherapy, Boston, October 1-5, 1979, No. 153.
33. D.T. Connor and M.von Strandmann,J.Med.Chem., 22, 1144 (1979).
34. D.T. Connor and M.von Strandmann,ibid., 22, 1055 (1979).
35. D.A.L.Davies and A.M.J.Pope, Nature, 273, 235 (1978).
36. Y.Iwai, A.Kora, Y.Takahashi, T.Hayaski, J.Awaya, P.Masuma, R.Oiwa and S.Ōmura, J.Antibiot., 31, 959 (1978).
37. S.K.Singh and T.B.Samanta, Indian.J.Exp.Biol., 26, 708 (1978).
38. J.V.Uri, P.Actor, L.Phillips and J.A.Weisbach, J.Antibiot., 31, 580 (1978).
39. K.Ando, J.Matsuura, Y.Nawata, H.Endo, H.Sasaki, T.Okytomi, T.Sachi and G.Tamura, ibid., 31, 533 (1978).
40. H.Baker, A.Sidorawitz, S.M. Sehgal and C.Vezina, ibid., 31, 539 (1978).
41. H.Kaminura, Y.Onu and Y.Shiobara, J.Chromatogr.: Biomedical Applications, 163, 271 (1979).
42. E.Drouhet and B.Dupont, Bull.Soc.Fr.Myc.Med., 7, 175 (1978).
43. R.R.Brodie, L.D.Chasseaud and L.M.Wamsley, J.Chromatogr., 155, 209 (1978).
44. R.F.Cosgrove and G.J.Jones, J.Pharm.Pharmacol., 28, 334 (1978).
45. J.G.Grendahl and J.P.Sung, Antimicrob.Ag.Chemother., 14, 509 (1978).
46. J.Haller and M.Plempel, Sabouraudia, 16, 47 (1978).
47. M.Borgers, M.De Brabander, H.Van den Bossche and J.Van Cutsem, Postgrad.Med.J., 55, 687 (1979).
48. M.Borgers, M.De Brabander and H.Van den Bossche, Roy.Soc.Med. International Congress and Symposium Series, Acad. Press, London, 1979, No. 7, p. 21.
49. M.De Brabander, F.Aerts, J.Van Cutsem, H.Van den Bossche and M.Borgers, to be published in Sabouraudia.
50. D.Dixon, S.Shadomy, H.J.Shadomy, A.Espinell-Ingroff and T.M.Kerkering, J.Infect.Dis., 138, 245 (1978).
51. H.B.Levine and J.M.Cobb, Am.Rev.Respir.Dis., 118, 715 (1978).
52. D.M.Williams, J.E.Graybill and D.J.Drutz, 11th International Congress of Chemotherapy, Boston, Ocober 1-5, 1979, No. 145.
53. M.Hatala, Z.Modr, and M.Livka, ibid, No. 141.
54. B.Dupont and E.Drouhet, Bull.Soc.Fr.Myc.Méd., 7, 181 (1978).

55. D.Thienpont, J.Van Cutsem and D.A.Gough, Mykosen, 21, 417 (1978).
56. M.W.Balk, M.H.Crumrine and G.W.Fisher, Antimicrob.Ag.Chemother., 13, 321 (1978).
57. S.M.Ringel, ibid., 13, 762 (1978).
58. S.Shadomy, D.M.Dixon, A.Espinell-Ingroff, G.E.Wagner, H.P.Yu and H.S.Shadomy, ibid, 14, 99 (1978).
59. S.Shadomy, C.J.Utz and S.White, ibid., 14, 95 (1978).
60. H.B.Levine, S.M.Ringel and J.M.Cobb, Chest, 73, 202 (1978).
61. A.C.Huston and P.D.Hoeprich, Antimicrob.Ag.Chemother., 13, 905 (1978).
62. A.R.Mayrer, A.Brown, R.A. Weintraub, M.Ragni and B.Postic, Chest, 73, 546 (1978).
63. A.Polak and W.H.Wain in "Current Chemotherapy" Proceedings 10th International Congress of Chemotherapy, Eds. W.Siegenthaler and R.Lüthy, American Society for Microbiology, Washington, D.C., 1978, p. 213.
64. R.B.Diasio, J.E.Bennett and C.E.Myers, Bioch.Pharmacol., 27, 703 (1978).
65. J.E.Bennett, Ann.Intern.Med., 86, 319 (1977).
66. C.Ernst, J.Lematre, H.Rinnert, G.Dupont and J.Grange, C.R.Acad.Sci. (Paris), 289, 1145 (1979).
67. Kh.M.Kasumov, M.P.Borisova, L.N.Ermishkin, V.M.Potseluyev, A.Ya Silberstein and V.A. Vainshtein, Biochim.Biophys.Acta, 551, 229 (1979).
68. A.E.Elkhouly, Mykosen, 21, 318 (1978).
69. A.E.Elkhouly, ibid., 21, 300 (1978).
70. J.Kotler-Brajtburg, G.Medoff, G.S.Kobayashi, S.Boggs, D.Schlessinger, R.C.Pandey and K.L.Rinehart,Jr., Antimicrob.Ag.Chemother., 15, 716 (1979).
71. J.R.Little, T.J.Blanke, F.Valeriote and G.Medoff, in "Immune modulation and control of neoplasia by adjuvant therapy", Ed. M.A.Chirigos, Raven Press, New York, 1978, p. 381.
72. S.F.Shirley and J.R.Little, J.Immunol., 123, 2878 (1979).
73. S.F.Shirley and J.R.Little, ibid., 123, 2883 (1979).
74. H.J.Preuser and H.Rosteck, Sabouraudia, 17, 389 (1979).
75. H.Yamagushi and K.Iwata, ibid., 17, 311 (1979).
76. H.Van den Bossche, G.Willemsens, W.Cools, W.F.J.Lauwers and L.Le Jeune, Chem.Biol. Interact., 21, 59 (1978).
77. H.Van den Bossche, G.Willemsens, W.Cools, W.F.J.Lauwers and L.Le Jeune, in "Current Chemotherapy" Eds. W.Siegenthaler and R.Lüthy, American Society for Microbiology, Washington, D.C., 1978, p. 228.
78. M.J.Henry and H.D.Sisler, Antimicrob.Ag.Chemother., 15, 603 (1979).
79. M.R.Siegel and N.N.Ragsdale, Pestic.Biochim.Physiol., 9, 48 (1978).
80. H.Buchenauer, J.Plant Dis.Protec., 84, 440 (1977).
81. P.Leroux and M.Gredt, C.R. Acad.Sci.(Paris), 286, 427 (1978).
82. I.Haller, Abstracts XII International Congress Microbiology, Münich, Sept. 1978,No. 38/3.
83. H.Van den Bossche, G.Willemsens, W.Cools and F.Cornelissen, Abstract 109th meeting Belgian Biochemical Society, Arch.Int.Physiol.Biochim., 87, 849 (1979).
84. W.R.Nes, B.C.Sekula, W.D.Nes and J.H.Adler, J.Biol.Chem., 253, 6218 (1978).
85. K.E.Bloch, Crit.Rev.Biochem., 7, 1 (1979).
86. H.Yamaguchi, Antimicrob.Ag.Chemother., 13, 423 (1978).
87. H.Yamaguchi and K.Iwata, ibid., 15, 706 (1979).
88. I.J.Sud, D.L.Chou and D.S. Feingold, ibid., 16, 660 (1979).
89. K.Sakurai, T.Sakaguchi, H.Yamaguchi and K.Iwata, Chemotherapy, 24, 146 (1978).
90. K.Sakurai, T.Sakaguchi, H.Yamaguchi and K.Iwata, ibid., 24, 68 (1978).
91. A.L.Kisch, R.P.Maydew and A.P. Evan, J.Infect.Dis., 137, 789 (1978).
92. B.Dupont and E.Drouhet, Postgrad.Med.J., 55, 683 (1979).
93. W.H.Beggs, F.A.Andrews and G.A.Sarosi, Antimicrob.Ag.Chemother., 13, 266 (1978).
94. J.G.Grendahl and J.P.Sung, Milit.Med., 144, 474 (1979).
95. S.J.Davis, W.H.Donovan, Chest, 76, 235 (1979).
96. R.F.Cosgrove, A.E.Beezer and R.J.Miles, J.Inf.Dis., 138, 681 (1978).
97. G.A.Stern, Am.J.Ophtalmol., 86, 359 (1979).
98. B.E.Beyt,Jr., R.O. Cannow and P.G.Tuteur, South.Med.J., 71, 1164 (1978).
99. A.Polak, Chemotherapy, 24, 2 (1978).
100. J.S.Kivinen, T.Tavkila, L.Laakso and K.Laakso, Curr.Med.Res.Opin., 6, 88 (1979).
101. W.M.Artis, B.M.Odle and H.E.Jones, Archi.Dermatol., in press (1979).
102. J.T.Fell, R.T.Calvert and P.Riley-Bentham, J.Pharmac.Pharmacol., 30, 479 (1978).
103. C.F.Lopes, R.J.Alvarengo, E.O.Cisalpino, M.A.Resende and L.G.Oliveira, Int.J.Dermat., 17, 414 (1978).
104. C.A.Kaufmann, K.S.Israel, J.W.Smith, A.C.White, J.Schwarz and G.F.Brooks, Am.J.Med., 64, 923 (1978).
105. D.J.Drutz and R.I.Lehrer, Am.J.Med.Sci., 276, 77 (1978).
106. A.J.Atkinson,Jr. and J.E.Bennett, Antimicrob.Ag.Chemother., 13, 271 (1978).
107. E.Goldstein, Ann.Intern.Med., 89, 365 (1978).
108. R.J.Holt and A.Azmi, Infection, 7, 154 (1979).
109. M.Seo, H.Iida and J.Miura, Curr.Med.Res.Opin., 5, 169 (1977).
110. K.Kelleler and H.M.Gubbauer, Wien.Klin.Wochenschr., 90, 13 (1978).
111. C.H.Kirkpatrick and D.W.Alling, N.Engl.J.Med., 299, 1201 (1978).
112. R.Stigl and K.Lappy, Mykosen, 22, 341 (1979).
113. K.Blomqvist and M.Horsmanheimo, Acta Derm.Venereol., 58, 784 (1979).
114. T.R. Wade, H.E.Jones and J.J.Chanda, Arch.Intern.Med., 139, 784 (1979).

115. J.Wallin, Curr.Ther.Res., 23, 661 (1978).
116. J.Van Cutsem and A.Reyntjens, Mykosen, 21, 87 (1978).
117. G.W.Ratzmann, Z.Arztl.Fortbild., 71, 657 (1977).
118. L.D. Lilien, R.S. Ramanuerthy and R.S.Pildes, Pediatrics, 61, 57 (1978).
119. P.J.Chesney, R.A.Justman and W.M.Bogdanowitz, Johns Hopkins Med.J., 142, 155 (1978).
120. D.A.Stevens, A.Restapo-M, A.Cortes, J.Betancourt, J.N.Galgiani and J.Gomez, Am.J.Trop. Med.Hyg., 27, 801 (1978).
121. N.S.Lima, G.A.Teixeira, J.R.Miranda and A.C.F.Da Valle, Rev.Inst.Med.Trop.São Paulo, 20, 347 (1978).
122. L.J.Lutwick, M.W.Rytel, J.P.Yañez, J.N.Galgiani and D.A.Stevens, J.A.M.A., 241, 272 (1979).
123 R.Pradinaud, Mykosen, 21, 99 (1978).
124. J.F.Fischer, R.J.Duma, S.M.Markowitz, S.Shadomy, A.Espinell-Ingroff and W.H.Chew, Antimicrob.Ag.Chemother., 13, 965 (1978).
125. A.E.F.Davidson and J.K.Oates, Abstract 11th International Congress of Chemotherapy, Boston, October 1-5, 1979, No. 150.
126. E.Drouhet and B.Dupont, Mykosen, Suppl. 1, 192 (1978).
127. D.Hantschke, R.Schulte, S.Dabag and D.Greschuna, ibid., Suppl. 1, 230 (1978).
128. J.Bambule and D.Grigoriu, ibid., Suppl. 1, 87 (1978).
129. B.S.Verma, Curr.Ther.Res., 26, 634 (1979).
130. P.E.R.Rhemrev, M.C.Lestienne and K.Karoussos, J.Int.Med.Res., 7, 463 (1979).
131. L.Tanenbaum, C.Anderson, M.Chaplin, R.Jones, T.Matthews and K.Walker, Abstract 11th International Congress of Chemotherapy, Boston, October 1-5, 1979, No. 148.
132. J.Symoens, Postgrad.Med.J., 55, 697 (1979).
133. M.P.J.M.Bisschop, J.M.W.M.Merkus, H.Scheygrond, J.Van Cutsem and A.van de Kuy, Eur.J. Obstr.Gynec.Reprod.Biol., 9, 253 (1979).
134. A.A.Botter, F.Dethier, R.L.J.Mertens, J.Morias and W.Peremans, Mykosen, 22, 274 (1979).
135. D.Borelli, H.Rodriguez and C.Marcano, Rev.Fundacion. J.M. Vargas (Caracas), 3, 19 (1979).
136. R.Negroni, L.J.Conzalez Montaner, O.Palma Beltian, M.A.Tuculet and D.Rey, Rev.Arg.Micologia, 2, 12 (1979).
137. C.Brass, J.N.Galgiani, S.C.Campbell, R.A. O'Reilly and D.A.Stevens, Curr.Chemother. Infect.Dis., in press.
138. D.Lundberg, R.Graybill, W.Donovan, H.B.Levine, D.J.Drutz and M.Diaz, Abstract 11th International Congress of Chemotherapy, Boston, October 1-5, 1979, No. 147.
139. J.R.Graybill, H.B.Levine, J.H.Henderson and W.T.Kuiker, ibid., No. 153.

Chapter 16. Antiviral Agents

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The last review on antiviral agents in this series by C.E. Hoffman[1] concluded with the remark that "Antiviral chemotherapy has come of age." With the recent FDA approval of vidarabine for systemic use and the appearance of numerous books and review articles on antiviral drugs, this indeed appears to be the case. In fact, there have been so many new developments in the chemotherapy of DNA virus diseases alone that this review is confined to agents active against DNA viruses. Broader coverage of antiviral agents is provided by the excellent reviews of DeClercq[2] and Sidwell and Witkowski.[3] Additional reviews and books by Bauer,[4] Gauri,[5] Galasso et al.,[6] Oxford,[7] Suhadolnik,[8] Whitley and Alford,[9] Swallow[10] and Tamm and Sehgal[11] provide in depth coverage of many aspects of antiviral chemotherapy.

Despite the optimism of the preceeding paragraph, much remains to be done in the development of effective antiviral drugs. Although hundreds of compounds with antiviral activity have been identified,[3] few possess sufficient selectivity toward the virus to give good therapeutic responses in vivo. Because many antiviral agents act by inhibition of macromolecular biosynthesis, both cellular and viral functions can be affected. Inhibition of viral or cellular functions in virus-infected cells leads to an inhibition of virus replication. In contrast, inhibition of cellular functions in uninfected cells produces cytotoxicity in vitro that is manifested as bone marrow suppression, gut ulceration, immune suppression, etc. in vivo. Suppression of the immune system can be especially devastating in attempted antiviral therapy.[12] The need, therefore, is for antiviral drugs that inhibit viral replication without interfering with normal cell biosynthetic mechanisms.

Even though viruses depend on many host cellular functions to replicate, viruses also utilize a limited number of unique macromolecules such as cell surface receptors, nucleic acid sequences and virus-packaged or induced enzymes. Exploitation of such unique viral functions appears to be involved in the action of the only two drugs approved by the U.S. Food and Drug Administration for systemic administration (amantadine and vidarabine). The current challenge to medicinal chemists, therefore, is to utilize viral macromolecules as targets for chemotherapeutic agents.

DRUGS ACTIVE AGAINST DNA VIRUSES

The herpesviruses are the most important human pathogens among the DNA-containing viruses. There are five types of human herpesviruses. Herpes simplex virus (HSV) type 1 is responsible for a spectrum of infections which range from herpes labialis ("cold sores") to sight and life-threatening diseases such as herpes keratitis and encephalitis. HSV type 2 causes genital lesions in both males and females and may be transmitted to newborn during delivery. HSV type 2 also has been implicated as the

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etiologic agent in cervical neoplasia.[13] Varicella-zoster virus (VZV) is responsible for childhood chickenpox and may occur as shingles in adults. Cytomegalovirus causes an in utero or early postnatal infection that often is fatal or results in motor disability and mental retardation. Epstein-Barr virus is responsible for infectious mononucleosis and also may be the etiologic agent in Burkitt's lymphoma and postnasal carcinoma. All five of the herpesviruses can become latent after a primary infection and be reactivated at a later time. This pattern of dormacy and reactivation, despite the presence of circulating antibody, presents unique treatment problems.

One key to the treatment of herpesvirus infections is the presence in the infected cell of virus-induced enzymes. The bulk of the coding potential of new viral DNA can be described in terms of structural proteins and a few key enzymes involved in nucleic acid biosynthesis.[14-16] Several enzymes clearly have been identified as virus-coded, including DNA polymerase,[14] pyrimidine deoxyribonucleoside ("thymidine") kinase,[15] and deoxyribonuclease.[16] The first two enzymes now are known to have a role in the selective antiviral activity of several compounds. In the past few years a large amount of experimental work has been performed to develop drugs which utilize virus-induced enzymes as the basis for selective antiviral activity. These specific inhibitors fall into two general classes: (*i*) compounds that preferentially inhibit HSV-coded DNA polymerase, and (*ii*) fraudulent nucleosides that are converted to the active nucleotide only by HSV-coded thymidine kinase. In addition, there are specific inhibitors that act by other mechanisms such as disruption of the virus envelope and by yet undefined mechanisms.

Inhibitors of HSV DNA Polymerase

Vidarabine (9-β-D-arabinofuranosyladenine, ara-A, Vira-A™) - This drug has a firmly established role in the management of human herpesvirus infections. Previous studies with vidarabine, or adenine arabinoside as it has been improperly called,[17] have shown that the drug produces several biochemical actions.[18,19] The best evidence to date indicates that inhibition of HSV DNA synthesis most likely is responsible for the antiviral activity of the drug.[20] Inhibition of HSV DNA synthesis is a result of inhibition of HSV DNA polymerase by the 5'-triphosphate of vidarabine (aATP). Inhibition of HSV-1 DNA polymerase is potent and competitive with dATP.[21,22] The antiviral activity of vidarabine itself is antagonized in a competitive manner by deoxyadenosine,[23] thereby providing an additional link between DNA polymerase inhibition and antiviral activity. An earlier hypothesis that vidarabine acted as a chain-terminating nucleoside in viral DNA[24] was not confirmed by more recent work which found the compound is uniformly distributed throughout the viral genome and not concentrated at chain termini.[25] Even though vidarabine does not act as an absolute chain terminator, it is possible that incorporation of aAMP into DNA retards subsequent chain elongation. Recent work with mammalian DNA polymerase δ strengthens this possibility.[26] DNA polymerases α, β and γ from uninfected mammalian cells also are inhibited by aATP.[21,22,27,28] Inhibition of DNA polymerase α is more potent than inhibition of polymerases β and γ, but not as potent as inhibition of HSV-1 DNA polymerase.[21,22] Therefore, the selective action of vidarabine against HSV may be a consequence of the preferential action of aATP against HSV DNA polymerase. Because aATP also inhibits cellular DNA polymerases, the antiviral selectivity of vidarabine is not absolute. Inhibition of cellular DNA polymerases undoubtedly is related to the in vitro cytotoxicity[18] and in vivo teratogenicity,[29] and toxicity[30] noted at higher drug concentrations. Besides inhibiting DNA

polymerase, aATP is a potent inhibitor of polyadenylation of RNA.[31] Vidarabine also may exert some toxic effects directly, that is without metabolism to aATP, by suicide inactivation of S-adenosylhomocysteine hydrolase.[32]

Extensive evidence reviewed in previous articles has clearly established the antiviral efficacy of vidarabine in cell culture, in animal models and in humans.[1-10] Continuing in vitro studies have examined combinations of vidarabine and other agents. Combining the adenosine deaminase inhibitors coformycin or pentostatin with vidarabine resulted in enhanced activity against adenovirus replication,[33] as well as against mouse L5178Y cells,[34] and increased biological half-life of aATP.[35] Combinations of vidarabine and deaminase inhibitors administered to mice caused increased plasma levels and urinary recovery of vidarabine.[36] Other animal work showed vidarabine ointment is not as effective as phosphonoacetate ointment in preventing the establishment of latent ganglionic infection in mice.[37]

The clinical efficacy of vidarabine ointment against ocular herpes keratitis is well established.[4,6,18] Recent studies also found that a water-miscible gel preparation reduced lesion size in herpes labialis.[38] Vidarabine ointment, however, is inactive against genital herpes in men.[39] These data illustrate the need for work on topical antiviral drug delivery formulations. The carefully documented activity of vidarabine against human herpes encephalitis and herpes zoster in immunosuppressed patients[1,6,9] has been expanded in the last two years.[40] In addition, results of preliminary studies in a limited number of patients suggest that hepatitis B virus replication may be blocked by vidarabine.[41]

Despite the efficacy and safety of vidarabine in its antiviral dose range, the drug has several disadvantages. First, it is metabolized to the less active 9-β-D-arabinofuranosylhypoxanthine (ara-H) by adenosine deaminase; second it does not readily penetrate intact skin and mucous membranes; and third, its low aqueous solubility necessitates the i.v. administration of large fluid volumes for systemic therapy. In attempts to circumvent these problems, a large number of analogs have been synthesized. Adenosine deaminase-resistant nucleosides have been prepared and tested for antiviral and antineoplastic activity. The more interesting deaminase-resistant analogs include 2-fluorovidarabine,[42] carbocyclic vidarabine,[43] and 2'-azido-vidarabine.[44] In attempts to overcome poor topical penetrability of vidarabine, a number of O-acyl ester prodrugs have been prepared. One of these, 2',3'-di-O-acetyl vidarabine, is active in a guinea pig model of genital herpes.[45] Another, vidarabine-5'-valerate, showed good penetrability through skin in a model system.[46] In addition, this prodrug is an inhibitor but not a substrate of adenosine deaminase, thereby providing a powerful combination of activities.[47] The 5'-monophosphate of vidarabine (aAMP) also functions as a highly water soluble prodrug. Its effectiveness in vitro and in animal models[1,3,6,45,48] has now been demonstrated clinically. Results from initial, uncontrolled studies indicate that the water-soluble prodrug is as safe and effective as vidarabine in patients with severe herpesvirus infections including herpes encephalitis and herpes zoster.[49,50] The compound also has been tested as a topical preparation. It is not effective against herpes labialis when used as a 10% cream in a large, well controlled study.[51] However, when aAMP was applied topically and delivered by cathodal iontophoresis, it was highly efficacious in two animal models.[52] The 5'-monophosphate of ara-H (aHMP) also is active against experimental herpesvirus infections.[3]

Cytarabine (1-β-D-arabinofuranosylcytosine, ara-C, Cytosar™) - Cytarabine is a valuable antineoplastic drug but generally is not considered to be a useful antiviral agent. This is because cytarabine is more effective as an inhibitor of cellular DNA synthesis than of herpes virus DNA synthesis.[20,53] Although aCTP shows some selectivity toward HSV DNA polymerase,[22] the advantage is more than offset by lower aCTP levels in virus-infected cells compared to uninfected cells.[53] Lower aCTP levels could result from virus induction of deoxycytidine deaminase,[15] an enzyme which may be capable of deaminating and thereby detoxifying cytarabine.[18] The net result of such reverse biochemical selectivity is suppression of host virus defense mechanisms and drug failure in systemic antiviral therapy. Controlled clinical studies have found, in fact, that cytarabine was either no better than or worse than placebo in treating herpes zoster.[54]

Although cytarabine has not proven to be an effective antiviral agent, a new series of 5-substituted 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)pyrimidines have exciting antiviral potential.[55] In particular, 2'-fluoro-5-iodo-ara-C has shown exceptional potency against HSV-1 (ID_{50} <0.01 μg/ml) while exhibiting only moderate cytotoxicity in uninfected cells (ID_{50} = 14 - 48 μg/ml). Although the mode of action is unknown, HSV-induced enzymes probably are responsible for such antiviral selectivity.

Phosphonates - Phosphonoacetate (PA) is an effective inhibitor of the replication of human herpesviruses, both in cell culture and in experimental animals.[56] Detailed studies on PA-resistant mutants of HSV and their revertants provide convincing evidence that PA inhibits HSV replication by specific inhibition of HSV DNA polymerase.[56-58] Interaction occurs at the pyrophosphate binding site. Inhibition appears to be selective but the compound does inhibit cellular DNA synthesis[20] and produce cytotoxicity at higher concentrations.[56] Limited toxicity and metabolic disposition studies have been performed which indicate potential difficulty for systemic use of PA.[56] Clinical trials have not been undertaken.

An analog of PA, phosphonoformate (PF), also is an inhibitor of DNA polymerase induced by herpesviruses.[59-61] PF, but not PA, also inhibits DNA polymerase associated with hepatitis B Dane particles.[62] PF inhibits the replication of HSV-1 and HSV-2[60,63] at concentrations which are not toxic to uninfected cells.[60,64] Although PF is only minimally effective when administered systemically to mice,[63] it has excellent activity when applied topically to cutaneous[60,65,66] and vaginal HSV infections.[63,67] Based upon these favorable topical efficacy and safety studies in animals, a clinical trial of locally-applied PF cream against recurrent herpes labialis now is underway.[68]

Inhibitors Activated by HSV Pyrimidine Deoxyribonucleoside Kinase

Background - Most, if not all, fraudulent nucleosides must be metabolized to nucleotides to be active antiviral agents. Several fraudulent nucleosides recently have been identified as selective antiviral agents. Selectivity against herpesviruses arises because HSV-coded pyrimidine deoxyribonucleoside kinase (HSV "thymidine" kinase) recognizes them as substrates whereas kinases in uninfected cells do not. Upon conversion to nucleotides these compounds are potent inhibitors of viral and cellular functions -- but only in HSV-infected cells. Other fraudulent nucleosides, e.g., vidarabine, that are phosphorylated by cellular kinases either are not selective against HSV or derive specificity from other biochemical mechanisms.

Idoxuridine (5-iodo-2'-deoxyuridine, IDU, Stoxil™) - Idoxuridine is phosphorylated by both cellular and viral thymidine kinases;[69] thus the active nucleotide forms in both uninfected and HSV-infected cells. HSV-infected cells do, however, have markedly elevated levels of thymidine kinase[15] so that idoxuridine exhibits some antiviral specificity in vitro.[70] Rapidly proliferating normal tissues also have elevated thymidine kinase activity and consequently are susceptible to the action of the drug. As a result, toxicity and teratogenicity have been observed in animals,[69,71] and bone marrow suppression has been reported in humans following parenteral administration of the drug.[72] Such toxicity is not significant, however, when the drug is applied topically. Therefore, because of its potent antiviral activity, IDU is an established, important drug for treating herpes keratitis.[3,4,6] Recent additional interest in idoxuridine involves the topical application of the drug as a dimethylsulfoxide (DMSO) solution. In DMSO, idoxuridine readily penetrates the skin. In one study, a 20% solution in DMSO was active against genital herpes infections.[73] In another recent controlled trial, 30% idoxuridine in DMSO shortened viral shedding time in recurrent genital herpes in men but not in women. There was no positive impact on a number of clinical parameters in either men or women.[74]

Trifluridine (5-trifluoromethyl-2'-deoxyuridine, trifluorothymidine, F_3TdR) Like idoxuridine, trifluridine is phosphorylated by both cellular and viral kinases although there may be some specific dependence on the viral enzyme.[75] Ultimately, trifluridine is incorporated into viral and cellular DNA thereby interfering with messenger RNA synthesis.[76] Although trifluridine is preferentially incorporated into vaccinia virus DNA,[76] the nucleotides of trifluridine also inhibit cellular and viral DNA polymerases and cellular thymidylate synthetase.[76] The net result of these actions is potent antiviral activity and cytotoxicity.[70,76] Trifluridine has not been tested extensively by systemic administration in vivo, but has been critically evaluated topically against both experimental and clinical herpes and adenovirus eye infections.[76] In human clinical trials, drops of 1% trifluridine have generally been superior to idoxuridine drops and somewhat better than vidarabine ointment.[76] In addition, trifluridine is effective in patients with herpes keratitis that are unresponsive to idoxuridine or vidarabine.[76,77] FDA approval is being sought to market the drug in the U.S.

Aminoidoxuridine (5-iodo-5'-amino-2',5'-dideoxyuridine, AIU) - An analog of idoxuridine, AIU inhibits the replication of HSV-1, HSV-2, and VZV without significant host cell toxicity.[2,3,69,78] The reason for this remarkable specificity is the direct phosphorylation of the 5'-amino group by the HSV-coded kinase creating a phosphoramidate bond.[79] Conversion to the di-[79] and triphosphate follows with ultimate incorporation into DNA.[69] Unlike idoxuridine and trifluridine, phosphorylation does not occur in uninfected cells thereby sparing them from the toxic effects of the compound. Chemotherapy trials in animals have been positive, but the potency of the compound is less than that of idoxuridine or trifluridine.[2,69] Initial systemic toxicity studies have been encouraging,[80] but have not progressed far enough to support human clinical trials.

Other 5-Substituted-2'-deoxyuridines (5-substituted-dUrd) - Based on the antiviral activity of idoxuridine and trifluridine, a number of additional 2'-deoxyuridines have been prepared and tested. These include 5-ethyl-, 5-propyl-, 5-nitro-, 5-allyl-, 5-methylthiomethyl-, 5-ethynyl- and substituted 5-vinyl-2'-deoxyuridines.[70,81,82,83,84] In general, these compounds have very good in vitro antiviral selectivity and potency against HSV-1

and HSV-2. The selectivity and potency of E-5-(2-bromovinyl)-2'-deoxyuridine and its iodo analog are truly exceptional; 7000-10,000-fold higher concentrations are required to produce cytotoxicity than are needed for antiviral activity.[70] The antiviral selectivity of these congeners most likely is a result of selective phosphorylation by the virus-coded kinase.[83] The actual biochemical basis for the antiviral activity is unknown,[70] however, with the exception of recent data on the 5-propyl analog. This compound inhibits the synthesis of virus-specified deoxyribonuclease and DNA polymerase in a manner that correlates with inhibition of virus replication.[83] In addition, the monophosphates of 5-nitro-dUrd, 5-cyano-dUrd, etc. are inhibitors of thymidylate synthetase; it is doubtful, however, that this is the basis of antiviral activity.[2]

The development of the 5-ethyl compound is most advanced. In addition to its activity in vitro,[70,81] it is active against disseminated HSV-2 infections in mice[81] and herpes keratitis in rabbits[5,85] and humans.[85] Nonetheless, the 2-bromovinyl derivative[70] may be the most promising compound in the series. Not only is it very potent and highly selective in vitro,[70] it also is active topically and intraperitoneally against cutaneous HSV-1 infections.[70,86] In preliminary acute toxicity experiments, 600 mg/kg was well tolerated in mice.[70] The drug also appears to be well absorbed by the oral, subcutaneous and intraperitoneal routes of administration.[87] Blood levels were approximately 10-times higher than those produced by an equivalent dose of idoxuridine.

5-Substituted-arabinosyluridines (5-substituted ara-U) - The most extensively studied member in this series is the naturally-occurring 5-methyl compound, arabinosylthymine (1-β-D-arabinofuranosylthymine, ara-T).[88] Selectivity against herpesvirus-infected cells is a consequence of the preferential phosphorylation of ara-T by HSV thymidine kinase,[88] although some cellular kinase(s) may phosphorylate the nucleoside as well.[89] In most cases, ara-T is highly potent and remarkably selective against herpesviruses, producing little toxicity in uninfected cells.[70,88] Antiviral activity of ara-T, and cytotoxicity in those cases where it occurs,[90] most likely is a consequence of incorporation of the analog into cellular DNA[89] and inhibition of viral and cellular DNA polymerase by aTTP.[89,91] To date, only limited in vivo data, and no clinical data, are available. These show that ara-T is effective in protecting hamsters from lethal infection with equine herpesvirus.[2,88] Ara-T also may have immunosuppressive activity.[88,92]

Other ara-U analogs that have been prepared and tested include the 5-ethyl, 5-propyl, 5-methoxymethyl, 5-cyano-and 5-nitro compounds.[93,94] Of these, 5-ethyl-ara-U is nearly as active as ara-T against HSV-1 and even less cytotoxic in uninfected cells.[93,94] Antiviral specificity probably is the result of selective phosphorylation by HSV thymidine kinase, because the triphosphates of 5-ethyl- and 5-propyl-ara-U inhibit mammalian DNA polymerases.[95] Efficacy data from animal models have not yet been published.

Acyclovir [acycloguanosine, 9-(2-hydroxyethoxymethyl)guanine, ACG, Zovirax] Acyclovir is a new compound, first described in late 1977, that has unique biochemical specificity for herpesviruses.[96] The compound is a very potent inhibitor of HSV-1, HSV-2 and VZV replication which produces little cytotoxicity in uninfected cells.[70,96-99] It is highly specific because it is phosphorylated by herpesvirus thymidine kinase, but not by cellular kinases.[96,100] The phosphorylation of this purine analog by a virus-coded pyrimidine kinase is quite surprising. Viral specificity also is a conse-

quence of more potent inhibition (2-50 fold) of HSV DNA polymerase than of mammalian DNA polymerase α by acyclo-GTP.[96,101] The involvement of two different herpesvirus-coded enzymes in eliciting selective antiviral activity is unique among currently recognized inhibitors. Additional evidence for the involvement of two virus-coded enzymes has been provided by studies with HSV mutants resistent to acyclovir.[102]

In vivo, the drug is active against cutaneous HSV infections when administered topically[97,103] or systemically.[104] It also is effective against experimental herpes keratitis when administered by both these routes.[105,106] In all of these studies,[103-106] acyclovir prevented establishment of latent ganglionic infection. In another study, the systemic administration of either acyclovir or vidarabine actually reduced established latent infections.[107] Intravenous administration is effective in preventing death from herpes encephalitis in mice[97] or rabbits.[105] Toxicity studies show acyclovir is well tolerated by the oral and intraperitoneal routes of administration (acute LD_{50} in mice=10,000 and 1,000 mg/kg, respectively).[97] The drug is incompletely absorbed by the oral route when administered to mice, but is distributed to all tissues following subcutaneous administration. Highest concentrations are found in kidney and intestine, lowest in the brain.[97] In humans, plasma concentrations of acyclovir decline biphasically following intravenous administration of 0.5 to 5.0 mg/kg.[108] Urinary excretion of unchanged drug accounts for 30 to 69% of the dose; a metabolite, 9-carboxymethoxymethyl-guanine, accounts for an additional 2-14%.[108]

Initial clinical work in humans already has been performed. An eye ointment of 3% acyclovir was effective against herpes keratitis in a controlled study.[109] In an open study, acyclovir administered intravenously at 5 mg/kg appears to be effective against localized and disseminated HSV and VZV infections.[110] In another study, no toxicity was observed at this dose level.[108] Additional controlled clinical trials are underway.

5,6-Dihydro-5-azathymidine (DHAdT) - DHAdT is a nucleoside antibiotic that inhibits HSV-1 replication at concentrations not toxic to uninfected cells. Although the mechanism of selectivity is unknown, the compound is active against HSV-1 and HSV-2 infections in experimental animals.[111]

Inhibitors That Act by Other Mechanisms

2-Deoxy-D-glucose - This glucose analog interferes with the replication of enveloped viruses including HSV. The compound is incorporated into cellular and viral glycoproteins and glycolipids,[112] thereby preventing the formation of longer chain homologs and disrupting the integrity of the lipid-containing viral envelope. In the only clinical trial to date, application of a 0.19% concentration of 2-deoxy-D-glucose in a miconazole nitrate cream reduced lesion duration and time of positive virus culture in women with genital HSV infections.[113]

Other Agents that Disrupt Viral Envelopes - Nonionic surfactants destroy the infectivity of HSV in vitro by dissolving the lipid-containing envelope.[114] Proprietary vaginal contraceptive formulations containing nonionic surfactants also inactivate HSV in vitro.[114] No beneficial effect was noted, however, in a well controlled clinical study that used a cream preparation of one of the surfactants (Nonoxynol 9) to treat genital herpes.[115]

Diethyl ether is a lipid solvent that will dissolve virus envelopes. On the basis of anecdotal evidence, it has been widely recommended for the treatment of herpes labialis. Two recent well controlled clinical studies found no evidence, however, that ether was of value in either lip[116] or genital[117] HSV infections.

Agents that Disrupt DNA - Certain dyes, after excitation by visible light, inactivate microorganisms in the presence of molecular oxygen. This "photodynamic inactivation" has been used with DNA intercalating dyes to inactivate HSV.[118] The dyes may not act by DNA intercalation, however, and may not be selective for the viruses. Nonetheless, intercalating dyes have been used for the treatment of HSV-1 and HSV-2 lesions.[118] Initial clinical work with topically applied neutral red dye appeared to be efficacious and popularized the treatment modality. More recent clinical studies, however, have failed to demonstrate efficacy for either neutral red or proflavine dyes.[119] In addition, photoinactivated HSV-1 and HSV-2 are able to transform cultured cells to the neoplastic state.[118] Continued use of the treatment seems unjustified.[119]

Certain divalent cations, especially zinc, may crosslink DNA. Based on this observation, various zinc salts have been evaluated against HSV in cell culture and in experimental animals. When applied topically by ultrasound or in vaginal sponges saturated with a zinc solution, HSV-2 titers in animal lesions were reduced and rates of healing increased.[120,121] In an initial clinical trial in men, application with ultrasound of a zinc sulfate, urea and tannic acid cream may have been beneficial.[121]

BROAD SPECTRUM ANTIVIRAL AGENTS

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxmide, Virazole™) - This synthetic, truncated nucleoside inhibits the replication of both RNA and DNA viruses *in vitro* and *in vivo*.[122] Activity has been confirmed against more than 12 DNA viruses and 27 RNA viruses. In clinical trials the drug has shown promise against type A hepatitis, influenza A, herpes zoster and herpes genitalis.[122] In both *in vitro* and *in vivo* studies, ribavirin has produced minimal toxicity in its antiviral dose range.[122]

The exact biochemical basis for the selective antiviral action of ribavirin is unknown. It is known, however, that the mode of action of ribavirin involves inhibition of nucleic acid biosynthesis.[122] Inhibition of DNA and RNA synthesis apparently is caused by nucleotide metabolites of ribavirin inhibiting IMP dehydrogenase and/or influenza virus RNA polymerase.[1,122] Although selective inhibition of the viral RNA polymerase by ribavirin triphosphate could explain antiviral selectivity against influenza virus, inhibition of neither enzyme explains selectivity against other viruses, especially DNA viruses. Recent data that ribavirin triphosphate inhibits capping of messenger RNA (mRNA)[123] provides new insight into why the drug acts against both DNA and RNA viruses. Because blockage of capping was demonstrated by inhibition of vaccinia virus mRNA guanylyltransferase (K_i = 32 μM),[123] it is possible that this inhibition also may contribute to antiviral selectivity. Beside affecting RNA synthesis, ribavirin also inhibits DNA synthesis.[122] However, inhibition of DNA synthesis in uninfected cells is not nearly as potent as previously assumed.[20] Recent data show that the use of [^{3}H]thymidine incorporation does not correctly measure DNA synthesis in ribavirin-treated mammalian cells.[124]

In addition to antiviral activity, extensive animal and human studies have explored the toxicity, teratogenicity and metabolic disposition of the drug.[122] Inadequate drug distribution to the respiratory tract following oral administration, in fact, may be responsible for the variable clinical efficacy of ribavirin against influenza.[122] In contrast, if the drug is administered as a small particle aerosol to experimentally infected animals, dramatic results are seen.[122,125] In the most recent study where the drug was given by inhalation, ribavirin significantly increased the number of mice surviving influenza A or B infections. In the same studies, amantadine was not as effective, but a combination of amantadine and ribavirin was more effective than either drug alone. Human clinical trials are underway.

(S)-9-(2,3-Dihydroxypropyl)adenine [(S)-DHPA] - (S)-DHPA is one of two antiviral nucleosides in which the sugar is replaced by an aliphatic side chain. Unlike acyclovir, whose reported activity is restricted to herpesviruses,[96] (S)-DHPA is active against both DNA and RNA viruses.[126,127] The compound is active against herpes, vaccinia, vesicular stomatitis (VSV) and measles virus at concentrations that do not affect cellular viability or DNA, RNA and protein synthesis. A number of other aliphatic nucleosides, including the (R)-enantiomer, (R)-DHPA, are not active.[126,127] Neither the mode of action nor the basis for antiviral selectivity of (S)-DHPA are known. In preliminary animal work, (S)-DHPA reduced by one-half mortality caused by intranasal inoculation of mice with VSV.

Arildone (4-[6-(2-chloro-4-methoxyl)phenoxyl]hexyl-3,5-heptanedione, Win 38020) - Arildone is one of a series of β-diketones that are active against DNA and RNA viruses.[1] These compounds are potent inhibitors of herpes, rhino, parainfluenza and respiratory syncytial viruses in cell culture.[128] At concentrations needed for antiviral activity, arildone does not adversely affect uninfected cells.[128,129] In vivo, arildone and analogs are particularly effective against HSV. Topical application in DMSO or a vanishing cream base is effective in a guinea pig cutaneous model and a mouse vaginal model.[128]

Like ribavirin, arildone is not virucidal and does not induce interferon production in cultured cells or in mice.[128] In HSV-2 infected cells it selectively inhibits viral DNA and protein synthesis by an undetermined mechanism.[130] Arildone also is not virucidal against polioviruses and does not interfere with adsorption or penetration. It does, nonetheless, inhibit the uncoating of poliovirus and thereby prevents virus-induced blockage of host cell protein synthesis.[129]

Interferon, Interferon Inducers and Immunomodulators - A discussion of these agents is beyond the scope of the present review. Detailed, additional information can be found in reviews on the antiviral activity of interferons,[131] the antiviral effects of polynucleotides,[132] the potential of oligonucleotides to inactivate specific viral nucleotide sequences,[132,133] and the antiviral activity of immunopotentiating substances.[134] In summary, these agents have had various degrees of success in well controlled clinical trials. For example, human leukocyte interferon was found to be effective against localized herpes zoster in cancer patients,[135] whereas levamisole (an immunomodulator) was without benefit in recurrent herpes labialis[134,136] and herpes genitalis.[137]

CONCLUSIONS

An enormous number of compounds have been synthesized and tested as potential antiviral agents yet very few are suitable for human use.[138] It now seems clear only those that act by selectivity utilizing or inhibiting unique viral functions will be developed into drugs. In order to detect such compounds, biochemical, in vitro and in vivo tests must be used that distinguish between viral and cellular functions. Furthermore, as the final step in the developmental process, well controlled and blinded clinical studies are required to establish the efficacy of antiviral drugs in humans.

REFERENCES

1. C. E. Hoffman, Ann. Rep. Med. Chem., 13, 139 (1978).
2. E. De Clercq, Arch. Int. Physiol. Biochem., 87, 353 (1979).
3. R. W. Sidwell and J. T. Witkowski in "Burger's Medicinal Chemistry," 4th ed., Part II, M. E. Wolff, Ed., John Wiley and Sons, New York, NY, 1979, Chapter 23, pp. 543-593.
4. D. J. Bauer, "The Specific Treatment of Virus Diseases," University Park Press, Baltimore, MD, 1977.
5. K. K. Gauri, Ed., "Anti-Herpesvirus Chemotherapy: Experimental and Clinical Aspects," Vol. 38 of Adv. Opthalmol., S. Karger, Basel, 1979.
6. G. J. Galasso, T. C. Merigan and R. A. Buchanan, Eds., "Antiviral Agents and Viral Diseases of Man," Raven Press, New York, NY, 1979.
7. J. S. Oxford, Ed., "Chemoprophylaxis and Virus Infections of the Respiratory Tract," Vols. I and II, CRC Press, Cleveland, OH, 1977.
8. R. J. Suhadolnik, "Nucleosides as Biological Probes," John Wiley and Sons, New York, NY, 1979.
9. R. J. Whitley and C. A. Alford, Ann. Rev. Microbiol., 32, 285 (1978).
10. D. L. Swallow, Prog. Drug Res., 22, 268 (1978).
11. I. Tamm and P. B. Sehgal, Adv. Virus Res., 22, 187 (1978).
12. T. C. Merigan and H. E. Renis in "Nucleoside Analogues; Chemistry, Biology and Medical Applications," R. T. Walker, E. De Clercq and F. Eckstein, Eds., Plenum Press, New York, NY, 1979, pp. 395-407.
13. F. Rapp, Amer. Scientist, 66, 670 (1978).
14. K. L. Powell and D. J. M. Purifoy, J. Virol., 24, 618 (1977).
15. S. Kit, Pharmacol. Therap., 4, 501 (1979).
16. P. J. Hoffman and Y-C. Cheng, J. Biol. Chem., 253, 3557 (1978).
17. J. C. Drach, Chem. Eng. News, 55 (40), 55 (1977).
18. T. W. North and S. S. Cohen, Pharmacol. Therap., 4, 81 (1979).
19. C. E. Cass in "Antibiotics, Mechanisms of Action," Vol. 5, F. E. Hahn, Ed., Springer-Verlag, Berlin, 1979.
20. J. C. Drach and C. Shipman, Jr., Ann. N.Y. Acad. Sci., 284, 396 (1977).
21. W. E. G. Müller, R. K. Zahn and D. Falke, Virology, 84, 320 (1978).
22. C. M. Reinke, J. C. Drach, C. Shipman, Jr., and A. Weissbach in "Oncogenesis and Herpesviruses III," Part 2, G. de The, W. Henle and F. Rapp, Eds., IARC Press, Lyon, France, 1978, pp. 999-1005.
23. S. H. Smith, C. Shipman, Jr. and J. C. Drach, Cancer Res., 38, 1916 (1978).
24. W. E. G. Müller, R. K. Zahn, R. Beyer and D. Falke, Virology, 76, 787 (1977).
25. J. C. Pelling, J. C. Drach and C. Shipman, Jr., Abstracts, 79th Annual Meeting of the American Society for Microbiology, Los Angeles, CA, May 1979, No. A26.
26. M. Y. W. Tsang Lee, J. J. Byrnes, K. M. Downey and A. G. So, Biochemistry, 19, 215 (1980).
27. A. Okura and S. Yoshida, J. Biochem., 84, 727 (1978).
28. K. Ono, A. Ohashi, A. Yamamoto, A. Matsukago, T. Takahasi, M. Saneyoshi and T. Ueda, Cancer Res. 39, 4673 (1979).
29. J. L. Schardein, D. L. Hentz, J. A. Petrere, J. E. Fitzgerald and S. M. Kurtz, Teratology, 15, 231 (1977).
30. B. Hafkin, R. B. Pollard, M. L. Tiku, W. S. Robinson and T. C. Merigan, Antimicrob. Agents Chemother., 16, 781 (1979).
31. T. B. Leonard and S. T. Jacob, Biochim. Biophys. Acta, 563, 150 (1979).
32. M. S. Hershfield, J. Biol. Chem., 254, 22 (1979).
33. R. Wigand, J. Med. Virol., 4, 59 (1979).
34. W. E. G. Müller, R. K. Zahn, J. Arendes, A. Maidhof and H. Umezawa, Z. Physiol. Chem., 359, 1287 (1978).
35. D. S. Schewach and W. Plunkett, Biochem. Pharmacol., 28, 2401 (1979).
36. W. J. Suling, L. S. Rice and W. M. Shannon, Cancer Treat. Rep., 62, 369 (1978).

37. R. J. Klein, A. E. Friedman-Kien and P. B. Yellin, Infect. Immun., 20, 130 (1978).
38. N. H. Rowe, S. L. Brooks, S. K. Young, J. Spencer, T. J. Petrick, R. A. Buchanan, J. C. Drach and C. Shipman, Jr., Oral Surg., 47, 142 (1979).
39. A. L. Hilton, T. E. C. Bushell, D. Waller and J. Blight, Brit. J. Venereal Dis., 54, 50 (1978).
40. G. M. Maxwell, Y. H. Thong, J. I. Manson and C. F. Robertson, Med. J. Australia, 1, 181 (1978).
41. R. B. Pollard, J. L. Smith, E. A. Neal, P. B. Gregory, T. C. Merigan and W. S. Robinson, J. Amer. Med. Assoc., 239, 1648 (1978).
42. R. W. Brockman, F. M. Schabel, Jr. and J. A. Montgomery, Biochem. Pharmacol., 26, 2193 (1977).
43. R. Vince and S. Daluge, J. Med. Chem., 20, 612 (1977).
44. C. M. Cermak-Mörth, R. Christian and F. M. Unger, Biochem. Pharmacol., 28, 2105 (1979).
45. D. C. Baker, T. H. Haskell, S. R. Putt and B. J. Sloan, J. Med. Chem., 22, 273 (1979).
46. C. D. Yu, J. L. Fox, N. F. Ho and W. I. Higuchi, J. Pharm. Sci., 11, 1347 (1979).
47. R. A. Lipper, S. M. Machkovech, J. C. Drach and W. I. Higuchi, Molec. Pharmacol., 14, 366 (1978).
48. M. G. Falcon and B. R. Jones, J. Gen. Virol., 36, 199 (1977).
49. O. Sauer, G. T. Werner, H. Schneider, H. G. Lenard, L. V. Haselberg and H.-J. Nettesheim, Klin. Pädiat., 191, 566 (1979).
50. D. L. J. Tyrrell, T. Ti and G. A. Le Page in "Current Chemotherapy: Proceedings of the 11th International Congress of Chemotherapy," America Society for Microbiology, Washington, D.C., 1980, in press.
51. S. L. Spruance, C. J. Crumpacker, H. Haines, C. Bader, K. Mehr, J. MacCalman, L. E. Schnipper, M. R. Klauber, J. C. Overall and the Collaborative Study Group, N. Engl. J. Med., 300, 1180 (1979).
52. B. S. Kwon, L. P. Gangarosa, N.-H. Park, D. S. Hull, E. Fineberg, C. Wiggins and J. M. Hill, Invest. Ophthamol., 18, 984 (1979).
53. T. Ben-Porat, M. Brown and A. S. Kaplan, Molec. Pharmacol., 4, 139 (1968).
54. C. E. Orfanos, A. Weese, U. Runne, J. Kratka and G. Goerz, Dtsch. Med. Wochenschr., 102, 312 (1977).
55. J. J. Fox, K. A. Watanabe, R. S. Klein, C. K. Chu, S. Y.-K. Tam, U. Reichman, K. Hirota, I. Wempen, C. Lopez and J. H. Burchenal in "Nucleosides, Nucleotides and their Biological Applications," INSERM Symposia Series, Vol. 81, J. L. Barascut and J. L. Imbach, Eds., INSERM, Paris, 1979, pp. 241-270.
56. J. A. Boezi, Pharmacol. Ther., 4, 231 (1979).
57. D. M. Knipe, W. T. Ruyechan and B. Roizman, J. Virol., 29, 698 (1979).
58. R. G. Duff, E. E. Robishaw, J. C.-H. Mao and L. R. Overby, Intervirol., 9, 193 (1978).
59. J. M. Reno, L. F. Lee and J. A. Boezi, Antimicrob. Agents Chemother., 13, 188 (1978).
60. E. Helgstrand, B. Ericksson, N. G. Johansson, B. Lannerö, A. Larsson, A. Misiorny, J. O. Norén, B. Sjöberg, K. Stenberg, G. Stening, S. Stridh, B. Öberg, S. Alenius and L. Philipson, Science, 201, 819 (1978).
61. B. Eriksson and B. Öberg, Antimicrob. Agents Chemother., 15, 758 (1979).
62. E. Nordenfelt, E. Helgstrand and B. Öberg, Acta Path. Microbiol. Scand. Sect. B, 87, 75 (1979).
63. E. R. Kern, L. A. Glasgow, J. C. Overall, Jr., J. M. Reno and J. A. Boezi, Antimicrob. Agents Chemother., 14, 817 (1978).
64. K. Stenberg and A. Larsson, ibid, 14, 727 (1978).
65. S. Alenius, Z. Dinter and B. Öberg, ibid, 14, 408 (1978).
66. R. J. Klein, E. De Stefano, E. Brady and E. E. Friedman-Kien, ibid, 16, 266 (1979).
67. S. Alenius and H. Nordlinder, Arch. Virol., 60, 197 (1979).
68. J. Wallin, E. Lycke, E. Helgstrand, B. Öberg and J.-O. Lernestedt in "Current Chemotherapy: Proceedings of the 11th International Congress of Chemotherapy," American Society for Microbiology, Washington, D.C., 1980, in press.
69. W. H. Prusoff, M. S. Chen, P. H. Fischer, T-S. Lin, G. T. Shiau, R. F. Schinazi and J. Walker, Pharmacol. Therap., 7, 1 (1979).
70. E. De Clercq, J. Descamps, P. DeSomer, P. J. Barr, A. S. Jones and R. T. Walker, Proc. Nat. Acad. Sci. U.S., 76, 2947 (1979).
71. B. Goz, Pharmacol. Rev., 29, 249 (1978).
72. Boston Interhospital Virus Study Group, New Engl. J. Med., 292, 599 (1975).
73. J. D. Parker in "Chemotherapy of Herpes Simplex Virus Infection," J. S. Oxford, F. A. Drasar and J. D. Williams, Eds., Academic Press, London, 1977, pp. 131-137.
74. D. L. Silvestri, L. Corey, C. Winter, M. Remington and K. K. Holmes in "Current Chemotherapy: Proceedings of the 11th International Congress of Chemotherapy," American Society for Microbiology, Washington, D.C., 1980, in press.
75. E. De Clercq, E. Krajewska, J. Descamps and P. E. Torrence, Molec. Pharmacol., 13, 980 (1977).
76. C. Heidelberger and D. H. King, Pharmacol. Therap., 6, 427 (1979).
77. R. A. Hyndiuk, R. E. Charlin, T. V. P. Alpren and R. O. Schultz, Arch. Ophthalmol., 96, 1839 (1978).

78. J. P. Iltis, T-S. Lin, W. H. Prusoff and F. Rapp, Antimicrob. Agents Chemother., 16, 92 (1979).
79. M. S. Chen and W. H. Prusoff, J. Biol. Chem., 254, 10449 (1979).
80. D. M. Albert, D. H. Percy, C. A. Puliafito, E. Fritsch, D. Pavan-Langston, T-S. Lin, D. C. Ward and W. H. Prusoff, Adv. Ophthalmol., 38, 89 (1979).
81. W. B. Davis, J. E. Oakes and J. A. Taylor, Antimicrob. Agents Chemother., 14, 743 (1978).
82. E. De Clercq, J. Descamps and D. Shugar, ibid, 13, 545 (1978).
83. Y-C. Cheng, S. Grill and G. Dutschman, Biochem. Pharmacol., 28, 3529 (1979).
84. E. De Clercq, J. Descamps, C. L. Schmidt and M. P. Mertes, Biochem. Pharmacol., 28, 3249 (1979).
85. K. K. Guari and K-L. Elze, Klin. Monats. Augenheilk., 171, 459 (1977).
86. J. Descamps, E. De Clercq, P. J. Barr, A. S. Jones, R. T. Walker, P. F. Torrence and D. Shugar, Antimicrob. Agents Chemother., 16, 680 (1979).
87. E. De Clercq, J. Descamps, P. de Somer, P. J. Barr, A. S. Jones and R. T. Walker, ibid, 16, 234 (1979).
88. G. Gentry, J. McGowan, J. Barnett, R. Nevins and G. Allen, Adv. Opthalmol., 38, 164 (1979).
89. W. E. G. Müller, R. K. Zahn, J. Arendes and D. Falke, J. Gen. Virol., 43, 261 (1979).
90. W. E. G. Müller, R. K. Zahn, A. Maidhof, R. Beyer and J. Arendes, Chem. Biol. Interactions, 23, 141 (1978).
91. A. Matsukage, K. Ono, A. Ohashi, T. Takahashi, C. Nakayama and M. Saneyoshi, Cancer Res., 38, 3076 (1978).
92. J. M. Barnett, J. J. McGowan and G. A. Gentry, Infec. Immun., 26, 294 (1979).
93. H. Machida, S. Sakata, A. Kuninaka, H. Koshino, C. Nakayama and M. Saneyoshi, Antimicrob. Agents Chemother., 16, 158 (1979).
94. T. Kulikowski, Z. Zawadzki, D. Shugar, J. Descamps and E. De Clercq, J. Med. Chem., 22, 647 (1979).
95. K. Ono, A. Ohashi, A. Yamamoto, A. Matsukage, T. Takahashi, C. Nakayama and M. Saneyoshi, Nuc. Acids Res., Special Publication No. 5, s429 (1979).
96. G. B. Elion, P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp and H. J. Schaeffer, Proc. Nat. Acad. Sci. U.S., 74, 5716 (1977).
97. H. J. Schaeffer, L. Beauchamp, P. de Miranda, G. B. Elion, D. J. B. Bauer and P. Collins, Nature, 272, 583 (1978).
98. P. Collins and D. J. Bauer, J. Antimicrob. Chemother., 5, 431 (1979).
99. C. S. Crumpacker, L. E. Schnipper, J. A. Zaia and M. J. Levin, Antimicrob. Agents Chemother., 15, 642 (1979).
100. J. A. Fyfe, P. M. Keller, P. A. Furman, R. L. Miller and G. B. Elion, J. Biol. Chem., 253, 8721 (1978).
101. P. A. Furman, M. H. St. Clair, J. A. Fyfe, J. L. Rideout, P. M. Keller and G. B. Elion, J. Virol., 32, 72 (1979).
102. H. J. Field, G. K. Darby and P. Wildy in "Current Chemotherapy: Proceedings of the 11th International Congress of Chemotherapy," American Society for Microbiology, Washington, D.C., 1980, in press.
103. R. J. Klein, A. E. Friedman-Kien and E. De Stefano, Antimicrob. Agents Chemother., 15, 723 (1979).
104. H. J. Field, S. E. Bell, G. B. Elion, A. A. Nash and P. Wildy, ibid, 15, 554 (1979).
105. H. E. Kaufman, E. D. Varnell, Y. M. Centifanto and S. D. Rheinstrom, ibid, 14, 842 (1978).
106. D. J. Bauer, P. Collins, W. E. Tucker, Jr. and A. W. Macklin, Brit. J. Ophthamol., 63, 429 (1979).
107. D. Pavan-Langston, N. H. Park and J. H. Lass, Arch. Ophthamol., 97, 1508 (1979).
108. P. Demiranda, R. J. Whitley, M. R. Blum, R. E. Keeney, N. Barton, D. M. Cocchetto, S. Good, G. P. Hemstreet, L. E. Kirk, D. A. Page and G. B. Elion, Clin. Pharmacol. Therap., 26, 718 (1979).
109. B. R. Jones, P. N. Fison, L. M. Cobo, D. J. Coster, G. M. Thompson and M. G. Falcon, Lancet, 3 Feb., 243 (1979).
110. P. J. Selby, B. Jameson, J. G. Watson, G. Morgenstern, R. L. Powles, H. E. M. Kay, R. Thornton, H. M. Clink, T. J. McElwain, H. G. Prentice, M. G. Ross, R. Corringham, A. V. Hoffbrand and D. Brigden, Lancet, 15 Dec., 1267 (1979).
111. H. E. Renis and E. E. Eidson, Antimcrob. Agents Chemother., 15, 213 (1979).
112. E. K. Ray and H. A. Blough, Virology, 88, 118 (1978).
113. H. A. Blough and R. L. Giuntoli, J. Amer. Med. Assoc., 241, 2798 (1979).
114. S. S. Asculai, M. T. Weis, M. W. Rancourt and A. B. Kupferberg, Antimicrob. Agents Chemother., 13, 686 (1978).
115. L. A. Vontver, W. C. Reeves, M. Rattray, L. Corey, M. A. Remington, E. Tolentino, A. Schweid and K. K. Holmes, Amer. J. Obstet. Gynecol., 133, 548 (1979).
116. M. E. Guinan, J. MacCalman, E. R. Kern, J. C. Overall, Jr. and S. L. Spruance, J. Amer. Med. Assoc., in press (1980).
117. L. Corey, W. C. Reeves, W. T. Chiang, L. A. Vontver, M. Remington, C. Winter and K. K. Holmes, New Engl. J. Med., 299, 237 (1978).
118. L. E. Bockstahler, T. P. Coohill, K. B. Hellman, C. D. Lytle and J. E. Roberts, Pharmacol. Therap., 4, 473 (1979).

119. R. H. Kaufman, E. Adam, R. R. Mirkovic, J. L. Melnick and R. L. Young, Amer. J. Obstet. Gynecol., 132, 861 (1978).
120. P. O. Tennican, G. Z. Carl and M. Chvapil, Life. Sci., 24, 1877 (1979).
121. M. Fahim, T. Brawner, L. Millikan, M. Nickell and D. Hall, J. Med., 9, 245 (1978).
122. R. W. Sidwell, R. K. Robins and I. W. Hillyard, Pharmacol. Therap., 6, 123 (1979).
123. B. B. Goswami, E. Borek, O. K. Sharma, J. Fujitaki and R. A. Smith, Biochem. Biophys. Res. Commun., 89, 830 (1979).
124. J. C. Drach, J. W. Barnett, M. A. Thomas, S. H. Smith and C. Shipman, Jr. in "Ribavirin: A Broad Spectrum Antiviral," R. A. Smith and W. Kirkpatrick, Eds., Academic Press, New York, NY, 1980, in press.
125. V. Knight, S. Z. Wilson, P. R. Wyde, S. Drake, R. B. Couch and G. A. Galegov, ibid, in press.
126. E. De Clercq, J. Descamps, P. De Somer and A. Holy, Science, 200, 563 (1978).
127. E. De Clercq and A. Holy, J. Med. Chem., 22, 510 (1979).
128. G. D. Diana, P. M. Carabateas, R. E. Johnson, G. L. Williams, F. Pancic and J. C. Collins, J. Med. Chem., 21, 889 (1978).
129. J. J. McSharry, L. A. Caliguiri and H. J. Eggers, Virology, 97, 307 (1979).
130. M. F. Kuhrt, M. J. Fancher, V. Jasty, F. Pancic and P. E. Came, Antimicrob. Agents Chemother., 15, 813 (1979).
131. W. E. Stewart II and L. S. Lin, Pharmacol. Therap., 6, 443 (1979).
132. N. Stebbing, ibid, 6, 291 (1979).
133. J. Summerton, J. Theor. Biol., 78, 77 (1979).
134. G. H. Werner, Pharmacol. Therap., 6, 235 (1979).
135. T. C. Merigan, K. H. Rand, R. B. Pollard, P. S. Abdallah, G. W. Jordan and R. P. Fried, New Engl. J. Med., 298, 981 (1978).
136. S. L. Spruance, G. G. Krueger, J. MacCalman, J. C. Overall, Jr. and M. R. Klauber, Antimicrob. Agents Chemother., 15, 662 (1979).
137. T-W. Chang and N. Fiumara, ibid, 13, 809 (1978).
138. E. C. Herrmann and J. A. Herrmann, Pharmacol. Therap., 7, 35 (1979).

Section IV - Metabolic Diseases and Endocrine Function

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Chapter 17. Recent Developments in Lipoprotein Research and Antihyperlipidemic Agents

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Introduction - Atherosclerosis is characterized by the deposition of cholesterol esters in the inner layers of the arterial wall. For this reason, extensive research has focused on the transport and metabolism of cholesterol. Evidence has accumulated in recent years that an understanding of the control of lipid transport necessitates the elucidation of the role of the individual lipoproteins and apoproteins. In this way, therapeutic approaches to the prophylaxis and treatment of atherosclerosis can be more thoroughly evaluated. In last year's chapter of this series, developments in atherosclerosis regression, high density lipoproteins, antihyperlipidemic drugs and the therapeutic control of cholesterol gallstones were discussed.[1] In this chapter, selected recent advances in lipoprotein research relevant to lipid disorders are reviewed, and an update is presented on the therapeutic control of hyperlipidemia.

Lipoproteins - All plasma lipoproteins have the same basic configuration comprising a hydrophobic core of triglyceride and cholesterol ester surrounded by an amphiphilic monolayer of cholesterol, phospholipid, and various proteins called apoproteins. The triglyceride-rich chylomicrons (synthesized in the intestine) and very low density lipoproteins (VLDL) (originating in the liver and intestine) are metabolized in the plasma by lipoprotein lipase, and the liberated triglycerides are taken up by peripheral tissues. The resultant remnant lipoproteins are ultimately metabolized to low density lipoproteins (LDL). Chylomicrons, VLDL and LDL are atherogenic while high density lipoproteins (HDL), synthesized in the liver and intestine, may be anti-atherogenic. The various lipoproteins undergo molecular exchanges of both their protein and lipid components within the vascular system and are thus metabolically related.

HDL have been reported to have a protective role in coronary heart disease (CH)[2-5] and cerebrovascular disease.[6] In 400 patients, coronary artery occlusion, as determined by arteriography was significantly more frequent in subjects with HDL-cholesterol (HDL-C) levels less than 50 mg/dl.[7] As more information accumulates, the hypotheses regarding the control and function of HDL are being modified. For example, although an inverse relationship between plasma triglycerides and HDL-C has been shown, there is no evidence which demonstrates that plasma triglycerides regulate HDL concentrations.[8] Thus, in patients with Type IV[9] or Type V[10] hyperlipoproteinemia and in obese patients[11] who respond to therapy and weight reduction by a significant lowering of serum triglycerides, no rise in HDL-C took place. Therapeutic interventions which reduce the secretion of triglyceride-rich lipoproteins may not raise HDL-C, but increased catabolism of these lipoproteins may elevate HDL-C, due to transfer of surface components from triglyceride-rich lipoproteins to HDL.[12]

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Moderate exercise raises HDL-C;[13-16] however, in one report, calisthenic-type exercise lowered HDL-C.[17] The effect of dietary manipulation on HDL-C is also being closely examined. Only certain types of dietary fiber reduce total serum cholesterol in man.[18,19] Data on the effects of fiber on HDL-C are conflicting and remain to be clarified.[19-22] Increased ingestion of vegetable and fish protein may elevate HDL-C.[23-25] A potential complicating factor concerns the several forms of HDL. Thus, HDL_2, a subfraction of HDL, increases in exercise[16] and is higher in women than in men;[26] HDL_2 is thought to be more beneficial than HDL_3 in protecting against atherosclerosis.[5,26] In addition, the HDL fraction which contains the arginine-rich apoprotein, similar to that produced in animals by feeding a high cholesterol diet, is thought to have atherogenic properties.[27] It would thus seem relevant either to measure cholesterol in the different HDL subclasses or to quantitate HDL in terms of the apoprotein content.

Apoproteins - In the past few years, many changes which occur in apoproteins in various lipid disorders have been elucidated. The structure, physicochemical properties, and metabolism of the various apoproteins have recently been reviewed (e.g. refs. 28-30). Each lipoprotein can be characterized not only by its apoprotein content, but also by the proportion of each apoprotein. Apoprotein A-I (apoA-I) and apoA-II comprise the major protein portion of HDL and are also found in VLDL and chylomicrons.[29] These apoproteins are synthesized in the liver and intestine[31,32] and are present in different proportions in HDL_2 and HDL_3.[33] The absolute amounts and/or the ratio of apoA-I to apoA-II are altered in lecithin-cholesterol acyl transferase (LCAT) deficiency,[34] cholestatic liver disease,[35] Tangier disease,[36] hyperlipidemia,[37] peripheral vascular disease[38] and in survivors of myocardial infarction (MI).[39] These changes may have diagnostic value. ApoA-I activates LCAT[40] and is reduced in several of these lipid disorders. The role of apoA-II appears to be mainly structural.[28,41]

ApoB, the primary protein in LDL and a major constituent of VLDL and chylomicrons,[29] is synthesized in the liver and intestine.[28] Its chemical composition and properties have recently been described.[42,43] A high cholesterol diet (in the form of egg yolk) produced marked increases in LDL-cholesterol (LDL-C) and apoB levels.[44] ApoB was raised in familial Type II hyperlipoproteinemia[28] and in MI survivors.[39] It has been suggested that elevated apoB and/or decreased apoA-I is a better indicator of MI risk than elevated plasma cholesterol.[38] ApoB is thought to regulate the uptake and internalization of LDL by peripheral tissue,[45,46] while cholesterol turnover within the cells is controlled by the entire LDL particle.[46]

The cellular uptake of LDL and the delivery of cholesterol to the cell have been studied extensively in tissue culture mainly by Goldstein and Brown.[45,47] The sequence of events has been described in the following hypothesis. All mammalian cells contain a surface glycoprotein which acts as a specific receptor for LDL. The LDL receptor complex is taken up by the cell by endocytosis. The vesicles fuse with lysosomes, the lysosomal enzymes hydrolyze the LDL-cholesterol esters to cholesterol and fatty acid and the protein to amino acids. The cholesterol is utilized to maintain the functional integrity of the cellular membranes or is reesterified with fatty acid for storage. The hypothesis provides various control mechanisms which prevent excessive cholesterol accumulation: the number of cell-surface LDL receptors is regulated by the amount of cholesterol which enters the cell; also, internalized cholesterol suppresses cellular cholesterol synthesis. It was therefore proposed that the main function of the LDL receptor in man is to protect against atherosclerosis but that unphysiologically high plasma levels of LDL-C exceed the capacity of the receptor-mediated process, thereby producing uncontrolled accumulation of cholesterol

esters in the smooth muscle cells.[45,47]

ApoC-I, C-II and C-III are the major protein constituents of VLDL and chylomicrons; small amounts are present in HDL.[29] These apoproteins are thought to be primarily synthesized in the liver and exchange in the plasma between HDL and VLDL or chylomicrons.[28,30,32] ApoC-II activates lipoprotein lipase.[28] This effect has led to the suggestion that HDL may regulate plasma triglyceride levels, rather than vice-versa.[8] Also, the amount of apoC-II per unit VLDL protein was shown to be inversely related to the severity of hypertriglyceridemia.[48] A rare disorder resembling Type I hyperlipoproteinemia is characterized by a genetic deficiency in apoC-II.[49] This syndrome has also been observed in relatives of the original patient[50] and in a pair of Japanese twins.[51] The condition is readily controlled by administration of either HDL or the missing apoprotein.[49] The active fragment of this apoprotein has been synthesized.[52] ApoC-I activates LCAT, though to a lesser extent than does apoA-I.[40] ApoC-III is thought to inhibit lipoprotein lipase,[53] and thus excess apoC-III has been associated with hypertriglyceridemia.[53,54]

In less abundance are apoD, apoA-III and apoE.[28] ApoA-III has also been reported to activate LCAT.[28] ApoD along with A-II, C-II and C-III inhibited LCAT in vitro in the presence of A-I or C-I.[40] The role in vivo is unknown. ApoE is synthesized in the liver[32] and is normally a minor component of lipoproteins.[28] ApoE enters the circulation with nascent HDL particles and is transferred to VLDL.[28] HDL_C, a lipoprotein enriched in apoE, has been isolated from cholesterol-fed dogs and swine.[27] HDL_C binds in vitro to fibroblasts and smooth muscle cells in a manner more resembling LDL than normal HDL.[27] A lipoprotein with similar binding activity, HDL_I, has been isolated from humans.[55] ApoE has been further subdivided by isoelectric focusing into various subspecies.[56] ApoE-III was found to be deficient in Type III hyperlipoproteinemia and may serve as an additonal marker for this disease.[56,57]

The HDL which is synthesized in the intestine contains mainly apoA but is devoid of apoC and apoE. These latter apoproteins are acquired by interaction with particles originating in the liver.

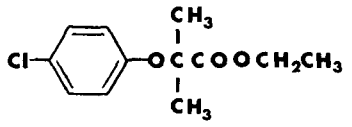
Other minor apoproteins have been found in man: apoA-IV,[58] a major protein component of human lymph chylomicrons; apoF,[59] a minor component of HDL; and apoG,[60] associated with very high density lipoproteins (VHDL) and HDL.

In certain disorders, lipoproteins may appear which either contain abnormal apoproteins or abnormal amounts of normal apoproteins. For example, LpX appears in obstructive jaundice and LCAT deficiency.[61] LpX identification may be useful in the diagnosis of certain liver disorders.[62,63] Lp(a) occurs in higher amounts in patients with CHD than in controls.[64] Lp(a) contains apoB and an abnormal apoprotein called apoLp-(a)[65] and may serve as a quantitative genetic marker for CHD.[64,65]

The development of techniques for the routine analysis of specific apoproteins will greatly facilitate their use as diagnostic parameters. Several such screening techniques have been reported.[53,56,66,67]

Antihyperlipidemic Agents - Clofibrate and Nicotinic Acid - Clofibrate (1) and nicotinic acid (5) continue to be widely used antihyperlipidemic drugs; a combination of the two agents produced greater reductions in serum lipids than did either drug alone.[68] Two nicotinic acid esters of clofibric acid

have been studied: etofibrate (2), reported to possess greater antihyperlipidemic activity in man than clofibrate,[69] produced in rats greater lipid lowering than clofibrate or nicotinic acid alone;[70] I-612 (3) reduced serum lipids in patients with Types IIa, IIb and IV hyperlipoproteinemia.[71,72]



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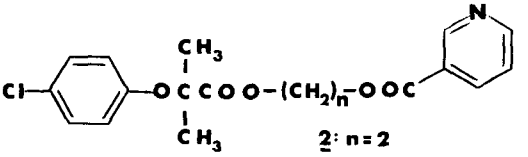
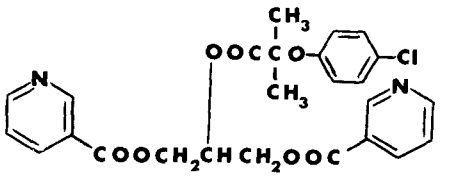


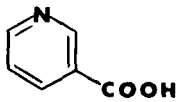
2: n=2
3: n=3

Another compound which contains both clofibric acid and nicotinic acid moieties, WAC-104 (4), was reported to possess greater antihyperlipidemic activity than clofibrate in rats and rabbits and to have an antiatherogenic effect in rabbits.[73]



4

Data are conflicting regarding the effect of nicotinic acid and its analogs on HDL-C. HDL-C was unchanged in subjects with Type II hyperlipoproteinemia given β-pyridylcarbinol (6), although the LDL/HDL ratios were improved.[74,75] In another study, niceritrol (7) increased HDL-C.[68] Also, in normal volunteers, nicotinic acid raised HDL-C and increased the plasma HDL_2/HDL_3 ratio.[76] The latter was due to an elevation in HDL_2 coupled with a fall in HDL_3. This change was associated with a lower molar ratio of apoA-I/apoA-II in HDL_3, possibly due to a transfer of apoA-I from HDL_3 to HDL_2 and/or a decrease in apoA-II synthesis;[76] such changes may be associated with decreased incidence of CHD. Regarding side effects, nicotinic acid, like clofibrate, increased biliary cholesterol concentration.[77] Indomethacin pretreatment substantially reduced the nicotinic acid-induced increase in forearm blood flow without affecting plasma free fatty acids; thus, it was concluded that the skin flush induced by nicotinic acid was due to the release of prostaglandins.[78]



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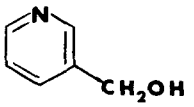

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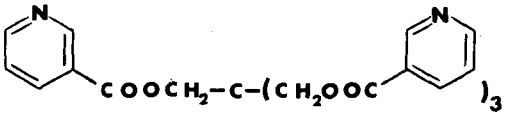
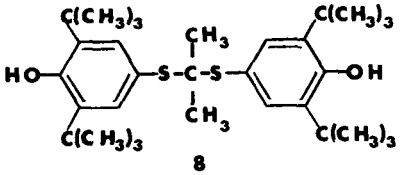


7

Ion-exchange Resins - The ion-exchange resins (cholestyramine, colestipol, polidexide) are highly effective in lowering LDL-C in Type II hyperlipoproteinemia. Although the resins do not elevate HDL-C,[79-82] the drugs may produce favorable changes in HDL subfractions and apoprotein ratios. Colestipol treatment caused no change in apoA-I levels but decreased those of apoA-II,[80] while cholestyramine increased apoA-I but did not alter apoA-II.[81] Thus, in both studies, an increase in apoA-I/apoA-II ratio occurred, while in the latter study, the changes were accompanied by an increase in the HDL_2/HDL_3 ratio. Cholestyramine treatment produced similar percentage decreases in both LDL-C and apoB.[82] Cholestyramine reduced the cholesterol saturation of bile but, in combination with clofibrate, produced no change in the saturation index of bile as compared to pretreatment levels.[77]

Probucol - Probucol (8) lowers elevated levels of cholesterol but not of triglycerides. However, probucol lowers HDL-C,[83,84] and its long-term effect on CHD remains to be evaluated.

Bezafibrate - Bezafibrate (9) was reported both to elevate[85] and to have no effect[86,87] on HDL-C in subjects with Type IIa hyperlipoproteinemia. Bezafibrate appears to exhibit similar therapeutic activity as clofibrate but at lower doses. In man, bezafibrate has a half-life of 2 hr, is highly protein bound (94-96%) and is excreted primarily in the urine.[88]



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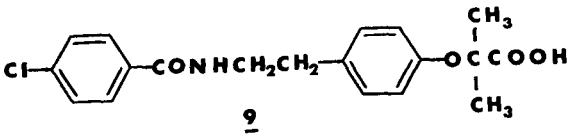
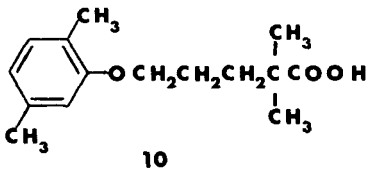


9

Gemfibrozil - Clinical studies continue to show that gemfibrozil (10) lowers LDL-C and VLDL-triglycerides and increases HDL-C;[89,90] many subjects have been treated for 5 years.[90] As with clofibrate and bezafibrate, the extent of gemfibrozil-induced decrease in LDL and VLDL lipid levels correlated with the pretreatment values.[91] Gemfibrozil lowered serum triglycerides in hypertriglyceridemic diabetics but did not affect glucose tolerance, serum insulin or ADP-induced platelet aggregation.[92] The lithogenic index of bile was unchanged in normal volunteers given therapeutic doses of gemfibrozil for 4 weeks.[93] Although both clofibrate and gemfibrozil elevated liver weight in the rat, gemfibrozil produced smaller increases in liver mitochondrial α-glycerophosphate dehydrogenase and carnitine acyltransferases than did clofibrate.[94]



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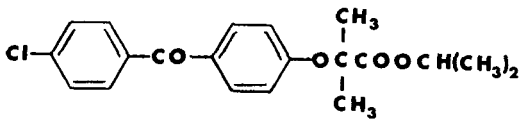


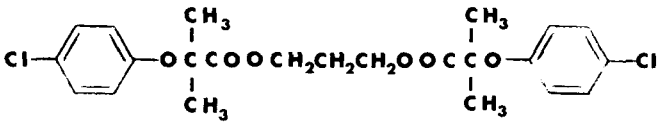
11

Fenofibrate (Procetofene) - Several clinical studies have corroborated the LDL-C and VLDL-triglyceride lowering activity of fenofibrate (11) in patients with hyperlipoproteinemia, although HDL-C remained virtually unaltered.[95-98] A frequent finding with fenofibrate treatment was increased SGOT and SGPT;[98-100] increased saturation index of bile has also been observed.[101] Fenofibrate normalized platelet function in Type IIb hyperlipoproteinemia.[97] Pharmacokinetic studies in normal volunteers have shown that procetofenic acid, the active metabolite of fenofibrate, was eliminated from plasma in two phases, with half-lives of 5 and 22 hr, respectively.[102] The steady-state drug levels in plasma which were associated with hypocholesterolemia in Type IIa hyperlipoproteinemia averaged 5 μg/ml.[103] In rats, fenofibrate inhibited hepatic cholesterol and fatty acid synthesis,[104] produced liver enlargement,[104-106] and elevated liver cytochrome P450 activity.[106] Like clofibrate and tibric acid, fenofibrate in the rat increased liver mitochondrial α-glycerophosphate dehydrogenase, catalase and palmitoyl-CoA oxidation; the two latter effects are associated with peroxisome proliferation.[105]

Simfibrate - Simfibrate (12), a closely related analog of clofibrate, lowered serum cholesterol and triglycerides[107,108] and inhibited platelet aggregation[107] in hyperlipidemic subjects.

2-[3-(2-Thiazolyl thio)phenyl]propionic Acid (TPA) - Agents, which both inhibit platelet aggregation and lower blood lipids, offer an attractive approach to thrombotic problems frequently associated with atherosclerosis. TPA (13) reduced aggregation of human platelets in vitro and, in guinea

pigs, reduced collagen-induced platelet aggregation.[109] TPA at a dose of 0.003% of the diet lowered serum triglycerides in rats rendered hyperlipidemic with sucrose.[109] TPA also possessed uricosuric properties in chimpanzees.[109]



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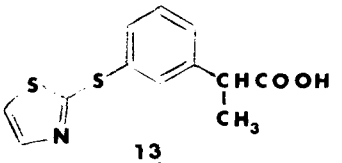
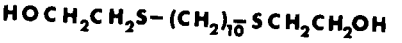


13

Plafibride - Plafibride (14), a morpholinomethylurea derivative of clofibrate, inhibited platelet aggregation in rats and dogs,[110,111] lowered serum triglycerides in hypertriglyceridemic rats,[111] and decreased LDL-C in rats.[112] In hyperlipidemic subjects given plafibride, platelet aggregation was lowered, serum triglycerides and LDL-C were reduced, while HDL-C was increased.[112]

Tiadenol - In patients with Type IIa hyperlipoproteinemia, tiadenol (15) lowered LDL-C in parallel with a decrease in apoB; however, HDL-C was also significantly lower.[113] Tiadenol in Type IV hyperlipoproteinemia reduced serum triglyceride and apoE concentrations; unlike clofibrate, tiadenol did not elevate lipoprotein lipase activity.[114]

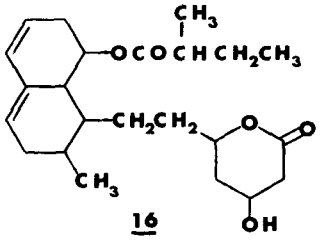
14



15

Compactin (ML-236B) - Compactin (16), isolated from cultures of Penicillium citrinum,[115] is a competitive inhibitor of hydroxymethylglutaryl (HMG) CoA reductase.[116] The compound lowered serum cholesterol in dogs,[117] cynomolgus monkeys[118] and rats rendered hyperlipidemic with Triton WR-1339[119] but not in normal rats,[120,121] mice[120] or chicks;[121] serum triglycerides were unaltered.[117,118] In patients with Type IIa or Type IIb hyperlipoproteinemia, compactin lowered serum cholesterol but did not alter serum triglycerides.[122] Compactin suppressed HMG CoA reductase in human fibroblasts[123] and inhibited cholesterol biosynthesis in isolated lymphocytes and intestinal mucosa in normal subjects and those with familial hypercholesterolemia.[124]

Ciprofibrate - In patients treated with ciprofibrate (17), LDL-C was reduced in Type II and VLDL-triglycerides were lowered in Type IV hyperlipoproteinemia.[125] In subjects with Type II hyperlipoproteinemia, ciprofibrate was reported to produce a slight increase[125] or no change[126] in HDL-C. In rats, ciprofibrate had an elimination half-life of 82 hr, and serum cholesterol and triglycerides remained suppressed for 3 days after cessation of treatment.



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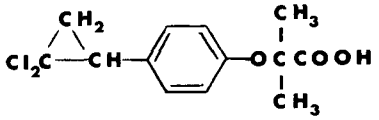
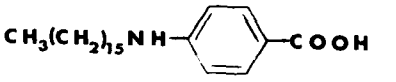


17

Cetaben - Cetaben (18) was initially reported to possess antiatherosclerotic properties in rabbits[128] and monkeys.[129] Recently, cetaben was shown to inhibit lipid biosynthesis and cholesterol esterification in rhesus monkey aortic smooth muscle cells.[130] Cetaben also lowered serum cholesterol in rats, rabbits and monkeys and inhibited the incorporation of [^{14}C]-acetate and [^{3}H]-glycerol into rat liver cholesterol, phospholipids and triglycerides.[131]

Terbufibrol - In baboons with diet-induced hypercholesterolemia, terbufibrol (19) lowered both LDL-C and HDL-C.[132] The results of two Phase I clinical trials with terbufibrol have been described in a preliminary report.[133] After two weeks treatment, serum cholesterol was reduced an average of 19% in normocholesterolemic healthy volunteers. The serum elimination half-life in man averaged 5 hr.[133]



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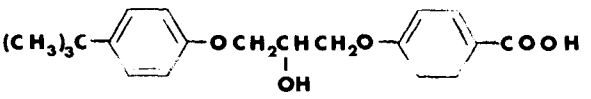
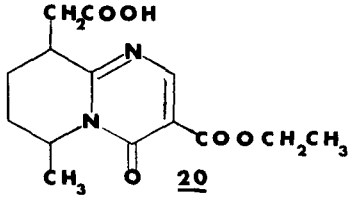


19

Chinoin-123 - Chinoin-123 (20) has been reported to be an antiatherosclerotic agent in cholesterol-fed rabbits.[134] The compound also suppressed the development of vascular lesions induced by a combination of a cholesterol diet and adjuvant arthritis in rats.[135]

Acipimox - An analog of nicotinic acid, acipimox (21),[136] was 20 times more active than nicotinic acid in inhibiting lipolysis in fasting volunteers. Unlike nicotinic acid, acipimox did not cause a rebound in plasma free fatty acids.[137] A three-month trial showed that acipimox reduced serum triglycerides in Type IV and cholesterol in Type II hyperlipoproteinemia. It was proposed that acipimox has a mechanism of action similar to that of nicotinic acid but with a more prolonged effect on lipolysis.[137]



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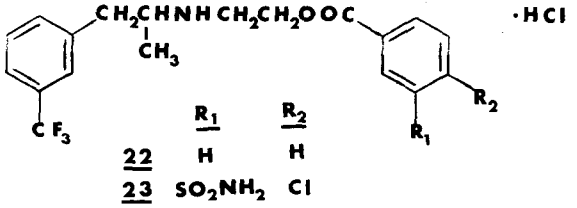
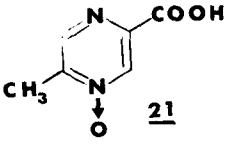


	R_1	R_2
22	H	H
23	SO_2NH_2	Cl

S-1204 - Structurally related to 780 SE (22),[138,139] the fenfluramine derivative S-1204 (23) inhibited the incorporation of labelled acetate into arterial lipids of chow-fed rabbits but had no effect on lipid levels in plasma, liver and aortic tissue.[140]



21

References

1. M.N. Cayen, in "Annual Reports in Medicinal Chemistry", Vol. 14, H.J. Hess, Ed., Academic Press, New York, NY, 1979, p 198.
2. N.E. Miller, Lipids, 13, 914 (1978).
3. R. Beaglehole, I.A.M. Prior, E. Eyles and V. Sampson, N.Z. Med. J., 90, 139 (1979).
4. V. Goldbourt and J.H. Medalie, Am. J. Epidemiol., 109, 296 (1979).
5. J. Witztum and G. Schonfeld, Diabetes, 28, 326 (1979).
6. H. Taggart and R.W. Stout, Eur. J. Clin. Invest., 9, 219 (1979).

7. J.J. Barboriak, A.J. Anderson, A.A. Rimm and J.F. King, Metabolism, 28, 735 (1979).
8. M.K. Chan, Z. Varghese, J.W. Persaud and J.F. Moorhead, Lancet, 2, 305 (1979).
9. J. Witztum, M. Dillingham, W. Giese, G. Schonfeld and S. Weidman, Circulation, 58, II-38 (1978).
10. C.J. Glueck, E.A. Stein and M.L. Kashyap, Artery, 5, 463 (1979).
11. P.D. Thompson, R.W. Jeffery, R.R. Wing and P.D. Wood, Am. J. Clin.Nutr.,32,2016(1979).
12. A.R. Tall and D.M. Small, New Eng. J. Med., 299, 1232 (1978), and refs. cited.
13. J.K. Huttunen, E. Lansimies, E. Voutilainen, C. Ehnholm, E. Hietanen, I. Penttila, O. Siitonen and R. Rauramaa, Circulation, 60, 1220 (1979).
14. D.W. Erkelens, J.J. Albers, W.R. Hazzard, R.C. Frederick and E.L. Bierman, J. Am. Med. Assoc., 242, 2185 (1979).
15. D. Streja and D. Mymin, J. Am. Med. Assoc., 242, 2190 (1979).
16. P.D. Wood and W.L. Haskell, Lipids, 14, 417 (1979), and refs. cited.
17. R.A. Moore, W.A.F. Penfold, R.D. Simpson, R.W. Simpson, J.I. Mann and R.C. Turner, Ann. Clin. Biochem., 16, 76 (1979).
18. D. Kritchevsky, Am. J. Clin. Nutr., 31, S65 (1978), and refs. cited.
19. J.M. Munoz, H.H. Sandstead, R.A. Jacob, G.M. Logan Jr., S.J. Reck, L.M. Klevay, F.R. Dintzis, G.E. Inglett and W.C. Shuey, Am. J. Clin. Nutr., 32, 580 (1979).
20. R.M. McDougall, L. Yakymyshyn, K. Walker and O.G. Thurston,Can.J.Surgery,21, 433(1978).
21. M. Stasse-Wolthius, M.B. Katan, R.J.J. Hermus and J.G.A.J. Hautvast, Atherosclerosis, 34, 87 (1979).
22. G.P. van Berge-Henegouwen, A.W. Huybregts, S. van de Werf, P. Demacker and R.W.Schade, Am. J. Clin. Nutr., 32, 794 (1979)
23. J. Burslem, G. Schonfeld, M.A. Howald, S.W. Weidman and J.P. Miller, Metabolism, 27, 711 (1978).
24. C.R. Sirtori, E. Gatti, O. Mantero, F. Conti, E. Agradi, E. Tremoli, M. Sirtori, L. Fraterrigo, L. Tavazzi and D. Kritchevsky, Am. J. Clin. Nutr., 32, 1645 (1979).
25. T.O. von Lossonczy, A. Ruiter, H.C. Bronsgeest-Schoute, C.M. van Gent and R.J.J. Hermus, Am. J. Clin. Nutr., 31, 1340 (1978).
26. G.J. Miller and N.E. Miller, in "High Density Lipoproteins and Atherosclerosis", A.M. Gotto Jr., N.E. Miller and M.F. Oliver, Eds., Elsevier, Amsterdam, 1978, p 95, and refs. cited.
27. R.W. Mahley and T.L. Innerarity, in "Drugs, Lipid Metabolism and Atherosclerosis", D. Kritchevsky, R. Paoletti and W.L. Holmes, Eds., Adv. Exp. Med. Biol., Vol. 109, Plenum Press, New York, NY, 1978, p 99.
28. E.J. Schaefer, S. Eisenberg and R.I. Levy, J. Lipid Res.,19,667(1978), and refs.cited.
29. W.W. Mantulin and A.M. Gotto Jr., in "International Conference on Atherosclerosis", L.A. Carlson, R. Paoletti, C.R. Sirtori and G. Weber, Eds., Raven Press, New York, NY, 1978, p 57.
30. H.J. Pownall, J.D. Morrisett, J.T. Sparrow, L.C. Smith, J. Shepherd, R.L. Jackson and A.M. Gotto Jr., Lipids, 14, 428 (1979).
31. E.J. Schaefer and R.I. Levy, in "Lipoprotein Metabolism", S. Eisenberg, Ed., Progr. Biochem. Pharmacol., Vol. 15, S. Karger, New York, NY, 1979, p 200.
32. A.-L. Wu and H.G. Windmueller, J. Biol. Chem., 254, 7316 (1979).
33. J.J. Albers, R.G. Warnick and M.C. Cheung, Lipids, 13, 926 (1978).
34. J.A. Glomset, in "Lipoprotein Metabolism", S. Eisenberg, Ed., Progr. Biochem. Pharmacol., Vol. 15, S. Karger, New York, NY, 1979, p 41.
35. K. Yamamoto, S. Koga and H. Ibayashi, Clin. Chim. Acta, 87, 85 (1978).
36. G. Assmann, A. Capurso, E. Smootz and U. Wellner, Atherosclerosis, 30, 321 (1978).
37. G. Schonfeld, A. Bailey and R. Steelman, Lipids, 13, 951 (1978).
38. G.V.H. Bradby, A.J. Valente and K.W. Walton, Lancet, 2, 1271 (1978).
39. G. Bittolo Bon, G. Cazzolato, G.B. Quinci and F. Belussi, Artery, 4, 385 (1978).
40. J.J. Albers, J. Lin and G.P. Roberts, Artery, 5, 61 (1979).
41. C. Edelstein, F.J. Kezdy, A.M. Scanu and B.W. Shen, J. Lipid Res., 20, 143 (1979).
42. S. Goldstein and M.J. Chapman, Biochem. Biophys. Res. Commun., 87, 121 (1979).
43. L. Socorro and G. Camejo, J. Lipid Res., 20, 631 (1979).
44. D. Applebaum-Bowden, W.R. Hazzard, J. Cain, M.C. Cheung, R.S. Kushwaha and J.J. Albers, Atherosclerosis, 33, 385 (1979).
45. J.L. Goldstein and M.S. Brown, Ann. Rev. Biochem., 46, 897 (1977).
46. R.B. Shireman and W.R. Fisher, J. Lipid Res., 20, 594 (1979).
47. M.S. Brown and J.L. Goldstein, in "Disturbances in Lipid and Lipoprotein Metabolism", J.M. Dietschy, A.M. Gotto Jr. and J.A. Ontko, Eds., Amer. Physiol. Soc., Bethesda, MD., 1978, p 173.
48. M.L. Kashyap, L.S. Srivastava, B.A. Hynd, G. Perisutti, D.W. Brady, P. Gartside and C.J. Glueck, Lipids, 13, 933 (1978).
49. W.C. Breckenridge, J.A. Little, G. Steiner, A. Chow and M. Poapst, New Engl. J. Med. 298, 1265 (1978).
50. D.W. Cox, W.C. Breckenridge and J.A. Little, New Engl. J. Med., 299, 1421 (1978).
51. T. Yamamura, H. Sudo, K. Ishikawa and A. Yamamoto, Atherosclerosis, 34, 53 (1979).
52. A.L. Catapano, P.K.J. Kinnunen, W.C. Breckenridge, A.M. Gotto Jr., R.L. Jackson, J.A. Little, L.C. Smith and J.T. Sparrow, Biochem. Biophys.Res.Commun.,89,951(1979).
53. G. Schonfeld, P.K. George, J. Miller, P. Reilly and J. Witztum, Metabolism, 28, 1001 (1979).
54. J. Stocks, G. Holdsworth and D. Galton, Lancet, 2, 667 (1979).

55. T.L. Innerarity, R.W. Mahley, K.H. Weisgraber and T.P. Bersot, J. Biol. Chem., 253, 6289 (1978).
56. S.W. Weidman, B. Suarez, J.M. Falko, J.L. Witztum, J. Kolar, M. Raben and G. Schonfeld, J. Lab. Clin. Med., 93, 549 (1979).
57. J.M. Falko, G. Schonfeld, J.L. Witztum, J. Kolar and S.W. Weidman, Metabolism, 28, 1171 (1979).
58. G. Utermann and U. Beisiegel, Eur. J. Biochem., 99, 333 (1979).
59. S.-O. Olofsson, W.J. McConathy and P. Alaupovic, Biochemistry, 17, 1032 (1978).
60. M. Ayrault-Jarrier, J. -F. Alix and J. Polonovski, Biochemie, 60, 65 (1978).
61. J. Agorastos, C. Fox, D.S. Harry and N. McIntyre, Clin. Sci. Mol. Med., 54, 369(1978).
62. R. Fellin, E. Manzato, S. Zotti, G. Baggio, G. Briani and M. Rugge, Clin. Chim. Acta, 85, 41 (1978).
63. J.R. Poley, D.B. Caplan, H.N. Magnani, P. Alaupovic, E.I. Smith, D.P. Campbell, M. Bhatra, M. Burdelski and D. Bojanovski, Eur. J. Clin. Invest., 8, 397 (1978).
64. F. Krempler, G. Kostner, K. Bolzano and F. Sandhofer, Atherosclerosis, 30, 57 (1978) and refs. cited.
65. F. Krempler, G. Kostner, K. Bolzano and F. Sandhofer, Biochim. Biophys. Acta, 575, 63 (1979).
66. D.J. Nazir, P.L. Brown and M.J. McQueen, Clin. Chim. Acta, 88, 31 (1978).
67. W.F. Riesen, R.C. Mordasini and G.W. Middlehoff, FEBS Letters, 91, 35 (1978).
68. B. Vessby, H. Lithell, I.-B. Gustafsson and J. Boberg, Atherosclerosis,33,457(1979).
69. H. Kaffarnik, J. Schneider and W. Haase, Dtsch. Med. Wschr., 100, 2486 (1975).
70. J.G. Priego, M.L. Maroto, M. Pina and R.E. Catalan, Gen. Pharmacol., 10,215,315(1979).
71. A. Bucalossi, G. Buzzelli and A. D'Alessandro, Clin. Ter., 89, 127 (1979).
72. G. Buzzelli, A. Doni, G. Lippi and A. Resina, Clin. Ter., 89, 251 (1979).
73. C. Figols, Drugs of the Future, 4, 681 (1979).
74. C.A. Dujovne, D.L. Azarnoff, P. Pentikainen, C.V. Manion and A. Hurwitz, Amer. J. Med. Sci., 277, 255 (1979).
75. P. Schwandt, P. Weisweiler and G. Neureuther, Atherosclerosis, 34, 35 (1979).
76. J. Shepherd, C.J. Packard, J.R. Patsch, A.M. Gotto Jr. and O.D. Taunton, J. Clin. Invest., 63, 858 (1979).
77. B. Angelin, K. Einarsson and B. Leijd, Eur. J. Clin. Invest., 9, 185 (1979).
78. L. Kaijser, B. Eklund, A.G. Olsson and L.A. Carlson, Med. Biol., 57, 114 (1979).
79. P.T. Kuo, K. Hayase, J.B. Kostis and A.E. Moreyra, Circulation, 59, 199 (1979).
80. J.L. Witztum, G. Schonfeld, S.W. Weidman, W.E. Giese and M.A. Dillingham, Metabolism, 28, 221 (1979).
81. J. Shepherd, C.J. Packard, H.G. Morgan, J.L.H.C. Third, J.M. Stewart and T.D.V. Lawrie, Atherosclerosis, 33, 433 (1979).
82. G. Assmann and G. Kladetzky, Therapiewoche, 29, 2615 (1979).
83. J.L. Anderson, and J.S. Schroeder, J. Cardiovasc. Pharmacol.,1, 353 (1979).
84. T.A. Miettinen, J.K. Huttunen, T. Kumlin, V. Naukkarinen, S. Mattila and C. Ehnholm, Eur. J. Clin. Invest., 9, 24 (1979).
85. H.R. Arntz, U.H. Klemens, P.D. Lang and J. Vollmar, Med. Klin., 73, 1731 (1978).
86. M. Kohlmeier, P. Oster, G. Schlierf, C.C. Heuck and B. Schellenberg, Eur. J. Clin. Invest., 9, 19 (1979).
87. J.G. Wechsler, V. Hutt, H.-U. Klor and M. Ditschuneit, Fifth Int. Symp. on Atherosclerosis, Houston, TX, Nov. 6-9, 1979, Abstr. No. 151.
88. U. Abshagen, W. Bablok, K. Koch, P.D. Lang, H.A.E. Schmidt, M. Senn and H. Stork, Eur. J. Clin. Pharmacol., 16, 31 (1979).
89. P. Schwandt, P. Weisweiler and G. Neureuther, Artery, 5, 117 (1979).
90. V. Manninen, A. Eisalo, M. Malkonen, J. Virtamo and J. Tuomilehto, Fifth Int. Symp. on Atherosclerosis. Houston, TX, Nov. 6-9, 1979, Abstr. No. 155.
91. L.A. Carlson and A.G. Olsson, in "Lipoprotein Metabolism", S. Eisenberg, Ed., Progr. Biochem. Pharmacol., Vol. 15, S. Karger, New York, NY, 1979, p 238.
92. J. Marks, J. Macpherson and A.N. Howard, Fifth Int. Symp. on Atherosclerosis, Houston, TX, Nov. 6-9, 1979, Abstr. No. 273.
93. M.J. Hall, L.M. Nelson, R.L. Russell and A.N. Howard, Scot. Med.J.,24,175(1979).
94. M.T. Kahonen and R.H. Ylikahri, Atherosclerosis, 32, 47 (1979).
95. P.L. Lauwers, Curr. Ther. Res., 26, 30 (1979).
96. P. Drouin, L. Mejean, D. Lambert, J.P. Sauvanet and G. Debry, ibid., p 350.
97. S. Renaud, R. Morazain, J.P. Sauvanet, E. Dumont and P. Drouin, Haemostasis,8,82(1979).
98. R. Mordasini, E.M. Grandjean, P.C. Nobile, G. Paumgartner and G. Riva, Schweiz. Med. Wschr., 109, 1140 (1979).
99. A. Rossi, Nouv. Presse. Med., 7, 3153 (1978).
100. J.L. de Gennes, J. Truffert and P. Periac, ibid., p 2398.
101. E.M. Grandjean, R. Mordasini and G. Paumgartner, Schweiz. Med. Wschr. 109, 601 (1979).
102. J.P. Desager and C. Harvengt, Int. J. Clin. Pharmacol., 16, 570 (1978).
103. P. Drouin, L. Mejean, D. Lambert, J.P. Sauvanet and G. Debry, Curr. Ther. Res., 26, 357 (1979).
104. D. Kritchevsky, S.A. Tepper and J.A. Story, Pharmacolog. Res. Commun., 11, 635 (1979).
105. B.R. Holloway, J.M. Thorp and M.S. Rose, Toxicol. Appl. Pharmacol., 48, A125 (1979).
106. T.C. Orton, J.E. Higgins and M.S. Rose, ibid., p A126.
107. G. de Rosa, L. Savi, E. de Rosa, P. Pola and P. Boni, Clin. Ter., 88, 267 (1979).

108. A. Giuliani, C. Trezzini, G. Urbainati and R. Conti, ibid., p 481.
109. D.H. Minsker, G.M. Fanelli Jr., J.L. Gilfillan, J.W. Huff, P. Jordan and P. Kling, J. Pharmacol. Exp. Ther., 210, 37 (1979).
110. L. Bruseghini and J. Vilageliu, Arch. Farmacol. Toxicol., 4, 132 (1978).
111. L. Burseghini and J. Vilageliu, in "International Conference on Atherosclerosis", L.A. Carlson, R. Paoletti, C.R. Sirtori and G. Weber, Eds., Raven Press, New York, NY, 1978, p 701.
112. M. Ribalta and L. Bruseghini, Drugs of the Future, 4, 42 (1979), and refs. cited.
113. G. Baggio, G. Briani, R. Fellin, S. Martini, M.R. Baiocchi and G. Crepaldi, Artery, 5, 486 (1979).
114. G. Franceschini, A. Poli, M. Sirtori, G. Gianfranceschi and C.R. Sirtori, Fifth Int. Symp. on Atherosclerosis, Houston, TX, Nov. 6-9, 1979, Abstr. No. 408.
115. A. Endo, M. Kuroda and Y. Tsujita, J. Antibiotics, 29, 1346 (1976).
116. K. Tanzawa and A. Endo, Eur. J. Biochem., 98, 195 (1979).
117. Y. Tsujita, M. Kuroda, K. Tanzawa, N. Kitano and A. Endo, Atherosclerosis, 32, 307 (1979).
118. M. Kuroda, Y. Tsujita, K. Tanzawa and A. Endo, Lipids, 14, 585 (1979).
119. M. Kuroda, K. Tanzawa, Y. Tsujita and A. Endo, Biochim. Biophys. Acta, 489, 119 (1977).
120. A. Endo, Y. Tsujita, M. Kuroda and K. Tanzawa, Biochim, Biophys. Acta, 575, 266 (1979).
121. R. Fears, D.H. Richards and H. Ferres, Fifth Int. Symp. on Atherosclerosis, Houston, TX, Nov. 6-9, 1979, Abstr. No. 27.
122. A. Yamamoto, A. Endo and H. Sudo, ibid., Abstr. No. 152.
123. M.S. Brown, J.R. Faust, J.L. Goldstein, I. Kaneko and A. Endo, J. Biol. Chem., 253, 1121 (1978).
124. D.J. Betteridge, W. Krone, J.P.D. Reckless and D.J. Galton, Lancet, 2, 1342 (1978).
125. A.G. Olsson, L. Oro and L.A. Carlson, Fifth Int. Symp. on Atherosclerosis, Houston, TX, Nov. 6-9, 1979, Abstr. No. 160.
126. D.F. Brown, A. Beyler and K. Daudiss, Amer. J. Cardiol., 43, 409 (1979).
127. J. Edelson, D.P. Benziger, A. Arnold and A.L. Beyler, Atherosclerosis, 33, 351 (1979).
128. A.S. Katocs Jr. and S.A. Schaffer, Fed. Proc., 36, 1160 (1977).
129. W. Hollander, S. Prusty, S. Nagraj, B. Kirkpatrick, J. Paddock and M. Colombo, Atherosclerosis, 31, 307 (1978).
130. E.E. Largis, C.-H. Wang and S.A. Schaffer, Fifth Int. Symp. on Atherosclerosis, Houston, TX, Nov. 6-9, 1979, Abstr. No. 36.
131. J.D. Albright, S.A. Schaffer and R.G. Shepherd, J. Pharmaceut. Sci., 68, 936 (1979).
132. A.N. Howard, R. Zschocke, R. Loser and G. Hofrichter, Atherosclerosis, 32, 367 (1979).
133. P. St. Janiak, Drugs of the Future, 4, 140 (1979).
134. G. Ecsedi, I. Hermecz and S. Virag, Acta Pharm. Hung., 48, 40 (1978).
135. S. Virag, C. Vertesi and I. Welner, Ther. Hung., 26, 171 (1978).
136. L. de Angelis, Drugs of the Future, 4, 545 (1979).
137. M. Fuccella, L. Musatti, G.C. Descovich and C. Sirtori, Clin. Pharmacol. Ther., 25, 226 (1979).
138. B. Riveline, Postgrad. Med. J., 51, 162 (1975).
139. G. Marquie, Eur. J. Pharmacol., 45, 1 (1977).
140. G. Marquie, Atherosclerosis, 32, 253 (1979).

Chapter 18. Recent Advances in the Design and Development of Antiobesity Agents

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Introduction - In the intervening years since the last review,[1] progress has been made in the identification and development of novel antiobesity agents. Major efforts continue to be directed towards the development of appetite suppressants. A monograph[2] and several comprehensive reviews[3,4] document our current knowledge of the alteration of the central mechanisms involved in the regulation of food intake by anorectic drugs. Novel pharmacological approaches which function by modulating peripheral energy metabolism have received increasing attention.[5-7] These include, in particular, a) reducing the availability of dietary carbohydrate or lipid by altering intestinal absorption and b) modifying lipid metabolism.

The etiology, metabolic characteristics and treatment of obese patients were reviewed[8] and important recent advances in obesity research were summarized.[9] Monographs summarizing the relationships between obesity and diabetes mellitus including the role of a) adipose cellularity, b) metabolic, neuroendocrine, and nutritional factors, and c) membrane insulin receptors and transport mechanisms have also appeared.[10,11]

Anorectic Agents

Fenfluramine and Derivatives - Recent double-blind studies have demonstrated the superiority of sustained-release fenfluramine (1) over the rapid-release form or placebo as an adjunct to diet restriction in effecting weight loss in obese patients.[12-14] Improved glucose tolerance and insulin response[15] and significant decreases in serum cholesterol and triglycerides[13,14] were observed. There was a significant correlation between weight loss and plasma concentrations of fenfluramine and its metabolite norfenfluramine.[16,17]

The pharmacology and mechanism of action of fenfluramine have been reviewed.[18,19] Although fenfluramine is chemically related to amphetamine, it differs in that it increases brain serotonin (5-HT) concentration by stimulating release of 5-HT and blocking its neuronal uptake. The effect of fenfluramine on food intake can be abolished by 5-HT antagonist drugs that lower 5-HT brain concentrations. Studies in man, monkeys and rats showed that fenfluramine was metabolized to at least four compounds with pharmacological activity, d- and l-fenfluramine and d- and l-norfenfluramine.[19] Concentrations of fenfluramine were higher in the blood and brain of obese goldthioglucose mice compared to lean mice, whereas no difference in norfenfluramine levels was observed.[20] Fenfluramine appeared to suppress carbohydrate consumption while sparing protein intake in rats allowed to choose between diets that vary in protein content.[21,22] In contrast, amphetamine produced a dose-related restriction of protein and other nutrient intake.[22] Fluoxetine (Lilly 110140), a specific inhibitor of 5-HT uptake, produced a potent anorectic effect of short duration in rats.[23] Like fenfluramine, fluoxetine spared protein consumption while reducing caloric intake.[24]

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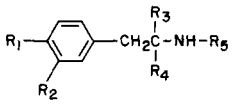
In addition to anorexia, peripheral effects may play an important role in the antiobesity action of fenfluramine. An increase in the peripheral utilization of carbohydrate and a decrease in lipid biosynthesis were demonstrated. Fenfluramine and norfenfluramine in therapeutic concentrations increased glucose uptake by isolated human skeletal muscle.[25] In addition, fenfluramine decreased the rate of triacylglycerol synthesis in the liver, intestine and adipose tissue through the inhibition of phosphatidate phosphohydrolase.[26,27] These effects of fenfluramine on energy metabolism may have increased its usefulness in the treatment of maturity-onset diabetes, particularly in obese patients.[28-31] Improved glucose tolerance and weight loss were demonstrated which were significantly greater than that produced by diet alone.[28,31] No lactic acid acidosis was observed with fenfluramine.[29] Chronic administration of fenfluramine resulted in significant decreases of plasma norepinephrine and dopamine-β-hydroxylase in depressed patients, and a reversal of the hydrochlorothiazide-induced increase in plasma norepinephrine in obese hypertensive patients.[32]



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H	SCF_3	H	CH_3	C_2H_5	2
H	CF_3	H	CH_3	$-(CH_2)_2OC(=O)-C_6H_5$	3
H	H	H	CH_3	SO_2CF_3	4
$NHSO_2CH_3$	H	H	CH_3	SO_2CH_3	5
Cl	H	H	C_6H_5	H	6
Cl	H	H	C_6H_4-4-Cl	H	7
H	Cl	H	C_6H_5	H	8
H	H	H	CH_3	$CH_2C_6H_4$-4-F	9
H	H	CH_3	CH_3	$CH_2C_6H_4$-4-F	10
H	Cl	$-(CH_2)_6-$		H	11
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A double-blind study showed that flutiorex (2) was twice as potent as fenfluramine in reducing food intake in normal volunteers.[33] Stimulation of the α-adrenergic and central nervous systems, as indicated by an elevation of systolic blood pressure, the development of mydriasis, and an increase in critical flicker frequency was also noted. However, a sustained-release formulation of flutiorex, tiflorex (TFX-SR), reduced hunger and food intake five hours after administration without causing cardiovascular effects or central nervous system stimulation.[34] A sex-linked difference in the pharmacokinetics of flutiorex was observed which could explain the greater clinical activity of this compound in males.[35] Benfluorex, S-780 (3), depressed food consumption and body weight in rats and these effects were associated with a decrease in hepatic triacylglycerol synthesis through the inhibition of phosphatidate phosphohydrolase.[36,37] This compound also produced some weight loss in obese subjects.[38] Several β-methoxy analogs of fenfluramine have been found to possess anorectic activity in rats without CNS stimulation.[39]

Other Phenethylamine Derivatives - A double-blind trial demonstrated that diethylpropion at a single dose of 75 mg/day and mazindol at 1 mg t.i.d. were equally effective in producing weight loss in obese patients on a

1000 cal./day diet.[40] A comprehensive review of diethylpropion has appeared.[41] A study of fenproporex in obese women showed that the drug had a distinct anorectic action without central or sympathomimetic effects.[42]

Chemical syntheses have yielded several interesting new phenethylamine derivatives with anorectic activity. Of a number of sulfur-containing amphetamine derivatives synthesized, compounds 4 and 5 significantly reduced food intake and body weight in rats.[43] Anorexigenesis of varying degrees without motor stimulation was demonstrated in rats fed various 1,2-diphenylethylamines 6, 7, and 8.[44] Several compounds demonstrated anorectic activity similar to amphetamine or fenfluramine. Among a series of β-phenethylamine derivatives synthesized and tested for anorectic activity in mice, F1697 (9) and F1698 (10) were found to be comparable to amphetamine, clobenzorex, and chlorphentermine.[45] They had a low stimulating effect but therapeutic indices were unfavorable.

1-Benzylcycloalkylamines with cycloalkyl rings of five, six or seven carbons produced varying degress of hypophagia in rats generally without stimulant effects.[46] Of eighteen 1-benzylcycloalkylamines synthesized, eight compounds with halogen substituents on the benzyl ring were found to reduce food intake by 50% or more when injected i.p. into rats at one-tenth of the mouse LD_{50}. The most potent nonstimulant anorectic compounds of the series were 11 and 12, which resembled chlorphentermine in their activity.

Non-Phenethylamine Derivatives - Double-blind studies have continued to attest to the efficacy of mazindol (13) as an adjunct to dietary restrictions in promoting weight loss in obese patients.[47-51] Mazindol was found to be of no additional clinical advantage when used in conjunction with a very low caloric formula diet (260 kcal/day).[52] A multicenter trial demonstrated that mazindol plus behavioral modification resulted in a greater mean weight loss than either mode of therapy alone.[53]

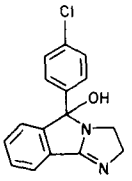
It has been suggested that the anorectic action of mazindol is mediated by activation of a dopaminergic mechanism.[54,55] Like amphetamine, mazindol causes the release of dopamine and blocks its re-uptake.[54,55] Mazindol's anorectic effect is antagonized by α-methyltyrosine (a catecholamine synthesis inhibitor) and pimozide (a dopaminergic receptor blocker).[54,55] A recent study supported the hypothesis that mazindol and amphetamine may be acting through perifornical hypothalamic catecholamine neurons consisting of dopaminergic and β-adrenergic receptors.[56]

Mazindol administered to obese diabetics or obese women resulted in a significant reduction in serum cholesterol[50,51] and triglycerides,[50] and a significant increase in nonesterified fatty acids.[51] Acute studies suggested that mazindol impaired absorption of glucose from the gut.[57] Mazindol increased glucose uptake in isolated human skeletal muscle whether insulin was present or not,[58] and inhibited glucose oxidation and insulin binding by rat isolated fat cells.[59] However, these effects are not likely to be relevant to the antiobesity action of mazindol in man. Other effects of mazindol on energy metabolism include an increase in hydroxyacyl CoA dehydrogenase and a decline in malic dehydrogenase in striated muscle, an enhancement of urinary norepinephrine elimination, and an elevation of triiodothyronine binding globulin.[51]

DITA (3',4'-dichloro-2-[2-imidazolin-2-yl-thio]acetophenone) produced anorexia and reduction of body weight in several animal species. Its anorexigenic activity was comparable to diethylpropion and, in mice, was

associated with significantly increased spontaneous motor activity.[60] The anorectic effect of DITA was suggested to be mediated mainly through the dopaminergic system, since blockade of the adrenergic and serotonergic systems failed to alter its activity whereas the dopamine antagonist haloperidol or α-methyltyrosine antagonized anorexigenesis.[61]

(−)-Hydroxycitrate and (±)-trans-epoxyaconitate differ from the previously described anorectic agents in that both compounds produced anorexia and promoted weight loss apparently by modifying peripheral rather than central mechanisms regulating appetite.[63] The (−)-hydroxycitrate induced anorexia was not associated with conditioned aversion[62] but resulted from an alteration in peripheral metabolite flux since fatty acid synthesis[63] was inhibited and, concomitantly, glycogen synthesis and levels were increased, even under pair-feeding conditions.[64] (−)-Hydroxycitrate significantly reduced body weight due to a selective reduction in body fat and food intake in several obese rodent models.[65] No alterations in fatty acid or glycogen synthesis were produced by (±)trans-epoxyaconitate, however, its anorectic activity appeared to be related to a selective reduction in the rate of gastric emptying.[6]



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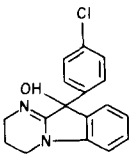

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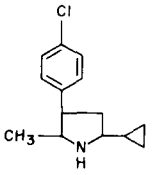

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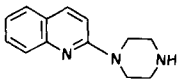
Ciclazindol (14) depressed appetite in human subjects after a single oral dose of 50 mg,[66] and caused a dose-related, insulin-independent increase in glucose uptake in both isolated human skeletal muscle[67] and rat hemidiaphragm.[68] UP 507-04 (15) decreased food intake and body weight in mice and rats with experimental obesity.[69]

Quipazine (16), a central 5HT agonist, induced anorexigenesis in rats.[70,71] Anorexia was prevented by pretreatment with methergoline, a drug showing central anti-5-HT activities, but not by electrolytic lesioning of the median raphe nucleus, an important site of origin of serotonergic neurons in the brain.[70,71]



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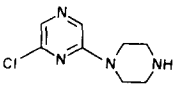

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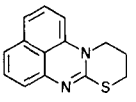
MK-212 (17) preferentially decreased carbohydrate consumption in rats but maintained protein intake.[72] Food intake was suppressed in rats and cats.[73] MK-212 appeared to have a shorter duration of action than fenfluramine, and a minor effect on motor activity; it was not self-administered by rats. Serotonin antagonists such a methergoline, cyproheptadine and cinanserin attenuated the anorectic effect of MK-212.[74] Reduction of brain serotonin by intraventricular injection of 5,6-dihydroxytryptamine, i.p. injection of p-chloroamphetamine, or lesioning the median raphe nucleus similarly diminished the anorectic activity induced by MK-212. These findings indicate that MK-212 has a serotoninmimetic mechanism of action similar to that of fenfluramine.[73,74]

Species differences in plasma levels, urinary elimination and metabolism of the anorectic agent 11698 JL (2-[1-aminopropyl]-2-indanol) have been demonstrated in the rat, dog and man after oral administration.[75] 11698 JL appeared to reduce plasma levels of immunoreactive insulin as well as insulin secretion *in vitro* by pancreatic endocrine cells.[75]

Among a series of fused pyrimidine derivatives, 18 was found to be the most effective and was devoid of central nervous system effects.[76] Among a series of substituted oxazines, 19 and 20 produced significant anorexia and anticonvulsant activities in rats and were devoid of significant motor depressant activity.[77] In a series of trifluoromethylphenyl piperazines, 21 was found to be the most potent, however, teratogenic effects in rats and mice precluded its further development.[78]



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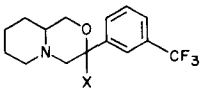
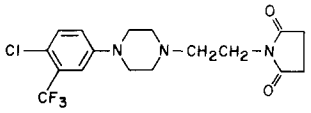


X = H 19
X = OH 20



21

Peptides - The satiety inducing effect of the gastrointestinal hormone cholecystokinin (CCK) in rats was confirmed in Rhesus monkeys[79] and in man.[80,81] It is believed that CCK may act on peripheral rather than central nervous system substrates[82] and that CCK may perform a regulatory function in a neurohumoral feedback mechanism.[83] Electrophysiological alterations in specific brain structures involved in appetite regulation were produced by CCK but not by gastrin or secretin.[83] CCK and CCK octapeptide (CCK_{26-33}), have been found in cerebrospinal fluid and in large amounts in selective areas of the brain of humans.[84] Endogenous CCK peptides extracted from the brain are immunologically similar to extracts of CCK from the jejunum. In rat brain, high concentrations of CCK octapeptide cells are located in the hypothalamus, particularly in dorsomedial areas.[85]

Other naturally occuring peptides isolated from human or animal sources were found to have anorexigenic or antiobesity properties when tested in mice or rats. Bombesin, a tetradecapeptide originally isolated from amphibian skin, suppressed food but not water intake in rats[86] and may be a putative satiety signal. Another unidentified gastric satiety signal was recently demonstrated in rats.[87] The action of this gastric signal does not depend on vagal innervation and it may therefore be hormonal in nature.[88]

Satietin is a highly potent anorexigenic substance isolated from human plasma which reduced food intake markedly after i.v. administration to rats deprived of food for four days.[89] Neither CCK octapeptide nor pyro-glu-his-glyOH, two endogenous peptides described as possessing satiety effects, had an effect in this system. Satietin also decreased food intake when given intraventricularly suggesting a direct action on the central regulation of feeding. The anorexia was more potent and of longer duration than that produced by amphetamine.[89]

Avian pancreatic polypeptide and bovine pancreatic polypeptide given i.p. returned the hyperinsulinemia, hyperglycemia and weight gain of New Zealand obese mice to normal.[90] This reversion was also produced by the intraperitoneal implantation of islet cells from white mice to New Zealand obese mice; the implanted islets secreted mouse pancreatic polypeptide.

A tripeptide (pyro-glu-his-glyOH) isolated from the urine of patients with anorexia nervosa, reduced food intake after s.c. administration to mice.[91,92] After a 20-day treatment period, food intake and body weight did not normalize in these mice until six months later. Thyrotropin releasing hormone (pyro-glu-his-proNH$_2$) whether injected i.p. or intraventricularly elicited hypophagia in food-deprived rats.[93] Water ingestion was also reduced and locomotor activity was increased. In contrast to amphetamine, reduction of food intake produced by TRH was enhanced by prior 6-hydroxydopamine treatment. Subcutaneous or intracerebral injection of calcitonin inhibited feeding in rats. Calcitonin was postulated to inhibit feeding by acting directly on the CNS.[94]

Other Agents Influencing Appetite - Weight loss was observed in aged Parkinsonian patients after long-term l-dopa treatment.[95] L-dopa stimulated growth hormone secretion in normal subjects but obese subjects were unresponsive.[96,97] However, plasma growth hormone values were markedly increased in obese subjects treated with l-dopa plus propranolol.[98] During dietary restriction in obese patients, l-dopa increased energy expenditure and produced a greater reduction of body weight than diet alone.[99] The possible mechanisms of action of the hypophagic effects of amphetamine and l-dopa appeared to be similar.[100] L-dopa exerted its appetite suppressant effects through greater catecholamine synthesis specifically within dopaminergic and adrenergic neurons of the periforni-cal hypothalamic region.[101] Recent evidence demonstrated that the sustained body weight decline in genetically obese rats treated with l-dopa could not be accounted for by reduced food consumption alone.[102]

Prostaglandin $F_{2\alpha}$ injected intraventricularly reduced food consumption in food deprived and in satiated rats and it was suggested that the hypophagic effect of prostaglandin $F_{2\alpha}$ occurred at both peripheral and central sites.[103] The involvement of prostaglandin generating systems was demonstrated, since i.p. and i.g. administration of the prostaglandin precursors arachidonic, linolenic and linoleic acids also inhibited food intake in hungry rats.[104] Prior treatment with indomethacin and paracetamol reversed the anorexia and behavioral satiety induced by the three fatty acids, but had no effect on prostaglandin $F_{2\alpha}$-induced suppression of food intake.

The elevation of body glycerol concentration in rats by multiple daily injections of glycerol led to hypophagia and body weight loss, followed by normal food intake and normal rate of body weight increase.[105] A reduction in food consumption was also demonstrated in rats after the injection of adenosine, and to a lesser extent inosine.[106]

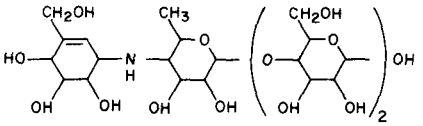
In acute experiments in rats, atropine methyl nitrate inhibited the sham feeding of a liquid diet, but not drinking, and elicited behavioral satiety.[107] Scopolomine methyl nitrate suppressed food intake and caused weight loss in ventromedial hypothalmic lesioned obesity, but food intake recovered and a significant amount of obesity was maintained.[108]

The opiate antagnist, naloxone produced a dose-related suppression of eating and drinking in rats.[109-111] Excessive food consumption by genetically obese strains of rats and mice was diminished by naloxone.[112] These particular rodent strains have abnormally high pituitary and plasma levels of endorphin which is consistent with the view that the opioid peptides are involved in the modification of eating behavior. Furthermore, β-endorphin injected intrahypothalmically in normal and satieted rats appeared to stimulate food consumption.[113] A modulating effect of endorphins on feeding behavior has been suggested.[111]

Agents Which Modify Energy Metabolism

Inhibitors of Intestinal Absorption - Retardation or inhibition of digestion of dietary carbohydrate should lead to a dimunition of hyperglycemia and hyperinsulinemia in subjects suffering from obesity or diabetes. In rats, Bay g 5421 (22), a complex oligosaccharide of microbial origin,[114] competitively inhibited intestinal glucosidases, and retarded the digestion of both sucrose and starch.[115] The postprandial increment of blood glucose and serum insulin was inhibited by Bay g 5421 in rats and in man. Body weight as well as body and blood lipids were decreased in obese rats. Results from a study in healthy volunteers suggested that a dose of 100-200 mg of Bay g 5421 per meal might be effective for clinical trials in diabetes, hypertriglyceridemia and obesity.[116]

Among a series of p-alkanoyl toluenes synthesized as potential anti-obesity agents, SaH 50-283 (23) inhibited weight gain and food efficiency while only slightly reducing food intake.[117] This effect was attributed to an inhibition of maltase activity in the brush border. Cycloleucine (1-amino-1-cyclopentane-carboxylic acid) decreased body weight gain and food efficiency.[118] Food consumption was not significantly depressed except at the highest dose (0.1% in diet). This decreased food efficiency was attributed specifically to an inhibition of pancreatic α-amylase activity; the activities of maltase, sucrase, lactase and pancreatic lipase were not significantly affected.



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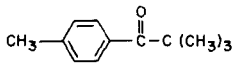

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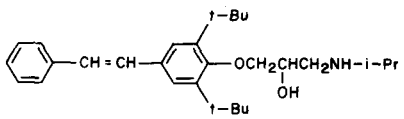
The hydrophobic nonionic surfactant Pluronic L-101, a copolymer of polyoxethylene (90%) and polyoxpropylene (10%), is a potent inhibitor of human pancreatic lipase in vitro.[119] When fed to rats as a 1% or 3% dietary admix, it decreased weight gain through a reduction of body fat without affecting food consumption. Elimination of dietary fat in feces was enhanced significantly during Pluronic L-101 treatment. Perfluorooctyl bromide, a radioopaque contrast medium, reduced body weight gain in rats by coating the stomach and intestines, thus preventing the absorption of ingested food.[120]

Inhibitors of Lipid Biosynthesis - In a series of linked aryl aryloxypropanolamines synthesized with the aim of identifying compounds which would reduce body weight by decreasing body fat without affecting appetite, (24) significantly suppressed body weight in rats and dogs.[121] The compound, however, possessed considerable hematological toxicity.



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Dehydroepiandrosterone, a mammalian glucose-6-phosphate dehydrogenase inhibitor, prevented the development of obesity by limiting the accumulation of triacylglycerol in a strain of mice prone to maturity-onset obesity.[122] Hepatic lipogenesis and body weight were decreased, but food consumption was not affected. The structurally similar 5α-androstan-

17-one had a similar antiobesity effect.[123] The compound did not suppress appetite or affect lipogenic rates in liver and carcass, although there was a decrease in the accumulation of triacylglycerol.

References

1. A.C. Sullivan, L. Cheng, J.G. Hamilton. Ann. Rep. Med. Chem., 11, 200 (1976).
2. "Central Mechanisms of Anorectic Drugs", S. Garattini, R. Samanin, Eds. Raven Press, New York, 1978, 487 pp.
3. B.G. Hoebel, in "Handbook of Psychopharmacology" Vol. 8, L.L. Iversen, S.D. Iversen, S.H. Snyder, Eds. Plenum Press, New York, 1977, p 55.
4. A.C. Sullivan, L. Cheng, in "Appetite Regulation and Its Modulation by Drugs", J.N. Hathcock, J. Coon, Eds. Academic Press, New York, 1978, p 21.
5. A.C. Sullivan, K. Comai, Int. J. Obes. 2, 167 (1978).
6. A.C. Sullivan, J. Triscari, in "Recent Advances in Obesity Research: II", G.A. Bray, Ed. Newman Publishing, Ltd. (London), 1978, p 442.
7. P. Turner, Int. J. Obesity 2, 343 (1978).
8. "Obese Patient", G.A. Bray. (Major Problems in Internal Medicine Ser.: Vol. 9). W. B. Saunders Co., Philadelphia, 1976.
9. "Recent Advances in Obesity Research: II", G.A. Bray, Ed. Newman Publishing, Ltd. (London), 1978.
10. "Advances in Modern Nutrition" Vol. 2 (Part 1), H.M. Katzen, R.J. Mahler, Eds. John Wiley & Sons, New York, 1978.
11. "Advances in Modern Nutrition" Vol. 2 (Part 2), H.M. Katzen, R.J. Mahler, Eds. John Wiley & Sons, New York, 1978.
12. R.W. Dent, in "Central Mechanisms of Anorectic Drugs", S. Garattini, R. Samanin, Eds. Raven Press, New York, 1978, p 451.
13. R.E. Noble, Curr. Med. Res. Opin. 6 (Suppl. 1), 169 (1979).
14. J. Vague, M. Tramoni, Curr. Med. Res. Opin. 6 (Suppl. 1), 194 (1979).
15. R. Ranquin, H.M. Brems, Curr. Med. Res. Opin. 5, 341 (1977-78).
16. J.A. Innes, M.L. Watson, M.J. Ford, J.F. Munro, M.E. Stoddart, D.B. Campbell, Brit. Med. J. 2, 1322 (1977).
17. P.G. Preston, M.J. Ford, J.F. Munro, Curr. Med. Res. Opin. 6 (Suppl. 1), 188 (1979).
18. J. Duhault, L. Beregi, R. du Boistesselin, Curr. Med. Res. Opin. 6 (Suppl. 1), 3 (1979).
19. S. Garattini, S. Caccia, T. Mennini, R. Samanin, S. Consolo, H. Ladinsky, Curr. Med. Res. Opin. 6 (Suppl. 1), 15 (1979).
20. A. Bizzi, M.T. Tacconi, G. Tognoni, P.L. Morselli, S. Garattini, Int. J. Obesity 2, 1 (1978).
21. J.J. Wurtman, R.J. Wurtman, Curr. Med. Res. Opin. 6 (Suppl. 1), 28 (1979).
22. J.E. Blundell, C.J. Latham, E. Moniz, R.A. McArthur, P.J. Rogers, Curr. Med. Res. Opin. 6 (Suppl. 1), 34 (1979).
23. A.J. Goudie, E.W. Thornton, T.J. Wheeler, J. Pharm. Pharmacol. 28, 318 (1976).
24. J.J. Wurtman, R.J. Wurtman, Science 198, 1178 (1977).
25. P. Turner, Curr. Med. Res. Opin. 6 (Suppl. 1), 101 (1979).
26. D.N. Brindley, M. Bowley, R.G. Sturton, P.H. Pritchard, S.L. Burditt, J. Cooling, in "Central Mechanisms of Anorectic Drugs", S. Garattini, R. Samanin, Eds. Raven Press, New York, 1978, p 301.
27. D.N. Brindley, R.G. Sturton, P.H. Pritchard, J. Cooling, S.L. Burditt, Curr. Med. Res. Opin. 6 (Suppl. 1), 91 (1979).
28. J.W.H. Doar, M.E. Thompson, C.E. Wilde, P.F.J. Sewell, Curr. Med. Res. Opin. 6 (Suppl. 1), 247 (1979).
29. H. Forster, E. Koch, Curr. Med. Res. Opin. 6 (Suppl. 1), 207 (1979).
30. J.K. Wales, Curr. Med. Res. Opin. 6 (Suppl. 1), 226 (1979).
31. B. Riveline, Curr. Med. Res. Opin. 6 (Suppl. 1), 236 (1979).
32. C.R. Lake, M.D. Coleman, M.G. Ziegler, D.L. Murphy, Curr. Med. Res. Opin. 6 (Suppl. 1), 63 (1979).
33. J.F. Giudicelli, C. Richer, A. Berdeaux, Br. J. Clin. Pharmacol. 3, 113 (1976).
34. T. Silverstone, J. Fincham, J. Plumley, Br. J. Clin. Pharmacol. 7, 353 (1979).
35. J.F. Giudicelli, C. Richer, A. Berdeaux, N. Guessous, Eur. J. Clin. Pharmacol. 10, 325 (1976).
36. D.N. Brindley, M. Bowley, S. Burditt, P.H. Pritchard, K.A. Lloyd-Davies, P. Boucrot, J. Pharm. Pharmacol. 28, 676 (1976).
37. D.N. Brindley, P.H. Pritchard, R.G. Sturton, Brit. J. Pharmacol. 64, 377P (1978).
38. A.C. Asmal, W.P. Leary, W. Deppe, C.J. Lockett, Res. Commun. Chem. Pathol. Pharmacol. 18, 95 (1977).
39. S.L. Beregi, J. Duhault, Arzneim.-Forsch. 27, 116 (1977).
40. G.S. Allen, Curr. Ther. Res. Clin. Exp. 22, 678 (1977).
41. M.T. Hoekenga, R.H. Dillon, H.M. Leyland, in "Central Mechanisms of Anorectic Drugs", S. Garattini, R. Samanin, Eds. Raven Press, New York, 1978, p 391.
42. G. Hertel, W. Fallot-Burghardt, Fortschr. Med. 96, 2380 (1978).
43. W.O. Foye, J.N. Sane, J. Pharm. Sci. 66, 923 (1977).
44. P. Ghosh, A.G. Bolt, R.I. Mrongovius, Arzneim. Forsch. 28, 1561 (1978).

45. S. Casadio, H. Cousse, G. Mouzin, A. Stenger, M. Charveron, P. Vilain, H. Lauressergues, Boll. Chim. Farm. 117, 83 (1978).
46. R.I. Mrongovius, P. Ghosh, A.G. Bolt, Clin. Exp. Pharmacol. Physiol. 6, 81 (1979).
47. P.J. Miach, W. Thomson, A.E. Doyle, W.J. Louis, Med. J. Aust. 2, 378 (1976).
48. G. Enzi, A. Baritussio, E. Marchiori, G. Crepaldi, J. Int. Med. Res. 4, 305 (1976).
49. A.G. Wallace, Med. J. Aust. 1, 343 (1976).
50. G. Slama, A. Selmi, M. Hautecouverture, G. Tchobroutsky, Diabete Metab. 4, 193 (1978).
51. R. Rath, K. Vondra, A. Bass, V. Kujalova, J. Wenkeova, Int. J. Obesity 3, 133 (1979).
52. I. McLean Baird, A.N. Howard, Int. J. Obesity 1, 271 (1977).
53. B.R. Walker, I.M. Ballard, J.A. Gold, J. Int. Med. Res. 5, 85 (1977).
54. Z.L. Kruk, M.R. Zarrindast, Br. J. Pharmacol. 58, 367 (1976).
55. M.O. Carruba, F. Zambotti, L. Vicentini, G.B. Picotti, P. Mantegazza, in "Central Mechanisms of Anorectic Drugs", S. Garattini, R. Samanin, Eds. Raven Press, New York, 1978, p 145.
56. S.F. Leibowitz, C. Rossakis, Eur. J. Pharmacol. 53, 69 (1978).
57. L.C. Harrison, A.P. King-Roach, K.C. Sandy, Metabolism 24, 1353 (1975).
58. M.J. Kirby, P. Turner, J. Pharm. Pharmacol. 28, 163 (1976).
59. L.C. Harrison, A.P. King-Roach, Clin. Exp. Pharm. Phys. 3, 503 (1976).
60. A.H. Abdallah, Toxicol. Appl. Pharmacol. 41, 329 (1977).
61. A.H. Abdallah, D.M. Roby, W.H. Boeckler, C.C. Riley, Eur. J. Pharmacol. 40, 39 (1976).
62. J. Panksepp, A. Pollack, R.B. Meeker, A.C. Sullivan, Pharmacol. Biochem. Behav. 6, 683 (1977).
63. A.C. Sullivan, J. Triscari, H.E. Spiegel, Am. J. Clin. Nutr. 30, 777 (1977).
64. A.C. Sullivan, J. Triscari, in "Hunger: Basic Mechanisms and Clinical Implications", D. Novin, W. Wyrwicka, G. Bray, Eds. Raven Press, New York, 1976, p 115.
65. A.C. Sullivan, J. Triscari, Am. J. Clin. Nutr. 30, 767 (1977).
66. R.S.B. Ehsanullah, M.J. Kirby, M. Leighton, V.M.S. Oh, Br. J. Clin. Pharmacol. 4, 400P (1977).
67. M.J. Kirby, P. Turner, Br. J. Clin. Pharmacol. 4, 459 (1977).
68. M.J. Kirby, P.J. Williams, Int. Congr. Pharm., 7th, Paris, July 16-21, Abstracts, pt 1, p 268 (1978).
69. G. Dumeur, B. Hüe, J.M. Lwoff, M.A. Mouries, D. Tremblay, Br. J. Pharmacol. 58, 437P (1976).
70. R. Samanin, C. Bendotti, G. Candelaresi, S. Garattini, Life Sci. 21, 1259 (1977).
71. R. Samanin, C. Bendotti, F. Miranda, S. Garattini, J. Pharm. Pharmacol. 29, 53 (1977).
72. J.J. Wurtman, R.J. Wurtman, Life Sci. 24, 895 (1979).
73. B.V. Clineschmidt, H.M. Hanson, A.B. Pflueger, J.C. McGuffin, Psychopharmacology 55, 27 (1977).
74. B.V. Clineschmidt, J.C. McGuffin, A.B. Pflueger, J.A. Totaro, Br. J. Pharmacol. 62, 579 (1978).
75. A. Louis-Broillet, M. Strolin Benedetti, J. Maillard, E. Niesor, Ann. Pharm. Fr. 36, 309 (1978).
76. K.-C. Liu, H.-H. Chen, L.-C. Lee, J.-W. Chern, Naunyn-Schmiedeberg's, Arch. Pharmacol. 312, 776 (1979).
77. G.O. Rankin, T.N. Riley, J.C. Murphy, J. Med. Chem. 21, 460 (1978).
78. P.E. Cross, R.P. Dickinson, G. Halliwell, J.E.G. Kemp, Eur. J. Med. Chem.-Chim. Ther. 12, 173 (1977).
79. J.D. Falasco, G.P. Smith, J. Gibbs, Physiol. Behav. 23, 887 (1979).
80. R.A.L. Sturdevant, H. Goetz, Nature 261, 713 (1976).
81. G. Stacher, H. Bauer, H. Steinringer, Physiol. Behav. 23, 325 (1979).
82. C.B. Nemeroff, A.J. Osbahr, III, G. Bissette, C. Jahnke, M.A. Lipton, A.J. Prange, Jr., Science 200, 793 (1978).
83. M.C. Schanzer, E.D. Jacobson, N. Dafny, Neuroendocrinology 25, 329 (1978).
84. J.F. Rehfeld, C. Kruse-Larsen, Brain Res. 155, 19 (1978).
85. R.B. Innis, F.M.A. Correa, G.R. Uhl, B. Schneider, S.H. Snyder, Proc. Natl. Acad. Sci., USA 76, 521 (1979).
86. J. Gibbs, D.J. Fauser, E.A. Rowe, B.J. Rolls, E.T. Rolls, S.P. Maddison, Nature 282, 208 (1979).
87. F.S. Kraly, G.P. Smith, Phys. Behav. 21, 405 (1978).
88. F.S. Kraly, G. Gibbs, Abstr. Soc. Neurosci. 4, 176 (1978).
89. J. Knoll, Physiol. Behav. 23, 497 (1979).
90. R.J. Gates, N.R. Lazarus, Hormone Res. 8, 189 (1977).
91. O. Trygstad, I. Foss, P.D. Edminson, J.H. Johansen, K.L. Reichelt, Acta Endocrinol. 89, 196 (1978).
92. K.L. Reichelt, I. Foss, O. Trygstad, P.D. Edminson, J.H. Johansen, J.B. Boler, Neuroscience 3, 1207 (1978).
93. R.A. Vogel, B.R. Cooper, T.S. Barlow, A.J. Prange, Jr., R.A. Mueller, G.R. Breese, J. Pharmacol. Exp. Ther. 208, 161 (1979).
94. W.J. Freed, M.J. Perlow, R.J. Wyatt, Science 206, 850 (1979).
95. J. Vardi, Z. Oberman, I. Rabey, M. Stre'fler, D. Ayalon, M. Herzberg, J. Neurol. Sci. 30, 33 (1976).
96. G. Gragnoli, V. Palazzuoli, R. Favilli, I. Tanganelli, G. Migliarese, Acta Diabetol. Lat. 14, 137 (1977).

97. B. D'Alessandro, A. Bellastella, M.R. Cafaro, R. De Luca, V. Esposito, Boll. Soc. Ital. Biol. Sper. 53, 2333 (1977).
98. A. Barbarino, L. De Marinis, L. Troncone, Metab. Clin. Exp. 27, 275 (1978).
99. P.S. Shetty, R.T. Jung, W.P.T. James, Lancet 1, 77 (1979).
100. I.S. Sanghvi, G. Singer, E. Friedman, S. Gershon, Pharmacol. Biochem. Behav. 3, 81 (1975).
101. S.F. Leibowitz, C. Rossakis, Psychopharmacology 61, 273 (1979).
102. R.B. Hemmes, H.M. Pack, J. Hirsch, Fed. Proc. Fed. Am. Soc. Exp. Biol. 38, 277 (1979).
103. N.S. Doggett, K. Jawaharlal, Br. J. Pharmacol. 60, 409 (1977).
104. N.S. Doggett, K. Jawaharlal, Br. J. Pharmacol. 60, 417 (1977).
105. D. Wirtshafter, J.D. Davis, Science 198, 1271 (1977).
106. M.C. Capogrossi, A. Francendese, M. DiGirolamo, Am. J. Clin. Nutr. 32, 1762 (1979).
107. D. Lorenz, P. Nardi, G.P. Smith, Pharmacol. Biochem. Behav. 8, 405 (1978).
108. R.G. Carpenter, B.A. Stamoutsos, L.D. Dalton, L.A. Frohman, S.P. Grossman, Physiol. Behav. 23, 955 (1979).
109. S.G. Holtzman, Life Sci. 24, 219 (1979).
110. D.R. Brown, S.G. Holtzman, Pharmacol. Biochem. Behav. 11, 567 (1979).
111. B. Brands, J.A. Thornhill, M. Hirst, C.W. Gowdey, Life Sci. 24, 1773 (1979).
112. D.L. Margules, B. Moisset, M.J. Lewis, H. Shibuya, C.B. Pert, Science 202, 988 (1978).
113. L. Grandison, A. Guidotti, Neuropharmacology 16, 533 (1977).
114. D.D. Schmidt, W. Frommer, B. Junge, L. Mueller, W. Wingender, E. Truscheit, D. Schaefer, Naturwissenschaften 64, 535 (1977).
115. W. Puls, U. Keup, H.P. Krause, G. Thomas, F. Hoffmeister, Naturwissenschaften 64, 536 (1977).
116. I. Hillebrand, K. Boehme, G. Frank, H. Fink, P. Berchtold, Diabetologia 15, 239 (1978).
117. R.S. Ho, C.G. Aranda, Arch. Int. Pharmacodyn 237, 98 (1979).
118. C.G. Aranda, R.S. Ho, W.R. Sterling, Proc. Soc. Exp. Biol. Med. 162, 401 (1979).
119. K. Comai, A.C. Sullivan, Int. J. Obesity (in press).
120. M. Hussain, S. Niazi, A. Arambulo, D.M. Long, J. Pharm. Sci. 66, 907 (1977).
121. M.T. Cox, S.E. Jaggers, G. Jones, J. Med. Chem. 21, 182 (1978).
122. T.T. Yen, J.A. Allan, D.V. Pearson, J.M. Acton, M.M. Greenberg, Lipids 12, 409 (1977).
123. T.T. Yen, J.A. Allan, D.V. Pearson, J.M. Acton, Experientia 34, 1542 (1978).

Chapter 19. Modulation of Cyclic Nucleotide Metabolism and Function by Xenobiotics

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Introduction - Adenosine 3',5 cyclic monophosphate (cAMP) is an important intracellular mediator of the actions of hormones.[1,2] There has been increasing interest over the past decade in the therapeutic potential of agents capable of modulating the activity of the enzymes of cAMP metabolism and function. Such effectors would act at the level of (a) adenylate (adenylyl) cyclase, which forms cAMP from ATP upon hormonal stimulation; (b) cyclic nucleotide phosphodiesterase (PDE), which hydrolyzes both cAMP and its guanosine analog, cGMP, to the corresponding 5' nucleotide; and (c) cAMP-dependent protein kinase, which uses ATP to phosphorylate particular proteins and enzymes in the process of eliciting the appropriate cellular response to the hormonal signal. Guanylate cyclase and cGMP-dependent protein kinase, which are functionally similar to their counterparts mentioned above, have also been described and investigated, but there is much less understanding of the role of cGMP in either physiology or pathophysiology.

Comprehensive reviews on PDE effectors were published in 1975-1977.[3-5] The therapeutic implications of cyclic nucleotide metabolism were last discussed in this series in 1975,[6] although specific topics in cyclic nucleotide biochemistry and chemistry have been surveyed more recently.[7,8] Accordingly, this chapter will review the identification and development of exogenous effectors of cyclic nucleotide metabolism and function since 1977.

Adenylate Cyclase - Some 2-aminotetralins were found to stimulate dopamine-responsive adenylate cyclase from rat brain striatum, inhibit binding of (^{3}H)haloperidol, and induce stereotypy in the rat.[9] Morphine at high concentrations can stimulate dopamine-responsive cyclase from mouse brain caudate nuclei.[10] D-lysergic acid diethylamide (LSD) and some derivatives can increase activity of a serotonin-responsive adenylate cyclase from the liver fluke.[11] A large number (40-50) of catecholamines and related analogs have been tested for modulation of catecholamine-responsive cyclase and binding to the β-adrenergic receptor from the turkey erythrocyte; structure-activity relationships have been analyzed.[12,13]

Isoboldine, an aporphine, was a good inhibitor of dopamine-stimulated cyclase from rat caudate nuclei (I_{50} ≈ 1 uM).[14] The related compound, nuciferine, inhibited the β-adrenergic-responsive cyclase from the rat erythrocyte. On the other hand, some protoberberines (e.g., canadine) were potent inhibitors of the dopamine-activated enzyme (I_{50} <1 uM) without marked effects on the erythrocyte cyclase.[14] RMI 12330A (1) inhibited adenylate cyclase activity from rat liver plasma membranes in the presence of NaF, glucagon, epinephrine plus GTP, and in the absence of activators, suggesting interaction of RMI 12330A at the catalytic subunit. Inhibition was not competitive with magnesium ATP (MgATP) and was irreversible.[15] Cyclases from rat spleen, brain, heart, and kidney were

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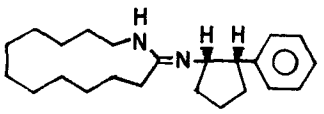

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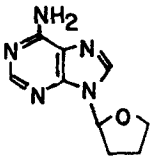

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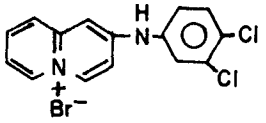

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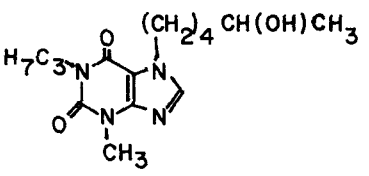
also inhibited, but Mg^{2+}-dependent ATPase and other enzymes were affected as well, suggesting a non-specific, membrane-perturbing action.[16]

The ribose-altered adenosine analogs, 2',5'-dideoxyadenosine and 9-(tetrahydro-2-furyl)adenine (SQ 22536, 2), inhibited prostaglandin E_1 (PGE_1)-activated adenylate cyclase from human platelets (I_{50} = 4 and 13 uM, respectively). Both compounds also antagonized the inhibition by PGE_1 of platelet aggregation induced by ADP or (arginine) vasopressin, and the stimulation by PGE_1 of cAMP formation. Neither compound induced aggregation by itself or in the presence of suboptimal levels of ADP, collagen, arachidonate, or vasopressin.[17] Nolinium bromide (3), an antispasmodic and antisecretory agent, inhibited histamine-responsive cyclase in similar fashion to metiamide (though not as well as cimetidine). Cyclase stimulation by NaF, PGE_2, or a GTP analog was not affected by nolinium.[18] The ability of a number of nonsteroidal anti-inflammatory agents to inhibit PG-sensitive adenylate cyclase from human gastric mucosa might underlie their ulcerogenic activity.[111] Of a number of 1,3-dialkylxanthines, 8-phenyltheophylline was the most effective inhibitor of adenosine-stimulated cAMP formation in adenine-prelabelled guinea pig cerebral cortical slices (I_{50} = 6 uM).[19] 1,3-Dibutylxanthine and 1,3-dipropylxanthine were also more potent than theophylline. Chlorimipramine is the most potent inhibitor, of a number of imipramine analogs, of dopamine-activated adenylate cyclase from rat brain striatum.[20] Serotonin-sensitive cyclase from monkey anterior limbic cortex is blocked by classical serotonin antagonists, with methiothepin (K_i = 10 nM) > cyproheptadine > methysergide. The antipsychotic agent molindone is unique in that it specifically blocks serotonin-sensitive rather than dopamine-responsive enzyme.[21] 2-Bromo-LSD is ten times as potent as cimetidine (or LSD) as an inhibitor of histamine-stimulated cyclase from guinea pig brain hippocampus and cortex.[22] (+) Butaclamol inhibited histamine-, norepinephrine-, and dopamine-responsive cyclase activity from rat and rabbit brain.[23]

Cyclic Nucleotide Phosphodiesterase - Xanthines continue to generate interest as inhibitors of PDE and as potential therapeutic agents. HWA 153 (4) inhibited cAMP PDE from bovine bronchi in more potent fashion than aminophylline. Oral administration of HWA 153 (25 mg/kg) to guinea pigs raised cAMP levels in lungs and bronchi by 35-45% after 1 hr.[24] Bronchospasm induced by histamine, acetylcholine, serotonin, and bradykinin was blocked by HWA 153 in vivo after intravenous or oral dosing.[25] CK-0383 (5) was a potent bronchodilator in vitro and in vivo and was more potent and selective for bronchial smooth muscle than theophylline. It also possessed anti-anaphylactic activity in the rat.[26] 3-Methylxanthine was somewhat less potent than theophylline against PDE from dog tracheal smooth muscle, but the two compounds were equivalent in ability to relax tracheal chain preparations and to contract the isolated guinea pig heart.[27] Benz-fused mesoionic xanthine analogs (6) are 5-15 times as potent as theophylline as PDE inhibitors.[28] Some 1,3-dialkylxanthines are relatively specific inhibitors of Ca^{2+}-dependent soluble cGMP PDE from rat brain. 7-Benzyl-3-isobutyl-1-methylxanthine was most potent in this

regard.[19] 1-Isoamyl-3-isobutylxanthine is a reasonably selective inhibitor of Ca^{2+}-independent PDE and has very low activity as an adenosine antagonist.[19]



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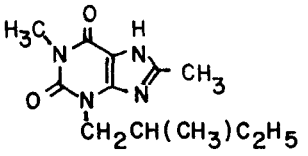

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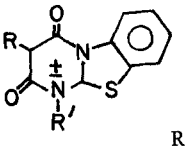


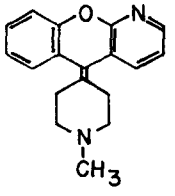
R = small alkyl

R' = cycloalkyl-methyl

6

Sch 15280 (7), a bronchodilator, weakly inhibited PDE from guinea pig lung and bronchi but failed to elevate cAMP levels in lung or potentiate the effects of isoproterenol.[29] Both cromoglycate and M&B 22,948 inhibited PDE from mast cells at higher concentrations than those necessary to block secretion of histamine.[30] On the other hand, good correlations between I_{50} values for inhibition of tracheal smooth muscle PDE and EC_{50} values for muscle relaxation have been noted for agents such as etazolate (SQ 20,009), ICI 58,301, 3-isobutyl-1-methylxanthine (IBMX), theophylline and caffeine.[31-33] Highly significant correlations were found between the ability to inhibit anaphylactic histamine release from human lung in vitro or in the rat passive cutaneous anaphylaxis (PCA) model and the ratio of inhibition (I_{50} or K_I values) of cAMP PDE to inhibition of cGMP PDE for 24 anti-allergic compounds.[34] A somewhat weaker correlation could be noted between cGMP PDE inhibition and inhibition of the rat PCA. This and the report that the antiallergic agents, cromoglycate, ICI 74,197, and M&B 22,948 were considerably more potent as inhibitors of cGMP PDE from human lung than as inhibitors of cAMP PDE[35] suggest that cGMP PDE inhibitors and perhaps cGMP levels may modulate anaphylaxis. FPL 55712, an antagonist of slow reacting substance of anaphylaxis (SRS-A), inhibited cGMP PDE from rat brain more potently than papaverine, theophylline, or etazolate, and was also a potent cAMP PDE inhibitor.[36]

PDE inhibitors can also affect cardiovascular function. UK 14,275 (8), a compound shown to inhibit cardiac PDE in vitro with a potency



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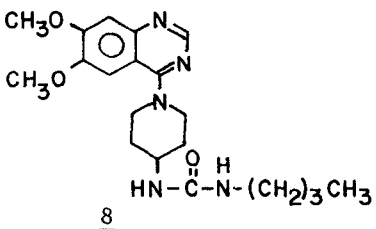

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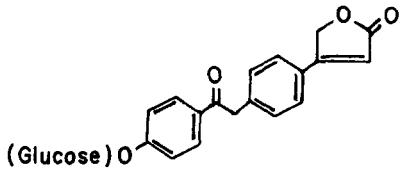
20-fold that of theophylline, showed positive inotropic activity in healthy volunteers without significant chronotropic activity.[37] LV dP/dt_{max} (an index of myocardial contractility) and cardiac output were increased in patients with suspected coronary artery diseases, again without chronotropic effects.[38] AP 10 (9), a cAMP PDE inhibitor, increased rat atrial cAMP levels and produced marked positive inotropic effects on the isolated heart.[49] YC-93 (10), a potent vasodilator, inhibited cAMP PDE from dog coronary artery better (I_{50} = 5 uM) than IBMX or papaverine.[39] Cyclic AMP levels in coronary artery were increased after 1 to 5 min of incubation with YC-93. Phthalazinol (EG 626, 11), an inhibitor of cAMP PDE, inhibited spontaneous phasic contractions of rabbit portal vein with



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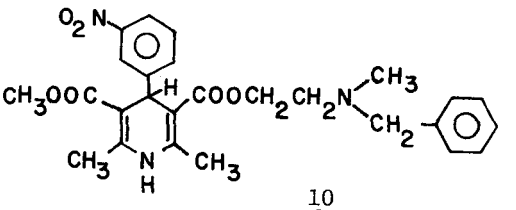

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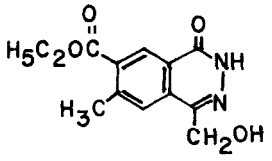
an I_{50} value of 35 uM.[40] EG-626 may act by affecting intracellular calcium pools. A series of 6,7-dialkoxyisoquinolines was synthesized as simplified papaverine analogs and was shown to inhibit PDE from bovine thoracic aorta and antagonize phenylephrine-induced contractions of isolated rat aorta.[41] In a series of di- and tetrahydroisoquinolines tested as inhibitors of PDE from dog heart, USV 2469 (12a) and USV 2776 (12b) were the most potent.[42] Some of these compounds also showed anti-allergic activity.[43] It has been hypothesized that dipyridamole and other PDE inhibitors act as antithrombotic agents by potentiating the elevation of cAMP levels induced by endogenous circulating prostacyclin (PGI_2).[44] On the other hand, prazosin, an antihypertensive agent which was designed as and found to be an inhibitor of PDE in vitro, did not raise cAMP levels in aorta or heart in vivo, which suggests that it does not produce smooth muscle relaxation by modulation of intracellular cAMP.[45] Treatment of rabbit platelets with cilostamide (OPC-3689), a potent and specific inhibitor of rabbit and human platelet cAMP PDE, enhances PGE_1-induced increases in cAMP levels and blocks aggregation induced by ADP, collagen or arachidonic acid.[115]



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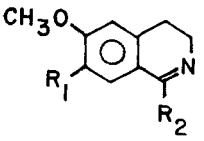


$R_1 = C_6H_5CH_2O$

$R_2 = C_6H_5$

12a

CH_3O

m-CF_3-C_6H_4

12b

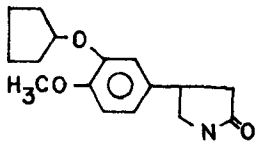
ZK 62711 (13), a potent inhibitor of PDE from rat gastric mucosa (I_{50} = 18 uM), stimulated gastric acid and pepsin secretion in the rat and enhanced the effect of histamine when given i.v.. The increase in cAMP levels in gastric mucosa effected by histamine was enhanced by ZK 62711.[46] The anti-ulcer agent, carbenoxolone, inhibits PDE from rat gastric mucosa in noncompetitive fashion.[47]

Antipsychotic drugs from a number of chemical classes displayed high-affinity, calcium-dependent binding to the calcium-dependent activator of cyclic nucleotide PDE. The relationship of this property to clinical efficacy is still unclear.[48]

Etazolate was more potent than caffeine, aminophylline, IBMX, and Ro 20-1724 as an inhibitor of cAMP PDE from human lung.[50] The polyene antibiotic, filipin, could eliminate the stimulation of rat adipocyte PDE by insulin, either by preincubation or by treatment following exposure of the cells to the hormone.[51] Other compounds and compound classes reported to inhibit PDE include atrazine,[52] progesterone and diethylstilbesterol,[53] 4-hydroxy-cyclophosphamide,[54] some bicyclic organophosphates,[55] a number of sulphonated aromatic dyes,[56] a series of flavonoids,[57,110] and

certain biphenylalkyl monoesters of succinic and related acids.[58]

Cyclic Nucleotide-dependent Protein Kinase - There has been relatively little reported on the modulation of cyclic nucleotide-dependent protein kinase (PK) by other than endogenous factors. It has been noted that AA 373 (14), a novel relaxant of the sphincter of Oddi, along with its 2' ethoxy derivative (AA 149), enhanced the phosphorylation of sarcoplasmic reticulum fractions from bile duct by PK. This property may, in part, explain the pharmacologic activity of these compounds.[59] Lin-benzoadenosine 3',5' monophosphate can maximally activate PK from rat brain and rabbit skeletal muscle, but was tenfold less potent than cAMP vs. brain PK and fivefold less potent vs. the muscle enzyme.[60] The benzyl ester of cAMP was found to diffuse into C6 glioma cells and cause morphologic



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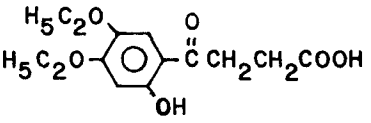

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changes characteristic of cAMP. The ester hydrolyzes exclusively to cAMP within the cell and appears to represent a cAMP "prodrug".[61]

Indomethacin inhibited PK activity from rabbit ileal mucosa, in the presence or absence of cAMP, with I_{50} values of 2-5 X 10^{-8}M. Endogenous protein phosphorylation was inhibited with I_{50} values of 0.6 - 1.0 x 10^{8}M. Inhibition of PK was time-dependent and not reversible by dilution. Indomethacin is 80 to 220 times more potent as a PK inhibitor than it is as a PG synthetase inhibitor.[62] The catalytic subunit of bovine cardiac muscle cAMP PK can be irreversibly and stoichiometrically inactivated by 5'-p-fluorosulfonylbenzoyladenosine; the compound interacts competitively with MgATP (K_i = 235 uM) and exhibits properties of an affinity label of the MgATP-binding site.[63] This reagent also inactivates the porcine skeletal muscle PK catalytic subunit by modification of a lysine residue.[64] Cibacron Blue F3GA, the blue chromophore of Blue Dextran 2000, irreversibly inhibited the bovine brain PK catalytic subunit. The dye appears to be an active-site-directed agent which requires cAMP to convert the insensitive holoenzyme to the sensitive species.[65] Cordycepin (3'-deoxyadenosine) inhibited PK from various sources in the presence or absence of cAMP or cGMP; the inhibition was competitive with ATP and required concentrations of 100-1000 uM. This action was not specific because cyclic nucleotide-independent PK was also inhibited.[66] On the other hand, diamide (15) reversibly and rapidly inhibited cAMP-dependent PK from bovine thyroid, but did not affect cyclic nucleotide-independent PK from the same organ. Inhibition was non-competitive with respect to ATP or histone, could be prevented by addition of 10 mM DTT, glutathione, or 2-mercaptoethanol, was half-complete at 800 uM, and was total at 10 mM.[67]

Guanylate Cyclase - It appears that the formation of nitric oxide (NO) can explain the stimulatory effects on guanylate cyclase and cGMP levels of azide, hydroxylamine, nitrite, nitroglycerin, nitroprusside, nitrosamines, and nitrosoureas.[68] Some of these agents may decompose or be metabolized to NO or other reactive nitrogen oxides; NO itself can activate guanylate cyclase.[69] Conversion of azide or hydroxylamine to NO occurs enzymically (peroxidatically) and requires H_2O_2 generated by superoxide dismutase from

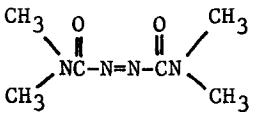
superoxide radical anion ($O_2^{\cdot -}$), which is in turn formed by the action of enzymes such as nitrate reductase or flavoenzymes.[68,70] Superoxide dismutase itself can stimulate guanylate cyclase, probably by furnishing H_2O_2 for the Haber-Weiss reaction leading to the formation of OH radical:[68,70]

$$O_2^{\cdot -} + H_2O_2 \longrightarrow OH\cdot + OH^- + O_2$$

Cyclase activation by this process is blocked by OH radical scavengers, such as hydroquinones, catecholamines, and butylated hydroxyanisole (BHA). Hydroxyl radicals may be formed nonenzymically by catecholamines in the presence of Mn^{2+} as well as during unsaturated fatty acid oxidation, leucocyte phagocytosis, PG formation, and platelet aggregation, and may modulate cGMP levels during these processes.[68] Nitroprusside (0.1 mM) raised soluble guanylate cyclase activity from rat liver tenfold; this effect could be abolished by 1 mM N-ethylmaleimide. This presumed oxidation of enzyme sulfhydryl groups could involve reactive nitrogen oxides.[71] Activation of enzyme from rat myometrium by nitroprusside was also reported.[72] Several nitroso carcinogens such as N-methyl-N'-nitro-N-nitrosoguanidine (MNNG),[73,74] N-methyl-nitrosourea (MNU),[73,75] and 1,3-bis (2-chloroethyl)-1-nitrosourea (BCNU)[75] activate soluble cyclase and raise cGMP levels in tissues dramatically at concentrations of 0.01-1.0 mM. Streptozotocin,[75] 4-nitroquinoline-1-oxide,[76] and hydrazine[73,77] have similar properties. The antipromoter activity of retinol may arise from its ability to block activation of cyclase by carcinogens, an ability shared by BHA.[73] Toxic agents have been reported to stimulate guanylate cyclase, including the herbicide, paraquat (which generates superoxide radical anion),[78] mandelonitrile and cyanide (but not amygdalin or neoamygdalin) [79], cytotoxic preparations from two marine sponges,[80] and phencyclidine.[81] The action of phencyclidine may be due to contamination by the synthetic intermediate, 1-piperidino-cyclohexane carbonitrile, which is labile and may liberate cyanide.[82]

An extract from the balsam pear inhibited guanylate cyclase from rat liver and other tissues almost completely, even if stimulated by streptozotocin or MNNG. Adenylate cyclase activity was unaffected. The active principle is acid-stable and heat-labile, and appears not to be lipid.[83] It blocked growth of an undifferentiated adenocarcinoma of the rat prostate, and concurrently lowered cGMP levels in the tumor cells in culture. It remains to be seen whether the antitumor and cyclase inhibitory properties derive from a single molecule.[84] The cardiotoxic anthracycline antitumor agents, doxorubicin (I_{50} = 0.6-0.8 mM) and daunorubicin decreased guanylate cyclase activity from rat heart, but not liver, lung, kidney, and spleen. Human heart guanylate cyclase was also inhibited but adenylate cyclase from rat heart was not. Some aspects of anthracycline cardiotoxicity may be related to inhibition of guanylate cyclase activity.[85] Very high concentrations (50 mM) of saccharin were necessary to inhibit the cyclase from liver, stomach, colon, kidney and urinary bladder.[86] Ethanol (2.5-5.0%) markedly lowered cyclase activity *in vitro* from several rat tissues, including pancreas, liver and cerebellum.[87]

Modulation of cAMP in Intact Cells and Tissue - The selective β_2-adrenergic agonist, procaterol (15a), when administered *i.v.* to conscious rats, caused a 2-3 fold increase in cAMP levels in heart and liver, but a 6-10 fold increase in cAMP concentrations in trachea, lung, and skeletal muscle. Thus, elevations of cAMP levels paralleled the β_2 specificity of procaterol.[88] Of a series of acylhydroxyalkanoic acids examined for their ability to raise cAMP levels in the mouse ovary, 16 was only one-thousandth as potent as PGE_1 but possessed renal vasodilatory and platelet anti-aggregatory properties *in vivo* in the guinea pig after oral adminis-



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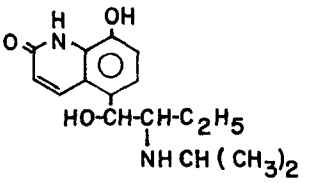
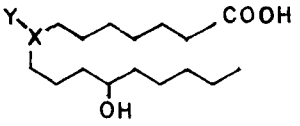


15a

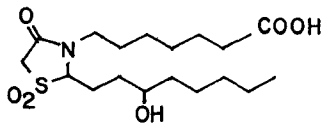
tration.[89] The sulfone analog (17) elevated cAMP concentrations in mouse ovary and human psoriatic skin and effectively blocked antigen-induced lymphocyte transformation in vitro.[116] Among a group of 8-aza-11,12-secoprostaglandins, 18 was one-hundredth as potent as PGE_1 in the mouse ovary assay, and showed some renal vasodilatory activity in the dog upon infusion.[90] The most active member of a series of oxoprostanoic acid isosteres was 19 which, though less active than PGE_1, compared favorably with tetrahydro PGA_1 in the mouse ovary test.[91]



X =	CH	CH	N
Y =	$COCH_3$	CH_3SO_2	$CONH_2$

16 17 18

Theophylline and salbutamol had supra-additive effects on inhibition of anaphylactic contractions and histamine release, and on elevated cyclic AMP levels in guinea pig lung parenchymal strips in vitro. This suggests that increases in cAMP levels may at least partially subserve the pharmacologic effects seen.[92] An i.v. bolus of the diuretic, piretanide (20),



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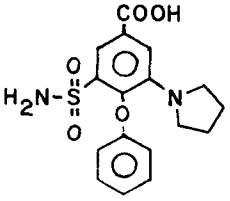

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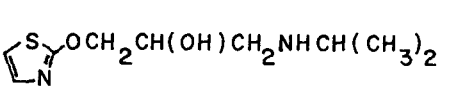
given to male volunteers caused a significant increase in urinary cAMP excretion rate 30-90 min after dosing.[93] Livers of rats treated with 2.5 and 0.5 ml/kg CCl_4 showed a rapid increase in cAMP levels (by 70-100%) without effect on cGMP levels.[94] Treatment with clofibrate (12 mg/day) for 7 days also increased hepatic cAMP content.[95] Indomethacin (5-10 mg/kg) blocked the inflammatory response associated with acetic acid-induced pleurisy in the rat, with concomitant elevations of cAMP concentrations and depressions of cGMP concentrations in the pleural exudate.[96] Quercetin, a flavone, increased levels in Ehrlich ascites tumor cells by threefold at 100 uM.[97]

(+) Tazolol (21) was shown to be a central β-adrenergic antagonist, as evidenced by its ability to block ℓ-isoproterenol-induced increases in

cAMP in rat brain cortical slices (I_{50} = 7 x 10^{-7}M). It was ten times as potent as (+) sotalol.[98] Phenytoin (10-100 uM) inhibited veratridine- and oubain-induced elevation of cAMP and cGMP levels in mouse cerebral cortex slices, but did not affect glutamate- or K^+-induced increases in cyclic nucleotide levels. Thus phenytoin is similar in behavior to tetrodotoxin, but 1000 times less potent.[99] (-)-trans-Δ^1-Tetrahydrocannabinol and cannabidiol inhibited the increases in cAMP levels in WI-38 fibroblasts exposed to PGE_1 and catecholamines. However, extended exposure of the cells to compound alone resulted in elevated cAMP levels.[113] Some phosphorylated derivatives of phloretin blocked the accumulation of cyclic AMP in CNS clonal cell lines stimulated by PGE_1, isoproterenol, and adenosine. The most potent antagonist of the PGE_1-induced increases in either neuroblastoma or human astrocytoma cells was di-4-phloretin phosphate.[114] Several flavonoids can block the increases in cAMP levels in neuroblastoma cell cultures stimulated by PGE_1, isoproterenol, and adenosine.[100] This result is distinct from the enhancement of cAMP levels by the compounds themselves.[57,97] Paraquat, a guanylate cyclase stimulator,[78] can, at higher concentrations, block the cyclic nucleotide increases in rat lung slices caused by histamine or isoproterenol.[101] Sodium nitroprusside, which decreases cyclic AMP levels in bovine parathyroid cells stimulated by isoproterenol, dopamine, and choleratoxin, elevates cGMP levels in these cells by itself.[102] The antilipolytic agent, N^6-phenylisopropyladenosine, at concentrations as low as 0.1 uM, reduced accumulation of cAMP in fat cells stimulated by norepinephrine and theophylline. Interestingly, N^6-phenyl-N^6-allyl-adenosine (BM 11,189) was antilipolytic without lowering cAMP levels.[103]

Modulation of cGMP in Intact Cells and Tissue - The guanylate cyclase activators azide, hydroxylamine, nitrite, nitroglycerin, and nitroprusside relaxed carbachol-contracted tracheal smooth muscle and raised cGMP, but not cAMP, levels. Since elevated cyclic AMP levels were previously postulated to relax smooth muscle, these results obscure the roles of cAMP and cGMP in mediating smooth muscle tone under non-adrenergic influences.[104] Harmaline, isoniazid and apomorphine elevate, whereas haloperidol, chlorpromazine, diazepam, and opiates lower cGMP concentrations in rat brain.[105,112] Some convulsant bicyclic organophosphates, of which the most toxic was 22, raised cGMP levels and lowered cAMP levels in mouse brain after intracerebroventricular application.[106] Rats dosed with ethanol acutely (6 g/kg) or chronically (11-15 g/kg/day for 4 days) were depleted of brain cGMP. Acutely intoxicated animals showed marked decreases of cGMP in cerebellum, brain stem, caudate nucleus and cerebral



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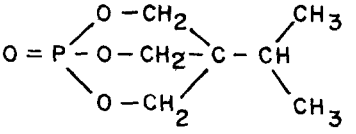

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cortex. Chronically intoxicated animals showed depressed but somewhat higher levels of brain cGMP. During withdrawal, cGMP levels returned to control values.[107] Adipocytes from rats treated with clofibrate (30 mg/kg/day for 31 days) showed an increased accumulation of cGMP and a somewhat reduced accumulation of cAMP in response to ACTH, compared to untreated cells. Thus, cyclic nucleotides were altered as with insulin,

another antilipolytic agent.[108] Several ginsenosides (saponins from Panax ginseng) increased DNA, RNA, protein and lipid synthesis in bone marrow cells. cGMP levels were increased in vivo by ginsenosides Rb_2, Re and Rg_1. cAMP levels in bone marrow cells in vitro were decreased by Rb_2, Rc, and Rg_1.[109]

References

1. G.A.Robison, R.W.Butcher and E.W.Sutherland, "Cyclic AMP", Academic Press, New York, N.Y. 1971.
2. I.Weinryb, Perspect. Biol. Med., 22, 415(1979).
3. M.S.Amer and W.E.Kreighbaum, J.Pharm. Sci., 64,1(1975).
4. M.Chasin and D.N.Harris, Adv. Cyclic Nucleotide Res., 7,225(1976).
5. B.Weiss and R.Fertel, Adv. Pharmacol. Chemother., 14,189(1977).
6. M.S.Amer and G.R.McKinney, Ann. Repts. Med. Chem., 10,192(1975).
7. J.P.Miller and R.K.Robins, Ann. Repts. Med. Chem., 11,291(1976).
8. H.Sheppard, Ann. Repts. Med. Chem., 12,172(1977).
9. J.G.Cannon, B.Costall, P.M.Laduron, J.E.Leysen and R.J.Naylor, Biochem. Pharmac., 27,1417(1978).
10. L.C.Tang and G.C.Cotzias, Proc. Natl. Acad. Sci. USA, 75, 1546(1978).
11. J.K.Northup and T.E.Mansour, Mol. Pharmacol. 14, 804 (1978).
12. J.P.Bilezikian, A.M.Dornfeld and D.E.Gammon, Biochem. Pharmac., 27, 1445 (1978).
13. J.P.Bilezikian, A.M.Dornfeld and D.E.Gammon, Biochem. Pharmac., 27, 1455 (1978).
14. H.Sheppard and C.R.Burghardt, Biochem. Pharmac., 27, 1113 (1978).
15. G.Guellaen, J.-L. Mahu, P.Mavier, P.Berthelot and J.Hanoune, Biochim. Biophys. Acta, 484, 465 (1977).
16. G.Guellaen, J.-L. Mahu, P.Mavier, J.Hanoune and P.Berthelot, Biochem. Pharmac., 27, 641 (1978).
17. R.J.Haslam, M.M.L.Davidson and J.V.Desjardins, Biochem. J., 176, 83 (1978).
18. T.P.Dousa, Y.S.F. Hui, T.E.Northrup and M.M.Goldenberg, Biochem. Pharmac., 28 343 (1979).
19. F.W.Smellie, C.W.Davis, J.W.Daly and J.N.Wells, Life Sci., 24, 2475 (1979).
20. G.C.Palmer, H.R.Wagner, S.J.Palmer and A.A.Manian, Res. Commun. Chem. Pathol. Pharmacol., 16, 573 (1977).
21. H.S.Ahn and M.H.Makman, Life Sci., 23, 507 (1978).
22. J.P.Green, C.L.Johnson, H.Weinstein and S.Maayani, Proc. Natl. Acad. Sci. USA, 74, 5697 (1977).
23. G.C.Palmer, H.R.Wagner, S.J.Palmer and A.A.Manian, Arch. int. Pharmacodyn., 233, 314 (1978).
24. V.Stefanovich and E.Porsche, Arzneim.-Forsch./Drug Res., 29, 917 (1979).
25. V.C.Anagnostopoulos and J. Komarek, Arzneim.-Forsch./Drug Res., 29, 1013 (1979).
26. M.Belej, J.Elenewich, B.Touhey, A.DeFelice, A.Smart and J.Diamond, Pharmacologist, 20, 197 (1978).
27. J.F.Williams, S.Lowitt, J.B.Polson and A.Szentivanyi, Biochem. Pharmac., 27,1545 (1978).
28. J.J.Gaines, R.A.Glennon and M.E.Rogers, ABSTRACT MEDI CHEM-72, 178th ACS National Meeting, Washington, D.C., Sept. 9-14, 1979.
29. W.Kreutner and J.E.Sherwood, Biochem.Pharmac., 26, 951 (1977).
30. H.Bergstrand, B.Lundquist and A.Schurmann, Mol. Pharmacol., 14, 848 (1978).
31. J.B.Polson, J.J.Krzanowski, D.F.Fitzpatrick and A.Szentivanyi, Biochem. Pharmac., 27, 254 (1978).
32. D.J.Newman, D.F.Colella, C.B.Spainhour, Jr., E.G.Brann, B.Zabko-Potapovich and J.R.Wardell, Jr., Biochem. Pharmac., 27, 729 (1978).
33. J.B.Polson, J.J.Krzanowski, W.H.Anderson, D.F.Fitzpatrick, D.P.C.Hwang and A.Szentivanyi, Biochem. Pharmac., 28, 1391 (1979).
34. C.J.Coulson, R.E.Ford, S.Marshall, J.L.Walker, K.R.H.Wooldridge, K.Bowden and T.J.Coombs, Nature, 265, 545 (1977).
35. H.Bergstrand, J.Kristoffersson, B.Lundquist and A.Schurmann, Mol. Pharmacol., 13, 38 (1977).
36. M.Chasin and C.Scott, Biochem. Pharmac., 27, 2065 (1978).
37. P.G.Jackson, G.Jackson, D.Kitson and D.E.Jewitt, Br. J. Clin. Pharmac., 5, 7 (1978).
38. K.Jennings, P.G.Jackson, M.Monaghan and D.E.Jewitt, Br. J. Clin. Pharmac., 5, 13 (1978).
39. N.Sakamoto, M.Terai, T.Takenaka and H.Maeno, Biochem. Pharmac., 27, 1269 (1978).
40. M.Kaiman, S.Shibata and T.Shimamoto, Arch. int. Pharmacodyn., 228, 23 (1977).
41. C.Lemoulinier, J.-M.Scheftel, G.Leclerc, C.-G.Wermuth and J.-C.Stoclet, Eur.J.Med. Chem. Chim. Thèr., 13, 289 (1978).
42. R.G.Van Inwegen, P.Salaman, V.St.Georgiev and I.Weinryb, Biochem. Pharmac., 28, 1307 (1979).
43. V.St.Georgiev, R.P.Carlson, R.G.Van Inwegen and A.Khandwala, J. Med. Chem., 22, 348 (1979).
44. S.Moncada and R.Korbut, Lancet, 1, 1286 (1978).
45. H.Sands and R.Jorgensen, Biochem. Pharmac., 28, 685 (1979).

46. J.Puurunen, C.Lücke and U.Schwabe, Naunyn-Schmiedeberg's Arch. Pharmacol., 304, 69 (1978).
47. H.Vapaatalo, I.-B. Lindén, T.Metsä-Ketelä, M.Kangasaho and K.Laustiola, Experientia, 34, 384 (1978).
48. R.M.Levin and B.Weiss, J.Pharmacol. Exp. Therap., 208, 454 (1978).
49. A.F.Prigent, G.Nemoz, M.Roche and H.Pacheco, Arch. int. Pharmacodyn., 241, 131 (1979).
50. W.F.Glass, II and J.B.Moore, Jr., Biochem. Pharmac., 28, 1107 (1979).
51. W.I.Ling and W.Y.Cheung, Molec. Cell. Endocrinol., 14, 113 (1979).
52. B.Messner, J.Berndt and J.Still, Biochem. Pharmac., 28, 207 (1979).
53. F.Ferre, D.DePariente, M.Breuiller and L.Cedard, Biochem. Pharmac., 27, 1292 (1978).
54. M.J.Tisdale, Biochem. Pharmac., 26, 1469 (1977).
55. D.B.Coult and R.G.Wilkinson, Biochem. Pharmac., 26, 887 (1977).
56. A.R.Ashton and G.M.Polya, Biochem. J., 175, 501 (1978).
57. M.Ruckstuhl, A.Beretz, R.Anton and Y.Landry, Biochem. Pharmac., 28, 535 (1979).
58. M.E.Rogers, S.G.Boots and M.R.Boots, J. Pharm. Sci., 68, 903 (1979).
59. M.Kimura, I. Kimura and S.Kobayashi, Biochem. Pharmac., 26, 994 (1977).
60. M.J.Schmidt, L.L.Truex, N.J.Leonard, D.I.Scopes and J.R.Barrio, J.Cyclic Nucleotide Res., 4, 201 (1978).
61. J.Engels and E.-J.Schlaeger, J. Med. Chem., 20, 907 (1977).
62. H.S.Kantor and M.Hampton, Nature, 276, 841 (1978).
63. C.S.Hixon and E.G.Krebs, J.Biol. Chem., 254, 7509 (1979).
64. M.J.Zoller and S.S.Taylor, J. Biol. Chem., 254, 8363 (1979).
65. J.J.Witt and R.Roskoski, Jr., Biochemistry, 19, 143 (1980).
66. R.I.Glazer and J.F.Kuo, Biochem. Pharmac., 26, 1287 (1977).
67. M.McClung and J.Miller, Biochem. Biophys. Res. Commun., 76, 910 (1977).
68. C.K.Mittal and F.Murad, J. Cyclic Nucleotide Res., 3, 381 (1977).
69. S.Katsuki, W.Arnold, C.Mittal and F.Murad, J. Cyclic Nucleotide Res., 3, 23 (1977).
70. F.Murad, C.K.Mittal, W.P.Arnold, S.Katsuki and H.Kimura, Adv. Cyclic Nucleotide Res., 9, 145 (1978).
71. F.R.DeRubertis and P.A.Craven, J. Biol. Chem., 252, 5804 (1977).
72. D.Leiber and S.Harbon, Biochem. Biophys. Res. Commun., 89, 598 (1979).
73. P.A.Craven and F.R.DeRubertis, Cancer Res., 37, 4088 (1977).
74. F.R.DeRubertis and P.A.Craven, Cancer, 40, 2600 (1977).
75. F.R.DeRubertis and P.A.Craven, Biochim. Biophys. Acta, 499, 337 (1977).
76. F.R.DeRubertis and P.A.Craven, J.Natl. Cancer Inst., 59, 1741 (1977).
77. D.L.Vesely and G.S.Levey, Biochem. Biophys. Res. Commun., 74, 780 (1977).
78. D.L.Vesely, B. Watson and G.S.Levey, J. Pharmacol. Exp. Therap., 209, 162 (1979).
79. D.L.Vesely, W.R.Benson, E.B.Sheinin and G.S.Levey, Proc. Soc. Exper. Biol. Med., 161, 319 (1979).
80. P.J.Lad, J.W.Brown and W.T.Shier, Biochem. Biophys. Res. Commun., 85, 1472 (1978).
81. D.L.Vesely, Biochem. Biophys. Res. Commun., 88, 1244 (1979).
82. W.H.Soine, W.C.Vincek and D.T.Agee, New Engl. J. Med., 301, 438 (1979).
83. D.L.Vesely, W.R.Graves, T.M.Lo, M.A.Fletcher and G.S.Levey, Biochem. Biophys. Res. Commun., 77, 1294 (1977).
84. A.J.Claflin, D.L.Vesely, J.L.Hudson, C.B.Bagwell, D.C.Lehotay, T.M.Lo, M.A.Fletcher, N.L.Block and G.S.Levey, Proc. Natl. Acad. Sci. USA, 75, 989 (1978).
85. G.S.Levey, B.A.Levey, E.Ruiz and D.C.Lehotay, J.Molec. Cell. Cardiol., 11, 591 (1979).
86. D.L.Vesely and G.S.Levey, Biochem. Biophys. Res. Commun., 81, 1384 (1978).
87. D.L.Vesely and G.S.Levey, Res. Commun. Chem. Pathol. Pharmacol., 17, 215 (1977).
88. Y.Saitoh, T.Hosokawa, T.Igawa and Y.Irie, Biochem. Pharmac., 28, 1319 (1979).
89. J.B.Bicking, C.M.Robb, R.L.Smith, E.J.Cragoe, Jr., F.A.Kuehl, Jr. and L.R.Mandel, J.Med. Chem., 20, 35 (1977).
90. J.H.Jones, W.J.Holtz, J.B.Bicking, E.J.Cragoe, Jr., L.R.Mandel and F.A.Kuehl, Jr., J.Med. Chem., 20, 44 (1977).
91. R.L.Smith, T.Lee, N.P.Gould, E.J.Cragoe, Jr., H.G.Oien and F.A.Kuehl, Jr., J. Med. Chem., 20, 1292 (1977).
92. H.W.Mitchell, H.Hau and M.A.Denborough, Eur. J. Pharmacol., 57, 399 (1979).
93. H.L.Elliott, A.F.Ansari, J.R.Lawrence, B.C.Campbell and B.Whiting, Drugs Exp. Clin. Res., 5, 51 (1979).
94. L.Paradisi and M.U.Dianzani, Chem.-Biol. Interactions, 26, 1 (1979).
95. C.Landriscina, F.M.Ruggiero, G.V.Gnomi and E.Quagliariello, Biochem. Pharmac., 26, 1401 (1977).
96. A.Bertelli and M.L.Schinetti, Drugs Exp. Clin. Res., 2, 177 (1977).
97. Y.Graziani and R.Chayoth, Biochem. Pharmac., 26, 1259 (1977).
98. P.Skolnick, L.P.Stalvey, J.W.Daly, T.W.Stone and D.Taylor, Life Sci., 21, 1655 (1977).
99. J.A.Ferrendelli and D.A.Kinscherf, J.Pharmacol. Exp. Therap., 207, 787 (1978).
100. R.Ortman, D.Nutto and J.Waldmeyer, Biochem. Pharmac., 28, 2357 (1979).
101. S.N.Giri and M.A.Hollinger, Experientia, 35, 1219 (1979).
102. D.G.Gardner, E.M.Brown and G.D.Aurbach, Endocrinology, 105, 360 (1979).
103. P.B.Wieser and T.S.Pendleton, Biochem. Pharmac., 28, 693 (1979).
104. S.Katsuki and F.Murad, Mol. Pharmacol., 13, 330 (1977).
105. G.Biggio, E.Costa and A.Guidotti, J.Pharmacol. Exp. Therap., 200, 207 (1977).
106. D.B.Coult, D.J.Howells and A.P.Smith, Biochem. Pharmac., 28, 193 (1979).

107. W.A.Hunt, J.D.Redos, T.K.Dalton and G.N.Catravas, J. Pharmacol. Exp. Therap., 201, 103 (1977).
108. C.Gianoulakis, M.Lis, and M.Chrètien, Can. J. Physiol. Pharmacol., 57, 738 (1979).
109. M.Yamamoto, M.Masaka, K.Yamada, Y.Hayashi, A.Hirai and A.Kumagai, Arzneim.-Forsch./ Drug Res., 28, 2238 (1978).
110. J.E.Ferrell, Jr., P.D.G.Chang Sing, G.Loew, R.King, J.M.Mansour and T.E.Mansour, Mol. Pharmacol., 16, 556 (1979).
111. B.Simon and H.Kather, Pharmacology, 19, 96 (1979).
112. J.P.O'Callaghan, Q.Chess, C.McKimmey and D.H.Clouet, J.Pharmacol. Exp. Therap., 210, 361 (1979).
113. L.A.Kelly and R.W.Butcher, J.Cyclic Nucleotide Res., 5, 303 (1979).
114. R.Ortmann, D.Nutto and R.Jackisch, Naunyn-Schmiedeberg's Arch. Pharmacol., 305, 233 (1978).
115. H.Hidaka, H.Hayashi, H.Kohri, Y.Kimura, T.Hosokawa, T. Igawa and Y.Saitoh, J.Pharmacol. Exp. Therap., 211, 26 (1979).
116. R.L.Smith, J.B.Bicking, N.P.Gould, T.-J.Lee, C.M.Robb, F.A.Kuehl, Jr., L.R.Mandel and E.J.Cragoe, Jr., J. Med. Chem., 20, 540 (1977).

Chapter 20. Complement Inhibitors

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The purpose of this chapter is to assemble the disjointed literature regarding inhibitors of the complement system. Chemical control of complement mediated processes holds promise for the treatment of a variety of diseases associated with acute inflammatory events. Complement activation has clearly been shown to occur in rheumatoid arthritis,[1] lupus erythematosus,[2] and glomerulonephritis[3] and is believed to be an important effector mechanism in these diseases.

It is not the purpose of this review to discuss detailed mechanisms of complement reactions. This Annual Report has previously presented the serum complement system[4] and two recent excellent reviews of complement physiology are recommended.[5,6] A review of earlier literature with special attention to aromatic amino acids is also valuable.[7]

As Becker[8] has pointed out, the reasons for seeking synthetic complement inhibitors may be manifold, the potential therapeutic value being only one. It is anticipated therefore that much of the complement inhibitor literature deals with agents requiring unrealistically high effective concentrations to be of therapeutic usefulness.

It is generally accepted that treatment of certain diseases may be effected through control of complement consumption by interrupting the generation of or the action of cellular stimuli attending inflammatory processes. Such stimuli include chemoattractants for polymorphonuclear leukocytes, inducements for noncytotoxic enzyme secretion, histamine releasing agents, and permeability factors. To a large degree these collective activities are expressed by the pharmacologic action of C5a and C3a, cleavage products of the fifth(C5) and the third(C3) components of complement. It logically follows, therefore, that arresting complement utilization at a point prior to or including C5 consumption would diminish concomitant inflammatory events.

In accordance with this rationale we have chosen to categorize, where feasible, the site(s) of action of a compound into four major phases of complement utilization (Fig. 1). These four phases (I-IV) are defined as follows. I. Activation of the first complement component (C1) from a proenzymic to an active enzymic state ($\overline{C1}$): Inhibitors affecting the subcomponents C1q, C1r, or C1s will be included in this group. II. Assembly of the complex enzyme, C3 convertase: Reaction steps resulting in the formation of the classical C3 convertase ($\overline{C4b,2}$) will be designated IIc and those reactions leading to assemblage of the alternative pathway convertase ($\overline{C3b,B}$) will be termed IIa. III. Compounds that inhibit C3 convertase, whether classical (IIIc) or alternative (IIIa), or alter the generation of a C3 site, will be incorporated into these categories. IV. Inhibitors of C5 convertase(s) (IVc, IVa) that alter the generation of a membrane-bound C5 site (C5b), or alter the cleavage of C5 will be included in these groups.

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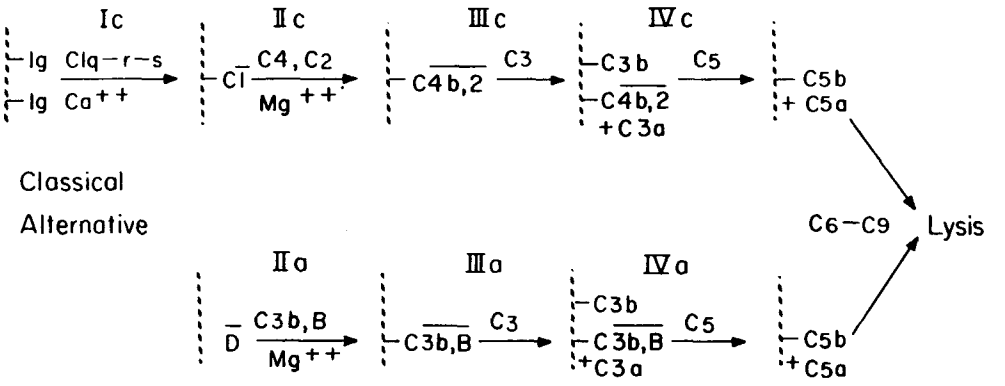


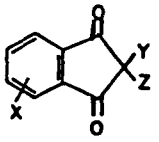
Figure 1

Antiinflammatory Drugs - Three fenamic acids, flufenamic, mefanamic, and niflumic, block classical pathway activity.[9-12] Flufenamic acid was the most active of this group, showing significant inhibition at 0.05 mM with human complement.[12] Whole guinea pig complement was inhibited (0.7-0.9 mM)[9,12] and blockage of terminal component activity (C3-C9) at 0.1 mM was observed as well.[10] Flufenamic acid also demonstrated inhibition at multiple sites. Diminished C2 utilization (IIc) was observed at 0.9 mM with lesser activities against C5 and C3 by their respective convertases (IIIc, IVc) being noted. Enhanced decay of the C5 site by flufenamic acid was noted at 1.8 mM and preincubation with C5, C3, or C2 resulted in loss of their hemolytic activities.[9] Inhibitory concentrations were apparently attainable in vivo since suppression of the reverse passive Arthus reaction (RPAR) at 5 mg/animal (mouse) was effective.[11]

Gold sodium thiomalate (GST) clearly alters both pathway activities. Direct inactivation of $C\bar{1}$ and $C1\bar{s}$ (0.01-0.05 mM)(IIc) was established.[13] Similar amounts of GST were shown to interfere with assembly of the alternative pathway C3 convertase (IIa), most likely by affecting the binding site of C3b for factor B.[14] Effective concentrations of GST are within steady state limits of individuals undergoing chrysotherapy.

Hydrocortisone succinate and prednisolone sodium phosphate suppress classical complement at relatively high concentrations, 1.6-13.0 mM,[15] and 6.0 mM,[16] respectively. Relatively high concentrations (5-10 mM) of chloroquine and plaquenil inhibit classical pathway activity.[16,17] Aspirin was reported not to be inhibitory at 9.0 mM to terminal component activity (C3-C9) nor whole complement activity at 5.5 mM.[12] Very high concentrations (31 mM) of aspirin show some inhibitory activity.[16]

Phenylbutazone and its analogues, p-hydroxyphenylbutazone and sulfinpyrazone, were reported to be anticomplementary at 3-10 mM,[12,16] 1.8 mM, and 3.0 mM,[17] respectively. Other classical non-steroidal antiinflammatory agents reported to inhibit complement include indomethacin at 0.9 mM,[10,12] and colchicine at 10 mM.[17] The investigational drugs 043/63 (1) and 043/13 (2) are reportedly inhibitory at 0.32 mM and 0.66 mM, respectively.[18] Diftalone (3) in the 0.5-1.0 mM range inhibits both the classical and alternative pathways.[19]



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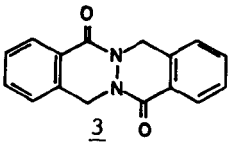
1,	5-Br	Br	2-naphthyl
2,	4-Br	H	C_6H_5



3

Amino Acids - Epsilon aminocaproic acid (EACA) influences complement action in a variety of ways. EACA significantly inhibits C1 activation (Ic) in normal human serum (NHS) from 25 to 100 mM.[20,21] EACA prevention of C1 activation is time and temperature dependent (>50% inh., 60'/30°). Early studies showed that the presence and position of the amino group was critical to inhibitory activity, inhibition being absent with α-aminocaproic and valeric acids.[20] Decarboxylation of EACA resulted in greater inhibition than that observed with the parent compound. This structure-activity relationship (SAR) is compromised since whole complement lytic activity was utilized and more recent studies indicate that EACA acts at more than one site. EACA (100 mM) interferes with assembly of the alternative pathway C3 convertase[22] (IIa) and therefore reduces C3 turnover in serum. High concentrations of EACA (1.0 M) inhibit serum carboxypeptidase (anaphylatoxin inactivator, AI) and particulate C5 convertase formation (IIIa).[24] In a study on the inhibitory activity of three EACA analogues on serum-derived proteases, p-GB-DBiG (4), p-GB-DBoG (5), and ε-GcA-CEP (6), $\overline{C1}$ subcomponent inhibition was observed.[25] Effective inhibition of $C1\overline{r}$ esterolytic activity (Ic) was reported for 4 at 4.4 x 10^{-6}M. All three analogues inhibited $C1\overline{s}$ esterolytic activity (IIc) in the range of 0.03 to 0.50 mM. Aromatic ester analogues of EACA inhibited both the activation of (Ic) and action of (IIc) human C1 at 1.0 mM.[26] EACA has been used therapeutically and is considered to prevent the activation of several plasma zymogens, including C1.[27] It should be kept in mind that EACA may indirectly alter complement by inhibiting plasminogen activation.[28] Plasmin is known to directly activate $C1\overline{s}$, factor $\overline{D}$, and C3.

$NH_2C(=NH)NHXCOOYCOOZ$

	X	Y	Z
4,	1,4-C_6H_4	1,4-$C_6H_4CH_2$	$CH_2CON(CH_3)_2$
5,	1,4-C_6H_4	1,4-C_6H_4	$CH_2CON(CH_3)_2$
6,	$(CH_2)_5$	1,4-C_6H_4	C_2H_5

Aromatic amino acids represent another class of compounds that alter complement activity at more than a single reaction step. Aromatic amino acids possessing a phenolic hydroxyl group were shown to be especially active in inhibiting the generation of C4 sites (IIc).[29,30] Significant inhibition (50%) was effected at 1.1 mM with glycyl-L-tyrosine, carbobenzoxy-L-tyrosine, carbobenzoxy-L-tryptophan, N-acetyl-L-tyrosine, and N-acetyl-L-tyrosine ethyl ester (ATEE). L-tyrosine and carbobenzoxy-L-phenylalanine effected 50% inhibition at 2.0 and 5.0 mM, respectively. That the phenolic hydroxyl group was important was demonstrated by showing that N-acetyl-L-tyrosine was 7X more active than N,O-diacetyl-L-tyrosine and 11X more active than N-acetyl-L-phenylalanine. Similarly, 5-hydroxy-L-tryptophan was found to be 5X more effective than L-tryptophan. ATEE was found to alter the generation of C3 sites by action of the classical C3 convertase on C3 (IIIc), 50% inhibition occurring at 2 mM.[31] A synthetic substrate of certain proteases, N-benzyl-L-arginine ethyl ester, inhibits lytic complement activity at

20 mM.[32] Tosyl arginine methyl ester (TAME) inhibits $C\bar{1}$-directed C4 and C2 site formation at 10 mM.[29] It is noteworthy that TAME inhibits factor $\bar{D}$ activity (IIa) at the same concentration. This factor is believed to be the alternative pathway counterpart of $C1\bar{s}$.[27]

Sulfhydryl-containing amino acids, cysteine, and homocysteine, and the tripeptide glutathione, were shown to possess inhibitory activity in the 10 to 40 mM range as assessed by whole complement activity, generation of classical C3 convertase, $C1\bar{s}$ esterolysis, and alternative pathway lysis.[28]

Some amino acids have been reported to inhibit binding of C1q to immune complexes.[33] Aspartic acid, glutamic acid and lysine (2-5 mM) inhibited this phase of complement action (Ic) in decreasing potency.

Certain peptides have been shown to interfere with complement function. Leupeptin (acetyl-leucyl-leucyl-arginal), a protease inhibitor derived from actinomycete fermentation, is an effective complement inhibitor. Leupeptin suppresses $C1\bar{s}$ esterolysis at low concentrations (60% at 0.03 mM).[28] In line with this activity, leupeptin effects 50% suppression of classical convertase generation (IIc) at 0.25 mM. Cloxacillin was reported to alter whole complement activity at 4.0 mM.[16] Polymyxin B, several hours after i.v. administration (1 mg/kg), apparently reduced rat complement by 50%.[34] In vitro inhibition (50%) was effected at .5 mM.

Polyanionic Substances - Heparin inhibition of complement action was first observed several decades ago.[35] Heparin has long been known to act early[36] in the complement sequence and was utilized by Osler et al.[37] to suppress in vitro generation of anaphylatoxin. At a heparin concentration of 0.3 μg/ml, inhibition occurs through interference of C1q binding of immune complexes (Ic).[38] Heparin, at 100 μg/ml, inhibits $C1\bar{s}$-mediated consumption of C4 and C2[39] (IIc) but is without effect on $C1\bar{s}$-mediated esterolysis of TAME, indicating possible interference with $C1\bar{s}$ binding of C4 and C2.[40] Heparin (2-200 μg/ml) indirectly inhibits complement action by potentiating C1 inactivator (C1INA) in serum or in a purified state.[41,42] Strong inhibition (50%, 2 μg/ml) of $C\overline{567}$-induced lysis has also been reported.[43] Microgram quantities of both commercial and native heparin inhibit formation of alternative pathway C3 convertase (IIa) and prevent βlH-mediated decay-dissociation of this enzyme.[44]

Pentosan-polysulfo-ester (SP 54) inhibits complement in a fashion similar to heparin. Microgram quantities of this polyanion show inhibition of most reaction steps except C1 activation (Ic).[45] The most studied effect of this polyanion is the blockage of C4 and C2 utilization by $C\bar{1}$ or $C1\bar{s}$ (IIc) with effective inhibition being demonstrable in the 10-100 μg/ml range.[38,40,46] By use of immunochemical techniques, SP 54 showed blockage of the alternative pathway (IIa) at similar concentrations.[47] Low molecular weight dextran sulfate (5,000 daltons) is similarly (IIa) anticomplementary at high concentrations (30% inh., 10 mg/ml).[48]

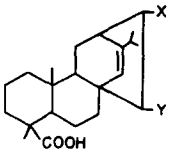
One potent, widely used complement inhibitor is the antitrypanosomal agent, suramin.[49,50] Suramin was reported to diminish whole complement activity by 25% at 5 μM and 75% at 50 μM. At 0.1 mM suramin blocks C1 activation (Ic) whether assessed with purified C1 or with C1 residing in hereditary angioedema serum.[49] Suramin blocks $C\bar{1}$ activity at 0.5 mM and may interfere with later reaction steps as well.[50]

Carrageenan, a sulphated polysaccharide, is a well known complement inactivator.[51,52] Carrageenan binds to C1 and removes it from fluid phase.[51] Intravenous administration of this agent (5 mg/kg) has been reported to diminish hemolytic complement by 99%.[52]

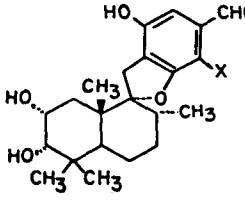
The sulfonic acid azo dye, chlorazol fast pink alters serum complement activity through C1INA potentiation in a fashion similar to heparin.[53,54]

Polynucleotides - Early work with polynucleotides showed that Poly I, Poly G, Poly GU(G/U, 85/15) and Poly IU(I/U, 83/17) inactivated complement at 0.0073, 0.054, 0.25 and 1.23 μM phosphate (P), respectively, and that DNA and RNA were inactive.[55,56] Poly I exclusively prevented C1q binding which was reversed by appropriate additions of Poly A.[57,58] Injections of Poly I into rats (10 μM P/rat) gave a precipitous decline in hemolytic complement activity.[59] More recent *in vitro* work indicates that Poly I samples with higher molecular weights are more inhibitory, and that Poly I, Poly G, Poly AG(A/G, 1.2/1), Poly U_z (poly 2'-azido-2'-deoxyuridylic acid), Poly C_{cl}(poly 2'-chloro-2'-deoxycytidylic acid), and Poly dC_z(poly 2'-azido-2'-deoxycytidylic acid) inhibit whole complement at 10-40 μg/ml.[60]

Acids - Maleopimaric acid (MPA, 7), fumaropimaric acid (FPA, 8), and several of their analogues inhibit whole complement in the range of 0.8-7.0 mM.[61,62] MPA reversibly inhibits C3 convertase assembly (IIc) by interfering with the interaction of sensitized erythrocytes with $C\bar{1}$. Utilization of C2 was also inhibited (IIc). FPA inhibits the chemotactic activity of $C(\overline{567})$ at 7 mM and suppresses the cutaneous and systemic Forssman reactions and the RPAR in guinea pigs (300-600 mg/kg).[62,63]



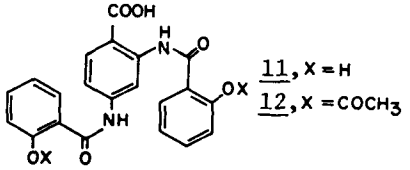
7, X,Y=cis-COOCO-
8, X,Y=trans-COOH,COOH



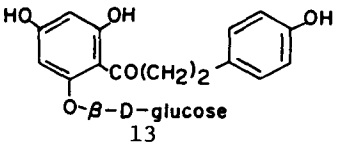
9, X=CHO
10, X=COOH

A fungal metabolite (K-76, 9), and its oxidation product (K-76 COOH, 10), represent interesting complement inhibitors that were recently reported.[64] The mechanism of action of (0.5-2.0 mM) 10 was selective for C5 reactivity, strongly inhibiting C5 utilization by C5 convertase (IVc) and accelerating the decay of the C5 intermediate.

2,4-Bis(2-hydroxybenzamido)benzoic acid (AB-23, 11) and its diacetylated derivative AB-50 (12) inhibit *in vitro* and *in vivo* complement activity.[66] AB-23 suppresses both pathways of complement utilization in the 0.125-0.50 mM range. AB-50, which rapidly reverts to AB-23 *in vivo*, suppressed the passive Arthus reaction in guinea pigs and diminished proteinuria in experimental nephrotoxic rats (100-500 mg/kg).[65]



11, X=H
12, X=COCH3

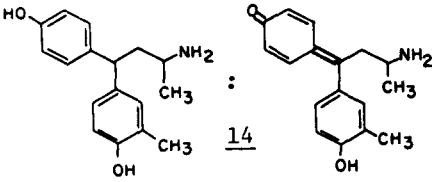


13

The diuretics furosemide and ethacrynic acid inhibit hemolytic complement at 3 mM and 1.9 mM respectively.[66]

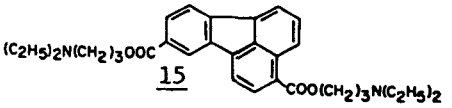
Phenols - Many phenols are anticomplementary. Phloridizin (13) blocks whole complement at 0.31-5 mM[9,6,7] by altering C3 utilization (IIIc).[6,7] Several less potent phenols, salicylaldoxime, catachol, L-epinephrine, resorcinol, and phenol, inhibit complement via the same mechanism as 13.[67]

The histamine releasing agent, 1935L (14), inhibited whole complement at 0.12 mM and lowered in vivo complement levels in rats.[34] Warfarin was anticomplementary at 3 mM[16] and the vitamin B_6-type compounds, pyridoxime, pyridoxal, pyridoxamine and pyridoxal-5-phosphate, inhibited Clq fixation at 0.40-0.48 mM.[33] The importance of phenolic substitution on aromatic amino acids has already been described.[29,30]



14

Amines - Several amines with diverse structures demonstrate anticomplementary activity. An analogue of tilerone (RMI 9563, 15) suppressed (50%) whole complement at 0.03 mM, probably acting on Cl̄s and assembly of C3 convertase (IIc).[68] This compound showed activity in vivo in several antiinflammatory models, including the direct passive Arthus reaction (10-30 mg/kg).



15

Cinnarizine demonstrated complement inhibition in both pathways at 0.07 mM, apparently through chelation of magnesium ions.[69,70]

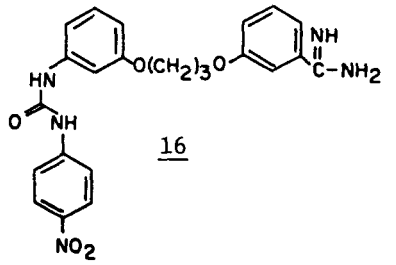
Eight phenothiazines irreversibly inhibited hemolytic complement at 0.27-2.2 mM.[71] Thioridazine and chlorpromazine were inhibitory at 0.27 and 2.2 mM, respectively. These agents acted by suppressing utilization of C2 and C4 (IIc).

Nine diamines were studied for their ability to inhibit Clq binding to insoluble IgM (Ic).[72] All diamines tested were inhibitory at 10 mM. The most potent agents were 2,5-diaminododecane and 2,5-diaminotoluene.

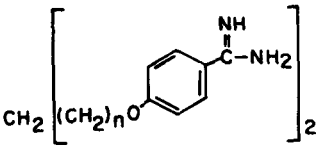
Although many simple primary amines were not inhibitory at 3.0 mM or less,[73] aniline inactivated complement at 2.6 mM.[30]

Benzamidines - NPUPPB (16) and propamidine (17) inhibit whole complement at 0.15 mM[74] and 2 mM,[75] respectively. NPUPPB blocks C2 (IIc) and C5 (IVc) utilization at 0.18 mM, and C1 fixation is inhibited at 0.13 mM.[74] Unlike 16, 17 enhances C1 activity at 2.0 mM yet effects inhibition of

C4 (IIc) and C5 (IVc) utilization. The alternative pathway is suppressed by 17 due to an effect on C5 turnover (IVa).[75,76] Hydrolytic assays indicated 17 inhibition of $C1\bar{r}$, $C1\bar{s}$ and formation of $C1\bar{s}$.[77] At 4 mM, 17 reversibly interacted with the B-determinant of C3, imparting hemolytic suppression.[78] The interaction of 17 with C3 may also explain alternative pathway inhibition.[76] Pentamidine (18), a close analogue of 17, was inactive in the systemic Forssman reaction.[62] Early work showed that benzamidine blocked whole complement at 3 mM[73] and C1 at 2 mM.[79] Placing N-substituents on benzamidine destroyed activity[73] and meta substitution relative to para substitution produced greater inhibition.[73,80-83] Bisbenzamidines are more active than monobenzamidines.[84] A quantitative SAR has been formulated for meta-substituted monobenzamidines based on whole complement inhibition.[85]

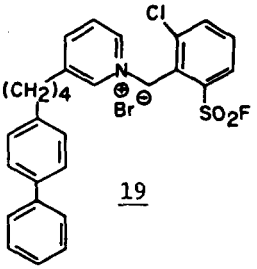


16

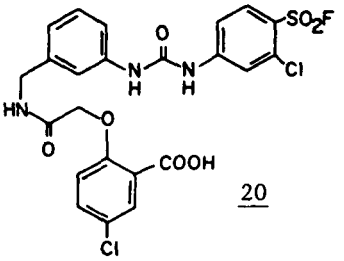


17, n=1
18, n=2

Sulfonyl Fluorides - Many diverse aryl sulfonyl fluorides inactivate complement at 0.015-1.00 mM.[32,73,82,83,86-89] Three of these 19, 20, and 21 are potent inhibitors.



19



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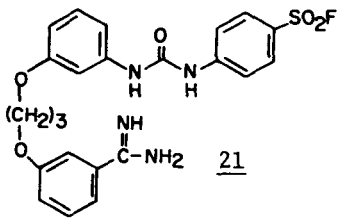

21

These compounds and several of their analogues have been shown to irreversibly bind to $C\bar{1}$ (IIc).[82,83,88-91] Quantitative SAR have been formulated for the arylpyridinium sulfonyl fluorides (19)[92] and the arylamidine sulfonyl fluorides (21).[85]

Inorganics - The zinc cation was reported to reversibly inhibit whole complement activity from 0.025 to 0.50 mM. Utilization of every complement component except C5 and C9 was inhibited (12-55%) from 0.025-0.50 mM. C5 function was enhanced (192%) from 0.025-0.1 mM but inhibited at concentrations of $ZnCl_2$ greater than 0.1 mM. In vivo Zn^{++} serum levels of 0.10 mM were obtained and caused diminished complement levels and the intensity of the RPAR was reduced as well.[93]

Sodium cyanate irreversibly inhibits normal and sickle cell serum complement, C3, C5, C6, and C7 being affected at 50 mM after 8 hours of incubation at 37°. C3b inactivator was significantly altered at 50 mM.[94]

Sodium azide has recently been reported to inhibit diluted complement activity from 7.5-60 mM.[95]

References

1. N. J. Zvaifler in "Inflammation: Mechanisms and Control," I. H. Lepow and P. A. Ward, Eds., Academic Press, New York, N.Y., 1972, p 223.
2. H. R. Holman in "Immunological Diseases," Vol. II, D. W. Talmage, B. Rose, and J. H. Vaughan, Eds., Little, Brown and Co., Boston, MA, 1965, p 995.
3. F. J. Dixon in "Immunological Diseases," Vol. II, D. W. Talmage, B. Rose, and J. H. Vaughan, Eds., Little, Brown and Co., Boston, MA, 1965, p 1225.
4. H. R. Colten in "Annual Reports in Medicinal Chemistry," Vol. 7, R. V. Heinzelman, Ed., Academic Press, New York, N.Y., 1972, p 228.
5. M. M. Frank, Rev. Infect. Dis., 1, 483 (1979).
6. H. J. Muller-Eberhard in "Molecular Basis of Biological Degradative Processes," R. D. Berlin, H. Herrmann, I. H. Lepow, and J. M. Tanzer, Eds., Academic Press, New York, N.Y., 1978, p 65.
7. E. L. Becker in "Complement", G. E. W. Wolstenholme and J. Knight, Eds., Little, Brown and Co., Boston, MA, 1965, p 58.
8. E. L. Becker in "Inflammation: Mechanisms and Control," I. H. Lepow and P. A. Ward, Eds., Academic Press, New York, N.Y., 1972, p 281.
9. T. W. Harrity and M. B. Goldlust, Biochem. Pharmacol., 23, 3107 (1974).
10. W. T. Jackson and A. Snlen, Fed. Proc., 30, 472 (1971).
11. P. F. Kohler and J. S. Martinez, Fed. Proc., 30, 472 (1971).
12. P. Orsolin, M. R. Milani, and C. Velgi, Arzneim. Forsch., 29, 179 (1979).
13. D. R. Schultz, J. E. Volanakis, B. I. Arnold, N. J. Gottlieb, K. Bakai and R. M. Stroud, Clin. Exp. Immunol., 17, 395 (1974).
14. J. J. Burge, D. T. Fearon and K. F. Austen, J. Immunol., 120, 1625 (1978).
15. H. Gewurz, P. R. Wernich, P. G. Quie and R. A. Good, Nature, 208, 755 (1965).
16. K. Whaley, D. J. P. Sloane, A. G. Davidson and P. M. Brooks, Br. J. Clin. Pharmacol., 2, 123 (1975).
17. J. Francios, F. T. Gagnon, Can. J. Microbiol., 16, 63 (1970).
18. S. Rosini, V. Mazzonicini, J. Hyg. Epidemiol. Microbiol. Immunol., 21, 309 (1977).
19. T. DiPerri, A. Auteri and A. Luvara, Drugs Exp. Clin. Res., 3, 1 (1977).
20. F. B. Taylor, Jr. and H. Fudenberg, Immunology, 7, 319 (1964).
21. N. A. Soter, K. F. Austen, and I. Gigli, J. Immunol., 144, 928 (1975).
22. W. Vogt, G. Schmidt, R. Lynen, and L. Dieminger, J. Immunol., 114, 671 (1975).
23. E. H. Vallota and H. J. Muller-Eberhard, J. Exp. Med., 137, 1109 (1973).
24. E. H. Vallota, Immunology, 34, 439 (1977).
25. Y. Tamura, M. Hirado, K. Okamura, Y. Minato, and S. Fujii, Biochim. Biophys. Acta., 484, 417 (1977).
26. M. Muramatu, S. Shiraishi, and S. Fujii, Biochim. Biophys. Acta., 285. 224 (1972).
27. D. T. Fearon and K. F. Austen, Ann. N.Y. Acad. Sci., 256, 441 (1975).
28. Y. Takada, Y. Arimoto, H. Mineda, and A. Takada, Immunology, 34, 509 (1978).
29. A. Shimada and N. Tamura, Immunology, 22, 723 (1972).
30. A. Shimada and N. Tamura, Immunology, 32, 251 (1977).
31. H. S. Shin and M. M. Mayer, Biochemistry, 7, 3003 (1968).
32. B. R. Baker and J. A. Hurlbut, J. Med. Chem., 12, 415 (1969).
33. R. Allan, M. Rodrick, H. R. Knobel, and H. Isliker, Int. Arch. Allergy Appl. Immunol., 58, 140 (1979).
34. J. P. Giroud, J. Timsit, C. R. Soc. Biol. (Paris), 165, 69 (1971).
35. E. E. Ecker and P. Gross, J. Infect. Dis., 44, 250 (1929).
36. E. E. Ecker and L. Pillemer, J. Immunol., 40, 73 (1941).
37. A. G. Osler, H. G. Randall, B. M. Hill, and Z. Ovary, J. Exp. Med., 110, 311 (1959).
38. E. Raepple, H. Hill, and M. Loos, Immunochemistry, 13, 251 (1976).
39. R. Strunk and H. R. Colten, Clin. Immunol. Immunopathol., 6, 248 (1976).
40. M. Loos, J. E. Volanakis, and R. M. Stroud, Immunochemistry, 13, 789 (1976).
41. R. Rent, R. Myhrman, B. A. Fiedel, and H. Gewurz, Clin. Exp. Immunol., 23, 264 (1976).
42. K. Nagaki and S. Inai, Int. Arch. Allergy Appl. Immunol., 50, 172 (1976).
43. P. J. Baker, T. F. Lint, B. C. McLeod, C. L. Behrends, and H. Gewurz, J. Immunol., 114, 554 (1975).
44. J. M. Weiler, R. W. Yurt, D. T. Fearon, and K. F. Austen, J. Exp. Med., 147, 409 (1978).
45. D. Walb, M. Loos, and U. Hadding, Z. Naturforsch. Teil B, 26, 403 (1971).
46. F. C. Berthoux, A. Freyria, and J. Traeger, Pathol. Biol., 25, 105 (1977).
47. F. C. Berthoux, A. Freyria, and J. Traeger, Pathol. Biol., 25, 179 (1977).
48. R. Burger, D. Bitter-Suermann, and U. Hadding, Immunochemistry, 15, 231 (1978).
49. V. Eisen and C. Loveday, Br. J. Pharmacol., 49, 678 (1973).
50. J. S. C. Fong and R. A. Good, Clin. Exp. Immunol., 10, 127 (1972).
51. T. Borsos, H. J. Rapp, and C. Crisler, J. Immunol., 94, 662 (1965).
52. G. E. Davies, Immunology, 6, 561 (1963).
53. N. Bauman and J. A. Brockman, J. Immunol., 120, 1764 (1978).
54. H. P. Lambert and J. Richley, Br. J. Exp. Pathol., 33, 327 (1952).
55. S. Yachnin, J. Clin. Invest., 42, 1947 (1963).
56. S. Yachnin, J. Immunol., 93, 155 (1964).
57. S. Yachnin, D. Rosenblum, and D. Chatman, J. Immunol., 93, 540 (1964).

58. S. Yachnin, D. Rosenblum, and D. Chatman, J. Immunol., 93, 549 (1964).
59. S. Yachnin, D. Rosenblum, and D. Chatman, J. Clin. Invest., 43, 1175 (1964).
60. E. De Clercq, P. F. Torrence, and J. Hobbs, B. Janik, P. DeSomer, and B. Witkop, Biochem. Biophys. Res. Commun., 67, 255 (1975).
61. M. M. Glovsky, E. L. Becker, and N. J. Halbrook, J. Immunol., 100, 979 (1968).
62. I. G. Otterness, A. J. Torchia, and H. D. Doshan, Biochem. Pharmacol., 27, 1873 (1978).
63. M. M. Glovsky, P. A. Ward, E. L. Becker, and N. J. Halbrook, J. Immunol., 102, 1 (1969).
64. K. Hong, T. Kinoshita, W. Miyazaki, T. Izawa, and K. Inoue, J. Immunol., 122, 2418 (1979).
65. Y. Ohsugi, T. Matsuno, and Y. Takagaki, Chem. Pharm. Bull., 25, 1202 (1977).
66. T. DiPerri, S. Forconi, A. Auteri, A. Vittora, F. L. Pasini, and F. Guercini, Minerva Nefrol., 21, 147 (1974).
67. E. Rodriquez and A. G. Osler, J. Immunol., 85, 347 (1960).
68. H. Megel, A. Roychaudhuri, M. Bayer, and T. H. Beaver, Agents and Action, 8, 218 (1978).
69. T. DiPerri and A. Auteri, Arch. Int. Pharmacdyn Ther., 203, 23 (1973).
70. T. DiPerri, A. Auteri, F. L. Pasini, and F. Mattioli, Arch. Int. Pharmacodyn. Ther., 226, 281 (1977).
71. T. S. S. Mao, J. J. Noval, P. Pellerin, and O. J. Plescia, Can. J. Biochem., 47, 547 (1969).
72. C. R. Sledge and D. H. Bing, J. Biol. Chem., 248, 2818 (1973).
73. B. R. Baker and E. H. Erickson, J. Med. Chem., 12, 408 (1969).
74. M. M. Glovsky, M. Cory, and A. Alenty, Immunolgy, 26, 819 (1974).
75. W. Vogt, B. Hinsch, G. Schmidt, and I. Von Zabern, Immunology, 36, 131 (1979).
76. W. Vogt, G. Schmidt, and B. Hinsch, Immunology, 36, 139 (1979).
77. S. S. Asghar, K. W. Pondman, and R. H. Cormane, Biochim. Biophys. Acta., 317, 539 (1973).
78. S. S. Asghar and R. H. Cormane, Immunochemistry, 13, 975 (1976).
79. D. H. Bing, J. Immunol., 105, 1289 (1970).
80. B. R. Baker and M. Cory, J. Med. Chem., 12, 1049 (1969).
81. J. D. Geratz, M. C.-F. Cheng, and R. R. Tidwell, J. Med. Chem., 19, 634 (1976).
82. B. R. Baker and M. Cory, J. Med. Chem., 14, 119 (1971).
83. B. R. Baker and M. Cory, J. Med. Chem., 14, 805 (1971).
84. J. Hauptmann and F. Markwardt, Biochem. Pharmacol., 26, 325 (1977).
85. C. Hansch and M. Yoshimoto, J. Med. Chem., 17, 1160 (1974).
86. B. R. Baker and J. A. Hurlbut, J. Med. Chem., 12, 902 (1969).
87. B. R. Baker and J. A. Hurlbut, J. Med. Chem., 12, 677 (1969).
88. M. H. Doll and B. R. Baker, J. Med. Chem., 19, 1079 (1976).
89. B. R. Baker and M. H. Doll, J. Med. Chem., 14, 793 (1971).
90. D. H. Bing, J. L. Mernitz, and S. E. Spurlock, Biochemistry, 11, 4263 (1972).
91. A. S. D. Pang, D. E. Schmidt, Jr., W. P. Aston, Life Sci., 13, 351 (1973).
92. C. Hansch, M. Yoshimoto, and M. H. Doll, J. Med. Chem., 19, 1089 (1976).
93. D. W. Montgomery, M. Chvapil, and C. F. Zukoski, Infect. Immun., 23, 424 (1979).
94. D. R. Schultz and P. I. Arnold, J. Immunol., 115, 1558 (1975).
95. D. R. Shaw, M. W. Shaw, S. E. Hickman, E. W. Lamon, and F. M. Griffin, Jr., Immunology, 39, 53 (1980).

Chapter 21. Agents that Affect Prolactin Secretion

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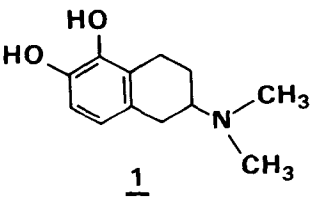
Introduction - This chapter will review the recent work on substances that have been shown to affect prolactin secretion. Upon consideration of the large number of physiological circumstances (stress, lactation, pregnancy, pseudopregnancy, cervical stimulation, estrogen levels, reproductive cycles, sleep, temperature) where prolactin secretion is modified, it is no surprise that many agents exist that are able to alter release of prolactin. This review will be divided into sections covering substances that inhibit prolactin release and substances that stimulate prolactin release.

Prolactin Release Inhibitors - The first class of compounds found to inhibit prolactin secretion is catecholamines. Specifically, dopamine has been proposed by a multitude of authors as the physiological prolactin inhibiting factor (PIF) of the hypothalamus. Dopamine is released by the tuberoinfundibular dopaminergic neurons into the hypophyseal portal circulation which carries it to the adenohypophysis where it inhibits the release of prolactin. The concentration of dopamine in hypophyseal portal blood is sufficient to inhibit prolactin release by a direct action on pituitary tissue, and substances such as amphetamine and progesterone inhibit the release of prolactin and concomittantly increase the concentration of dopamine in the hypophyseal portal circulation.[1,2] Dopamine agonists have been shown to uniformly inhibit the release of prolactin *in vivo* and act by a direct action on pituitary tissue *in vitro*.

A similarity in structure between dopamine and 2-aminotetralins prompted the synthesis of several of these compounds as potential dopamine agonists. The best known of these agents is 5,6-dihydroxy-2-dimethylaminotetralin (1).[3] A large number of variously N-substituted 2-aminotetralins having hydroxyl groups at 5 and 6 and at 6 and 7 positions have been shown to have dopaminergic activity.[4,5]



1

Interestingly, a series of N-substituted aminotetralins and aminoindanes lacking aromatic hydroxyl substituents have been found to be dopamine agonists *in vivo* and have been reported to inhibit prolactin release.[6] Thus, it is clear that compounds lacking the catechol moiety can be dopamine agonists. N-alkyl substitution of the above agents improves the dopamine agonist activity and prolactin inhibiting potency. Both N,N-dipropyl-2-aminotetralin (2) and N,N-dipropyl-2-aminoindane (3) were dopamine agonists and potent prolactin release inhibitors.[6] Since the *in vivo* activity of the above compounds could be antagonized with α-methyltyrosine, hydroxylation *in vivo* may be a necessary prerequisite for dopaminergic activity.

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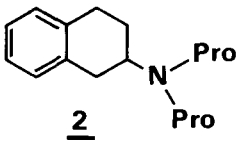

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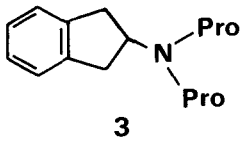

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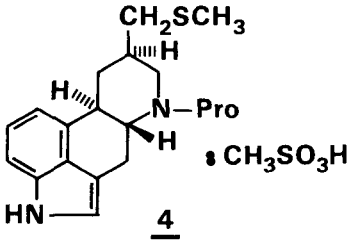
One group of dopamine agonists that shows a remarkably strong inhibitory effect on prolactin release both in vivo and on pituitary tissue in vitro is the ergolines. The best examples of these compounds are bromocryptine and lergotrile. Both compounds are potent inhibitors of prolactin release and have been reported to inhibit prolactin release in many different animal species and in man. The studies on these and other ergoline derivatives have been thoroughly reviewed in an extensive treatise by Berde and Schild,[7] and the reader who wishes to know more about the history of these compounds should consult the above review. One of the most significant recent developments in this area is the discovery that the ergoline derivatives can be made many-fold more potent as dopamine agonists and prolactin inhibitors by substitution of a propyl for a methyl group in the 6-position.[8] Pergolide mesylate (4) is probably the most potent inhibitor of prolactin release known at the present time[8] and is many-fold more potent than its corresponding 6-methyl analog.[9] Recently, another ergoline derivative, lisuride (5), has been shown to be a potent inhibitor of prolactin release and to be effective in lowering serum prolactin in man.[10,11]



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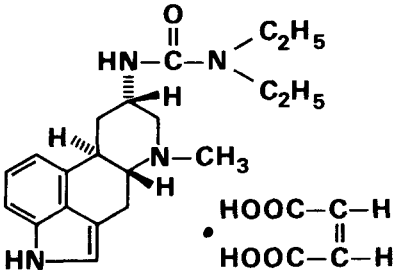
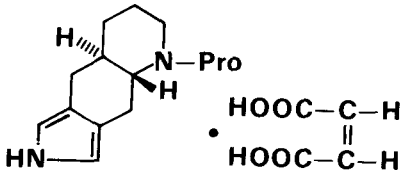


5

The portion of the ergoline nucleus that confers dopamine agonist activity upon the molecule has been the topic of much speculation. Many believe that the rigid phenylethylamine is the dopaminergic portion, but recently, the rigid pyrrolethylamine part of the molecule was shown to possess strong dopaminergic activity.[12] For example, (± trans)-4,4a,5,6,7,8,8a,9-octahydro-5-propyl-2H-pyrrol(3,4 -g) quinoline maleate (6) is an extremely potent inhibitor of prolactin release. Several compounds similar in structure to 6 also were found to inhibit prolactin release and possess dopamine agonist activity.[12]



6

Indirectly acting dopaminergic agents are also capable of blocking prolactin release. Amfonelic acid and methylphenidate are two agents that act by releasing dopamine from its stores. Both compounds inhibit prolactin

release.[13,14] In addition, it has been demonstrated that methylphenidate is unable to inhibit the release of prolactin when the dopamine stores were depleted by reserpine.[14]

Some investigators believe, that in addition to dopamine, other physiological inhibitors of prolactin release exist. One of the substances proposed to be a PIF is γ-aminobutyric acid (GABA).[15] GABA is a very weak inhibitor of prolactin release when incubated with pituitary tissue in vitro; however, the inhibitory action of GABA can be antagonized by GABA antagonists.[16] The GABA agonist, muscimol, is a much more potent inhibitor of prolactin release in vivo and in vitro than is GABA.[16,17] Marked suppression of prolactin release is also obtained after inhibition of GABA transaminase.[17,18] Thus, the possibility exists that, in addition to dopamine, GABA may be a physiological PIF (see Chapter 5).

A number of additional substances exist that are able to inhibit prolactin release. Acetylcholine and cholinergic agonists are inhibitory to prolactin release, while opioid substances are stimulatory. The opioids (endorphins, enkephalins, morphine, etc.) appear to stimulate prolactin release by preventing the release of dopamine from the tuberoinfundibular dopaminergic neurons.[19] Acetylcholine agonists have been shown to block the effects of opioids on prolactin release.[20] Presumably the opioids inhibit, while cholinergic substances stimulate, the tuberoinfundibular dopaminergic system.

In summary, it appears that the inhibitory control of prolactin release may be mediated by two substances: dopamine and GABA. These substances are able to inhibit prolactin release by acting directly at the level of the anterior pituitary. Substances that act on the central nervous system to increase the availability of dopamine and GABA to the anterior pituitary uniformly inhibit the release of prolactin. Several years ago, a number of groups proposed the existence of a polypeptide, PIF, but further characterization of this substance has not been reported.

Prolactin Release Stimulators - It is recognized that the predominant influence of the brain on prolactin secretion is inhibitory, mediated by one or more prolactin inhibiting factors. There is also strong evidence that a prolactin releasing factor may exist. Several endogenous substances such as thyrotropin releasing hormone, vasoactive intestinal polypeptide (VIP) and various other polypeptides have been shown to cause the release of prolactin by a direct action on anterior pituitary tissue. However, the physiological significance of their prolactin-releasing activity has not been determined. Prolactin releasing factor-like activity has been isolated in extracts made from hypothalamic tissue fragments of pregnant rats,[21] porcine hypothalami,[22] bovine hypophyseal stalk tissue[23] and methanol extracted plasma from rats pretreated with fluoxetine, a serotonin reuptake inhibitor, plus 5-hydroxytryptophan, the immediate precursor of serotonin.[24] The chemical structures of these proposed prolactin releasing factors remain, at least for now, unknown.

VIP, a 28 amino acid polypeptide, was originally isolated and purified from porcine small intestine.[25] VIP is present in the hypothalamus,[26] and radioimmunoassable VIP levels in hypothalamo-hypophyseal portal blood are 19 to as high as 180 times the concentration of the polypeptide in systemic arterial blood.[27] Synthetic VIP administered either intraventricularly or intravenously causes a dose-related elevation in plasma prolactin concentration in rats, an effect which is attenuated by simultaneous administration of the opiate antagonist, naloxone, or L-DOPA, a precursor of dopamine.[28] Incubation of hemipituitaries with doses of VIP ranging from 10^{-5}M to 10^{-8}M

causes significant stimulation of prolactin release *in vitro* by a direct action on anterior pituitary lactotrophs.[29,30]

The role of serotonergic pathways in controlling prolactin release has been extensively studied. Administration of serotonin (5HT), 5-hydroxytryptophan (5HTP) and synthetic 5HT agonists is stimulatory to prolactin secretion in rats, monkeys and man.[31,32] However, research into the function of the serotonergic pathway using 5HT antagonists such as methylsergide, cyproheptadine and metergoline has led to controversial results, due to the poor specificity of those agents.[34,35] Experimental and clinical data indicate that fluoxetine is a specific blocker of 5HT reuptake into synaptosomes.[36,37] Since reuptake is the main process in amine degradation, blockade of 5HT reuptake would lead to an enhanced tone of serotonergic pathways. While fluoxetine has no effect on prolactin when administered alone, it does markedly potentiate the release of prolactin in response to 5HTP in rats[24,38] and insulin in man.[39] Serotonergic stimulation produced by 5HTP plus fluoxetine treatment may augment prolactin release via a prolactin releasing factor.[24,38]

The discovery of the presence of endogenous opioid-like peptides in the brain and pituitary has created great interest in their potential physiological function. Recent neuroendocrine studies have demonstrated that methionine enkephalin, leucine enkephalin, β-endorphin and synthetic opioid pentapeptides stimulate prolactin release in rats[40,41] and humans.[42] These opioid-like peptides appear not to stimulate prolactin via a direct action on the anterior pituitary lactotrophs.[40] Morphine sulfate causes a significant elevation, and naloxone causes a significant reduction in basal serum prolactin concentrations in subhuman primates.[43] However, naloxone, while causing reductions in basal serum prolactin concentrations in rats[29] and monkeys,[43] does not influence basal serum prolactin concentrations in humans.[44] The fact that naloxone decreases serum levels of prolactin in rats and monkeys has led to the hypothesis that endogenous opiates may, in part, regulate basal prolactin secretion.

In summary, recent experimental data indicate that a hypothalamic prolactin releasing factor may exist. Prolactin release produced in response to serotonergic stimulation may be mediated by the release of a hypothalamic prolactin releasing factor. Several endogenously occurring polypeptides, including methionine enkephalin, leucine enkephalin, β-endorphin and VIP stimulate prolactin release. While the opioid-like peptides appear not to have a direct stimulatory effect, vasoactive intestinal polypeptide can act directly on anterior pituitary lactotrophs to promote prolactin release.

References

1. G.A. Gudelsky and J.C. Porter, Endocrinology, 104, 583 (1979).
2. O.M. Cramer, C.R. Parker, Jr. and J.C. Porter, Endocrinology, 105, 929 (1979).
3. J.G. Cannon, J.C. Kim and M.A. Aleem, J. Med. Chem., 15, 348 (1972).
4. J.G. Cannon, T. Lee and D. Goldman, J. Med. Chem., 20, 1111 (1977).
5. J. McDermed, Ann. Rep. Med. Chem., 14, 12 (1979).
6. D.B. Rusterholz, J.P. Long, J.R. Flynn, J.G. Cannon, T. Lee, J.P. Pease, J.A. Clemens, D.T. Wong and F.P. Bymaster, Europ. J. Pharmacol., 55, 73 (1979).
7. B. Berde and H.O. Schild, Ed., "Ergot Alkaloids and Related Compounds," Springer Verlag, Berlin, 1978.
8. R.W. Fuller, J.A. Clemens, E.C. Kornfeld, H.D. Snoddy, E.B. Smalstig and N.J. Bach, Life Sci., 24, 375 (1979).
9. J.A. Clemens and E.B. Smalstig in "Catecholamines: Basic and Clinical Frontiers," Vol. 2, E. Usdin, I.J. Kopin and J. Barchas, Eds., Pergamon Press, New York, 1979, p. 1248.
10. R. Horowski, H. Wendt and K.-J. Graf, Acta Endocrinol., 87, 234 (1978).
11. L. DeCeceo, P.L. Venturini, N. Ragni, P. Rossato, C. Maganza, G. Gaggero and R. Horowski, Br. J. Obstet. Gynaecol., 86, 905 (1979).

12. N.J. Bach, E.C. Kornfeld, N.D. Jones, M.O. Chaney, D.E. Dorman, J.W. Paschal, J.A. Clemens, E.B. Smalstig, D.T. Wong and F.P. Bymaster, J. Med. Chem., 23, in press (1980).
13. E. Vijayan, D.C. German and S.M. McCann, Life Sci., 22, 711 (1978).
14. J.A. Clemens and R.W. Fuller, Life Sci., 24, 2077 (1979).
15. A.V. Schally, T.W. Redding, A. Arimura, A. Dupont and G. Linthicum, Endocrinology, 100, 681 (1977).
16. L. Grandison and A. Guidotti, Endocrinology, 105, 754 (1979).
17. V. Locatelli, D. Cocchi, G. Racagni, F. Cattabeni, A. Maggi, P. Krogsgaard-Larsen and E.E. Muller, Brain Res., 145, 173 (1978).
18. G. Racagni, J.A. Apud, V. Locatelli, D. Cocchi, G. Nistico, R.M. diGiorgio and E.E. Muller, Nature, 281, 575 (1979).
19. G.A. Gudelsky and J.C. Porter, Life Sci., 25, 1697 (1979).
20. T. Muraki, Y. Tokunaga, T. Nakadate and R. Kato, Naunyn-Schmiedeberg's Arch. Pharmacol., 308, 249 (1979).
21. K. Takshashi, T. Wakai, N. Furuhashi, H. Hoshiai, Y. Wada, A. Saito, A. Haneda and M. Suzuki, Tohoku J. Exp. Med., 126, 77 (1978).
22. A.E. Boyd, III, F. Sanche-Franco, E. Spencer, Y.C. Patel, I.M.D. Jackson and S. Reichlin, Endocrinology, 103, 1075 (1978).
23. N. Yasuda and S.E. Greer, Biochem. and Biophys. Res. Comm., 85, 1291 (1978).
24. T.L. Garthwaite and T.C. Hagen, Neuroendocrinology, 29, 215 (1979).
25. V. Mutt and S.I. Said, Europ. J. Biochem., 42, 581 (1974).
26. P.C. Emson, J. Fahrenkrug, O.B. Schaffalitzky de Muckadell, T.M. Jessell and L.L. Iversen, Brain Research, 143, 174 (1978).
27. S.I. Said and J.C. Porter, Life Sci., 24, 227 (1979).
28. Y. Kato, Y. Iwasaki, J. Iwasaki, H. Abe, N. Yanaihara and H. Imura, Endocrinology, 103, 554 (1978).
29. C.J. Shaar, J.A. Clemens and N.B. Dininger, Life Sci., 25, 2071 (1979).
30. M. Ruberg, W.H. Rotsztejn, S. Arancibia, J. Besson and A. Enjalbert, Europ. J. Pharm., 51 319 (1978).
31. L. Krulich, E. Vijayan, R.J. Coppings, A. Giachetti, S.M. McCann and M.A. Mayfield, Endocrinology, 105, 276 (1979).
32. W.M. Fraser, H. St. Tucker, S.R. Gribb, J.P. Wigand and W.G. Blackard, Horm. Metab. Res., 11, 149 (1979).
33. R.R. Gala, J.A. Peters, D.R. Pieper and M.D. Campbell, Life Sci., 22, 25 (1978).
34. E.E. Muller, A.E. Panerai, D. Cocchi and P. Mantegazza, Life Sci., 21, 1545 (1977).
35. R.V. Gallo, J. Rabii and G.P. Moberg, Endocrinology, 97, 1096 (1975).
36. D.T. Wong, J.S. Horng, F.P. Bymaster, K.L. Hauser and B.B. Molloy, Life Sci., 15, 471 (1974).
37. L. Lemberger, H. Rowe, R. Carmichael, S. Oldham, J.S. Horng, F.P. Bymaster and D.T. Wong, Science, 199, 436 (1978).
38. J.A. Clemens, M.E. Roush and R.W. Fuller, Life Science, 22, 2209 (1978).
39. A. Masala, G. Delitala, L. Devilla, S. Alagna and P.P. Rovasio, J. Clin. Endocrinol. Metab., 49, 350 (1979).
40. C.J. Shaar, R.C.A. Frederickson, N.B. Dininger and L. Jackson, Life Sci., 21, 853 (1977).
41. C. Rivier, W. Vale, N. Ling, M. Brown and R. Guillemin, Endocrinology, 100, 238 (1977).
42. B.V. Graffenried, E. delPozo, J. Roubicek, E. Krebs, W. Poldinger, P. Burnmeister and L. Kerp, Nature, 272, 729 (1978).
43. M.S. Gold, D.E. Redmond, Jr. and R.K. Donabedian, Endocrinology, 105, 284 (1979).
44. D. Janowsky, L. Judd, L. Huey, N. Roitman and D. Parker, Psychopharmacology, 65, 95 (1979).

Section V - Topics in Biology

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Chapter 22. Scope and Mechanism of Enzymatic Monooxygenation Reactions

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Aerobic organisms make a living by oxidation of reduced organic or inorganic compounds and trapping ca. 40% of the oxidative energy released in chemically activated molecules such as ATP, acetylCoA, and NADH. Electrons removed from oxidizable substrates are ultimately channeled to O_2 which, as terminal acceptor, is reduced by 4 electrons to H_2O. About 90% of the flux of reductive oxygen metabolism is effected by the complex (seven polypeptides--two Cu, two heme cofactors) mitochondrial membrane protein cytochrome oxidase. Other enzymes such as flavoprotein α-hydroxy acid oxidases, α-amino acid oxidases, amine oxidases, glucose oxidase, and the cuproprotein galactose oxidase reduce O_2 by two electrons to H_2O_2 as the specific co-substrate is oxidized by two electrons (eq. 1). The species lying between O_2 and H_2O by sequential one-election addition are superoxide, peroxide, and hydroxyl radical respectively. All three intermediates are highly reactive and underscore the problem that although

$$O_2 \xrightarrow{1e^-} O_2^{-\cdot} \xrightarrow[2H^+]{1e^-} H_2O_2 \xrightarrow[1H^+]{1e^-} \underset{+H_2O}{OH\cdot} \xrightarrow[1H^+]{1e^-} H_2O \qquad (1)$$

enzymic dioxygen reduction is enormously favorable thermodynamically, the strategy is not without risk if the intermediates cannot be contained. To this end, surveillance enzymes for dismutation of superoxide, superoxide dismutase, and hydrogen peroxide, catalase, are a complement in aerobic organisms for protection.[1] The potential toxicity of these two compounds is in fact selectively exploitable in certain biological situations. For example, one killing mechanism leucocytes use on bacteria is to send out a burst of $O_2^{-\cdot}$ generated by a NADPH reductase in the white cell membrane.[2] Recent studies have shown that Trypanosoma brucei, one of the African sleeping sickness organisms, cannot synthesize heme and so does not have cytochromes or catalase. These organisms are killed by accumulation of intracellular H_2O_2 and by agents (e.g., free heme itself) that promote its homolytic cleavage to OH· or OOH· radicals.[3]

In addition to functioning as simple electron acceptor in biological redox reactions, dioxygen can also be activated for insertion of one (monooxygenation) or both (dioxygenation) of its atoms into a cosubstrate molecule by a wide variety of enzymes.[4-7] These enzymic oxygenations are often key metabolic events in assimilatory sequences, such as catechol amine or steroid hormone biosynthesis, as well as in degradative and detoxifying sequences in liver processing of drugs and toxins and occasionally in toxifying reactions such as precarcinogen activation in alkylnitrosamine or polycyclic hydrocarbon oxygenative metabolism.[8,9] We will briefly survey the scope and mechanism of monooxygenases in this chapter. Space limitations preclude discussion of dioxygenases such as those involved in catechol generation,[10] aromatic ring cleavage,[11] prostaglandin cyclooxygenation[12] and proline hydroxylations in collagen molecule maturation.[13]

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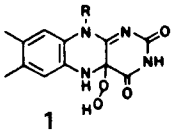
Since triplet O_2 is spin-unpaired and organic cosubstrates are spin-paired, enzymes have evolved mechanisms for selective acceleration of the kinetic sluggishness of hydroxylations in two ways, either use of redox active metals copper or iron, or use of a conjugated organic cofactor, such as flavin or pterin coenzymes.[6,14] There are no known oxygenases, with the possible exception of ribulose bisphosphate carboxylase acting in oxygenase mode,[15] which function without such a metal or cofactor. Iron and copper act as direct O_2 ligands and electron conduits, while the dihydroflavins and tetrahydropterins probably react via radical mechanisms with O_2 to yield semiquinone and $O_2^{-\cdot}$ with subsequent rapid radical recombination to yield flavin hydroperoxides 1[16-19] and pterin hydroperoxides 2[20,21] as proximal oxygenation agents.



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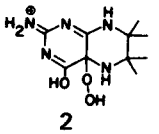

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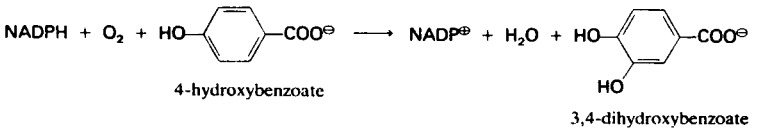
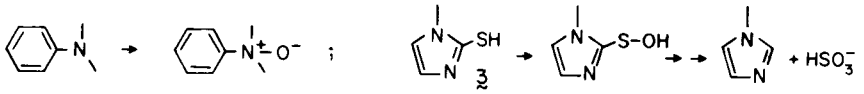
All four categories, iron-, copper-, flavin-, and pterin-dependent monooxygenases are known and fit into different chemical and physiological niches.[6]

Three types of molecules are oxygenated by flavoproteins: phenols are processed to catechols, ketones and aldehydes converted to lactones or acids, and amines and sulfur compounds converted to N-hydroxy and S-hydroxy products as noted below. A complete stoichiometry is indicated



for p-hydroxybenzoate hydroxylation, stressing the four-electron reduction experienced by O_2 and two-electron oxidation of cosubstrate and NADPH. The ketone to lactone conversion[22] appears to be a biological example of a Baeyer-Villiger reaction[23] with flavinhydroperoxide 1 as the nucleophilic peroxide equivalent. The N- and S-oxygenase enzyme is found

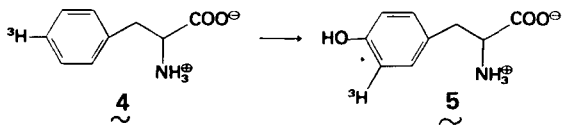


3

in animals and is important in drug metabolism, for example in conversion of the antithyroid methimazole 3 to N-methylimidazole and sulfite.[24] Thiocarbamates, thioamides, and some thiols and disulfides are also substrates at maximal velocities of ca. 1 molecule/sec. In these sequences, it is presumed the N and S atoms of substrates attack flavin hydroperoxide 1 as an electrophilic source of oxygen.

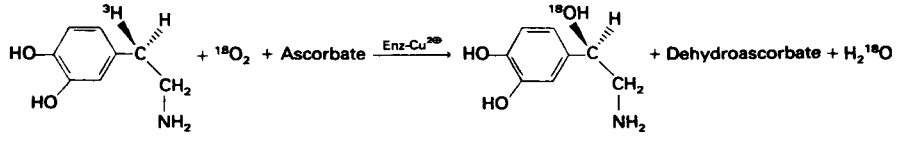
Three pterin-linked monooxygenases perform key aromatic ring hydroxylations in the biosynthesis of neurotransmitters adrenalin and serotonin, specifically in conversion of phenylalanine to tyrosine, tyrosine to L-DOPA, and conversion of tryptophan to 5-hydroxytryptophan. Phenylalanine hydroxylase, for example, effects regiospecific parahydroxylation (4→5) with characteristic migration of hydride as shown, the "NIH shift" consistent with an arene oxide intermediate.[26] The mechanism of

such arene oxide formation from 2 is unclear. Consistent with their placement in key biosynthetic pathways, these three hydroxylases appear to function under a number of metabolic and regulatory controls.[27]



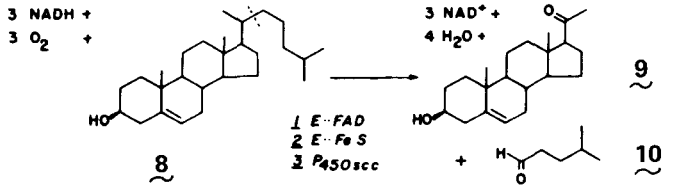
4 5

Further along the catechol hormone biosynthetic pathway in the adrenal medulla is the one well-characterized copper-dependent monooxygenase, dopamine-β-hydroxylase, in animal cells, converting dopamine, 6, to noradrenalin, 7. Ascorbate is exogenous two-electron reductant to generate two enzyme-bound cuprous atoms for incubation with O_2 to yield first a copper superoxide complex.[28] Subsequent steps are unclear mechanistically, including how the β-C-H bond is broken and the OH-functionality inserted with net retention[29] of configuration.



dopamine 6 norepinephrine (noradrenalin) 7

The largest collection of substrate monooxygenations carried out in animal (and bacterial) cells is effected by hemeprotein monooxygenases, the cytochrome P_{450} monooxygenases.[30,31] The active oxygenating agent is still in debate but may be a ferryl iron-oxygen species as a source of electrophilic oxygen.[32] Depending on biological niche, P_{450} monooxygenases fulfill different functions. Thus, pseudomonads can elaborate P_{450} enzymes which serve dissimilatory functions enabling growth on camphor via its oxygenation to the 5-exo alcohol[33] or growth on n-octane via its oxygenation to 1-octanol[34] as initial steps in catabolism. In animal cells, biosynthesis of adrenal steroid hormones such as corticosterone and hydrocortisone from cholesterol involve 4 sequential P_{450} monooxygenases, starting with the complex six-electron oxidative side chain cleavage of cholesterol, 8, to pregnenelone 9 and isocaprylaldehyde, 10, consuming three O_2 molecules, and then involving successive hydroxylations at C_{17}, C_{21}, and C_{11} at the β-face. The system actually requires three components: a flavoprotein reduced by NADPH, a small fe_2/S_2 protein, and adrenodoxin (that) which serve(s) as conduit for one electron at a time to the actual monooxygenase, the P_{450} enzyme.



8 9 10

In liver metabolism, and to a lesser extent in lung and intestine, P_{450} monooxygenases are used as the main apparatus for drug metabolism and detoxification of other xenobiotics by sequences which introduce hydroxyl functionality to increase polarity and so facilitate aqueous solubility and urinary excretion. The generalized stoichiometry, as in all monooxygenations discussed in this chapter, is use of an exogenous reductant, here again the readily available NADPH, and O_2 and cosubstrate.

$$R\text{-}H + NADPH + O_2 \xrightarrow[2.\ P_{450}]{1.\ E\text{-}FAD,FMN} R\text{-}OH + NADP + H_2O$$

Since the dihydronicotinamide reacts rapidly only by two-electron paths and the hemeprotein by one-electron paths, a two-electron/one-electron switch is needed and is provided by a flavin coenzyme, explaining the chemical logic for a coupled two-enzyme sequence.[6]

Exposure of a mammal to a wide variety of xenobiotics, classically such disparate compounds as phenobarbital and 3-methylcholanthrene, induces high levels (up to 10% of the protein of liver cell endoplasmic reticulum[35]) of a family of up to seven distinct P_{450} isozymes with overlapping specificities for hydroxylation of an enormous range of xenobiotic structures.[31] The primary structure of two rabbit liver isozymes designated LM2 and LM4 is in progress.[31] LM4 is most active on aromatic hydrocarbons, i.e., an "aryl hydrocarbon hydroxylase" while LM2 will carry out dealkylations of N-, O-, and S-methyl and ethyl compounds, alkane hydroxylations, N-hydroxylations, S-hydroxylations, including sulfenylations and sulfoxidations. While sp^3 C-H bonds are converted to alcohols, alkenes are converted to epoxides and aromatics are converted to arene oxides which may suffer several fates, among them isomerization to

C-oxygenation	N-oxygenation
alkenes → 1°,2°-alcohols	2°-amines → hydroxylamines
alkenes → epoxides	3°-amines → amine oxides

aromatics → arene oxides → phenols

$R\text{-}X\text{-}CH_3 \rightarrow R\text{-}X\text{-}CH_2OH \rightarrow RX + CH_2O$ (dealkylation)

S-oxygenation

thiols → sulfenates

thiolethers → sulfoxides → sulfones

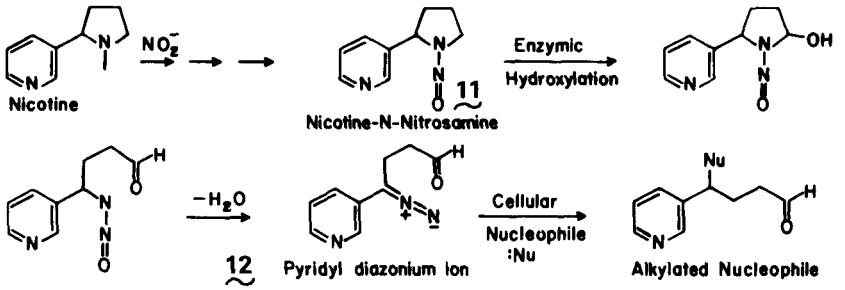
thioamides → amides

phenolic products. A distinct type of functional group processing involves azo group reduction[36] and nitro group[37] reduction where these functionalities, undergoing reduction rather than oxidation, must take the place of O_2 as electron acceptor in this enzyme's active site.

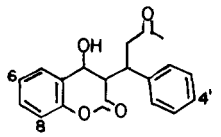
The detoxification strategy to make apolar molecules more readily excretable by introduction of polar oxygen functionalities is in general successful but not without its difficulties. In fact, the detoxification process occasionally leads to toxification by generation of activated electrophile intermediates[38-40] or initial products which are not captured with complete efficiency by such surveillance enzymes as epoxide hydrase[41] or the family of glutathionine S-transferases.[42] If one molecule in 100,000 escapes to react with cellular nucleophiles, proteins, and/or DNA, that may represent introduction of an eventual mutagenic and carcinogenic lesion. A strategy that cannot support even a very low percentage of failures is fraught with difficulty. Several examples illustrate some of the chemistry involved.

Nitrosamines, formed nonenzymically from amines and nitrite ion, can be activated to alkylating species by P_{450}-mediated hydroxylation at the

α-carbon to produce an initial α-hydroxynitrosamine product which can unravel to N_2 and a carbonium ion equivalent, an unwanted, adventitiously reactive alkylating agent towards cellular proteins and DNA. For example, nicotine present at 1-2% of commercial cigarette mass, undergoes nitrosation during curing and smoking of tobacco to yield nicotine-N-nitrosamine, 11.[43] This compound is hydroxylated at a carbon α- to the nitrosamine by P_{450} species to yield, ultimately the pyridyl diazonium ion, 12, by the indicated sequence.[43] Studies with D_6-dimethylnitrosamine show a deuterium isotope effect on enzymic decomposition, consistent with hydroxylation at carbon as an intermediate step.[44]

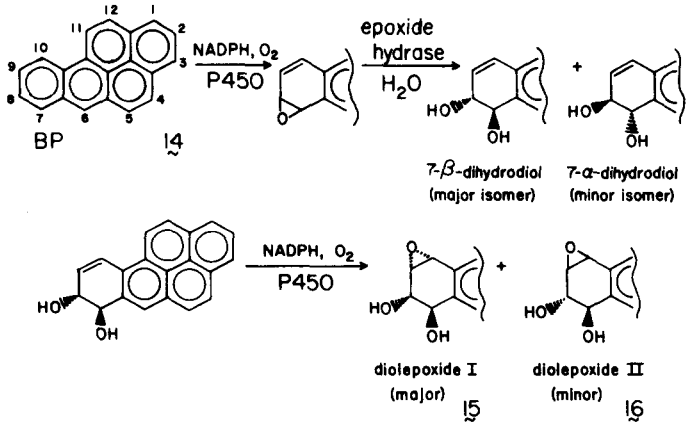


The coumarin anticoagulant warfarin, 13, is hydroxylated at a number of sites, and product analysis by HPLC assay has allowed detection of stereoselective and regioselective processing of R+S-warfarin by rabbit liver P_{450} isozymes. LM2 is stereoselective for S-warfarin, LM4 for the R-isomer. LM2 produces R-4'-OH and S-6-OH products, while LM4 produces R-6-OH and S-6-OH.[45] The anticoagulation activity of S-warfarin is 5-8-fold higher than the R-isomer and only the 4'-OH product among hydroxylated metabolites is active. Action of LM4 would metabolize the R-isomer preferentially on clinical administration of racemic warfarin; LM2 would remove the more active S-isomer.[45]



13

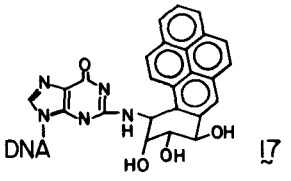
Benzo[a]pyrene, 14, a common polycyclic in the environment[8] (hundreds of tons/yr) is processed to varying extents by liver P_{450} isozymes with oxygen introduction at all carbon centers, except possibly C_{11}. At least four positional isomers of arene oxides 2,3; 4,5; 7,8; and 9,10 are implicated. These can open nonenzymically to phenols or be opened via catalytic action of epoxide hydrase to the trans dihydrodiols. Epoxide hydrase has presumably evolved as a scavenger enzyme with protective function to the liver, but here, epoxide hydrase opening of benzo[a]pyrene-7,8-epoxide puts the organism at greater risk because the BP-7,8-trans dihydrodiol is processed for a second P_{450} epoxidation cycle; the most reactive double bond is the 9,10-olefin. The major 7,8-dihydrodiol isomer is the 7-β and enzymic epoxidation is stereoselective to yield predominantly the 9,10-epoxide trans to the 7-OH, known as BP-diol epoxide I, 15. The minor diol epoxide II, 16, has the 7-β and 9,10-oxirane cis.[46] These



diolepoxides are more mutagenic than BP or intermediate metabolites by Ames test assay and may be ultimate carcinogens.[46] They are defused only poorly by either epoxide hydrase (or glutathionine S-transferases) to the harmless tetrols, so the diolepoxides last long enough to form covalent adducts with DNA. The toxic metabolite is a metabolic grandchild of the initial polycyclic BP and multiple oxygenative processing is a likely occurrence at low, substurating concentrations of xenobiotic substrates.[47] It is estimated that, at low chronic doses of BP, more than 80-90% of molecules will be oxygenated more than once. The 9,10-epoxide is in the "bay region" of the polycyclic and Jerina and colleagues have argued that such epoxides are most mutagenic, possibly more reactive in both S_N2 and S_N1 opening modes.[48]

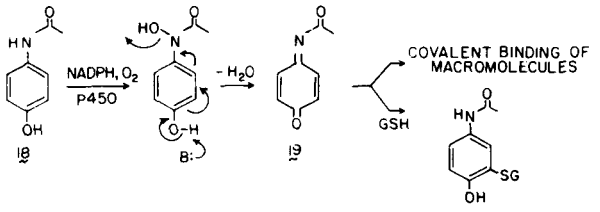
The lifetime of diolepoxide I in tissue culture medium is 8 min, while that for diolepoxide II is 0.5 min; in a 1:1 acetone-water mixture *in vitro* DNA modification from diolepoxides proceeded for up to six hours.[48] While the *in vivo* half time will depend on microenvironment, a lifetime of 5' is probably long enough for a metabolite to move from one organ to another.

The major adduct from diolepoxide I with DNA both in synthetic polyG and in DNA in human bronchial explants is attack of the N_2 amine of guanine residues on the β-face of the C_{10}-epoxide carbon to give the metabolite, 17, at *in vivo* levels of modification corresponding to 1 BP residue introduced per 10^4-10^5 nucleotides.[46,49] There is evidence for adducts to other bases and for reaction with internucleotide phosphodiester linkages[50] as well, leading to single strand breaks.

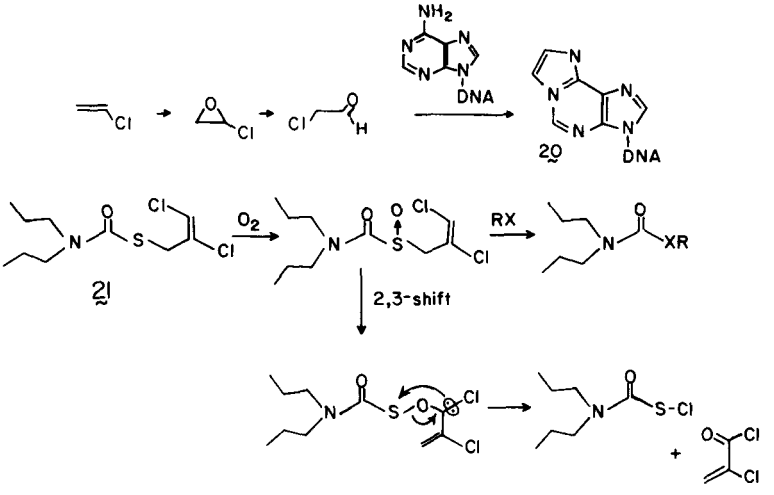


P_{450}-mediated hydroxylation at nitrogen atoms can also lead, adventitiously, to toxification, as exemplified in overdoses of acetaminophen, 18, where hepatic necroses or renal and pelvic tumors can develop.[51] Again, the problem is uncontrolled generation of a reactive electrophile, here an arylating iminoquinone, 19, from facile nonenzymic breakdown of the initial P_{450} N-hydroxylation product.[52] This electrophile may be

captured and detoxified by intracellular glutathionine, normally at 5-8 mM concentration, but a chronic or acute overdose may lead to GSH depletion and resultant covalent modification of cellular macromolecules. N-Hydroxylation of other arylamines, including the carcinogenic aminoacetylfluorene can have similar toxic consequences.[53]

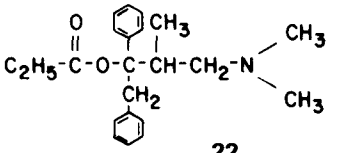


Additional examples of problems arising from reactivity of initial P_{450} monooxygenation products are vinylchloride and thiocarbamate herbicide processing. Vinylchloride oxygenation to chloroethylene oxide is probably followed by rapid nonenzymic ring opening and 1,2-chloride migration to yield chloroacetaldehyde, a known nucleoside base modifying agent.[54] Exposure of rats to 250 ppm of vinylchloride in drinking water led to isolation of etheno derivatives of deoxyadenosine, 20, and deoxycytosine presumably from imidazocyclization sequences from chloroacetaldehyde metabolites acting as intramolecular bifunctional alkylating agents.[54] The herbicide diallylate, 21, is S-oxygenated by liver microsomal enzymes to yield a product which is now activated in two modes, carbamylation of nucleophiles or a 2,3-sigmatropic rearrangement of allyl sulfoxide group to allylsulfenate.[55] The allylsulfenate in turn may rapidly rearrange to reactive sulfenyl chloride and chloroacrylylchloride, reactive metabolites.

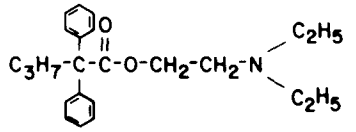


A recent study into molecular aspects of darvon (propoxyphene) toxicity in combination with other drugs has stressed the interactive processes between one xenobiotic and another.[56] Darvon, 22, has structural similarities to the classical P_{450} inhibitor SKF 525A, 23 (itself a slow hydroxylation substrate) and was found to have a K_i of 4.6×10^{-5}M compared to 4×10^{-6}M for SKF 525A in a P_{450} assay using aminopyrine as substrate. At a daily dose of 200-300 mg darvon, blood concentrations of 6×10^{-7}M accumulate, and liver concentrates the drug up to 20 30-fold, approaching the 10^{-5}M range intrahepatically, close to the K_I value.[56] Thus, darvon can titrate out P_{450} drug metabolism capacity, changing pharmacokinetic

disposition of other drugs, such as barbiturates, and generating profound pharmacological alterations.

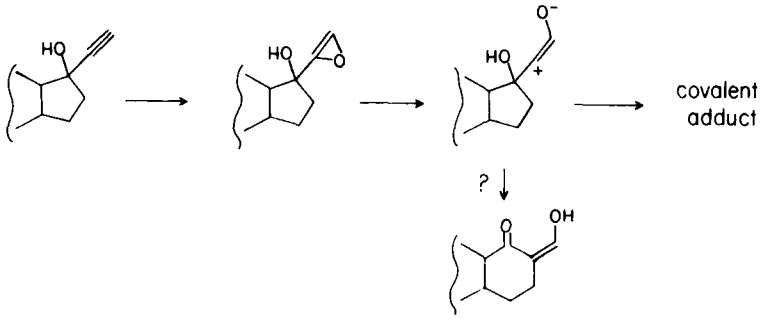


PROPOXYPHENE 22



SKF-525A 23

Given the production of reactive electrophilic species from enzymic oxygenation or from secondary processes as noted, one might expect liver cytochrome P_{450} monooxygenases to catalyze their own destruction on occasion.[57-59] Indeed, several recent reports have suggested that the isozyme induced by phenobarbital (e.g., LM2 in rabbits) is inactivated during suicidal processing of allylisopropylacetamide and related allylic substrates,[60] by ethynyl sterols such as norethisterone,[61] and by N-benzylcyclopylamine.[62] The first two types are thought to modify the heme group of the monooxygenase since profound alterations in the porphyrin visible spectra are observed. Proposed inactivation mechanisms are suggested below.[63]



Benzylcyclopropylamine may inactivate after C-hydroxylation to the hemiaminal, dehydration to the cyclopropylimine, and attack of an enzyme nucleophile to form a stable tetrahedral adduct.[62] The antidiuretic spironolactone inactivates adrenal and testicular P_{450}s possibly by S-oxygenation sequences and generation of electrophilic atomic sulfur,[63] a mechanism also suggested for microsomal protein labeling after enzymic S oxygenation of parathion,[64] CS_2,[65] and thiobenzamide.[66] Whether one can develop isozyme-selective suicide substrates of in vivo utility remains to be determined. This survey of recent P_{450} literature is eclectic and meant to illustrate some of the molecular hazards associated with enzymic reductive oxygen metabolism.

References

1. I. Fridovich, Ann.Rev.Biochem., 44, 147 (1975).
2. B. Babior, J. Curnutte and B. McMurrich, J.Clin. Invest., 58, 989 (1976).
3. S. Meshnik, S. Blobstein, R. Grady and A. Cerami, J.Exp.Med., 801, 569 (1978).
4. O. Hayaishi, Ed., "Molecular Mechanisms of Oxygen Activation," Academic Press: New York, NY, 1974.
5. W. Caughey, Ed., "Medical Aspects of Oxygen Metabolism," Academic Press: New York, NY, 1979.
6. C. Walsh, "Enzymatic Reaction Mechanisms," W.H. Freeman: San Francisco, CA, 1979.
7. C. Walsh, Ann.Rev.Biochem., 47, 881 (1978).
8. P. Ts'o and H. Gelboin, "Polycyclic Hydrocarbons and Cancer," Academic Press: New York, NY, 1978; Vols. 1 and 2.
9. G. Mulder, "Trends in Biochemical Sciences," Elsevier/North Holland: New York, NY, 1979; p. 86.
10. A. Jeffrey, H. Yeh, D. Jerina, T. Patel, J. Davey and D. Gibson, Biochemistry, 14, 575 (1975).
11. L. Que, J. Lipscomb, E. Munck and J. Wood, Biochem.Biophys.Acta, 485, 60 (1977).

12. B. Samuelsson and R. Paoletti, Eds., "Prostaglandins and Thromboxanes," Raven Press: New York, NY, 1975-1978; Vols. 1-3.
13. M. Abbott and S. Udenfriend in "Molecular Mechanisms of Oxygen Activation," O. Hayaishi, Ed., Academic Press: New York, NY, 1974; p. 168.
14. G. Hamilton, Ibid.; p. 405.
15. T. Andrews, G. Lorimer and N. Tolbert, Biochemistry, 12, 11, 18 (1973).
16. C. Kemal and T. Bruice, Proc.Natl.Acad.Sci.U.S.A., 73, 995 (1976).
17. B. Entsch, D. Ballou and V. Massey, J.Biol.Chem., 251, 2556 (1976).
18. S. Ghisla, B. Entsch, V. Massey and M. Husain, Eur.J.Biochem., 76, 149 (1977).
19. J.W. Hastings, C. Balny, C. LePeach and P. Douzou, Proc.Natl.Acad.Sci.U.S.A., 70, 3468 (1973).
20. S. Kaufman in "Iron and Copper Proteins," K. Yasunobo, H. Mower and O. Hayaishi, Eds., Plenum: New York, NY, 1976; p. 91.
21. G. Moad, C. Luthy, P. Benkovic and S. Benkovic, J.Am.Chem.Soc., 101, 6068 (1979).
22. M. Griffin and P. Trudgill, Biochem.J., 129, 595 (1972).
23. C. Walsh, C. Ryerson and F. Jacobson in "Advances in Chem. Series," D. Dolphin, Ed., American Chemical Society: Washington, D.C.; in press.
24. L. Poulsen, R. Hyslop and D. Ziegler, Biochem.Pharmacol., 23, 3431 (1974).
25. L. Poulsen and D. Ziegler, J.Biol.Chem., 254, 6449 (1979).
26. G. Guroff, D. Jerina, J. Rensen, S. Udenfriend and B. Witkop, Science, 157, 1524 (1967).
27. K. Kaufman and D. Fisher in "Molecular Mechanisms of Oxygen Activation," O. Hayaishi, Ed., Academic Press: New York, NY, 1974; p. 285.
28. W. Vaneste and A. Zuberbruhler, Ibid.; p. 371.
29. K. Taylor, J.Biol.Chem., 249, 494 (1974).
30. R. Sato and T. Omura, "Cytochrome P-450," Kodansha Press: Tokyo, Japan, 1978.
31. M. Coon and K. Vatsis, Ann.Rev.Biochem., 47, 336 (1978).
32. R. White, J. Groves and G. McCluskey, Acta Biol.Med.Germ, 38, 475 (1979).
33. I. Gunsalus, J. Mecks, J. Lipscomb, P. Debrunner and E. Munck in "Molecular Mechanisms of Oxygen Activation," O. Hayaishi, Ed., Academic Press: New York, NY, 1975; p. 561.
34. E. Loge and M. Coon in "Iron-Sulfur Proteins," W. Lovenberg, Ed., Academic Press: New York, NY, 1973; Vol. 1, p. 173.
35. L. Ernster, J. Capdevila, G. Dallerner, J. DePierre, S. Jakobsson and S. Orrenius in "The Structural Basis of Membrane Function," Y. Hatefi and D. Stigall, Eds., Academic Press: New York, NY, 1976; p. 389.
36. G. Labuc and J. Blunck, Biochem.Pharmacol., 28, 2367 (1979).
37. J. Gillette, J. Kamin and H. Sasame, Mol.Pharmacol., 4, 541 (1968).
38. J. Miller and E. Miller in "Origins of Human Cancer," H. Hiatt, J. Watson and J. Winsten, Eds., 1977; p. 605.
39. E. Miller, Cancer Res., 38, 1479 (1978).
40. J. Gillette, Biochem.Pharmacol., 23, 2785, 2794 (1974).
41. A. Lu, D. Ryan, D. Jerina, J. Daly and W. Levin, J.Biol.Chem., 250, 8283 (1975).
42. W. Habig, M. Pabst, and W. Jakoby, J.Biol.Chem., 249, 7130 (1974).
43. D. Hoffmann, I. Schmeltz, S. Hecht and R. Wynder in "Polycyclic Hydrocarbons and Cancer," P. Ts'o and H. Gelboin, Eds., Academic Press: New York, NY, 1978; p. 85.
44. W. Lijinsky, Nature, 218, 1174 (1968).
45. M. Dasco, K. Vatsis, L. Kaminsky and M. Coon, J.Biol.Chem., 253, 7813 (1978).
46. I. Weinstein, A. Jeffrey, S. Leffler, P. Pulkrabek, H. Yamasaki and D. Grunberger in "Polycyclic Hydrocarbons and Cancer," P. Ts'o and H. Gelboin, Eds., Academic Press: New York, NY, 1978; Vol. 2, p. 1.
47. B. Pullman, Ibid.; p. 419.
48. D. Jerina, H. Yagi, R. Lehr, D. Thakker, M. Schaefer-Ridder, J. Karle, W. Levin, A. Wood, R. Chang and A. Conney, Ibid.; p. 173.
49. S. Yang, D. McCourt, P. Roller and H. Gelboin, Proc.Natl.Acad.Sci.U.S.A., 73, 2594 (1976).
50. H. Gamper, A. Tung, K. Staub, J. Bartholomew and M. Calvin, Science, 197, 671 (1978).
51. G. Mulder, J. Hinson and J. Gillette, Biochem.Pharmacol., 26, 189 (1977).
52. J.W. Gorrod, "Biological Oxidation of Nitrogen," Elsevier/North Holland: New York, NY, 1978.
53. D. Miner and P. Kissinger, Biochem.Pharmacol., 28, 3285 (1979).
54. T. Green and D. Hathway, Chem.Biol.Interactions, 22, 211 (1978).
55. I. Schuphan, J. Rosen and J. Casida, Science, 205, 1013 (1979).
56. G. Peterson, R. Hostetter, T. Lehman and H. Covault, Biochem.Pharmacol., 28, 1783 (1979).
57. R. Rando, Science, 185, 320 (1974).
58. A. Maycock and R. Abeles, Acc.Chem.Res., 9, 313 (1976).
59. C. Walsh, "Horizons in Biochemistry and Biophysics," Addison-Wesley: Reading, MA, 1977; Vol. 3, p. 36.
60. P. Ortiz de Montellano, B. Mico and G. Yost, Biochem.Biophys.Res.Commun., 83, 132 (1978).
61. P. Ortiz de Montellano, K. Kunze, G. Yost and B. Mico, Proc.Natl.Acad.Sci.U.S.A., 76, 746 (1979).
62. R. Hanzlik, V. Kishore and R. Tullman, J.Med.Chem., 22, 759 (1979).
63. R. Menard, T. Guenther, H. Kon and J. Gillette, J.Biol.Chem., 254, 1726 (1979).

64. T. Kakatsugawa, N. Tolman and P. Dahm, Biochem.Pharmacol., 18, 1103 (1969).
65. C. Chengelis and R. Neal, Biochem.Biophys.Res.Commun., 90, 993 (1979).
66. I. Rau and P. Rockwell, Biochem.Biophys.Res.Commun., 90, 721 (1979).

Chapter 23. Recent Developments in Adrenergic Receptor Research

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Introduction - The biological effects of catecholamines, such as adrenalin and noradrenalin, are among the most diverse of any known pharmacological or hormonal agent. Virtually every system in the human organism is affected. More than thirty years ago, Raymond Ahlquist demonstrated that virtually all the physiological effects of catecholamines, generally termed adrenergic effects, could be classified as being of two major types. These two types of responses he termed "α"- and "β"-adrenergic, respectively, and hypothesized that they were mediated by two distinct types of adrenergic receptors, α- and β-adrenergic receptors.[1] The two receptor types were distinguished by their characteristic potency series for agonist drugs. For α-adrenergic responses, such as the ability to constrict smooth muscle, the order of potency of amines was epinephrine> norepinephrine> isoproterenol. For β-adrenergic effects, such as smooth muscle relaxation or the cardiac inotropic effect, a very different potency series was found with isoproterenol> epinephrine> norepinephrine. For each type of receptor there are characteristic competitive antagonists such as phentolamine for the α-adrenergic receptors and propranolol, for example, for the β-adrenergic receptors. A very wide variety of agonists and antagonists with relatively selective α- or β-adrenergic properties have been described.

Over the past six years, research in the area of adrenergic receptors has been in a very rapidly developing phase owing in large part to the successful development of direct ligand binding techniques for the study of these receptors. These techniques for studying the adrenergic receptors directly by ligand binding have in the space of a few years revolutionized research in this area. A variety of radioactively labelled ligands, both agonists and antagonists, have been developed and applied to successful study of α- and β-adrenergic binding sites in a wide variety of tissues from a wide variety of species. Research in this area has been the subject of a number of excellent and extensive reviews in the past few years.[2-5] A number of more general monographs and reviews have dealt with the general properties and characteristics of ligand binding to receptors.[6] Accordingly, here the more limited goal will be to review some of the most exciting and very recent developments in the area of ligand binding studies of adrenergic receptors. Most of the progress reviewed here has been reported in the literature only within the past year.

Adrenergic Receptor Subtypes - Subsequent to the classification of adrenergic receptors into α- and β- types by Ahlquist, it became clear that there were at least two major subtypes of each of these receptors. For the β-adrenergic receptors these are β_1 (cardiac and adipose tissue) characterized by a potency series, isoproterenol> epinephrine≥ norepinephrine and β_2 (smooth muscle) characterized by isoproterenol> epinephrine>> norepinephrine.[7] These two receptor subtypes both appear to be coupled to adenylate cyclase and are probably quite similar. There are antagonists such as metoprolol and practolol, which are somewhat selective for the β_1-subtype, and others, such as butoxamine, which are perhaps somewhat β_2-selective.

Subtypes of α-adrenergic receptors also exist,[8] and here the situation

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is somewhat more complex since there is a much greater physiological distinction between these two subtypes than is the case for the β-adrenergic receptor subtypes. The so-called α_1-adrenergic receptors, previously referred to as "postsynaptic," include all the typical postsynaptic α-adrenergic receptors such as those which mediate the vasoconstrictive effect of catecholamines, the effects in constricting other types of smooth muscle and some of the metabolic effects such as those on the liver. The so-called α_2-adrenergic receptors are found in a variety of locations, some apparently "presynaptic" and others "postsynaptic." The notion of presynaptic α-adrenergic receptors was developed some years ago to explain the ability of catecholamines to feedback inhibit the further release of norepinephrine from sympathetic nerve endings.[8] This feedback inhibition is mediated through typical α-adrenergic receptors which were initially called "presynaptic." These α-receptors can be pharmacologically distinguished from the typical postsynaptic α-receptors by their relatively greater affinity for certain agonists such as clonidine or antagonists such as yohimbine as opposed to the postsynaptic sites. Typical postsynaptic or α_1-adrenergic receptors have relatively much higher affinity for certain antagonists such as prazosin. Receptors with pharmacological characteristics very similar to those of the so-called "presynaptic" α-adrenergic receptors can also be found in postsynaptic locations such as, for example, on the human platelet. It has become popular within the past several years to refer to such receptors as α_2 as opposed to "presynaptic."

Radioligand binding studies are ideally suited to the task of delineating and quantitating adrenergic receptor subtypes. In principle, there are two different approaches which might be taken to the problem. The first is to use subtype-selective radioligands which will bind specifically and uniquely to only one of the two receptor subtypes. In the case of the α-adrenergic receptors, several of the available radioligands appear to be appropriate for this purpose. Thus, [^{3}H]prazosin appears to be quite α_1-selective (100-10,000 fold).[9] [^{3}H]WB4101, though α_1-selective in some systems, such as brain membranes (∿100 fold),[10] is much less selective in uterine membranes[11] and was found not to be adequate for distinguishing α_1- from α_2-receptors in that tissue. Several agonist ligands, such as [^{3}H]-epinephrine, [^{3}H]norepinephrine and [^{3}H]clonidine, appear to be somewhat more effective probes for labelling α_2-adrenergic receptors.[12] By contrast, the ligand [^{3}H]dihydroergocryptine appears to label α_1- and α_2-adrenergic receptors with similar affinity (∿5 nM).[13] For the β-adrenergic receptor, the two most commonly employed ligands, [^{3}H]-dihydroalprenolol (K_D ∿1-5 nM) and [^{125}I]hydroxybenzylpindolol (K_D ∿50 pM), are antagonists which appear to label β_1- and β_2-adrenergic receptors with equal affinity[14] (see Table for structures).

An alternate approach is to use a radioligand, which is itself nonsubtype-selective and then use selective unlabelled drugs to construct competition curves. The radioligand will label both subtypes, but the unlabelled competitor having equal affinity for the two subtypes will give a biphasic curve which, using one or another graphical or computational technique, can be dissected into the two component affinities. The latter approach has been used for both α- and β-adrenergic receptors. Several different analytical approaches for the evaluation of such data have been published, and some of the methods described are in actuality inappropriate. An initial report suggested that such biphasic competition curves could be transformed into a "pseudo-Scatchard" plot in which percent inhibition of binding was equated with the amount bound.[15] The selective drugs give biphasic plots which were then dissected into individual components. However, such graphical methods produce large errors which are further magnified by the data transformation involved. In addition, the graphical methods of analysis

are imprecise and have been shown to give misleading results.[16] A computer-assisted iterative method of analysis was presented in the literature by Minneman and Molinoff.[17] Although these procedures offer some advantage over those involving simple graphical analysis, they share a number of shortcomings of the graphical method since it is a very equivalent plot, the so-called Hofstee plot which is iteratively analyzed into individual components by the computer-assisted program described by Minneman and Molinoff.[17] Perhaps the most ideal way of analyzing such competition data is by nonlinear least squares curve fitting of the actual competition curves. DeLean et al[16] and Rodbard et al[18] have described such curve fitting procedures which are based solely on the law of mass action for the binding of multiple ligands to multiple classes of sites. The application of such methods yields rather precise estimates for the actual affinities and proportions of the different receptor subtypes in a particular set of membranes. These methods have been applied to the delineation of α-adrenergic receptor subtypes with [^{3}H]dihydroergocryptine[12] and β-adrenergic receptor subtypes with [^{3}H]dihydroalprenolol.[16]

Receptor Regulation - One of the most significant insights to come from recent direct ligand binding studies of adrenergic receptors is that the receptor binding sites rather than representing static entities in the plasma membrane are, in fact, subject to very dynamic regulation by a wide variety of hormonal, drug and other physiological and pathophysiological perturbations. In many cases, these receptor regulatory effects have been shown to importantly influence physiological sensitivity or responsiveness to catecholamine stimulation.[2] Several general principles seem to be emerging. It has been demonstrated that catecholamines themselves tend to decrease the number of α- and β-adrenergic receptors and thus diminish catecholamine responsiveness of a tissue.[19] In some cases, denervation or antagonists appear to increase receptor number and increase responsiveness.[20,21,22] Thus, as with several other hormone receptors, there appears to be an inverse relationship between the size of the functional receptor pool and the extent or level of ambient agonist stimulation. This may represent a homeostatic mechanism for protecting cells against excess or elevated levels of endogenous catecholamines.

In addition, a wide variety of other hormones have been shown to regulate the adrenergic receptors. This material has recently been reviewed in detail elsewhere[23] and will not be considered in detail here. Such diverse hormones as thyroid hormones, cortisone, estrogen and progesterone, among others have been shown to influence the concentration of adrenergic receptors in tissues.

Desensitization - One of the more interesting and heavily studied phenomena, which has been elucidated by studies of regulation of the adrenergic receptor binding sites, is referred to as desensitization. Other terms which are used include tachyphylaxis, tolerance and refractoriness. It is becoming increasingly clear that there are a number of distinct mechanisms by which such phenomena may occur. Perkins and his colleagues have suggested that these be classified as "homologous" and "heterologous" forms of desensitization. By homologous desensitization is meant a phenomenon whereby exposure of cells to a particular hormonal agent leads to subsequent refractoriness only to the stimulatory effects of that agent or closely related agents acting through the same receptor. By contrast, heterologous desensitization refers to a phenomenon whereby stimulation of the cells by a particular hormonal or pharmacological agent leads to subsequent refractoriness to stimulation by a wide variety of unrelated stimulators, even those working through distinct receptor sites. It is attractive to speculate that homologous forms of desensitization might be mediated through alterations in

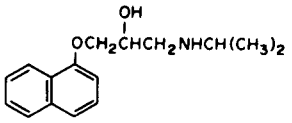
receptors, whereas heterologous forms of desensitization might be mediated by alterations in components of the system distal to the receptors. However, to date such conjectures remain speculative and have not been experimentally proved.

Data from a number of laboratories indicate that the homologous desensitization produced by β-adrenergic agonists appears to be accompanied by decreases in the number of β-adrenergic receptors in membranes as assessed by ligand binding with antagonists such as [^{3}H]dihydroalprenolol and [^{125}I]HYP.[19,25,26] However, it also seems clear that there are changes in the receptors which are not being reflected by the loss in antagonist binding capacity. For example, it has been shown that if binding studies are done with a radiolabelled agonist, there is a disproportionate and larger decrease in agonist binding than in antagonist binding activity.[27] It can also be shown that the ability of agonists to compete with radiolabelled antagonists for binding sites is diminished, that is, that their displacement curves are shifted to the right after desensitization.[28,29,34] Both of these findings suggest an "uncoupling" of the receptors. Although the molecular details have yet to be worked out, it is thought that these changes are indicative of some alteration on the inner or "coupling" face of the receptor which normally interacts with distal components of the adenylate cyclase system. Other studies, notably those of Perkins et al,[24] have indicated that this uncoupling change in some systems appears to temporally precede the actual loss of receptor binding capacity. The exact relationship of these two alterations in the receptors, as well as their respective contributions to the overall desensitization process, remains to be worked out.

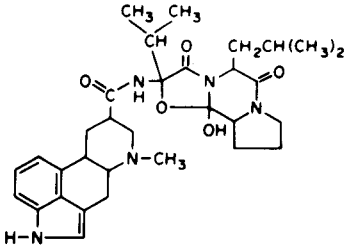
Guanine Nucleotide Regulation of Adrenergic Receptors - Rodbell and colleagues first indicated about ten years ago that guanine nucleotides such as GTP or their nonhydrolyzable analogs, for example Gpp(NH), are absolutely required for hormone stimulation of adenylate cyclase.[31] He also found that GTP diminished the affinity of glucagon receptor binding sites in liver.[32] Subsequently, in 1976, Maguire et al[33] and Lefkowitz et al[34] demonstrated that GTP also diminished agonist affinity for binding to β-adrenergic receptors, while having no effect on the binding of antagonists to the same receptors. Over the past year, it has also been demonstrated that α_2-adrenergic receptors, such as those found in the human platelet,[35,36] show similar agonist specific effects of GTP. This is particularly interesting because the α_2-, but not the α_1-adrenergic receptors, appear to be coupled to adenylate cyclase. Recently, by using nonlinear least squares curve fitting techniques, such as those described above, it has become possible to show that β-adrenergic agonists, but not antagonists, distinguish two affinity states of the receptors with respectively high and low affinity in binding experiments.[30] The high affinity sites appear to represent a stable complex of the receptor and a guanine nucleotide regulatory protein.[37] This complex has been shown to be an intermediate for stimulation of the enzyme adenylate cyclase.[38] Action of guanine nucleotides on this high affinity intermediate complex destabilize it, and now all receptors appear to display low affinity for agonists.[37] Guanine nucleotides are without effect on antagonist binding. Thus, these data suggest that the crucial property of β-adrenergic agonists, as opposed to antagonists, which may lead to activity, is the ability of the agonist to stabilize or promote the formation of a high affinity intermediate complex of the receptor and guanine nucleotide regulatory sites. In such a formulation, the nucleotide regulatory site, which is perturbed both by the agonist receptor complex on the one hand and by guanine nucleotides on the other, functions as a true coupling protein or transducer which shuttles between the receptor and the catalytic moiety of adenylate cyclase. It is tempting to speculate that

STRUCTURES OF SOME ADRENERGIC AGENTS

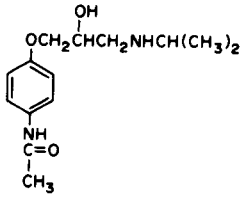
Propranolol – $\beta_1 + \beta_2$ antagonist



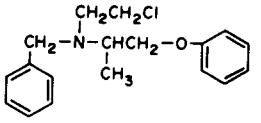
Dihydroergocryptine – $\alpha_1 + \alpha_2$ antagonist



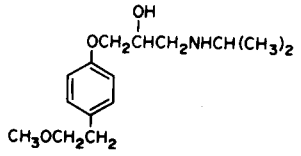
Practolol – β_1 selective antagonist



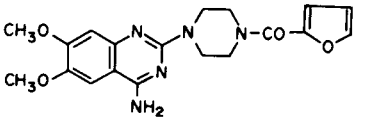
Phenoxybenzamine – irreversible α antagonist



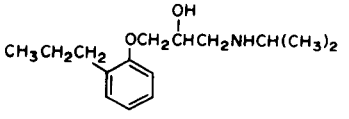
Metoprolol – β_1 selective antagonist



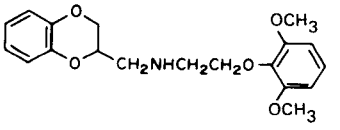
Prazosin – α_1 selective antagonist



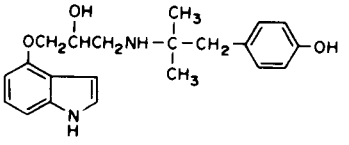
Dihydroalprenolol – $\beta_1 + \beta_2$ antagonist



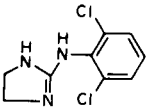
WB4101 – α_1 selective antagonist



Hydroxybenzylpindolol – $\beta_1 + \beta_2$ antagonist



Clonidine – α_2 selective agonist



closely related mechanisms may be involved in α_2-receptor-mediated inhibition of adenylate cyclase. How such similar mechanisms, however, lead to activation of the enzyme on the one hand (β-effect), or inhibition of enzyme activity on the other hand (α_2-effect), remains to be determined.

Solubilization and Purification of Adrenergic Receptors - An obvious long-range goal of adrenergic receptor research is the ultimate purification of the receptors and the determination of their detailed molecular structure. This goal remains far off due to a variety of technical complexities which confound attempts to purify the receptors. These include the vanishingly small concentration of the receptors in membranes, as well as the difficulties of solubilizing the receptors from their natural membrane-bound environment. Nonetheless, significant progress is being made. β-Adrenergic receptors can be solubilized from a variety of sources. So far, the most successful approach appears to be the use of the plant glycoside digitonin, which has mild detergent properties and which seems to be uniquely useful in solubilizing β-adrenergic receptors. Most work, thus far, has been reported using the frog[39] and turkey erythrocyte membrane model systems.[40] The receptors appear to be integral membrane proteins. Although the receptors have not yet been purified to homogeneity, they have been purified up to several thousand times using affinity chromatography with the β-adrenergic antagonist alprenolol covalently coupled to Sepharose.[40,41] It seems a reasonable expectation that within the next year or two the β-adrenergic receptors will be, in fact, purified to homogeneity using such techniques.

Even less is known about the detailed molecular properties of α-adrenergic receptors. The α_1-adrenergic receptors of the rat liver have been solubilized by two groups. Wood *et al*[42] reported solubilization with digitonin, as well as the further observation that the α- and β-adrenergic receptors in such solubilized liver membrane preparations could be separated by affinity chromatography on alprenolol Sepharose gels. This observation is of interest, since it suggests that the α- and β-adrenergic receptor binding sites cannot reside simultaneously on the same macromolecule. Guellaen *et al* reported solubilization of the [^{3}H]phenoxybenzamine prelabelled hepatic α-receptors with Lubrol PX.[43] This latter group also reported hydrodynamic parameters of this soluble [^{3}H]phenoxybenzamine detergent receptor complex which had a Stokes radius of 5.7 nm and a molecular weight of 128,000. When corrected for detergent binding, it was estimated that the α-adrenoreceptor of rat liver plasma membranes had a molecular weight of 96,000.[43]

Adrenergic receptor research appears to be in a log-phase of growth at the present time. The next few years should yield exciting information about the molecular nature of the receptors, the way in which they function to stimulate biological processes and the way in which their function modulates a variety of physiological and pathophysiological interventions.

References

1. R.P. Ahlquist, Am. J. Physiol., 153, 586 (1948).
2. L.T. Williams and R.J. Lefkowitz in "Receptor Binding Studies in Adrenergic Pharmacology," Raven Press, New York, 1978.
3. M.E. Maguire, E.M. Ross and A.G. Gilman, Adv. Cyclic Nucleotide Res., 8, 1 (1977).
4. B.B. Wolfe, T.K. Harden and P.B. Molinoff, Annu. Rev. Pharmacol., 17, 575 (1977).
5. R.J. Lefkowitz, Fed. Proc., 37, 123 (1978).
6. R.D. O'Brien, Ed., The Receptors: A Comprehensive Treatise, Plenum Press, New York, 1979.
7. A.M. Lands, A. Arnold, J.P. McAuliff, S.P. Luduena, T.G. Brown, Jr., Nature, 214, 597 (1967).
8. K. Starke, Rev. Physiol. Biochem. Pharmacol., 77, 1 (1977).
9. P. Greengrass and R. Bremmer, Eur. J. Pharmacol., 55, 323 (1979).
10. D.A. Greenberg, D. U'Prichard and S.H. Snyder, Life Sci., 191, 69 (1976).

11. B.B. Hoffman and R.J. Lefkowitz, Biochem. Pharmacol., in press (1980).
12. D. U'Prichard and S. Snyder, Life Sci., 24, 79 (1979).
13. B.B. Hoffman, A. DeLean, C.L. Wood, D. Schocken and R.J. Lefkowitz, Life Sci., 24, 1739 (1979).
14. R.J. Lefkowitz and B.B. Hoffman, Proceedings of the First International Conference on the Clinical Aspects of Cyclic Nucleotides, in press, Raven Press, New York, 1980.
15. B.B. Barnett, E.L. Rugg, S.R. Nahorski, Nature, 273, 166 (1978).
16. A.A. Hancock, A. DeLean and R.J. Lefkowitz, Mol. Pharmacol., 16, 1 (1979).
17. K.P. Minneman, L.R. Hegstrand and P.B. Molinoff, Mol. Pharmacol., 16, 34 (1979).
18. P.J. Munson and D. Rodbard, Endocrinology, 105, 1377 (1979).
19. R.J. Lefkowitz, C. Mukherjee, M.G. Caron, L.E. Limbird, R.W. Alexander, L.T. Williams, J.V. Mickey and R. Tate, Recent Progress in Hormone Research, 32, 597 (1976).
20. Y.F. Su, L. Cubeddu and J.P. Perkins, J. Cyclic Nucleotide Res., 2, 257 (1976).
21. G. Glaubiger, B.S. Tsai, R.J. Lefkowitz, B. Weiss and E.M. Johnson, Jr., Nature, 273, 240 (1978).
22. G. Glaubiger and R.J. Lefkowitz, Biochem. Biophys. Res. Commun., 78, 720 (1977).
23. R.J. Lefkowitz, Ann. Intern. Med., 91, 450 (1979).
24. Y.F. Su, T.K. Harden and J.P. Perkins, J. Biol. Chem., 254, 38 (1979).
25. M. Shear, P.A. Insel, K.L. Melmon and P. Coffino, J. Biol. Chem., 251, 7572 (1976).
26. G.L. Johnson, B.B. Wolfe, T.K. Harden, P.B. Molinoff and J.P. Perkins, J. Biol. Chem., 253, 1472 (1978).
27. M.R. Wessels, D. Mullikin and R.J. Lefkowitz, J. Biol. Chem., 253, 3371 (1978).
28. M.R. Wessels, D. Mullikin and R.J. Lefkowitz, Mol. Pharmacol., 16, 10 (1979).
29. T.K. Harden, Y.F. Su and J.P. Perkins, J. Cyclic Nucleotide Res., 5, 99 (1979).
30. R. Kent, A. DeLean and R.J. Lefkowitz, Mol. Pharmacol., 17, 14 (1980).
31. M. Rodbell, L. Birnbaumer, S.L. Pohl and H.M.J. Krans, J. Biol. Chem., 246, 1877 (1971).
32. M.C. Lin, S. Nicosia, P.M. Lad and M. Rodbell, J. Biol. Chem., 252, 2790 (1977).
33. M.E. Maguire, P.M. Van Arsdale and A.G. Gilman, Mol. Pharmacol., 12, 335 (1976).
34. R.J. Lefkowitz, D. Mullikin and M.G. Caron, J. Biol. Chem., 251, 4686 (1976).
35. B.S. Tsai and R.J. Lefkowitz, Mol. Pharmacol., 16, 61 (1979).
36. M.L. Steer, J. Khorana and B. Galgoci, Mol. Pharmacol., 16, 719 (1979).
37. L.E. Limbird, M. Gill and R.J. Lefkowitz, Proc. Natl. Acad. Sci. USA, 77, 775 (1980).
38. J. Stadel, A. DeLean and R.J. Lefkowitz, J. Biol. Chem., 255, 1436 (1980).
39. M.G. Caron and R.J. Lefkowitz, J. Biol. Chem., 251, 2374 (1976).
40. G. Vauquelin, T. Geynet, J. Hanoune and A.D. Strosberg, Proc. Natl. Acad. Sci. USA, 74, 3710 (1977).
41. M.G. Caron, Y. Srinivasan, J. Pitha, K. Kochiolek and R.J. Lefkowitz, J. Biol. Chem., 254, 2923 (1979).
42. C. Wood, M.G. Caron and R.J. Lefkowitz, Biochem. Biophys, Res. Commun., 88, 1 (1979).
43. G. Guellaen, M. Aggerbeck and J. Hanoune, J. Biol. Chem., 254, 10761 (1979).

Chapter 24. Chemotaxis

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Introduction - Leukocytes from blood accumulate more or less selectively in tissue sites of inflammation, whether the inflammation is caused by an infectious agent or an allergic or other inciting stimulus. The accumulation is the result of a whole series of events: increased blood flow, margination of cells, attachment of leukocytes to the endothelium of a blood vessel and locomotion of the leukocyte through the vessel and to the tissue site. The locomotion of leukocytes through blood vessels and into the inflammatory site is directed by a gradient of chemical substance either released from the inflammatory agent or formed endogenously. The directed locomotion is termed chemotaxis (a more precise definition is given below) and the agents responsible are called chemotactic factors.

Chemotaxis is not only of importance in the inflammatory accumulation of leukocytes but is thought to be significant in the metastasis of neoplastic cells and the migration of fibroblasts involved in wound healing.[1,2] Thus, drugs capable of either selectively enhancing or depressing chemotactic responsiveness of leukocytes and other cells are potentially of importance in a wide variety of diseases.

Chemotactic factors not only induce chemotaxis and other neutrophil functions (see below) but also cause enhancement of the bactericidal activity of neutrophils against various microorganisms.[3] Moreover, neutrophils removed from humans with microbial infections act very similarly to neutrophils exposed to chemotactic factors in vitro, suggesting that the mechanism of activation of neutrophils from many patients with acute bacterial infections may be, in part, an intravascular response to chemotactic factors produced as a result of the infection.[4]

In what follows, most attention will be directed to the chemotactic factor-stimulated locomotion of leukocytes, particularly neutrophils (polymorphonuclear leukocytes, PMNs). We will consider first assays of chemotaxis and of cell locomotion, then the various chemotactic factors, the mechanisms of leukocyte locomotion and lastly, the effect of drugs on cellular chemotactic responsiveness.

Recent reviews are available;[5-10] one, a report of a conference held in January 1977, covers many topics of basic, practical and clinical interest in leukocyte locomotion.[6]

Definitions and Techniques of Assay - Chemotaxis is defined as "a reaction by which the direction of locomotion of cells or organisms is determined by substances in their environment" and chemokinesis as "a reaction by which the speed or frequency of locomotion of cells...is determined by substances in their environment".[5] Most, if not all, chemotactic factors are both chemokinetic and chemotactic but some substances are only chemokinetic.

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The Boyden chamber technique of measuring the penetration of cells into or through the pores of a micropore filter in response to chemotactic factor placed below the filter continues to be the most popular, single method of assessment of cell locomotion both for clinical and research purposes. By itself, the method is incapable of distinguishing between chemotaxis and chemokinesis without a supplementary technique such as the Zigmond-Hirsch "checkerboard technique" or direct, microscopic visualization.[6] Most studies using the Boyden chamber or the "under agarose techniques" (see below) do not distinguish between chemotaxis and chemokinesis. Where this is not done we shall use the non-commital term "stimulation of locomotion".

Previous work has emphasized that Boyden chamber methods utilizing an incubation time long enough for cells to reach the bottom of the micropore filter are subject to error due to cells on the bottom falling off. To obviate this source of error, a second filter was recommended. Comparison of the double and single filter techniques showed no advantage of the double over the simpler single filter method.[11] Because of the labor involved in doing large numbers of chemotactic assays, automatic and semi-automatic methods of reading the number and distance of cells penetrating the micropore filter continue to be introduced.[12-14] Increasing the concentration of cells placed in the upper compartment increases the rate of human neutrophil migration through micropore filters,[15] confirming previous work. It was suggested that the increase in rate was due to the secretion of cationic protein by the migrating neutrophils. $^{51}Cr^{3+}$ has been used to label neutrophils for use in in vitro chemotaxis assays by the Boyden procedure and to follow the fate of the same cells in vivo.[6] However, the stimulated and unstimulated migration and other functions of Indium-111 labeled polymorphonuclear leukocytes are unimpaired, leading to the suggestion that Indium-III has significant advantages over $^{51}Cr^{3+}$ as a label for both in vitro and in vivo studies of neutrophil migration.[16]

The "under agarose technique" popularized by Nelson has been modified to avoid the use of serum or serum albumin in the medium and to utilize slides rather than Petri dishes.[17] Zigmond has estimated the gradients of various chemotactic factors present in the Boyden chamber, under agarose and visual systems, and has suggested modifications in the usual assay procedures which may be useful in testing chemotactic factors of widely differing diffusion coefficients.[18] Suddenly increasing the concentration of the synthetic chemotactic peptide causes neutrophils to transiently stop locomoting as demonstrated by employing a special chamber, direct microscopic visualization and time-lapse cinematography.[19] This was cited as evidence of "sensory adaptation" of neutrophils to chemotactic peptides.

Chemotactic Factors - Formylmethionyl Peptides - The discovery by Schiffmann that formyl methionine and related dipeptides were chemotactic,[6] led to the synthesis of a whole series of related oligopeptides (di- and tri-peptides), the so-called N-formylmethionyl peptides.[20] (Although other than N-formylmethionyl peptides are active, the group, as a whole will be so termed, for convenience.) A remarkable variation of chemotactic activity with structure was found. The N-terminal formyl group enhances activity 3 to 4 orders of magnitude; it cannot be substituted for by N-acetyl or by removing the NH_2-group from the methionine i.e. substituting the desamino methionine. Substituting norleucyl for methionine in the 1st position causes a 10 fold drop in activity. Increasing the length of side chain of the amino acid in the 1st position causes a regular increase in activity. Similarly, a leucyl residue in the 2nd position gives the maximum activity, whereas, in the 3rd position substituting a phenylalanine for

leucine increases activity 500 fold.[20] As these few examples show, the activity depends not only on the nature of the amino acid but its relative position in the peptide chain. Moreover, competitive antagonists specific for the formyl methionyl peptides have been discovered.[20]

The most active, until recently, and still the most widely used synthetic, chemotactic peptide is f-Met-Leu-Phe, with an ED50 for chemotaxis in the Boyden chamber assay of rabbit neutrophils of 7×10^{-11}M.[20] The classical synthesis of f-Met-Leu-Phe has been described.[23] The hexapeptide, f-Nle-Leu-Phe-Leu-Tyr-Lys is more active for human neutrophils and has the added advantage that it can be radiolabeled with ^{125}I in the tyrosyl moiety or made fluorescent by conjugating a fluorochrome through the ϵ-NH_2 group of the lysyl residue.[21,22] NMR analysis of f-Met-Leu-Phe has revealed an unusually high degree of conformational rigidity for so small a peptide; the peptide in solution is a monomeric, antiparallel, β-pleated sheet.[24] Pepstatin A, N-isovaleryl-L valyl-AHMA-L-alanyl-AHMA (AHMA=4-amino-3 hydroxy-6 methyl-heptanoic acid), a protease inhibitor, is chemotactic for human neutrophils, monocytes and eosinophils.[25] It acts at the formyl methionyl peptide receptor (see below).[26]

Arachidonic Acid Metabolites - The monohydroxy derivatives of arachidonic acid (HETEs) stimulate the locomotion of human neutrophils in the rank order of 5-HETE >> 8 HETE = 9 HETE >11 HETE = 12 HETE;[27] the labile intermediate, 12 L-hydroperoxy 5, 8, 10, 14 eicosatetraenoic acid (12-HPETE) is more potent than the corresponding HETE.[28] The stimulatory effect of 12 HETE and 12-hydroxy 5, 8, 10 heptadecatrienoic acid (HHT) is eliminated by methylation.[29] Both methyl esters competitively inhibit the stimulating effect of each of the free acids on neutrophil locomotion but are without effect on formylmethionyl peptides or chemotactic fragments of C5a, the fifth component of complement. Intraperitoneal injection of HETE into guinea pigs results in a migration of eosinophils into the site within 30 minutes and neutrophils by five hours.[28]

Complement-Derived Fragments - C5a, the anaphylatoxin derived from the fifth component of human complement, and C5a des arg, (with the carboxyl terminal Arg 74 removed) are both potent neutrophil chemotactic factors when assessed by the under agarose technique,[17] but C5a des Arg requires the addition of a small amount of serum to be active in the Boyden chamber assay.[17,30] The explanation suggested for this apparent anomaly is that an anionic polypeptide in normal serum serves as helper for C5a des Arg.[31] Sera of some systemic lupus erythematosus patients suffering from recurrent infections and depressed chemotactic reactivity of their neutrophils contain an anionic peptide of approximately 69,000 daltons which inhibits C5a-des Arg.[31] Following elucidation of the primary amino acid sequence of C5a, the C-terminal pentapeptide Met-Gln-Leu-Gly-Arg, was shown neither to be active by itself nor to inhibit the chemotactic activity of C5a.[32] Formylation of the amino terminus of the pentapeptide or the tetrapeptide lacking the C-terminal arginine, resulted in a low level of activity which was attributed to interaction of these derivatives with the neutrophil formylmethionyl peptide receptor rather than the C5a receptor. Procine C5a has been purified to homogeneity and the sequence of 10 of the first 12 amino acids from the NH_2-terminus is the same as human C5a.[33] Human and porcine C5a have very similar chemotactic activity. Trypsin cleavage of human C5a or C5a des arg yields a 6000 dalton fragment that stimulates the locomotion of Walker tumor cells, although neither of the undigested peptides were active against tumor cells.[1] Activation of either the classical or alternative pathways of complement conversion generates from

human serum an 80,000 dalton factor probably derived from C5 which stimulates the locomotion of human fibroblasts but not neutrophils or monocytes. [2]

Miscellaneous - Resorbing bone releases a factor that stimulates the *in vitro* locomotion of Walker carcinosarcoma cells. [34] It was suggested that the factor may play a role in the metastasis of malignant tumors to bone. Mixed lymphocyte cultures produce an activity that stimulates the locomotion of polymorphonuclear leukocytes and macrophages. [35] A dialyzable, heat stable peptide chemotactic for lymphocytes, probably B-lymphocytes, is released from rabbit IgG by action of a neutral thiol protease obtained from rabbit neutrophils. [36]

Mechanism - Receptor Interaction - The activity of the formylmethionyl peptides is due to their acting at a specific receptor on the neutrophil surface. [37] Chemotactic peptides not only induce chemotaxis and chemokinesis but also cause neutrophils to secrete granule enzymes, aggregate and induce all the manifestations of the respiratory burst. The peptides induce all of these neutrophil functions by interacting at a single population of receptors. [37] Both C5a and the "urate crystal induced chemotactic factor" from neutrophils act through specific receptors apparently differing from the formylmethionyl receptor. [38,39] C5a can induce the same neutrophil functions as the synthetic oligopeptides.

Receptor Modulation - The peptides show varying degrees of reversibility of binding as a function of the temperature and time of incubation that they have interacted with the neutrophil. [21,40] Total or almost total reversibility of the ligand-receptor combination is seen at 4°C, whereas temperatures above 23°C lead to a progressive loss of dissociability. Internalization of the ligand following binding has been demonstrated directly and indirectly. [22,40] One interpretation is that binding leads to an altered state of the receptor followed by internalization of the receptor-ligand complex. [21,40] The initial findings for the C5a-receptor interaction are similar to those for the synthetic oligopeptide receptor. [39]

Transduction of Surface Signal - Chemotactic peptides induce hyperpolarization of the neutrophil membrane. [41,42] Whether the change of membrane potential is the cause or the result of the subsequent events is unknown. The interaction of chemotactic peptides with the neutrophil results in the activation of the Na^+, K^+ ATPase of the neutrophil with a consequent influx of K^+ and efflux of Na^+, an influx of Ca^{2+} and of Na^+ and an efflux of Ca^{2+}. [43] Chemotactic factors induce two changes in cellular Ca^{2+} translocation: 1. An increase in neutrophil membrane permeability to Ca^{2+} and 2. A concentration-dependent release of Ca^{2+} from membranous intracellular stores into the cytoplasm. [43] Evidence for the former sequence is: 1. An increased uptake of $^{45}Ca^{2+}$. 2. An increase in steady state levels of $^{45}Ca^{2+}$ after stimulation with 10^{-10}M or greater of f-Met-LeuPhe in the presence of external Ca^{2+}. [44] 3. An increase in the specific activity of $^{45}Ca^{2+}$ of exchangeable Ca^{2+}. [44] Evidence for release of intracellular Ca^{2+} following stimulation with chemotactic factors is: 1. An increased efflux of $^{45}Ca^{2+}$. 2. A transient decrease in steady state levels of $^{45}Ca^{2+}$ either in the absence of external Ca^{2+} or in the presence of extremely low levels of f-Met-Leu-Phe ($\leq 10^{-11}$M). [45] It is noteworthy that the latter concentration is the lowest where one can detect a chemotactic response using a Boyden chamber system. 3. A corresponding transient decrease in the specific fluorescence of chlorotetracycline treated cells. [46]

Transmethylation inhibitors depress chemotactic factor stimulation of

locomotion of neutrophils, monocytes and macrophages, suggesting an important role for methylation processes. [47-50] The methylation process (or processes) involved is controversial. Rapid methylation and demethylation of the carboxyl groups of proteins (carboxy-O-methylation) of rabbit neutrophils has been reported by one group but failed to be detected in macrophages and in human neutrophils by another. [49,50] One group has it that chemotactic stimulation of rabbit neutrophils results in a fall in methylated phospholipids attributable to the release of arachidonic acid. The other has found that chemotactic factor stimulation of responsive leukocytes causes neither increased phospholipid turnover nor degradation but does inhibit phospholipid methylation. [48,50] Obviously, the resolution of these contradictory observations requires further work.

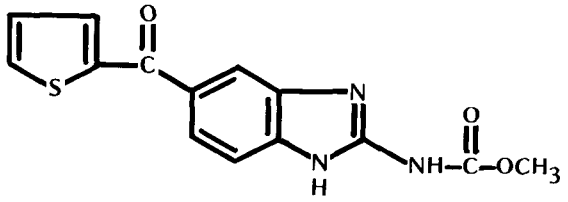
As pointed out above, HETES, products of the lipoxygenase pathway, stimulate neutrophil locomotion. Arachidonic acid, as well as chemotactic factors, stimulate cytochalasin B treated rabbit neutrophils to degranulate. [51] The stimulation by arachidonic acid and by chemotactic agents is blocked by 5, 8, 11, 14 eicosatetraynoic acid (ETYA) suggesting arachidonic metabolism is central to the stimulatory effects of chemotactic factors. Concentrations of indomethacin above those considered specific for the cyclooxygenase pathway also inhibit, whereas aspirin does not. The latter finding is in accord with the demonstration that arachidonic acid metabolism in rabbit and human neutrophils occurs very largely through the lipoxygenase pathway. [52-55] In a preliminary communication, Hirata et al. have reported that neutrophil phosphatidylcholine may be the source of the arachidonic acid in neutrophils following chemotactic factor stimulation but analysis of the total phospholipids and the effects of chemotactic stimulation upon the latter were not studied. [47] Labeled arachidonic acid incorporation into neutrophils is increased upon stimulation with f-Met-Leu-Phe. [56] Neutrophils can incorporate exogenously supplied HETES into triglyceride and phospholipid; the incorporation greatly increases in cells treated with the divalent cation ionophore A23187. [55] These observations raise important questions concerning the fate and role of endogenous arachidonic acid products when cells are stimulated. One partial answer to the latter is suggested by the finding that nordihydroguaiaretic acid, a lipoxygenase inhibitor, prevents the increase in the Ca^{2+} membrane permeability of neutrophils induced by chemotactic factors but has no effect on the chemotactic factor induced loss of intracellular Ca^{2+}. [57] These latter findings suggest not only that the loss of intracellular Ca^{2+} induced by chemotactic factor stimulation precedes the increase in Ca^{2+} membrane permeability but that one role for one or another lipoxygenase metabolite of arachidonic acid is to increase Ca^{2+} membrane permeability.

Cyclic Nucleotide Modulation of Chemotaxis - Agents that increase cAMP levels of leukocytes, e.g. theophylline, epinephrine, cholera toxin PGE_1, PGE_2 and PGA, histamine, etc., depress chemotactic responsiveness. [6] Agents that raise cellular cyclic GMP, acetyl-choline, ascorbic acid, serotonin, etc., enhance leukocyte responses to chemotactic factors. Chemotactic factors cause small increases in cyclic GMP levels of neutrophils. [6] They also induce a very rapid, transient rise in cAMP that peaks at one minute and returns to baseline by five minutes. [58,59] Removal of extracellular Ca^{++} from the medium abolishes the rise in cAMP, suggesting a role for Ca^{++}. [58] The prevailing view is that the cyclic nucleotides are not involved as necessary steps in the sequence of biochemical events leading from receptor interaction to leukocyte chemotactic response; rather, they serve as regulatory factors. However, our present concepts as to the role of cyclic nucleotides has so tenuous an experimental base that there is a need for further work, particularly studies of the role of cyclic nucleotide dependent and non-dependent protein phosphorylation in

chemotactic factor-induced leukocyte locomotion.

Microfilaments (actin) and Microtubules (tubulin) - Actin and myosin have been isolated from neutrophils, macrophages,[6] and most recently from B lymphocytes.[60] Recent fluorescent antibody studies have shown that actin concentrates in the leading edge of neutrophils oriented or locomoting toward a gradient of f-Met-Leu-Phe.[61] Scanning and transmission electron microscopy have further revealed the complexity of the microfilament associated processes occurring during neutrophil locomotion.[62] Gelsolin, a heat-labile protein of macrophages with a subunit molecular weight of 90,000, inhibits the gelation of actin and actin-binding protein in a Ca^{++}-dependent fashion.[63] This finding offers a possible link between the changes in Ca^{++}-cell translocation induced by chemotactic factors described above and those in microfilaments involved in locomotion.

Chemotactic factors induce assembly of neutrophil microtubules, microtubules orient themselves within neutrophils exposed to reversed gradients of chemotactic factors, and many studies have shown that anti-tubulin drugs such as colchicine, vinblastine, etc. depress *in vitro* leukocyte-stimulated locomotion.[6] These findings have led to the conclusion that microtubules regulate chemotaxis. This simple view has been contested by the finding that cells on glass orienting to chemotactic factors are unaffected by high levels of colchicine,[7] although oncodazole (R17934; methyl (5(2-thienyl-carbonyl) 1-H benzimidazol-2yl) carbamate), a new microtubule inhibitor, is reported to inhibit neutrophil orientation on glass.[64] Furthermore, the inhibitory activity of antitubulins on leukocyte locomotion has been attributed to the inhibition of the secretion of chemotactic factors from neutrophils and the resultant decrease in the stimulation of other leukocytes to locomote.[65] Despite these apparent contradictory results and conclusions, we still believe that the views of Allan and Wilkinson are largely, if not wholly, correct.[6] They conclude that leukocyte microtubules are not required for spatial orientation nor for locomotion toward a source. They are important, however, for accurate turning and/or maintenance of the shape and polarity of the moving cell. Because of the latter, cells with a natural or imposed deficit in microtubules may show decreased locomotion if the stimulus is weak or the leukocytes have to force their way through the pores of a filter *in vitro* or connective tissue interstices *in vivo*.[6]



oncodazole (R 17,934)

The Chediak-Higashi syndrome is a rare autosomal recessive disease characterized in humans by partial oculocutaneous albinism, frequent pyogenic infections, neutropenia, and characteristic giant lysosomes.[6] A similar syndrome is present in beige mice and other animals. Leukocytes from patients and mice show decreased locomotion in response to chemotactic factors. The defect in leukocyte locomotion has been attributed to a defect in microtubule assembly.[66] Boxer *et al.* have confirmed that there is defective assembly of the microtubules of Chediak-Higashi granulocytes in two additional affected children.[67] Moreover, as in their previously reported case, the defects in microtubule assembly, stimulated locomotion

and other leukocyte functions were corrected following the patients' ingestion of 20 mg/kg of ascorbic acid for two weeks. Ascorbic acid treatment also decreased the susceptibility of these patients to infection. The results in the three children are in contrast to the lack of effect of ascorbic acid on either leukocyte function or clinical course in two adults with the Chediak-Higashi syndrome.[68] The reason for the difference between the adults and children is not known. However, the latter authors found that administering ascorbic acid to beige mice did improve the function of their neutrophils, including the response to chemotactic factors and their ability to survive an experimental infection.

Deactivation - There is a correspondence between loss of biological responsiveness (deactivation) and of binding of f-Met-Leu-Phe that results when cells are first exposed to increasing concentrations of f-Met-Leu-Phe.[40] This sugggests that at least part of the decreased responsiveness of the cell may be related to the loss of free receptors on the cell surface. However, Gallin and coworkers have shown that limited exocytosis in neutrophils, induced by adding low levels of chemotactic factors and other secretogogues, increases the number of receptors on the cell surface for f-Met-Leu-Phe.[69] Spilberg *et al.* suggested that microtubule polymerization might be relevant to such a phenomenon, as colchicine appeared to reverse the deactivation;[70] we have been unable to demonstrate any effect of colchicine in this regard. Nelson *et al.* have attributed the deactivation, in part, to the formation of destructive oxidative metabolites.[71]

Drugs - Drugs, such as microtubule inhibitors, agents that increase cyclic AMP (see above), steroidal and non-steroidal anti-inflammatory agents and local and general anesthetics, can depress chemotactic factor-stimulated and unstimulated locomotion of leukocytes *in vitro* and in some instances have been shown to do so *in vivo*.[6] Other drugs, levamisole, agents that increase cGMP levels, e.g. carbachol, acetylcholine, ascorbic acid (see above), can enhance leukocyte locomotion.[6] The aminoglycoside antibiotics, gentamycin and amikacin administered in therapeutic doses to normal adults, cause a transient decrease in the locomotion of their neutrophils stimulated *in vitro* with chemotactic factors.[72] In few, if any, instances is the mechanism of the drug effects known. The difficulties of assessing the mechanism or mechanisms of drug actions on motility is illustrated by recent work on the effects of the β-adrenergic blockers propranolol and atenolol on leukocyte motility.[73] Propranolol consistently increased the non-stimulated, chemokinetic and chemotactic locomotory responses and inhibited the oxidative metabolic responses of human neutrophils, whereas atenolol was without effect. It was suggested that the activity of propranolol was due either to its ability to increase leukocyte cGMP or decrease superoxide (O_2^-) production.

No drugs are available that will selectively and specifically enhance or depress leukocyte responses to various chemotactic stimuli. Experimentally and therapeutically there are circumstances where it is desirable to interrupt the interaction between chemotactic factors and responsive cells. Experimental examples are numerous, *e.g.*, glomerulonephritis, experimental vasculitides such as the Arthus reaction,[5] and the acute pneumonitis produced by the instillation of chemotactic factors into the lungs or experimental animals.[74,75] Decomplementation has recently been demonstrated to decrease the tissue damage in the hearts of animals undergoing experimental myocardial infarction.[76,77] Ward and Hill earlier observed that C3 chemotactic fragments are present in such infarcts and apparently responsible for the influx of PMNs.[5] These findings suggest that it would be profitable to study whether a portion of the tissue damage of myocardial infarction can be prevented by preventing the chemotactic influx of inflam-

matory cells. Humans with rheumatoid arthritis and other arthritides with a large inflammatory component could also be expected to be helped by agents capable of depressing leukocyte chemotactic responsiveness. [5] Agents capable of enhancing the chemotactic responsiveness of leukocytes would also be valuable, as suggested by the large number of chemotactic defects in the leukocytes of patients with a lack of resistance to pyogenic infections. The ability of ascorbic acid to aid the clinical course of children with Chediak-Higashi syndrome is an illustration of this thesis.

References

1. W. Orr, S.H. Phan, J. Varani, P.A. Ward, R.O. Webster and P.M. Henson, Proc. Nat. Acad. Sci., 76, 1986 (1979).
2. A.E. Postlethwaite, R. Snyderman and A.H. Kang, J. Clin. Invest., 64, 1379 (1979).
3. A.C. Issekutz, K.Y. Lee and W.D. Biggar, J. Infect. Immun. 24, 295 (1979).
4. C.E. McCall, D.A. Bass, L.R. DeChatelet,A.S. Link, Jr. and M. Mann, J. Infect. Diseases, 140, 277 (1979).
5. E.L. Becker and P.A. Ward, in "Clinical Immunology", C.W. Parker, Ed., B.W. Saunders, Philadelphia, 1980 p. 272.
6. J.I. Gallin and P.G. Quie, Eds., "Leukocyte Chemotaxis, Methods, Physiology and Clinical Implications", Raven Press, NY (1978).
7. S.H. Zigmond, J. Cell Biol., 77, 269 (1978).
8. F.H. Valone, Clin. Immunol. and Immunopath., 15, 52 (1980).
9. E. Schiffmann and J.I. Gallin in "Current Topics in Cellular Regulation 15", B.L. Horecker, Ed., Academic Press, New York, NY, p. 203 (1979).
10. D.M.V. Parrott, Monogr. Allergy, 16, 173 (1980).
11. J.J. Cream and D.S. Pole, J. Immunol. Meth., 25, 193 (1979).
12. S.R. Turner, J. Immunol. Meth., 28, 355 (1979).
13. T.E. Van Dyke, A.A. Reilly, H. Horoszewicz, N. Gagliardi and R.J. Genco, J. Immunol. Meth., 31, 271 (1979).
14. V.A. Moss, H.K.L. Simpson and J.A. Roberts, J. Immunol. Meth., 27, 293-300 (1979).
15. P. Venge, J. Immunol., 122, 1180 (1979).
16. B. Zakhireh, M.L. Thakur, H.L. Malech, M.S. Cohen, A. Gottschalk and R.K. Root, J. Nuc. Med., 20, 741 (1979).
17. D.E. Chenoweth, J.C. Rowe and J.E. Hugli, J. of Immunol. Meth., 25, 337 (1979).
18. S.H. Zigmond in "Functional Aspects of Mononuclear Phagocytes", R. Van Furth, Ed., Martinus Nijhoff, The Hague. (1979).
19. S.H. Zigmond and S.J. Sullivan, J. Cell Biol., 82, 517 (1979).
20. R.J. Freer, A.R. Day, E.L. Becker, H.J. Showell, E. Schiffmann and E. Gross, in "Proceed. of the 6th Amer. Peptide Sympos." E. Gross and J. Meinenhofer, Eds. 749 (1979).
21. J. Niedel, S. Wilkinson and P. Cuatrecasas, J. Biol. Chem., 254, 10700 (1979).
22. J.E. Niedel, A. Kahane and P. Cuatrecasas, Science, 205, 1412 (1979).
23. A.R. Day and R.J. Freer, Int. J. Peptide Protein Res., 13, 334 (1979).
24. E.L. Becker, H.E. Bleich, A.R. Day, R.J. Freer, J.A. Glasel and J. Visintainer, Biochemistry 18, 4655 (1979).
25. S.K. Ackerman and S.D. Douglas, Clin. Immunol. and Immunol., 14, 244 (1979).
26. R.D. Nelson, S.K. Ackerman, V.D. Fiegel, M.P. Bauman and S.D. Douglas, Infec. and Immun., 26, 996 (1979).
27. E.J. Goetzl and F.F. Sun, J. Exp. Med., 150, 406 (1979).
28. E.J. Goetzl, F.H. Valone, V.N. Reinhold and R.R. Gorman, J. Clin. Inv., 63, 1181 (1979).
29. E.J. Goetzl, H.R. Hill and R.R. Gorman, Prostaglandins, 19, 71 (1980).
30. H.N. Fernandez, P.M. Henson, A. Otani and T.E. Hugli, J. Immunol., 120, 109 (1978).
31. H.D. Perez, I.M. Goldstein, D. Chernoff, R.O. Webster and P.M. Henson, Mol. Immunol., 17, 163 (1980).
32. D.E. Chenoweth, B.W. Erickson and T.E. Hugli, Biochem. Biophys. Res. Commun., 86, 227 (1979).
33. C. Gerard and T.E. Hugli, J. Biol. Chem., 254, 6346 (1979).
34. W. Orr, J. Varani, M.D. Bondek, P.A. Ward and G.R. Mundy, Science, 203, 176 (1979).
35. H.T. Cheung and G. Sundharadas, J. Immun., 123, 2189 (1979).
36. Y. Higuchi, M. Ishida and H. Hayashi, Cell. Immun., 46, 297 (1979).
37. E.L. Becker, J. Reticuloendothel. Soc. Sup., 26, 701 (1979).
38. I. Spilberg and J. Mehta, J. Clin. Invest., 63, 85 (1979).
39. D.E. Chenoweth and T.E. Hugli, Mol. Immunol., 17, 151 (1980).
40. G. Vitkauskas, H.J. Showell and E.L. Becker, Mol. Immunol., 17, 171 (1980).
41. H.M. Korchak and G. Weissmann, Proc. Natl. Acad. Sci. USA 75, 3818 (1978).
42. B. Seligmann and J.I. Gallin, Mol. Immunol., 17, 191 (1980).
43. E.L. Becker, P.H. Naccache, H.J. Showell and R.I. Sha'afi,"Proceed. 6th Amer. Peptide Sympos.", E. Gross and J. Meinenhofer, Eds, 743 (1979).
44. R.J. Petroski, P.H. Naccache, E.L. Becker and R.I. Sha'afi, Am. J. Physiol./Cell Physiol., 237, C43 (1979).

45. R.J. Petroski, P.H. Naccache, E.L. Becker and R.I. Sha'afi, FEBS Letters, 100, 161 (1979).
46. P.H. Naccache, H.J. Showell, E.L. Becker and R.I. Sha'afi, J. Cell Biol., 83, 179 (1979).
47. F. Hirata, B.A. Corcoran, K. Venkatasubramanian, E. Schiffmann and J. Axelrod, Proc. Nat. Acad. Sci. USA 76, 2640 (1979).
48. M.C. Pike, N.M. Krekick and R. Snyderman, Proc. Nat. Acad. Sci. USA, 76, 2922 (1979).
49. K. Venkatasubramanian, F. Hirata, C. Gagnon, B.A. Corcoran, R.F. O'Dea, J. Axelrod and E. Schiffmann, Mol. Immunol., 17, 201 (1980).
50. R. Snyderman and M.C. Pike, Mol. Immunol., 17, 209 (1980).
51. P.H. Naccache, H.J. Showell, E.L. Becker and R.I. Sha'afi, Biochem. Biophys. Res. Commun., 87, 292 (1979).
52. P. Borgeat and B. Samuelsson, Proc. Nat. Acad. Sci. USA, 76, 2148 (1979).
53. P. Borgeat and B. Samuelsson, J. Biol. Chem., 254, 2643 (1979).
54. P. Borgeat and B. Samuelsson, Proc. Nat. Acad. Sci. USA 76, 3213 (1979).
55. W.F. Stenson and C.W. Parker, J. Clin. Investig., 64, 1457 (1979).
56. R.P. Rubin, L.E. Sink, M.P. Schrey, A.R. Day, C.S. Leo and R.J. Freer, Biochem. Biophys. Res. Commun., 90, 1364 (1979).
57. P.H. Naccache, H.J. Showell, E.L. Becker and R.I. Sha'afi, Biochem. Biophy. Res. Comm., 89, 1224 (1979).
58. S. Jakowski and R.I. Sha'afi, Mol. Pharmac., 16, 473 (1979).
59. H.U. Keller, G. Gerisch and J.H. Wissler, Cell Biol. Intern. Reports. 3, 759 (1979).
60. M. Fechheimer and J.J. Cebra, J. Immunol, 122, 2590 (1979).
61. J.M. Oliver, J.A. Krawiec and E.L. Becker, J. Reticuloendoth. Soc., 24, 697 (1978).
62. J. Boyles and D.F. Bainton, J. Cell. Biol., 82, 347 (1979).
63. H.L. Yin and T.P. Stossel, Nature, 281, 583 (1979).
64. N.H. Valerius, Acta Path. Microbiol. Scand., Sect. C. 87, 83 (1979).
65. U.B. Soderstrom, G. Summingskold, B. Norberg, O. Back and L. Rydgren, Exp. Cell Res., 121, 325 (1979).
66. J.M. Oliver. Am. J. Path. 93, 220 (1978).
67. L.A. Boxer, D.F. Albertini, R.L. Baehner and J.M. Oliver, British J. of Haematol. 43, 207 (1979).
68. J.I. Gallin, R.J. Elin, R.T. Hubert, A.S. Fauci, M.A. Kaliner and S.M. Wolff, Blood, 53, 226 (1979).
69. J.I. Gallin, D.G. Wright and E. Schiffmann. J. Clin. Investig. 62, 1364 (1978).
70. I. Spilberg, B. Mandel and S. Hoffstein. J. Lab. Clin. Med. 94, 361 (1979).
71. R.D. Nelson, R.T. McCormack, V.D. Fiegel, M. Herron, R.L. Simmons and P.G. Quie, Inf. and Immun. 23, 282 (1979).
72. A.J. Khan, H.E. Evans, L. Glass, P. Khan, C.T. Chang and S.R. Nair, J. Lab. Clin. Med., 93, 295 (1979).
73. R. Anderson and A.J. van Rensburg, Immunol., 37 15 (1979).
74. U. Desai, D.L. Kreutzer, H. Showell, C.V. Arroyave and P.A. Ward, Amer. J. of Path., 96, 71 (1979).
75. P.M. Henson, K. McCarthy, G.L. Larsen, R.O. Webster, P.C. Giclas, B. Dreisin, T.E. King and J.O. Shaw, Amer. J. Path., 97, 93 (1979).
76. D. Maclean, M.C. Fishbein, E. Braunwald and P.R. Marko. J. Clin. Investig., 61, 541 (1978).
77. P.R. Moroki, C.B. Carpenter, M. Chiarello, M.C. Fishbein, P. Radvany, J.D. Knostman and S.L. Hale, ibid. 661 (1978).

Chapter 25. Antibodies as Drug Carriers and Toxicity Reversal Agents

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Introduction

The literature is replete with descriptions of the use of antibodies in a variety of pharmacologic and therapeutic procedures. Passively administered antibodies have been employed both prophylactically and therapeutically in infectious diseases, to prevent erythroblastosis fetalis, to prolong allograft survival and in the treatment of neoplastic diseases. Antibodies have also been used in numerous diagnostic and analytic operations. This review will focus on the application of: (i) tumor specific antibodies in transporting specific therapeutic agents to precise target sites and (ii) anti-drug antibodies in toxicity reversal. These two areas have been chosen because they represent some newer aspects of the application of antibodies that may be of particular interest to medicinal scientists.

Immunochemotherapeutic Complexes

Antibodies have been used to carry drugs, toxins, enzymes, radioactivity, and boron to specific tissue sites, particularly in the treatment of cancer where the employment of such therapeutic regimens is governed by severe constraints that arise from their generally cytotoxic nature. In efforts to maximize the therapeutic ratios of drugs, particularly those that have previously demonstrated promising anti-tumor activities as single agents, drug-antibody complexes have received the major share of attention. Less well studied as novel approaches to cancer chemotherapy have been immunochemotherapeutic complexes that utilize a) toxins and enzymes, which offer an opportunity of designing potential therapeutic regimens around highly specific biochemical mechanisms of action, b) radioiodinated antibodies, which deliver tumor destroying radioactivity to precise tumor sites, and c) boron derivatives of antibodies, which sensitize tumors to the subsequent effects of neutron radiation.

The preparation and utilization of complexes prepared from tumor specific antibodies plus a therapeutic agent can constitute more effective treatment than either of the component parts of the complex in terms of both efficacy and toleration. The mechanisms by which such complexes demonstrate synergistic effects have not been fully elucidated but may be related to a) "homing" effects where the antibody molecule causes the therapeutic agent to localize at a target tissue,[1] b) augmentation of the anti-tumor activity of one component of the complex by the other,[2-5] c) antibody facilitated cell penetration for those agents that act intracellularly,[6] d) prolongation of drug activity ("depot" effect) due to slow release from or slow degradation of the complex,[7] e) increased numbers of cells passing through a drug sensitive part of the cell cycle as a result of interaction with antibodies.[8]

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Chlorambucil - The most extensively studied drug-antibody complex is that derived from chlorambucil (1), a cytotoxic, alkylating agent that reacts with numerous biologically important nucleophiles. Ghose and Nigam[1] reported that a non-covalently bound chlorambucil-goat anti-Ehrlich ascites tumor antibody complex was a more effective inhibitor of tumor growth, both in vitro and in mice, than either drug or antibody alone. Chlorambucil bound to normal goat γ-globulin was no more effective than drug alone. In a complementary study, a complex of 1 and rabbit anti-EL4 antibodies exhibited greater activity in EL4-challenged mice than did 1 alone, antibody alone or 1 complexed to normal rabbit globulins.[9] Similar results using 1 and rabbit anti-Ehrlich ascites tumor antibodies were also reported.[10] A study in the Novikoff ascites tumor system with 1 and rabbit antibodies demonstrated that this non-covalent complex was far superior to drug or antibody alone or to a complex derived from drug and normal γ-globulin in inhibiting tumor formation and lowering mortality in tumor-challenged rats.[11]

The exact chemical nature of the non-covalently linked chlorambucil-antibody complexes has not been elucidated. However, 1 is protected from rapid hydrolysis and resultant loss of alkylating activity in the presence of serum from various species, presumably a consequence of non-specific binding to serum proteins.[12] A reduced rate of degradation can not, however, account entirely for the favorable effects observed using chlorambucil-antibody complexes since complexes prepared from 1 and normal immunoglobulins fail to demonstrate enhanced activities.

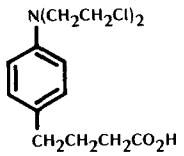
Davies and O'Neill,[13] following a protocol similar to that described above, also found that a chlorambucil-rabbit anti-EL4 antibody complex afforded protection against EL4-challenged mice that was greater than that provided by either drug or antibody alone. However, these authors could not attribute the heightened effects to a homing mechanism because similarly beneficial effects were obtained when the drug and antibody were administered separately.[2,3] Data substantiating this thesis were obtained from studying a complex derived from 1 and immune rabbit serum containing antibodies to polyoma transformed cells.[14] While the chlorambucil-antibody complex was more effective in inhibiting the cloning efficiency of these cells than was either serum or drug alone, the separate addition of drug and immune serum to the tissue culture afforded growth inhibition equal to that observed for the complex. Further support for the concept that the cytotoxic activity of 1 and antibodies used in tandem was equivalent at least to that of a drug-antibody complex was found in a system using cultured human melanoma cells.[15]

The apparent discrepancies between these sets of results, one requiring the use of pre-formed complexes for the expression of optimal anti-tumor activity and the other requiring the administration only of the constituents of the complexes, have not been resolved. Several groups of investigators have studied the nature of chlorambucil-protein interactions, but none appears to have examined complexes prepared under the strongly acidic conditions described by the Ghose group.[16] The rate of hydrolysis of 1 is retarded in the presence of bovine γ-globulin.[17] Similar findings were reported using human γ-globulin where it was shown that most protein-bound 1 results from alkylation.[18] In contrast, substantial non-covalent binding of 1 to rabbit immunoglobulin G was reported by Blakeslee and Kennedy.[19] These authors later showed that 1 forms a high molecular weight aggregate under the experimental conditions

of complex formation,[20] leading to the possibility that incorrect estimates may have been made concerning the degree to which 1 is non-covalently bound to antibodies.

The principal clinical application of chlorambucil-antibody complexes has been in the treatment of malignant melanoma.[9,12-24] Ghose et al.[24] found that 7 of 13 patients had objective responses or stabilized disease following treatment with this immunochemotherapeutic regimen, whereas none of 11 patients treated with DTIC, 5-(3,3-dimethyl-1-triazenyl)-imidazole-4-carboxamide, showed objective tumor regression. Significantly prolonged survival times were also observed for the drug-antibody complex treated group compared to the DTIC group.

In related experiments, the use of a complex prepared from 1 and anti-lymphocyte globulin led to marked delays in rejection times of skin and heart allografts in rats.[25]



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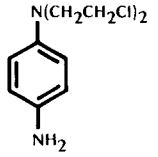

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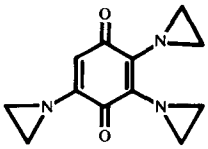

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p-Phenylenediamine Mustard - In contrast to 1, p-phenylenediamine mustard (2) has been linked covalently, through the interposition of spacer molecules, to rabbit anti-EL4 lymphoma antibodies.[26,27] This approach was utilized in order to synthesize complexes that contained relatively high drug to antibody ratios while avoiding the problems of reduced antibody specificity and diminished solubility which often accompany efforts to prepare heavily substituted antibodies.

Administration of a p-phenylenediamine mustard-polyglutamic acid-antibody conjugate to mice challenged with approximately 10,000 times the LD_{50} of EL4 tumor cells led to a median survival time of greater than 100 days. In contrast, mice given antibody alone or the drug-polyglutamic acid intermediate complex alone exhibited median survival times only slightly greater than those observed following saline treatment (13 days). The use of the intermediate complex plus specific antibody afforded results inferior to those obtained with the covalently linked tripartite complex (38 vs >100 day median survival times), suggesting that the latter may be functioning through a true homing mechanism.[26] A similar study, in which the survival times of mice challenged with EL4 cells were compared following treatment either with 2 covalently linked to antibody through dextran or with the drug-dextran complex plus antibody, showed that the fully covalently linked complex displayed significantly superior effects.[27]

Triaziquone - This cytotoxic agent (3) contains aziridine groups that are capable of alkylating tissue nucleophiles. The quinone moiety can also interact with cysteine and other thiols to afford thioether derivatives of aziridine-substituted hydroquinones which retain alkylating activity.[28] When 3 was linked covalently to rabbit antibodies against a methylcholanthrene-induced guinea pig sarcoma, the resulting complex displayed greater activity against monolayers of sarcoma cells than did drug alone, antibody alone or the same complex that had first been

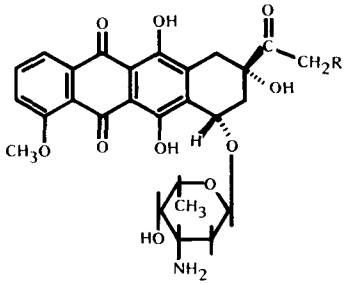
absorbed with sarcoma cells.[29] Conjugates prepared from 3 and non-immune γ-globulin, although cytotoxic, were much less active than those prepared using immunospecific globulins.[30]

Skin allograft survival times in mice were prolonged significantly following treatment with mixtures of 3 and rabbit anti-mouse thymocyte globulin. A covalently linked complex of drug and anti-thymocyte globulin was not as effective as the drug-antibody mixture.[31]

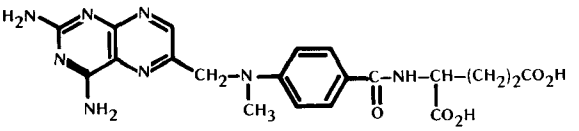
Daunomycin and Adriamycin - Daunomycin (4, daunorubicin) and adriamycin (5, doxorubicin) are chemically related natural products that are clinically effective anti-tumor agents.[32,33] Following incorporation into drug-antibody conjugates, both drugs showed retention of pharmacologic activity.[34]

Covalent attachment of 4 to rabbit anti-mouse B-cell leukemia antibodies was carried out in three different ways in order to study the effects on biologic activity of various types of drug-antibody combinations.[34] In the first example, periodate oxidation of 4 generated a carbonyl function-containing intermediate that was allowed to react with the amino substituents of the antibody, and the imine groups thus formed were reduced by sodium borohydride. A second method utilized glutaraldehyde to link the amino groups of the respective components of the complex. Finally, 1-dimethylaminopropyl-3-ethylcarbodiimide was used to forge an amide linkage between the drug and antibody. The complex which retained both drug and antibody activities to an optimal degree was that prepared using the periodate-sodium borohydride method.

Studies with complexes prepared from 4 and antibodies directed against either mouse B-cell leukemia or PC5 plasmacytoma showed that each conjugate displayed preferential cytotoxicity against homologous tumor. Daunomycin linked to irrelevant antibodies exhibited little or no activity against either tumor type.[35] The concentration of 4 within a drug-antibody complex was increased by using dextran as an intermolecular bridge.[36] A polyaldehyde derivative of dextran was allowed to react first with 4, then with anti-YAC lymphoma antibodies, and the resulting imine linkages were reduced with sodium borohydride. This complex was superior to 4 alone in increasing survival rates of YAC-challenged mice.



4, R = H
5, R = OH



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Methotrexate - This drug (6), which acts by inhibiting dihydrofolate reductase, was coupled, via diazotization, to hamster antibodies raised against mouse L1210 cells.[37] The drug-antibody complex, when compared

to 6 alone, specific antibodies alone, 6 plus specific antibodies or 6 coupled to normal immunoglobulins, demonstrated significant advantages in increasing survival rates in L1210-challenged mice. An analogous study, in which 6 was linked to rabbit anti-human reticulum cell sarcoma antibodies under similar chemical conditions, showed that the drug-antibody complex retained immunologic specificity in vitro.[38] Closer inspection of the chemistry involved in the diazotization of 6 revealed the occurrence of several competing reaction sequences that yield, depending upon reaction conditions, up to 14 products,[39] leading to difficulties in assessing the significance of earlier findings.

When 6 was conjugated to rabbit anti-L1210 antibodies through the formation of an amide link, the resulting complex displayed substantially greater activity than mixtures of 6 and antibodies in increasing the survival times of L1210-challenged mice.[39]

Cytosine Arabinoside - A non-covalently linked mixture of specific antibodies and cytosine arabinoside, an anti-viral and anti-neoplastic agent, was found to be more effective in inhibiting the growth of mouse L cells than was either component of the mixture.[40] The presence of antibody appeared to selectively stimulate uptake of drug into the nuclear DNA of the tumor cells.

Toxins and Enzymes - The extraordinarily toxic properties of diphtheria toxin[41] were exploited by showing that covalently linked conjugates of toxin and guinea pig anti-mumps virus antibodies exhibited much greater activity in lysing mumps infected rhesus monkey kidney cells than did antibodies alone.[42] The selectivity of the conjugate for mumps infected cells, in contrast to uninfected cells, was demonstrated by the low level of activity expressed by the conjugate against the latter. Similarly, a covalently linked complex consisting of diphtheria toxin and rabbit anti-trinitrophenyl (TNP) antibodies displayed significantly greater cytotoxicity against TNP-substituted Hela cells than it did against unmodified Hela cells.[43] Additional evidence for the selective cytotoxicity of the toxin-antibody complex was obtained by showing that the presence of a hapten, ε-dinitrophenyl (DNP)-lysine, inhibited the activity of the conjugate. Diphtheria toxin alone was non-selective, being equally toxic to both TNP-Hela cells and Hela cells.

Several reports have supported the concept that homing mechanisms are more important than synergy by showing that covalently linked complexes were more effective than simple toxin-antibody mixtures in delaying the appearance of tumors, in prolonging the lifespan of animals, in regression of established tumors, and in demonstrating in vitro cytotoxic activities.[44-46]

The report that enzymes could be coupled to antibodies with resultant retention of both immunologic and enzymatic activities was instrumental in the development of enzyme-antibody conjugates designed to demonstrate anti-tumor cytotoxic activities.[47] A covalently linked conjugate composed of glucose oxidase and anti-TNP antibodies was found to destroy TNP-substituted Hela and Hep-2 tumor cells in the presence of lactoperoxidase and potassium iodide.[48,49] The biochemical rationale of this cytotoxic reaction is based on the generation of hydrogen peroxide as a result of the oxidation of cellular glucose by the tissue targeted glucose oxidase. Lactoperoxidase, in the presence of iodide ion and the newly formed hydrogen peroxide, catalyzes the iodination of cellular constituents, thereby causing cell death. The presence

of each component of the toxic triad, the enzyme-antibody conjugate, lactoperoxidase and potassium iodide, was necessary for the manifestation of cytotoxic activity. Similar results were obtained when a combination of a glucose oxidase-anti-TNP antibody conjugate, horseradish peroxidase and arsphenamine, in which cytotoxicity is probably effected by an oxidized derivative of arsphenamine, was used to kill TNP-substituted Hela and Hep-2 cells.[50] A study in which glucose oxidase was coupled to antibodies elicited against a human colonic cancer line and against carcinoembryonic antigen, a specific marker for entodermally derived digestive tract tumors, showed that such enzyme-antibody conjugates, in the presence of lactoperoxidase and iodide ion, were significantly more cytotoxic to colonic tumor cells than were similar complexes prepared from normal immunoglobulins.[51]

A unique approach to the design of enzyme-antibody conjugates exhibiting anti-tumor activity involved the covalent linkage of alcohol dehydrogenase and anti-TNP antibodies.[52] Following incubation with this conjugate, nicotinamide adenine dinucleotide and allyl alcohol, which together can generate acrolein, the toxic oxidation product of allyl alcohol, a greater degree of killing was observed with TNP-substituted than with unsubstituted Hep-2 tumor cells. A non-covalently linked mixture of alcohol dehydrogenase and anti-TNP antibodies, together with the other factors required for the formation of acrolein, failed to demonstrate significant cytotoxicity, indicating the probable importance of the antibody in directing the enzyme to specific target sites.

Radioactivity - The use of antibodies as carriers of anti-tumor activity began with the work of Pressman et al. who found that specific antibodies labeled with ^{131}I retained immunologic specificity.[53,54] Following administration of radiolabeled anti-tumor antibodies to tumor bearing animals, localization of radioactivity was found to occur largely in tumor tissue,[55,56] but extensive purification of the labeled complex was necessary to improve selectivity.[57] As a result of the finding that antibodies raised against some tumor antigens cross-reacted with fibrinogen, efforts were initiated to treat experimental tumors with ^{131}I-labeled anti-fibrin antibodies.[58-60] Unfortunately, this type of therapeutic regimen was found to have limited effectiveness.[61-63]

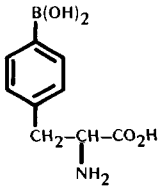
Mice inoculated with Ehrlich ascites cells that had first been incubated with ^{131}I-labeled rabbit anti-Ehrlich ascites cell antibodies failed to develop tumors in contrast to mice that were inoculated with cells that had been treated with non-iodinated antibodies or with ^{131}I-labeled normal immunoglobulins.[64] A comparison of radiolabeled specific and non-specific immunoglobulins showed that only the former localized at homologous tumor sites.[65] Preferential localization of radioiodinated specific antibodies was also observed using rabbit antibodies to rat mammary tumors,[66] and with antibodies raised against a microsome fraction of a hamster malignant melanoma.[67] The ability of ^{131}I-labeled specific antibodies to suppress tumor growth in mice was shown to be dependent upon the quantity of labeled antibody utilized.[68,69] Following treatment with ^{131}I-labeled specific antibodies, two of four tumor bearing individuals appeared to concentrate the radioactive complex in metastatic tissues.[69]

Boron - Bombardment of boron with slow neutrons leads to the following reaction:

$$^{10}_{5}B + ^{1}_{0}n \rightarrow ^{7}_{3}Li + ^{4}_{2}He$$

The nuclear fragments liberated during the fission process are of considerably higher energy than the incident neutrons and, furthermore, dissipate their energy over short distances, making boron fission an attractive procedure for destroying tumors while minimizing toxicity to normal tissues.[70] Early studies indicated the potential utility of this therapeutic approach by showing that irradiation of boron containing tumors in mice could lead to tumor regression.[70,71]

The feasibility of employing boron-containing amino acid derivatives to prepare boron-antibody conjugates was demonstrated by the successful coupling of D,L-4-boronophenylalanine (7) to bovine γ-globulin.[72] Incorporation of boron into antibodies was also accomplished by the diazotization process using 1-(4-aminophenyl)-1,2-dicarba-closo-dodecaborane (8) as the boron containing species.[73] Following covalent attachment to antibodies directed against human histocompatibility antigens, the resulting boron-antibody conjugate, upon neutron radiation, displayed significant cytotoxicity against human lymphocytes.



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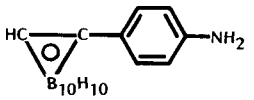

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Attempts to enhance biological activity by incorporating greater amounts of boron per antibody molecule without diminishing the aqueous solubility of the newly formed conjugate led to the preparation of numerous boron derivatives containing polar functional groups.[74,75] However, the increased levels of boron binding achieved were not sufficient to mediate tumor cell destruction in vivo.[76] Recently, boron containing intermediates that also display gluconamide groups were found to react with human γ-globulin to provide conjugates that not only incorporated high levels of boron but also exhibited good aqueous solubility.[77]

Antibody Reversal of Drug Effects

The principles by which the elicitation of antibodies to low molecular weight organic substances, such as drugs, is accomplished were established by Landsteiner,[78] who demonstrated that an antibody response to small molecules could be successfully elicited if such molecules were first coupled to carrier proteins. The development of the radioimmunoassay method by Yalow and Berson[79,80] has led to the routine elicitation of antibodies to drugs.[81] The systematic utilization of antibodies to reverse the effects of drugs is of more recent occurrence, but its development was foreshadowed by work on the neutralization of the activity of endogenous hormones.[82,83]

Digoxin - Antibody reversal of the effects of digoxin has been reviewed by Butler et al.[84,85] and Smith et al.[86] Digoxin, which is not sufficiently immunogenic to elicit antibodies by direct immunization, was coupled to bovine serum albumin to prepare a suitable immunogen.[87]

Immunization with this conjugate led, after several months, to the production of antibodies with affinity constants of the order of $10^{10}\ M^{-1}$.[88] Although the aglycone of digoxin may be considered a steroid-like structure, the antibody exhibited no significant cross reactivity in ligand displacement studies with a number of naturally occurring steroids.[88,89] This is not unexpected since digitoxin, which like the steroids lacks a hydroxyl group at position 12, shows a 40-50 fold lower affinity for anti-digoxin antibodies than digoxin.

Specific antibodies raised against digoxin were shown to reverse a number of digoxin effects *in vitro*. Antibodies antagonized the digoxin-induced increases in tension of guinea pig atrial strips;[90] reversed the toxic electrophysiological effects of digoxin on isolated Purkinje fibers;[91] counteracted the digoxin-mediated decrease in potassium transport in erythrocytes at a rate equivalent to the loss of membrane bound, but not intracellular, digoxin.[92] Anti-digoxin antibodies also were shown to reverse the effects of digoxin *in vivo*.[93]

Because Fab fragments of antibodies are excreted directly in the urine,[94] Butler[95] suggested that such fragments should be used in place of whole antibody to more rapidly eliminate bound digoxin. Compared to the intact antibody molecule, Fab fragments possess a number of potential advantages: a lack of species specific determinants which makes them less immunogenic and, therefore, less prone to induce anaphylactoid side effects;[84] a shorter half-life (4 to 12 hours) which leads to more rapid clearance;[94-97] little or no propensity to cause aggregation which results in greater safety following intravenous administration;[96] and a smaller molecular size which leads to a more rapid distribution and a larger volume of distribution.

The serum levels and urinary excretion patterns of ^{3}H-digoxin were compared in dogs receiving either sheep anti-digoxin serum or specifically purified sheep anti-digoxin Fab fragments.[98] Dogs treated with the specific Fab fragments achieved peak blood levels during the first day which decreased over a period of 6 days. Most of the digoxin was protein bound after treatment with either anti-digoxin antisera or Fab and thus pharmacologically inactive. While Fab-treated dogs excreted significant amounts of drug via the urine, antisera-treated dogs did not do so during the first 96 hours following treatment, presumably because of the long plasma half-life of the whole antibody molecule.

Advanced digoxin intoxication in the clinic was reversed through the use of specifically purified sheep anti-digoxin Fab fragments.[99] Within 10 minutes of completing an infusion of specific Fab fragments a stable sinus rhythm was restored in a single patient. The digoxin serum concentration rose rapidly, but the free digoxin concentration fell below measurable levels (less than 1 ng/ml). Renal excretion of digoxin was shown to be almost totally Fab bound, free digoxin levels remained low, and the patient recovered.

Digitoxin - No specific anti-digitoxin antibodies were elicited, but anti-digoxin antibodies which cross reacted with and bound digitoxin with an intrinsic association constant of $10^{10}\ M^{-1}$ were used to reverse toxicity in dogs.[100]

Ouabain and Acetyl Strophanthidin - The toxic effects of ouabain could be inhibited *in vivo* in rabbits following active immunization.[101] Gold and Smith[102] examined the effects of antibodies on the reversal of the

inotropic effects of ouabain and acetyl strophanthidin on cardiac muscle in vitro. Differences in reversal times for anti-ouabain antibody and anti-acetyl strophanthidin antibody were attributed to the differences in the rates of dissociation from the respective drug receptors.

Morphine - The elicitation of antibodies to morphine has been reported by a number of investigators as part of the development of radioimmunoassay procedures.[103-107] Morphine depresses the contractions of isolated electrically stimulated guinea pig ileum and this effect can be inhibited by specific antibody.[108,109] The excellent correlation between the morphine binding capacity and pharmacologic blocking capacity of the antisera supports the concept that morphine is neutralized by antibody and the activity remaining in the antibody-morphine mixture is attributable to free morphine.[109]

The passive administration of anti-morphine antibodies has a pronounced effect on the pattern of tissue distribution of morphine. After mice were treated with either anti-morphine antisera or control sera and subsequently injected with morphine, plasma levels of morphine were 90 fold higher in mice receiving the specific antisera. The tissue levels of morphine in the brains were calculated to be far lower. It appears that antibody preferentially binds morphine and the resultant complex circulates in the plasma, greatly diminishing the amount of drug available for tissue binding.[110]

Numerous studies have shown that active immunization against morphine inhibits the effects of subsequently administered drug.[110-113]

Barbiturates - Antibodies to the barbiturates were elicited by using the barbiturate hapten, 5-allyl-5-(β-carboxy-α-methyl)ethyl barbituric acid, coupled to bovine γ-globulin.[114,115] In actively immunized mice, the pharmacologic response (measured as depression of rotarod activity) to an active dose of pentobarbital was decreased[116] and serum levels following phenobarbital administration were increased.[117] Moreover, the absolute increase in the serum levels of pentobarbital was related directly to the binding capacity of the pentobarbital antisera.

Conclusion

Antibodies have been used to carry drugs and other therapeutic agents to specific tissue sites. To date, the application of this technique has been confined, almost exclusively, to neoplastic diseases. Medicinal chemical issues remaining for the future are: a) identification of a drug or class of drugs that may optimally be suited to the formation of immunochemotherapeutic complexes, b) comparative advantages of covalently and non-covalently bonded conjugates, c) relative importance of linking drugs to antibodies directly versus the use of spacer molecules, and d) the preparation of complexes with high drug to antibody ratios. The inability to elicit high titers of anti-tumor antibodies, together with difficulties encountered in removing antibodies directed against normal tissue, has limited the application of antibodies as carrier molecules. However, the recent advent of the monoclonal hybridoma technique[118] has made possible the production of pure, monospecific antibodies in high titer, which will have a profound impact on future research in this area.

The employment of modern protein purification techniques, coupled with the use of the non-complement fixing, relatively non-immunogenic,

and rapidly excreted Fab antibody fragment, has led to the establishment of a viable method for reversing the toxicity of drugs. However, because of the problems associated with obtaining and administering large quantities of antibodies, this method of toxicity reversal is presently largely applicable to the more potent classes of drugs such as the cardiac glycosides.

References

1. T. Ghose and S. P. Nigam, Cancer (Philadelphia), 29, 1398 (1972).
2. D. A. L. Davies, Cancer Res., 34, 3040 (1974).
3. D. A. L. Davies, S. Buckham, and A. J. Manstone, Brit. J. Cancer, 30, 305 (1974).
4. M. Segerling, S. H. Ohanian, and T. Borsos, Science, 188, 55 (1975).
5. R. D. Rubens, S. Vaughan-Smith, and R. Dulbecco, Brit. J. Cancer, 32, 352 (1975).
6. A. Guclu, J. Tai, and T. Ghose, Immunol. Commun., 4, 229 (1975).
7. M. Szekerke, R. Wade, and M. E. Whisson, Neoplasma, 19, 199 (1972).
8. I. Macpherson, Lancet, i, 1058 (1974).
9. T. Ghose, S. T. Norvell, A. Guclu, D. Cameron, A. Bodurtha, and A. S. MacDonald, Brit. Med. J., 3, 495 (1972).
10. I. Flechner, Eur. J. Cancer, 9, 741 (1973).
11. G. V. Smith, J. B. Grogan, J. Stribling, and J. Lockard, Amer. J. Surg., 129, 146 (1975).
12. L. G. Israels and J. H. Linford, Proc. Fifth Can. Cancer Conf., 399 (1963).
13. D. A. L. Davies and G. J. O'Neill, Brit. J. Cancer (Suppl. 1), 28, 285 (1973).
14. R. D. Rubens and R. Dulbecco, Nature (London), 248, 81 (1974).
15. C. Vennegoor, D. Van Smeerdÿk, and Ph. Rumke, Eur. J. Cancer, 11, 725 (1975).
16. A. Guclu, T. Ghose, J. Tai, and M. Mammen, Eur. J. Cancer, 12, 95 (1976).
17. W. J. Hopwood and J. A. Stock, Chem. Biol. Interact., 4, 31 (1971).
18. W. C. J. Ross, Chem. Biol. Interact., 8, 261 (1974).
19. D. Blakeslee and J. C. Kennedy, Cancer Res., 34, 882 (1974).
20. D. Blakeslee, M. Chen, and J. C. Kennedy, Brit. J. Cancer, 31, 689 (1975).
21. T. Ghose, S. T. Norvell, A. Guclu, and A. S. MacDonald, Eur. J. Cancer, 11, 321 (1975).
22. C. J. Oon, M. Apsey, H. Buckleton, K. B. Cooke, I. Hanham, P. Hazarika, J. R. Hobbs, and B. McLeod, Behring Inst. Mitt., 56, 228 (1974).
23. J. D. Everall, P. Dowd, D. A. L. Davies, G. J. O'Neill, and G. F. Rowland, Lancet, i, 1105 (1977).
24. T. Ghose, S. T. Norvell, A. Guclu, A. Bodurtha, J. Tai, and A. S. MacDonald, J. Nat. Cancer Inst., 58, 845 (1977).
25. D. Papachristou, A. F. Zaki, and J. G. Fortner, Transplant Proc., 9, 1059 (1977).
26. G. F. Rowland, G. J. O'Neill, and D. A. L. Davies, Nature (London), 255, 487 (1975).
27. G. F. Rowland, Eur. J. Cancer, 13, 593 (1977).
28. J. H. Linford, Chem. Biol. Interact., 6, 149 (1973).
29. J. H. Linford, G. Froese, I. Berczi, and L. G. Israels, J. Nat. Cancer Inst., 52, 1665 (1974).
30. J. H. Linford and G. Froese, J. Nat. Cancer Inst., 60, 307 (1978).
31. J. D. Beatty, E. Friesen, J. H. Linford, and L. G. Israels, Transplantation, 25, 197 (1978).
32. C. Tan, H. Tasaka, K. P. Yu, M. L. Murphy, and D. A. Karnosfsky, Cancer (Philadelphia), 20, 333 (1967).
33. R. M. O'Bryan, J. K. Luce, R. W. Talley, J. A. Gottlieb, L. H. Baker, and G. Bonadonna, Cancer (Philadelphia), 32, 1 (1973).
34. E. Hurwitz, R. Levy, R. Maron, M. Wilchek, R. Arnon, and M. Sela, Cancer Res., 35, 1175 (1975).
35. R. Levy, E. Hurwitz, R. Maron, R. Arnon, and M. Sela, Cancer Res., 35, 1182 (1975).
36. E. Hurwitz, R. Maron, A. Bernstein, M. Wilchek, M. Sela, and R. Arnon, Int. J. Cancer, 21, 747 (1978).
37. G. Mathe, T. B. Loc, and J. Bernard, C. R. Acad. Sci., 246, 1626 (1958).
38. E. Calendi, G. Costanzi, F. Indiveri, G. Lotti, and C. Zini, Boll. Chim. Farm., 108, 25 (1969).
39. D. A. Robinson, J. M. Whiteley, and N. G. L. Harding, Biochem. Soc. Trans., 1, 722 (1973).
40. W. T. Shearer and H. J. Mettes, J. Immunol., 123, 2763 (1979).
41. D. M. Gill, A. M. Pappenheimer, Jr., and T. Uchida, Fed. Proc., 32, 1508 (1973).
42. F. L. Moolten and S. R. Cooperband, Science, 169, 68 (1970).
43. G. W. Philpott, R. J. Bower, and C. W. Parker, Surgery, 73, 928 (1973).
44. F. L. Moolten, N. J. Capparell, and S. Cooperband, J. Nat. Cancer Inst., 49, 1057 (1972).
45. F. L. Moolten, N. J. Capparell, S. H. Zajdel, and S. R. Cooperband, J. Nat. Cancer Inst., 55, 473 (1975).
46. P. E. Thorpe, W. C. J. Ross, A. J. Cumber, C. A. Hinson, D. C. Edwards, and A. J. S. Davies, Nature (London), 271, 752 (1978).

47. S. Avrameas, Immunochemistry, 6, 43 (1969).
48. G. W. Philpott, R. J. Bower, and C. W. Parker, Surgery, 74, 51 (1973).
49. G. W. Philpott, W. T. Shearer, R. J. Bower, and C. W. Parker, J. Immunol., 111, 921 (1973).
50. G. W. Philpott, R. J. Bower, K. L. Parker, W. T. Shearer, and C. W. Parker, Cancer Res., 34, 2159 (1974).
51. W. T. Shearer, T. R. Turnbaugh, W. E. Coleman, R. D. Aach, G. W. Philpott, and C. W. Parker, Int. J. Cancer, 14, 539 (1974).
52. G. W. Philpott, E. H. Grass, and C. W. Parker, Cancer Res., 39, 2084 (1979).
53. D. Pressman and G. Keighley, J. Immunol., 59, 141 (1948).
54. A. Johnson, E. D. Day, and D. Pressman, J. Immunol., 84, 213 (1960).
55. D. Pressman and L. Korngold, Cancer (Philadelphia), 6, 619 (1953).
56. W. F. Bale, I. L. Spar, R. L. Goodland, and D. E. Wolfe, Proc. Soc. Exp. Biol. Med., 89, 564 (1955).
57. E. D. Day, J. Planinsek, L. Korngold, and D. Pressman, J. Nat. Cancer Inst., 17, 517 (1956).
58. E. D. Day, J. A. Planinsek, and D. Pressman, J. Nat. Cancer Inst., 22, 413 (1959).
59. I. L. Spar, R. L. Goodland, and W. F. Bale, Proc. Soc. Exp. Biol. Med., 100, 259 (1959).
60. W. F. Bale, I. L. Spar, and R. L. Goodland, Cancer Res., 20, 1488 (1960).
61. I. L. Spar, W. F. Bale, R. L. Goodland, G. W. Casarett, and S. M. Michaelson, Cancer Res., 20, 1501 (1960).
62. E. D. Day, J. A. Planinsek, and D. Pressman, J. Nat. Cancer Inst., 25, 787 (1960).
63. I. L. Spar, W. F. Bale, D. Marrack, W. C. Dewey, R. J. McCardle, and P. V. Harper, Cancer (Philadelphia), 20, 865 (1967).
64. T. Ghose, M. Cerini, M. Carter, and R. C. Nairn, Brit. Med. J., 1, 90 (1967).
65. M. J. Izzo, D. J. Buchsbaum, and W. F. Bale, Proc. Soc. Exp. Biol. Med., 139, 1185 (1972).
66. J. A. Kellen and J. S. Lo, Res. Commun. Chem. Pathol. Pharmacol., 5, 411 (1973).
67. H. J. Smith and M. Gökcen, Res. Commun. Chem. Pathol. Pharmacol., 7, 725 (1974).
68. T. Ghose and A. Guclu, Eur. J. Cancer, 10, 787 (1974).
69. T. Ghose, A. Guclu, J. Tai, A. S. MacDonald, S. T. Norvell and J. Aquino, Cancer (Philadelphia), 36, 1646 (1975).
70. P. G. Kruger, Proc. Nat. Acad. Sci., U.S.A., 26, 181 (1940).
71. P. A. Zahl, F. S. Cooper, and J. R. Dunning, Proc. Nat. Acad. Sci., U.S.A., 26, 589 (1940).
72. A. G. Mallinger, E. L. Jozwiak, Jr., and J. C. Carter, Cancer Res., 32, 1947 (1972).
73. M. F. Hawthorne, R. J. Wiersema, and M. Takasugi, J. Med. Chem., 15, 449 (1972).
74. H. S. Wong, E. I. Tolpin, and W. N. Lipscomb, J. Med. Chem., 17, 785 (1974).
75. E. I. Tolpin, H. S. Wong, and W. N. Lipscomb, J. Med. Chem., 17, 792 (1974).
76. R. L. Sneath, Jr., A. H. Soloway, and A. S. Dey, J. Med. Chem., 17, 796 (1974).
77. R. L. Sneath, Jr., J. E. Wright, A. H. Soloway, S. M. O'Keefe, and W. D. Smolnycki, J. Med. Chem., 19, 1290 (1976).
78. K. Landsteiner, The Specificity of Serological Reactions, Harvard Univ. Press, Boston, MA, 1945.
79. R. S. Yalow and S. A. Berson, Nature (London), 184, 1648 (1959).
80. R. S. Yalow and S. A. Berson, J. Clin. Invest., 39, 1157 (1960).
81. V. P. Butler, Jr., Pharm. Rev., 29, 103 (1977).
82. R. F. Clutton, C. R. Harington, and M. E. Yuill, Biochem. J., 32, 1119 (1938).
83. S. Lieberman, B. F. Erlanger, S. M. Beiser, and F. J. Agate, Jr., Rec. Prog. Horm. Res., 15, 165 (1959).
84. V. P. Butler, Jr., J. F. Watson, D. H. Schmidt, J. D. Gardner, W. J. Mandel, and C. L. Skelton, Pharm. Rev., 25, 239 (1973).
85. V. P. Butler, Jr., T. W. Smith, D. H. Schmidt, and E. Haber, Fed. Proc., 36, 2235 (1977).
86. T. W. Smith, V. P. Butler, Jr., and E. Haber, in "Antibodies in Human Diagnosis and Therapy" (Ed. E. Haber and R. M. Krause), Raven Press, NY, 1977, p. 365.
87. V. P. Butler, Jr. and J. P. Chen, Proc. Nat. Acad. Sci., U.S.A., 57, 71 (1967).
88. T. W. Smith, V. P. Butler, Jr., and E. Haber, Biochemistry, 9, 331 (1970).
89. T. W. Smith, V. P. Butler, Jr., and E. Haber, N. Engl. J. Med., 281, 1212 (1969).
90. J. Curd, T. W. Smith, J.-C. Jaton, and E. Haber, Proc. Nat. Acad. Sci., U.S.A., 68, 2401 (1971).
91. W. J. Mandel, J. T. Bigger, Jr., and V. P. Butler, Jr., J. Clin. Invest., 51, 1378 (1972).
92. J. D. Gardner, D. R. Kiino, T. J. Swartz, and V. P. Butler, Jr., J. Clin. Invest., 52, 1820 (1973).
93. D. H. Schmidt and V. P. Butler, Jr., J. Clin. Invest., 50, 866 (1971).
94. R. D. Wochner, W. Strober, and T. A. Waldman, J. Exp. Med., 126, 207 (1967).
95. V. P. Butler, Jr., N. Engl. J. Med., 283, 1150 (1970).
96. C. A. Janeway, E. Merler, F. S. Rosen, S. Salmon, and J. D. Crain, N. Engl. J. Med., 278, 919 (1968).
97. B. L. Lloyd and T. W. Smith, Circulation, 58, 280 (1978).

98. V. P. Butler, Jr., D. H. Schmidt, T. W. Smith, E. Haber, B. D. Raynor, and P. DeMartini, J. Clin. Invest., 59, 345 (1977).
99. T. W. Smith, E. Haber, L. Yeatman, and V. P. Butler, Jr., N. Engl. J. Med., 294, 797 (1976).
100. H. R. Ochs and T. W. Smith, J. Clin. Invest., 60, 1303 (1977).
101. F. Ciofalo and H. Ashe, Life Sci., 10, 341 (1971).
102. H. K. Gold and T. W. Smith, J. Clin. Invest., 53, 1655 (1974).
103. S. Spector and C. W. Parker, Science, 168, 1347 (1970).
104. S. Spector, J. Pharm. Exp. Therap., 178, 253 (1971).
105. H. van Vunakis, E. Wasserman, and L. Levine, J. Pharm. Exp. Therap., 180, 514 (1972).
106. B. H. Wainer, F. W. Fitch, R. M. Rothberg, and J. Fried, Science, 176, 1143 (1972).
107. B. H. Wainer, F. W. Fitch, J. Fried, and R. M. Rothberg, J. Immunol., 110, 667 (1973).
108. B. H. Wainer, F. W. Fitch, R. M. Rothberg, and C. R. Schuster, Nature (London), 241, 537 (1973).
109. L. DeCato, Jr. and F. L. Adler, Res. Commun. Chem. Pathol. Pharmacol., 5, 775 (1973).
110. B. Berkowitz, K. Cerreta, and S. Spector, Life Sci., 15, 1017 (1975).
111. B. Berkowitz and S. Spector, Science, 178, 1290 (1972).
112. K. F. Bonese, B. H. Wainer, F. W. Fitch, R. M. Rothberg, and C. R. Schuster, Nature (London), 252, 708 (1974).
113. K. D. Meisheri and G. E. Isom, Res. Commun. Chem. Pathol. Pharmacol., 19, 85 (1978).
114. S. Spector and E. J. Flynn, Science, 174, 1036 (1971).
115. E. J. Flynn and S. Spector, J. Pharm. Exp. Therap., 181, 547 (1972).
116. E. J. Flynn, K. V. Cerreta, and S. Spector, Eur. J. Pharmacol., 42, 21 (1977).
117. E. J. Flynn and K. V. Cerreta, Clin. Immunol. Immunopath., 9, 80 (1978).
118. Current Topics in Microbiology and Immunology, 81 (1978).

Section VI - Topics in Chemistry and Drug Design

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Chapter 26. Reactions of Interest in Medicinal Chemistry

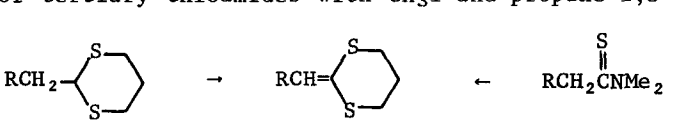
Daniel Lednicer,* Mead Johnson Pharmaceuticals, Evansville, Indiana

The sheer mass of this year's organic chemical literature obviously renders futile any attempt to present a comprehensive survey in the few pages allotted. What follows instead, is a personal view of developments deemed of potential interest to those engaged in the synthesis of biologically active compounds. Particular attention has been devoted to novel reagents, reactions or approaches which solve difficult transforms or which provide access to interesting structures.

REVIEWS - Interesting reviews have appeared entitled: "Electrophilic Cyclopropanes in Organic Synthesis",[1] "Pyrolysis of Sulfones as a Synthetic Method",[2] and "Synthesis of Aldehydes, Ketones and Carboxylic Acids from Lower Carbonyl compounds by C-C Coupling Reactions."[3] Additional articles summarized the use of 1,5-dipolar cycloadditions in the synthesis of heterocyclic compounds,[4] the chemistry of formamide acetals,[5] choice of hydrogenation catalysts,[6] and the use of organoselenium reagents in functional group manipulation.[7]

REAGENTS - Oxidations - The solution obtained from H_2O_2 and hexafluoroacetone hydrate is an effective epoxidizing agent for olefins.[8] Reaction of olefins with H_2O_2 in the presence of $CH_3C(OEt)_3$ similarly gives the epoxides[9]; this last reagent gives poor yields with terminal double bonds. Benzeneselenic anhydride oxidizes methyl groups to aldehydes in unsubstituted aromatic compounds as well as in compounds with electron donating groups.[10] Hydroquinones and catechols can be oxidized to the respective p- and o-quinones by means of $(C_6H_5)_2SeO$.[11]

The intermediates from alkylation of the anions from dithianes with 2-pyridyl disulfide spontaneously decompose to afford ketene acetals.[12] (The same functionality can be obtained by sequential treatment of tertiary thioamides with CH_3I and propane-1,3-dithiol.[13])



Treatment of enol phenylthioethers with $Pb(OAc)_4$ gives the allylic acetates[14] (the acetate group can be displaced with alkyl cuprates). Ene reaction of olefins with CH_3CONO (generated in situ from its anthracene adduct) gives the allylic hydroxylamine derivatives.[15]

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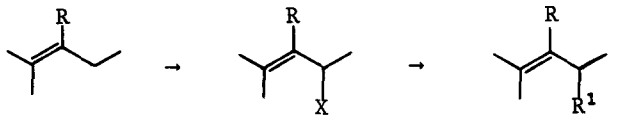
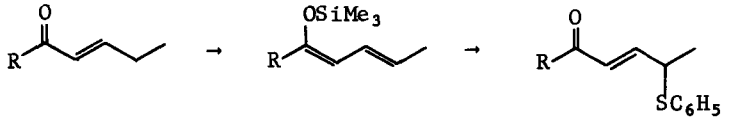


R=H; SC_6H_5 X = $OCOCH_3$ $N(OH)COCH_3$

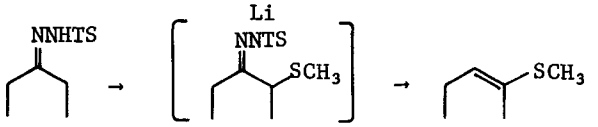
Acylation of the silyl enol ethers from enones with C_6H_5SCl gives exclusively the product of γ-attack; oxidation elimination serves to extend conjugation.[16]



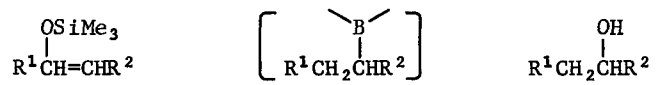
Reductions - Stilbenes and diphenylethylenes can be reduced by means of Mg and CH_3OH.[17] The same functionalities as well as enones, acrylates and Shiff bases can be reduced by K/graphite in protic solvents.[18] Hydrogenation of (nitrophenyl)acetylenes over Ru/C[19] or over RuS[20] results in selective reduction to anilinoacetylenes.

Ketones can be reduced selectively in the presence of aldehydes by use of $NaBH_4$ in the presence of aqueous $CeCl_3$, probably by formation of transient aldehyde acetals.[21] A complex of $NaBH_4$ with CuCl and triphenylphosphine will reduce acid chlorides to aldehydes.[22] Arylsuccinimides can be converted to N-arylpyrrolidines by means of $BF_3 \cdot NaBH_4$.[23]

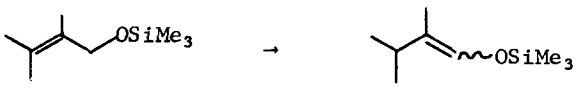
Transpositions - Alkylation of the α-anion from tosylhydrazones with CH_3SSCH_3 gives the intermediate sulfide; additional base at room temperature results in Bamford-Stevens elimination to afford the enolthioether of the transposed keytone.[24]



Treatment of silyl enol ethers with boranes such as 9-BBN gives the transposed organoboranes. These, on reaction with alkaline peroxide give the product of a reductive transposition.[25]



Allyl alcohols can be converted to aldehydes by transposition of the double bond. Thus, treatment of the silyl ether of allylic alcohols with $H_2Ru(C_6H_5)_3P)_4$ gives the silyl enol ether of the corresponding aldehyde.[26]



Other Reagents - Heating substituted methyl and ethyl malonates to 180° in the presence of boric acid gives high yields of the corresponding monocarboxylic esters.[27] In a related reaction, 4-substituted acetoacetates can be converted to methyl ketones with Me_3SiI at 100° in the absence of solvent. (Much of that reagent's chemistry can be carried out more conveniently with a mixture of Me_3SiCl and NaI).[29] Treatment of esters with Me_2AlNH_2 in refluxing xylene gives the corresponding nitriles.[30] The same products are obtained by reaction of aldehydes with NH_3 in the presence of nickel peroxide.[31]

Condensation of Grignard reagents with CS_2 in the presence of catalytic CuBr, followed by CH_3I, gives the homologated thiocarbonyl products.[32]

$$RMgX \longrightarrow RC(S)SMgX \longrightarrow RC(=S)SCH_3$$

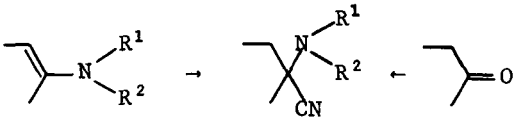
Aldehydes can be converted to the homologous bromomethyl ketones by condensation with the anion from the sulfoxide below, followed by thermal rearrangement of the resulting hydroxysulfone.[33]

$$RCHO + C_6H_5SOCH_2Br \longrightarrow RCH(OH)CH(SOC_6H_5)Br \longrightarrow RCOCH_2Br$$

Allyl and benzyl bromides will react with the organometallic reagent obtained from CF_3I and metallic Cu in HMPA to give the corresponding displacement products;[34] 3-bromoenones undergo an analogous displacement.

$$RCH_2Br + CF_3\ (CuI) \longrightarrow RCH_2CF_3$$

Eneamines can be converted to the corresponding α-aminonitriles by treatment with $(EtO)_2POCN$.[35] This same reagent will convert aldehydes and ketones to that same function in the presence of an amine.[36]

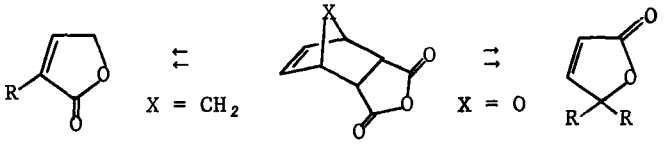


PROTECTING GROUPS - Pyridine toluenesulfonate has been found to be an effective, mild catalyst for formation of acetals; the same salt in aqueous acetone catalyzed the hydrolysis of those acetals.[37] Aqueous HF in CH_3CN constitutes a useful reagent for hydrolysis of dimethyl-tert-butylsilyl ethers.[38] Benzyl ethers of phenols and alcohols can be cleaved in good yield by treatment with $BF_3 \cdot Et_2O$ and C_2H_5SH.[39] Ethyl ethers are said to be more suitable protecting groups for phenols than the methyl counterparts, since the former are cleaved in better yields by BBr_3.[40] Trichloroethylmethoxy ethers of alcohols can be cleaved under neutral reductive conditions (Zu:Cu/MeOH or Zn/MeOH).[41]

$$ROH + CCl_3CH_2OCH_2OH \longrightarrow ROCH_2OCH_2CCl_3 \longrightarrow ROH$$

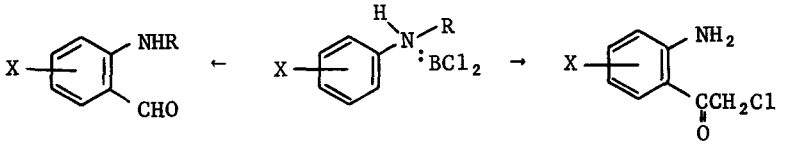
4,4'-Dimethoxybenzhydrylamine constitutes an ammonia equivalent, as the akylation products can be debenzhydrylated by means of formic acid.[42]

The olefin function in maleic anhydride can be protected as a Diels Alder adduct. Reaction of the furan adduct with Grignard reagents, followed by pyrolysis gives dialkylated butyrolactones.[43] Reduction of the cyclopentadiene adduct ($NaBH_4$), followed by anion chemistry and pyrolysis gives 2-alkylated lactones.[44]

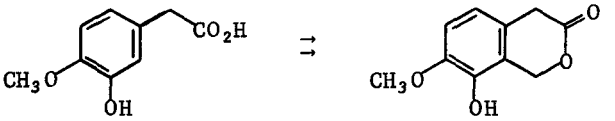


AROMATIC SUBSTITUTION - A complex of NBS and DMF constitutes a selective nuclear monobrominating agent for reactive aromatic compounds; for example, mesitylene, durene, aniline, phenol and resorcinol all give the corresponding monobromo compound.[45]

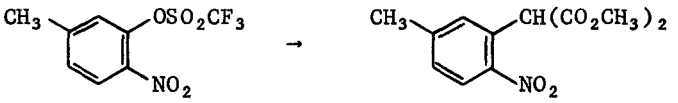
Boron trichloride complexes from alkylanilines react with alkylisonitriles to afford ortho-alkylated intermediates; these give the aldehydes on hydrolysis.[46] The corresponding complexes from primary anilines can be acylated with chloroacetonitriles.[47]



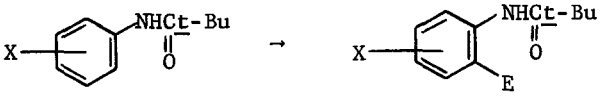
Sequential reaction of the substituted catechol acetic acid with $C_6H_5B(OH)_2$ and paraformaldehyde results in formylation ortho to the phenol. The intermediate borate ester gives a benzpyranone on hydrolysis.[48]



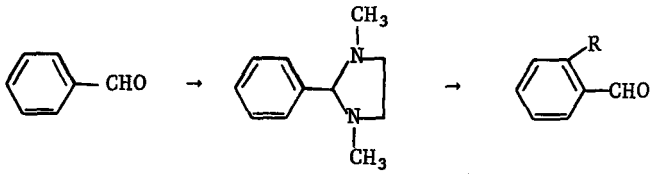
Phenols activated by nitro groups can be used as leaving groups by conversion to triflates. These derivates can, for example, be displaced by malonate anions.[49]



Pivaloyl amides of anilines have been found particularly suitable for formation of ortho-lithiated derivatives. Reactions with electrophiles give good yields of alkylation and carbonyl addition products.[50]



Conversion of benzaldehyde to its dimethylimidazoline derivative at the same time protects the carbonyl group and activates the ortho position to lithiation. Reaction of the dimethylimidazoline derivative with BuLi:TMEDA, followed by electrophiles, and then hydrolysis, gives the ortho-substituted benzaldehydes.[51]

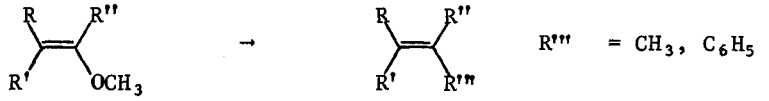


FORMATION OF CARBON-CARBON BONDS - Work continues apace on chiral syntheses. Recent examples report induction of chirality in alkylation of acetaldehyde with allylborane,[52] and assymetric induction in conjugate additon by use of chiral bases.[53] Schiff bases[54,55] or aminals[56] of carbonyl groups with chiral amines have been used to obtain chiroselective carbon bond formation. Additional examples involve use of chiral oxazolines[57] or esters with terpenes.[58]

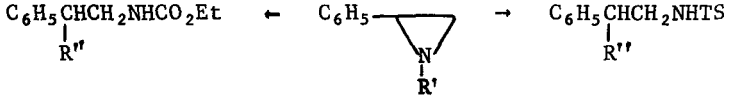
The storable solid sodium salt from methane tricarboxylate can be used to circumvent problems due to over alkylation of malonates; reaction with dibromobutane followed by decarboxylation, gives high yields of the difficultyly obtainable bromobutyl malonate.[59]

$$NaC(CO_2Et)_3 \longrightarrow Br(CH_2)_4C(CO_2Et)_3 \longrightarrow Br(CH_2)_4CH(CO_2Et)_2$$

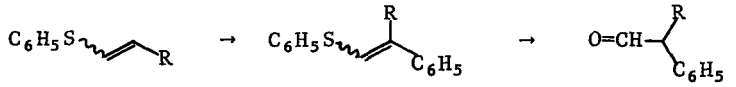
Though enol ethers are usually stable to Grignard reagents, addition of a catalytic amount of $((C_6H_5)_3P)_2NiCl$ leads to products resulting from displacement of the alkoxide group.[60]



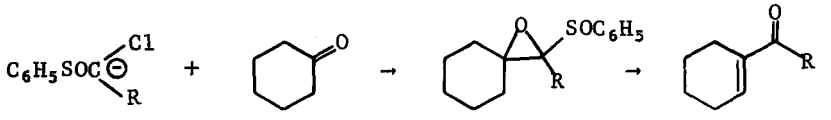
Tosylamides of phenylaziridines react with Grignard reagents to afford the corresponding β-phenethylamines. Reaction of the urethane derivatives with cuprates gives analogous ring opened products.[61]



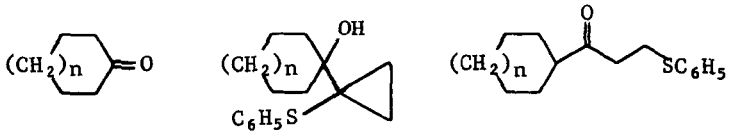
Phenylthioethers of aldehydes and ketones can be phenylated by reaction with bromobenzene in the presence of $Pd(OAc)_2$ and $(C_6H_5)_3P$.[62]



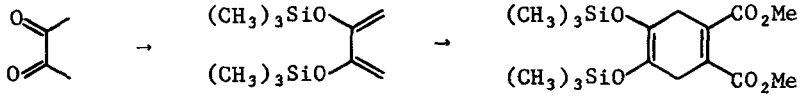
A reaction - similar to the glycidic ester syntheses - affords products representing formal addition of acyl reagents to ketones. Condensation of ketones with anions from α-chlorosulfoxides gives the corresponding epoxide. This opens to the enone on treatment with acid or heat.[63]



The anion from phenylcyclopropyl sulfide gives the saturated analogue of the above. The initial carbonyl addition product rearranges to the ketone on treatment with $ZnCl_2$ and HCl. The terminal sulfide group can be reduced (Raney Ni) or used to introduce a terminal double bond ($NaIO_4$, heat).[64]

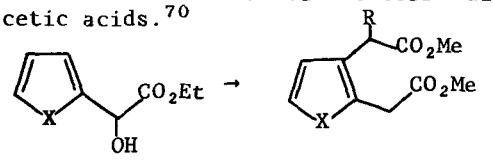


1,2-Diketones can be readily converted to the bis-silylenol-ethers. Enol ethers of 2,3-butanedione react with dienophiles to give cyclohexenes or cyclohexadienes.[65]



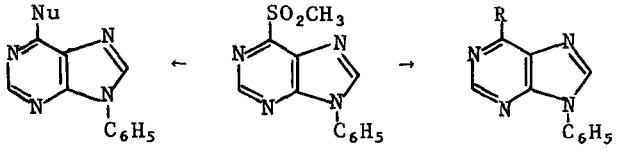
The prevalence of cyclopentanes in natural products has led to a number of synthetic approaches to such rings by annulation. These include, reaction of acryloyl chlorides with vinyl trimethylsilane,[66] followed by addition of dichloroketene to an olefin, ring expansion (CH_2N_2) and dehalogenation;[67] reaction of an enone with α-silylallyl-acetate catalyzed by palladium;[68] and a sequence involving γ-alkylation of a phosphorus reagent, followed by internal Wittig reaction.[69]

HETEROCYCLES - Electrocyclic rearrangement leading to ortho substitution has been applied to heterocycles. Thermal reaction of 2-hetero-2-hydroxy-acetic acids with orthoesters affords the corresponding 1,2-diacetic acids.[70]

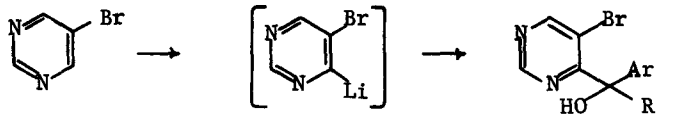


X=O, S, NTS R=H, Me, n-Pr

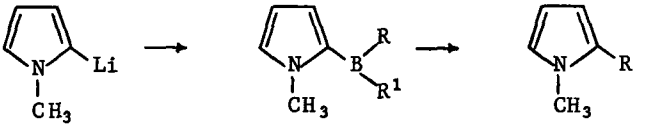
The methylsulfonyl group at the 4-position in purines can be displaced by means of Grignard reagents;[71] the same grouping can be replaced by nucleophiles (e.g. OH, OCH_3, NHR, $NHNH_2$) photolytically.[72]



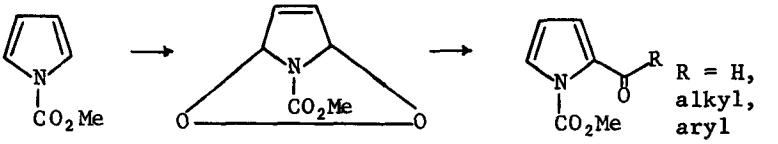
Treatment of 5-bromopyrimidine with LDA in the cold surprisingly leads to lithiation rather than halogen exchange. The resulting organometallic reacts with aromatic aldehydes and ketones to give modest yields of carbinols.[73]



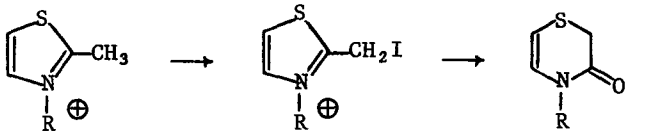
The organometallic reagent from lithiation of N-methyl-pyrrole reacts with alkylboranes to form adducts. These rearrange to 2-alkyl-pyrroles on treatment with NCS or I_2. Furan undergoes an analogous reaction.[74]



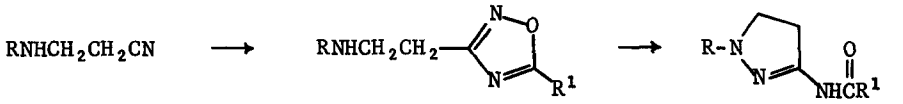
The adduct from N-carbethoxypyrrole and singlet oxygen (formulated as a bicycle) reacts with enolsilyl ethers in the presence of $SnCl_2$ to give products of formal acylation.[75]



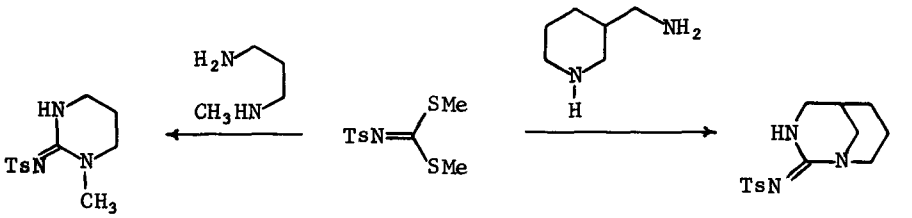
Sequential treatment of 2-methylthiazole with alkyl iodides and I_2/Et_3N affords the quaternary iodomethyl salts. These undergo ring expansion on treatment with KOH to form 1,4-thiazinones.[76]



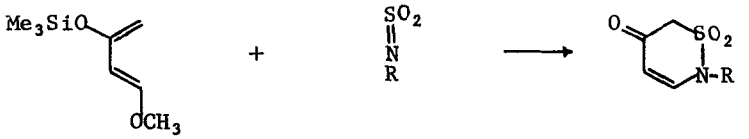
Thermal rearrangement of 2-aminoalkyloxadiazoles - available in 2-steps from the corresponding nitriles - affords unusual amino-pyrazolines.[77]



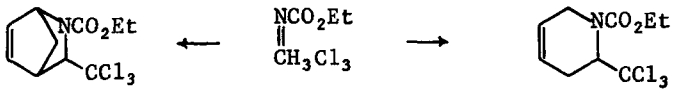
An interesting new bidentate reagent makes ring enclosed guanidines available in a single step. The utility of the procedure is expanded by the finding that the tosylamide function can be cleaved with liquid HF.[78]



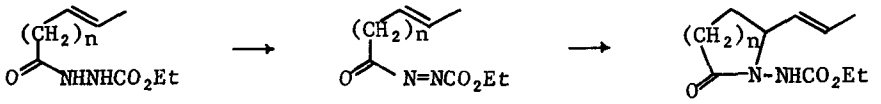
The availability of unusual dienes and dienophiles has led to novel syntheses for heterocycles. Condensation of the iminosulfoxide, obtained from treatment of the sulfamoyl chloride with Et_3N, with Danishevsky's trimethylsilyl ether diene gives the 1,2-thiazinone dioxides.[79]



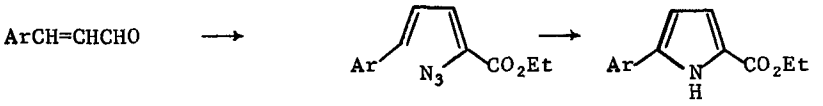
"Anhydro chloral urethane" acts as an effective dienophile. Subsequent manipulation of the trichloromethyl group makes available some unusually substituted compounds.[80]



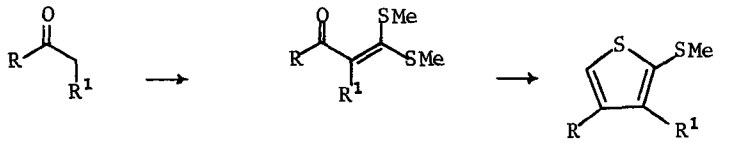
Oxidation of the diacylated hydrazides seen below (MnO_2) presumably affords diacylazines. These undergo electrocyclic closure to aminopyrrolidone or aminopiperidones.[81]



Condensation of cinnamaldehydes with azidoacetic ester gives the corresponding homologated products. The nitrene obtained by pyrolysis of this product inserts into the C-H bond adjacent to the aryl group to give moderate yields of the substituted pyrroles.[82] An analogous reaction starting with substituted furfurals (insertion in this case occurring onto the ring) affords pyrrolofurans.[83]



Treatment of the bis-(thiomethylene)ketones[84] with LDA in HMPA affords the corresponding 2-methylthiophenes.[85]



References

1. S. Danishefsky, Accts. Chem. Res. 12, 66 (1979).
2. F. Vogtle and L. Rossa, Angew. Chem. Int. Ed. in English, 18, 515 (1979).
3. S.F. Martin, Synthesis, 633 (1979).
4. E.C. Taylor and I.J. Turchi, Chem. Revs. 79, 181 (1979).
5. R.F. Abdulla and R.S. Brinkmeyer, Tetrahedron 35, 1675 (1979).
6. P.N. Rylander, Aldrichchimica Acta, 12, 22 (1979).
7. H.J. Reich, Accts. Chem. Res. 12, 22 (1979).
8. R. P. Heggs and B. Ganem, J. Am. Chem. Soc. 101, 2484 (1979).
9. J. Rebek, Jr. and R. McCready, Tetrahedron Lett. 1001 (1979).

10. D.H.R. Barton, R.A.H.F. Hui, D.J. Lester and S.V. Ley, Tetrahedron Lett. 3332 (1979).
11. J.P. Marino and A. Schwartz, Tetrahedron Lett. 3253 (1979).
12. Y. Nagao, K. Seno and E. Fujita, Tetrahedron Lett. 4403 (1979).
13. T. Harada, Y. Tamaru and Z. Yoshida, Tetrahedron Lett. 3524 (1979).
14. B. M. Trost and Y. Tamigawa, J. Am. Chem. Soc. 101, 4413 (1979).
15. G.E. Keck and J.B. Yates, Tetrahedron Lett. 4627 (1979).
16. I. Fleming, J. Goldhill and I. Patterson, Tetrahedron Lett. 3205 (1979).
17. J.A. Profitt and H.H. Ong, J. Org. Chem. 44, 3972 (1979).
18. M. Contento, D. Savoia C. Tombini and A. Umani-Rondi, Synthesis, 30 (1979).
19. A. Onopchenko, E.T. Sabourin and C.M. Selwitz, J. Org. Chem. 44, 1233 (1979).
20. A. Onopchenko, E.T. Sabourin and C.M. Selwitz, J. Org. Chem. 44, 3671 (1979).
21. J.-L. Luche and A.L. Gemal, J. Am. Chem. Soc. 101, 5848 (1979).
22. G.W.J. Fleet and P.J.C. Harding, Tetrahedron Lett. 975 (1979).
23. W. Merkel, D. Mania and D. Bormann, Ann. 461 (1979).
24. T. Nakai and T. Mimura, Tetrahedron Lett. 531 (1979).
25. G.L. Larson and L.M. Fuentes, Synthetic Commun. 9, 841 (1979).
26. H. Suzuki, Y. Koyama, Y. Moro-Oka and T. Iwa, Tetrahedron Lett. 1415 (1978).
27. T.-L. Ho, Synthetic Commun. 9, 609 (1979).
28. T.-L. Ho, J. Chem. Soc., Chem. Commun., 233 (1979).
29. G.A. Olah, S.C. Narang, B.G.B. Gupta and R. Malhotra, J. Org. Chem. 44, 1247 (1979).
30. J.L. Wood, N.A. Khatri and S.M. Weinreb, Tetrahedron Lett. 4907 (1979).
31. K. Nakagawa, S. Mineo, S. Kawamura and O. Mori, Synthetic Commun. 9, 529 (1979).
32. H. Westmijze, H. Kleijn, J. Meijer and P. Vermeer, Synthesis, 432 (1979).
33. V. Reutrakul, A. Tiensripojamarn, K. Kusamran and S. Nimigirawath, Chemistry Lett. 209 (1979).
34. Y. Kobayashi, K. Yamamoto and I. Kumadaki, Tetrahedron Lett. 4071 (1979).
35. S. Harusawa, Y. Hamada and T. Shioiri, Synthesis 716 (1979).
36. S. Harusawa, Y. Hamada and T. Shioiri, Tetrahedron Lett. 4663 (1979).
37. R. Sterzycki, Synthesis, 724 (1976).
38. R.F. Newton, D.P. Reynolds, M.A.W. Finch and S.M. Roberts, Tetrahedron Lett. 3981 (1979).
39. K. Fuji, K. Ichikawa, M. Node and E. Fujita, J. Org. Chem. 44, 1661 (1979).
40. J.B. Press, Synthetic Commun. 9, 407 (1979).
41. R.M. Jacobson and J. W. Clader, Synthetic Commun. 9, 57 (1979).
42. B.M. Trost and E. Keinan, J. Org. Chem. 44, 3451 (1979).
43. J.-C. Grandguillot and F. Rouessac, Synthesis, 607 (1979).
44. A. Ichihara, N. Nio and Y. Terayama, Tetrahedron Lett. 3731 (1979).
45. R.H. Mitchell, Y.-H. Lai and R.V. Williams, J. Org. Chem. 44, 4733 (1979).
46. T. Sugasawa, H. Hamana, T. Toyoda and M. Adachi, Synthesis, 99 (1979).
47. T. Sugasawa, M. Adachi, K. Sasakura and A. Kitagawa, J. Org. Chem. 44, 578 (1979).
48. M. Cushman and F. W. Dekow, J. Org. Chem. 44, 407 (1979).
49. J.G. Atkinson, B. Wasson, J. Fuentes, Y. Girard and C.S. Rooney, Tetrahedron Lett. 2857 (1979).
50. W. Fuhrer and H. W. Gschwend, J. Org. Chem. 44, 1133 (1979).
51. T.D. Harris and G.P. Roth, J. Org. Chem. 44, 2004 (1979).
52. R.W. Hoffmann and W. Ladner, Tetrahedron Lett. 4653 (1979).
53. K. Hermann and H. Wynberg, J. Org. Chem. 44, 2238 (1979).
54. R.F. Fraser, F. Akiyama and J. Banville, Tetrahedron Lett. 3929 (1979).
55. S. Hashimoto, H. Kogen, K. Tomioka and K. Koga, Tetrahedron Lett. 3009 (1979).
56. T. Mukaiyama, Y. Sakito and M. Asami, Chemistry Lett. 1253 (1979).
57. A.I. Meyers and R. K. Smith, Tetrahedron Lett. 2749 (1979).
58. T. Kaneko, D. L. Turner, M. Newcomb and D.E. Bergbreiter, Tetrahedron Lett. 103 (1979).
59. H.C. Padgett, I.G. Csendes and H. Rapoport, J. Org. Chem. 44, 3492 (1979).
60. E. Wenkert, E.L. Michelotti and C.S. Swindell, J. Am. Chem. Soc. 101, 2246 (1979).
61. A.P. Kozkowski, H. Ishida and K. Isobe, J. Org. Chem. 44, 2788 (1979).
62. B.M. Trost and T. Tanigawa, J. Am. Chem. Soc. 101, 4743 (1979).
63. D.F. Taber and B.P. Gunn, J. Org. Chem. 44, 450 (1979).
64. R.D. Miller and D.R. McKean, Tetrahedron Lett. 583 (1979).
65. M. T. Reetz and G. Neumeier, Chem. Ber. 112, 2209 (1979).
66. F. Cooke, J. Schwindeman and P. Magnus, Tetrahedron Lett. 1995 (1979).
67. A.E. Greene and J.-P. Depres, J. Am. Chem. Soc. 101, 4003 (1979).
68. B.M. Trost and D.M.T. Chan, J. Am. Chem. Soc. 101, 6429 (1979).
69. E. Piers, B. Abeyserkara and J. R. Scheffer, Tetrahadren Lett. 3279 (1979).
70. S. Raucher, A.S.-T. Lui and J.E. MacDonald, J. Org. Chem. 44, 1885 (1979).
71. E. Hayashi, N. Shimada and Y. Matsuoaka, J. Pharm. Soc. Japan 99, 114 (1979).
72. E. Hayashi and N. Shimada, J. Pharm. Soc. Japan 99, 201 (1979).
73. T.J. Kress, J. Org. Chem. 44, 2081 (1979).
74. E.R. Marinelli and A.B. Levy, Tetrahedron Lett. 2313 (1979).
75. M. Natsume and H. Muratake, Tetrahedron Lett. 3477 (1979).
76. M. Hojo, R. Masuda, S. Kosaka and N. Hayase, Synthesis, 272 (1979).

77. D. Korbonits, E.M. Bako and K. Horvath, J. Chem. Res. 801 (1979).
78. R.A. Houghten, R.A. Simpson, R.N. Hanson and H. Rapoport, J. Org. Chem. 44, 4536 (1979).
79. J.A. Kloek and K.L. Leschinsky, J. Org. Chem. 44, 305 (1979).
80. T. Imagawa and M. Kawanisi, Bull. Chem. Soc. Japan 52, 643 (1979).
81. E. Vedejs and G. P. Meier, Tetrahedron Lett., 4185 (1979).
82. J.P. Boukou-Poba, M. Farnier and R. Guilard, Tetrahedron Lett. 1717 (1979).
83. A. Krutosikova, J. Kovac and J. Kristofcak, Coll. Czech. Chem. Commun. 44, 1799 (1979).
84. J.P. Marino and J.L. Kostusyk, Tetrahedron Lett. 2439 (1979).
85. J.P. Marino and J.L. Kostusyk, Tetrahedron Lett. 2493 (1979).

Chapter 27. New Developments in Natural Products of Medicinal Interest

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Introduction - In 1978, 25% of the 200 most frequently prescribed drugs in the USA were of direct natural provenance or derived therefrom by a few simple chemical steps. Despite this, except for antibiotics and antitumor agents from microorganisms or from the sea, such work has been relatively neglected in recent years in this country. Recent advances in chromatography and in microspectrometric methods of structure analysis coupled with an increased reliance upon bioassay-directed fractionation present unusual opportunities for progress today. This, combined with a renewed enthusiasm for novel structures as potential pharmacodynamic agents, provided the inspiration for this review. Primary emphasis has been placed upon work appearing in the last 3 years in which the structure of the active agent is known. Because of adequate coverage elsewhere in this volume, hormones, antitumor agents and antibiotics from traditional sources have not been included. Even so, the remaining coverage is selective because of space consideration.

Natural products are the end result of enzymatic manipulation from normal primary metabolic pools and, as such, are inherently more likely to interact successfully with other biopolymers, such as receptors, than many of the compounds produced as the result of purely chemical imagination. Because bioactive natural products have been elaborated evolutionarily for other purposes, the average natural product is unlikely to be a clinically useful drug in its own right but rather serves as a chemical clue which, using modern principles of drug design and lead refinement, can lead to exciting new series for study and clinical development.

Non-Steroidal Antiinflammatory Agents - A number of leads, usually with inadequate pharmacological work-up, are available from higher plants. These compounds are of diverse structure, including terpenes (barbatosides A and B (1) from Dianthus Barbatus,[1] helenalin (2) from Eupatorium formosannum,[2] (-)-α-bisabolol (3) from camomile),[3] phenols (clitoriacetal (4) from Clitoria macrophylla),[4] acids (isoferulic acid (5) from Cimicifuga dahurica)[5] and alkaloids (taspine (6) from Croton lechleri[6] and ephedroxane (7) from Ephedra intermedia).[7] It is interesting to note that the majority of these leads enjoyed a folkloric reputation prior to scientific study. It is also interesting that ephedroxane was a well-known synthetic chemical prior to its discovery using bioassay-directed fractionation methods. From the limited amount of testing data available, taspine (6) appears to be the most potent of this group of agents, being 3-4 times more active orally than phenylbutazone vs carageenin-induced pedal edema. The use of α-methylenelactonic sesquiterpenes, such as helenalin (2), as antiinflammatory agents would seem to be precluded by their well-known activity as allergic contact dermatitis causing agents.[8]

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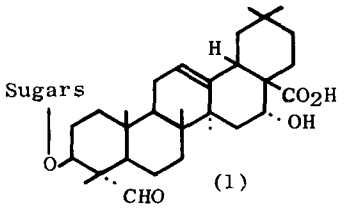

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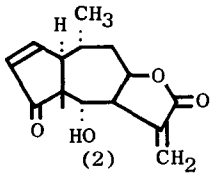

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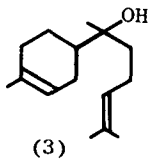
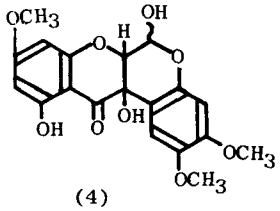


(3)



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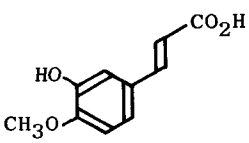

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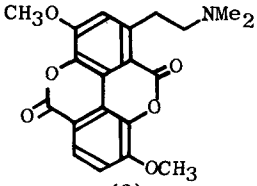

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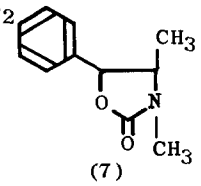
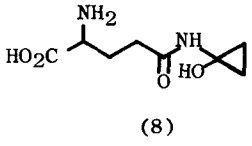


(7)

Antabuse-like Activity - Given the chronic problem many nations face with alcohol abuse and recent problems associated with the use of disulfuram, the finding that coprine (8), isolated from the inky cap mushroom Coprinus atramentarius, also interferes with alcohol dehydrogenase mediated alcohol metabolism has attracted considerable interest.[9,10]



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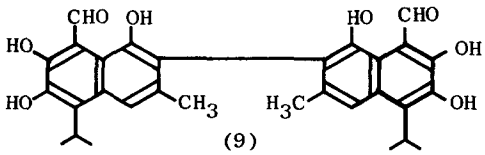

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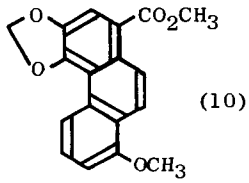
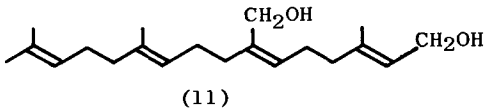


(10)

Antifertility Agents - Higher plant extracts frequently possess antifertility activity.[11,12] Recently, great interest was aroused by reports emanating from The Peoples Republic of China that gossypol (9), from cotton seed oil and from Thespesia populnea, inhibits sperm formation 4-5 weeks after oral administration is begun, without depressing testosterone levels. Use, therefore, as a male contraceptive is contemplated.[13] Aristolic acid and methyl aristolate (10) from the roots of Aristolochia indica show oral abortificient activity in experimental animals. It is more effective in mice than in rabbits.[14]

Antiulcer - Recent papers have drawn attention to the potential antiulcer activity of terpenes or widely different molecular complexity. A Thai medicinal plant, Croton sublyratus, contains (E,Z,E)-7-hydroxymethyl-3,11,15-trimethyl-2,6,10,14-hexadecatetraen-1-ol (18-hydroxygenanyl-geranol, 11) which is potent against rat Shay-ulcer and reserpine-induced ulcer in mice.[15] The diterpenes, plaunol A (12) and B (13), from the



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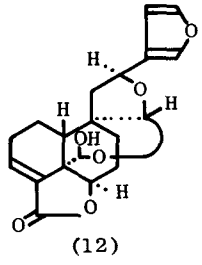

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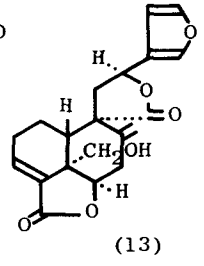
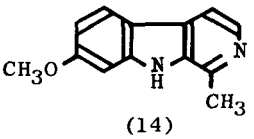


(13)

same species possess the same type of activity. Plaunol B is considerably more active than A.[16]

Cardiovascular Agents - It is generally accepted that adenosine plays an important role in the regulation of coronary blood flow even though the precise mechanism is not clear.[17] Therefore it is interesting to note that the major asystolic agents in the marine sponge Dashychakina cyathina turn out to be adenosine and 2'-deoxyadenosine.[18] The well-known indole alkaloids harmine (14), harmaline (15) and harmalol (16) from Peganum harmala have profound effects upon the heart[19] and the benzyltetrahydroisoquinoline alkaloid higenamine (17), from Aconitum japonicum, shows chronotropic activity.[20] A diterpene, forskolin (18), from Coleus forskolilii, has hypotensive activity as a consequence of its potent positive inotropic activity and vasodilator properties.[21]



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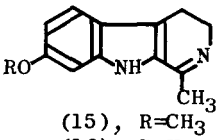
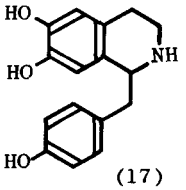


(15), R=CH_3
(16), R=H



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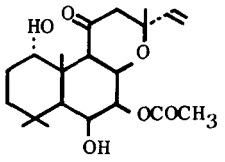
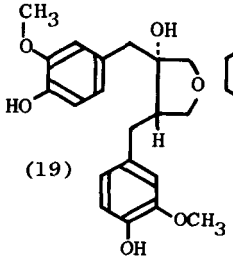


(18)

CNS Depressants - A lignan, (+)-nortrachelogenin (19), from Wikstroemia indica prolongs thiopental sleeping-time in mice and antagonizes methamphetamine action in rabbits.[22] The terpenes β-eudesmol (20) and hinesol (21) were isolated from Atractylodes lancea, A. ovata and A. japonica and proved to be the weakly potent CNS depressant agents in the crude drug "Zhu".[23] Among the alkaloids, gentianine (22), an isolation artifact derived from swertiamarin in studies upon Swertia japonica, shows moderate CNS depressant activity as well as antiulcerogenic activity when given p.o. or i.d. to mice.[24] Dactylyne (23), an unusual



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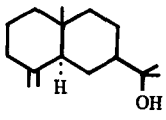

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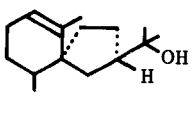

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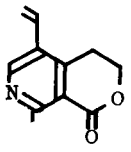

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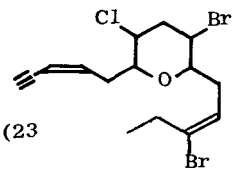
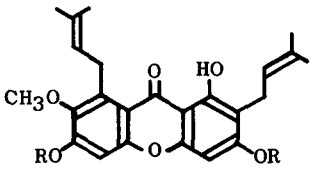


(23

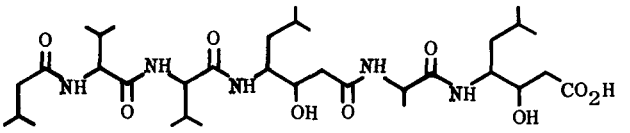
halogenated acetylene derivative isolated from the sea hare, Aplysia dactylomela, significantly potentiates pentobarbital sleep time when given i.p. to mice at 25 mg/kg. This was traced to inhibition of the metabolism of the barbiturate.[25] Finally, the xanthone diglucoside, mangostin-3,6-di-O-glucoside (24) isolated from Garcinia mangostana (mango) depresses CNS function as well as raises blood pressure.[26]

Diuretic - Pepstatin (25), an inhibitor of several acid proteases, has been investigated as a potential antiulcer and antihypertensive agent. Recently, it has been shown to be strongly diuretic at 80 mg/kg in mice when given in a single subcutaneous injection. This may be due to aldosterone antagonism by an indirect mechanism involving antagonism of

renin.[27] It is interesting to note that pepstatin was isolated from the fermentation products of a Streptomycete as part of a productive program which searches for pharmacodynamic agents other than antibiotics and antitumor agents from microorganisms.



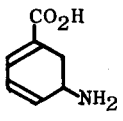
(24), R=β-D-glucosyl



(25)

Enzyme Inhibitors - Fermentation products of Streptomycetes continue to be fruitful sources of specific enzyme inhibitors of potential clinical value. One of the more important is gabaculine (26), from Str. toyocaensis, an inhibitor of the inhibitory neurotransmitter GABA.[28] p-Hydroxyphenylacetaldoxime (27) was isolated from Str. nigellus and shown to inhibit β-galatosidase, a widely distributed enzyme of uncertain biological function.[29]

Hypoglycemic Agents - Carboxyatractylate, 28, (LD_{50} = 10.7 mg/kg i.p. in mice), was found to be responsible for the hypoglycemic action of cocklebur extracts (Xanthium strumarium).[30]



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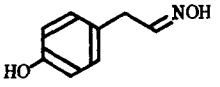

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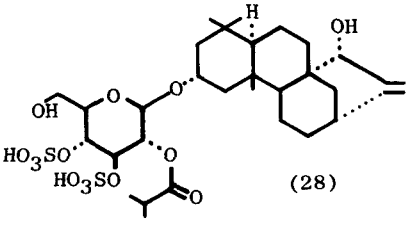
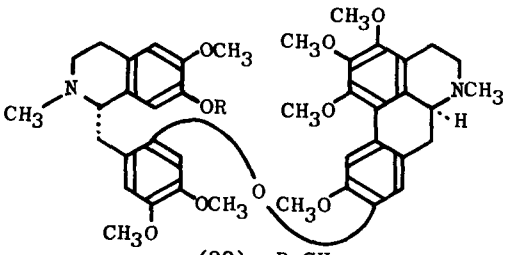


(28)

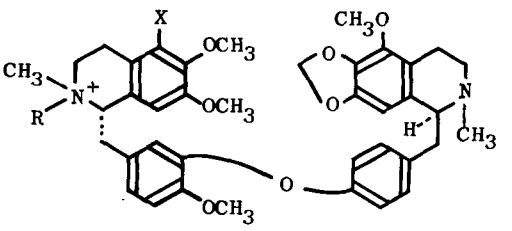
Hypotensive Agents - Many alkaloids have shown hypotensive activity over the years, and an intensive investigation of the genus Thalictrum has added many new agents to the list.[31-34] A number of these agents, notably thaliglucinone (37), also possess antimicrobial activity. The hypotensive alkaloids recently characterized are adiantifoline (29) and thaliadamine (30) from Thalictrum minus;[31] thaliracebine (31) and thalirabine (32) from a second study of T. minus;[32] thalicarpine (33), thalidasine (34), O-methylthalicberine (35), thalrugosamine (36), thaliglucinone (37) and thalphenine (38) from T. revolutum;[33] thaliglucinone (37) from T. longistylum;[34] staurosporine (39) from a Streptomyces species;[35] and ephadrine A (40) and B (41) from Ephedra vulgaris.[36]

Streptomyces species generally do not contain alkaloids in the classical sense. Staurosporine (39) is exceptional, and, in addition to its antimicrobial activity, is also strongly antihypertensive. Ephedra vulgaris has been extensively examined, yet continues to provide new alkaloids among which

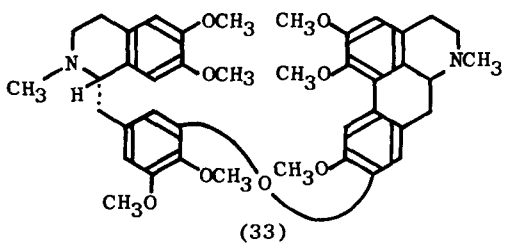
ephadrine A (40) is significantly hypotensive i.v. in mice (1.5-1.8 mg/kg).



(29), R=CH_3
(30), R=H



(31), R=CH_3, X=O
(32), R=X=H



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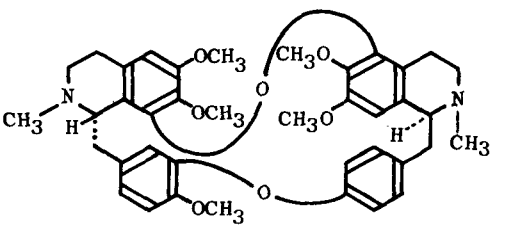

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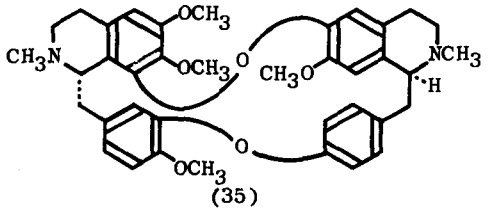

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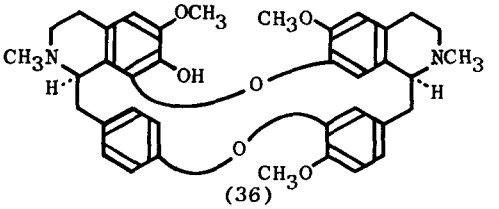

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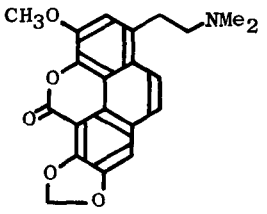

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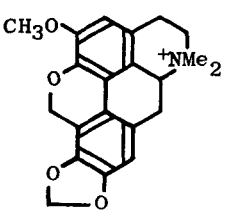

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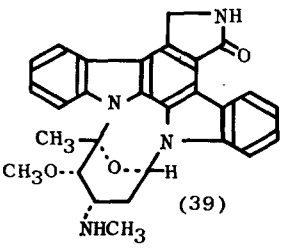

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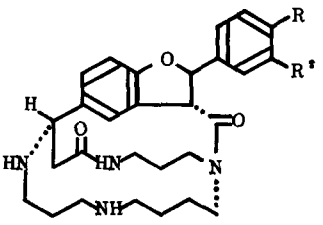
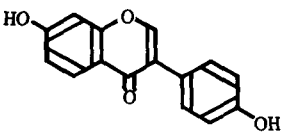


(40), R=R'=H
(41), R=OH, R'=OCH_3



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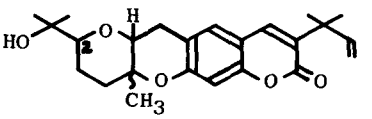
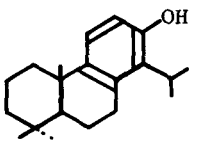


(43)

Spasmolytic - Daidzein (42), an isoflavone from Pueraria tuberosa and other plants, shows about a third of the spasmolytic effect of papaverine on mouse small intestine preparations,[37] and the more complex terpenoidal coumarins, clausmarins A and B (isomers at C2, 43) from Clausena pentaphylla, are potent spasmolytic agents in experimental animals.[38]

Hypocholesterolemic Agents - Totarol (44), from Thujopsis dolabrata, reduces blood levels about 27% when added at about 0.1% to a cholesterol enriched diet and fed to rats.[39] Several other terpenes are active -- notably abietic acid (45) -- presumably all by interfering with cholesterol absorption from the intestine. Breynin A and B, glycosides of breynogenin (46) of Breynia officinalis,[40] were effective in rats when given i.p. but they were also rather toxic (0.2-0.5 mg/kg). The fungus, Pythium ultimum produced an agent which interfered with hepatic cholesterol biosynthesis without hepatomegly. Fractionation showed this agent to be citrinin (47),[41] well known as a toxic antibiotic but not previously known to have this activity. A similar investigation of the metabolites of Penicillium citrinum led to three related metabolites, ML-236A (48), ML-236B (49) and ML-236C (50), of which B is the most potent inhibitor of in vitro cholesterol biosynthesis. Compound B is also active in rats.[42]



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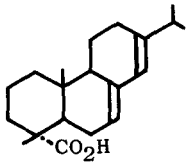

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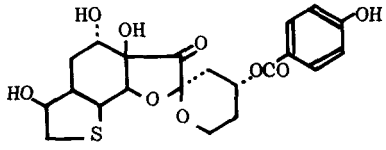
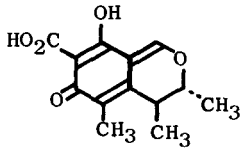


(46)

Antimuscarinics - Gephrytoxin (51), an acetylenic alkaloid from the skin of the Colombian frog Dendrobates histrionicus, joins the group of exotic neuroactive natural products from neotropical frog extracts which possess relatively potent muscarinic antagonist properties.[43]



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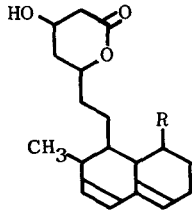
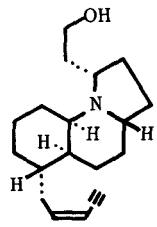


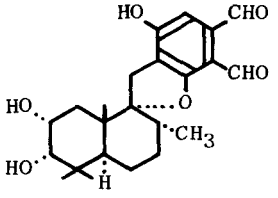
(48), R=OH
(49), R=OCOCH(CH_3)CH_2CH_3
(50), R=H



(51)

Immunosuppressive Agents - K-76 (52) is a compliment inhibitor from Stachybotrys complimenti which might be useful in immune-complex diseases and, indeed, appears to improve the symptoms of experimental glomerulonephritis.[44] Gamma-L-Glutaminyl-4-hydroxybenzene (53) was isolated from the edible mushroom Psalliota bisporous[45] and shown to inhibit DNA synthesis in human lymphocytes stimulated with phytohemagglutinin. It is active at 4 mcg/ml, not very toxic (LD_{50} = 5 g/kg), and is now being tested for its ability to delay skin homograft rejection.

Antifungal Agents from Higher Plants - Antifungal agents are widely distributed among the higher plants, especially agents elaborated in response to fungal attack (phytoalexins -- suicide metabolites). This accounts for the relative resistance many plants exhibit to fungal invasion. Unfortunately, few of these agents have been tested against human pathogens and almost none have been studied in animal models.



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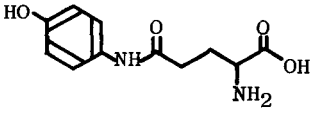

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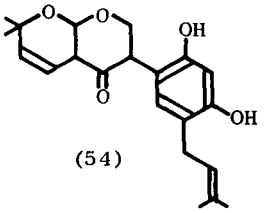
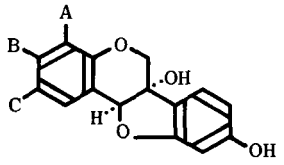
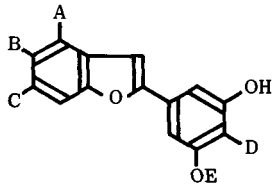


(54)

Among the many agents recently characterized are cajanone (54) from Cajanus cajan;[46] glyceollin I (55), II (56), III (57), IV (58) from Glycine max;[47] moracin A (59), B (60), C (61) and D (62) from Morus alba;[48] canthin-6-one (63) from Hibiscus syriacus;[49] tryptanthrin (64) from Strobilanthes cusia;[50] canellan (65) from Canella winterana;[51] carpesiolin (66) from Carpesium abrotanoides;[52] Ptilonia metabolite (67) from Ptilonia australasica;[53] and uliginosin A (68) from Hypericum uliginosum.[54] Tryptanthrin has a folkloric reputation in Taiwan and is active against athelete's foot infections.



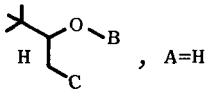


(55),  , C=H

(59), A=C=OCH_3, B=D=E=H

(56),  , A=H

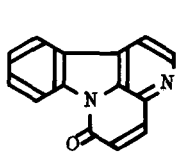
(60), A=D=H, B=OH, C=OCH_3, E=CH_3

(57),  , A=H

(61), A=B=H, C=OH, 

(58), A=H, B=OCH_3, C=CH_2 

(62), A=B=E=H, C=OH, D= CH_2 



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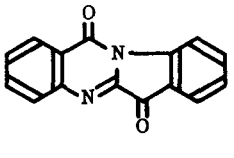

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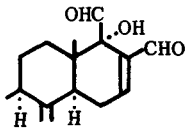

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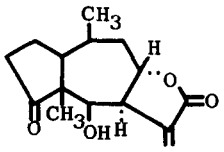

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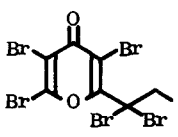

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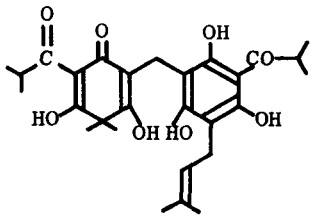
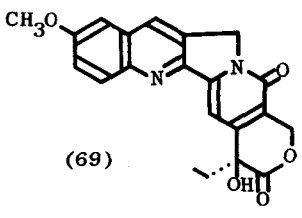
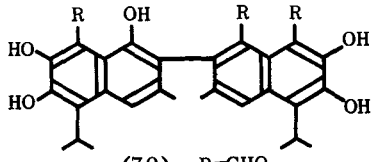


(68)

Antiviral Agents From Higher Plants - Antiviral activity is not often reported from higher plants and is rarely pursued beyond the in vitro stage. Recently, 10-methoxycamptothecin (69), from Ophiorrhiza mungos, has been shown to inhibit herpes virus in vitro,[55] and gossypol (70), and its less toxic deformylation product (71), from the pigment gland of cotton seed, have shown activity in mice against influenza virus.[56] Compound 69 had previously been known as an antileukemic agent.[57]

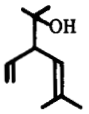


(69)



(70), R=CHO
(71), R=H

Antibacterial Agents from Higher Plants - Antibacterial compounds are widely distributed in the plant kingdom.[58] Unfortunately, the agents are rarely evaluated in vivo. The active agents are often new to the literature but usually belong to series structurally familiar to natural products chemists. The mildly antiseptic activity of alcohols (such as santolinol (72, from Artemesia herba-alba[59]) and simple phenolics (atylosol (73) from Atylosia trinervia);[60] pisiferic acid (74) from Chamaecyparis pisifera [the carboxy group is incidental since ferruginol (75) is more potent than 74];[61] xylopic acid (76) from Xylopia aethiopica;[62] various xanthones from Canscora decussata, of which 77 and 78 are 0.1 x as active as streptomycin vs Mycobacterium tuberculosis;[63] flavanoids; chalcones from Uvaria chamae, of which uvaretin (79) and dichamanetin (80) are representative, potent, and broad spectrum[64]; and the flavanone glabrol (81), the most potent principal of Glycyrrhiza glabra[65], has been known for a long time. The more complex members are more selective and more potent suggesting that further investigation would be rewarding. A number of alkaloids, such as the bisbenzylisoquinolines hernandezine (82), thalidezine (83) and thalistyline (84) from Thalictrum podocarpum[66] and the isolation artifacts from Hunnemannia fumariaefolia (the apparent prodrug pseudoalcoholates (85, 86) and nitrile (87) of sanguinarine[67]), are broad spectrum and representative. Compounds 85-87 are dramatically more potent than sanguinarine itself. Rounding out this group is a sentimental favorite, louisfieserone (88) from Indigofera suffruticosa.[68]



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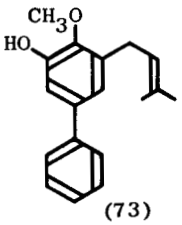

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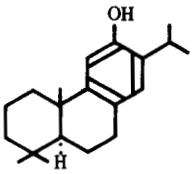
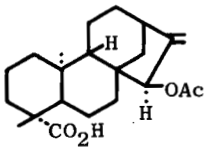


(74), R=CO_2H
(75), R=CH_3



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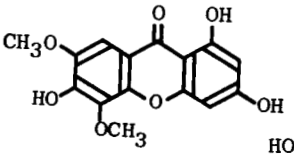

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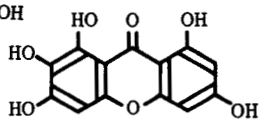

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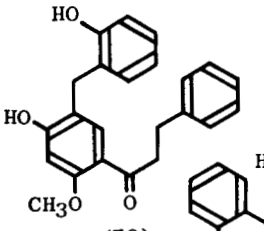

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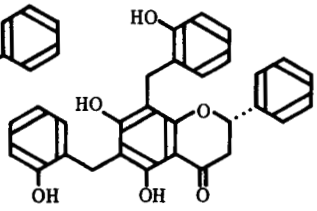

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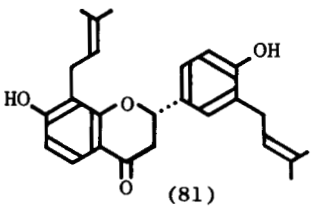

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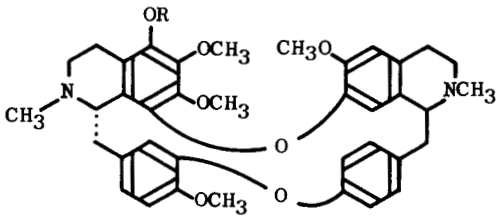
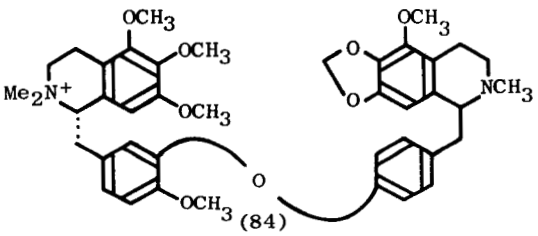
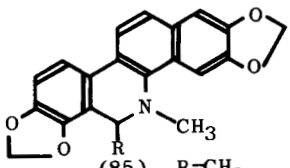


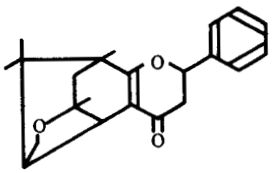
(82), R=CH_3
(83), R=H



(84)



(85), R=CH_3
(86), R=C_2H_5
(87), R=CN



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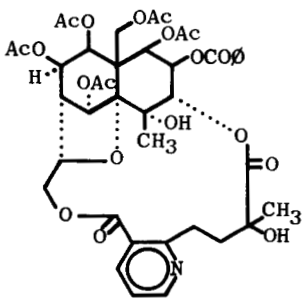

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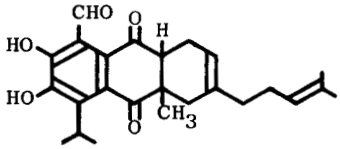
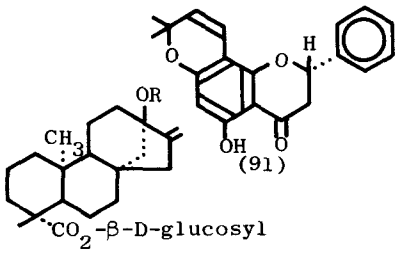


(90)

The antipseudomonal activity of ferruginol and xylopic acid, the antitubercular activity of uvaretin and the canscora xanthones and the potency of glabrol are of particular interest.

Miscellaneous - A heterogeneous pharmacological and structural group remains. Unfortunately, spatial restrictions prevent a detailed discussion of these compounds. Several of the more interesting substances falling into this category show promise of opening new areas of investigation, such as, for example, sclerosporin (97) and trigonelline (92).

Reason for Interest or Pharmacological Action	Name	Source
Insecticide	wilfordine (89)	Euonymous alatus[69]
Insecticide	helicoside H3 (90)	Gossypium hirsutum[70]
Piscicidal	obovatin (91)	Tephrosia obovata[71]
Antimitotic Hormone	trigonelline (92)	Pisium sativum[72]
Sweetening Agent	stevioside (93)	Stevia rebaudiana[73]
Sweetening Agent	rebaudioside A (94)	Stevia rebaudiana[74]
Sweetening Agent	dulcoside A (95)	Stevia rebaudiana[75]
Plant Growth Regulator	hydroxyencomic acid (96)	Cattleya trianaei[76]
Sporogenic in Fungi	sclerosporin (97)	Sclerotina fruiticola[77]
Antihepatatic Agent	unnamed (98)	Fructus schizandrae[78]
Chiral Prostaglandin Synthon	aucubin (99)	Aucuba japonica[79]
Chiral Prostaglandin Synthon	terrein (100)	Aspergillus fischeri[80]
Adriamycin synthon	aloe-emodin (101)	Aloe sp.[81]



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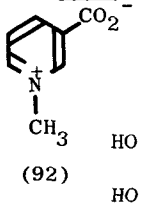

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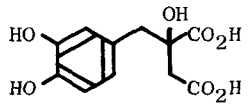
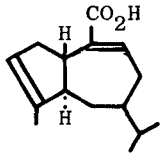
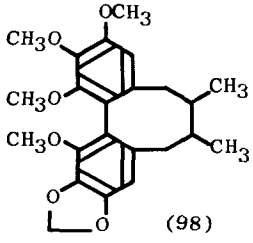


(96)

(93), R=β-D-glucosyl(1,2)-β-D-glucosyl
(94), R=β-D-glucosyl(1,2)-β-D-glucosyl(1,3)-β-D-glucosyl
(95), R=β-D-glucosyl



(97)



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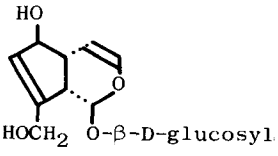

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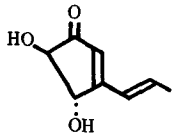

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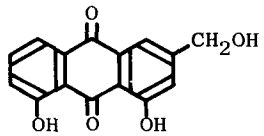

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References

1. G.A. Cordell, R.L. Lyon, H.H.S. Fong, P.S. Benoit and N.R. Farnsworth, The Journal of Natural Products, 40, 361 (1977).
2. I.H. Hall, K.H. Lee, C.O. Starnes, Y. Sumida, R.Y. Wu, T.G. Waddell, J.W. Cochran and K.G. Gerhart, J. Pharm. Sci., 68, 537 (1979).
3. V. Jakovlev, O. Isaac, K. Thiemer and R. Kunde, Planta Medica, 35, 125 (1979).
4. H. Taguchi and P. Kanchanapee, Chem. Pharm. Bull., 25, 1026 (1977).
5. M. Shibata, Y. Yamatake, Y. Amagaya and M. Fukushima, Yakugaku Zasshi, 95, 539 (1975).
6. G.P. Perdue, R.N. Blomster, D.A. Blake and N.R. Farnsworth, J. Pharm. Sci., 68, 1 (1979).
7. C. Konno, T. Taguchi, M. Tamada and H. Hikino, Phytochemistry, 18, 697 (1979).
8. E. Rodriguez, M.O. Dillon, T.J. Mabry, J.C. Mitchell and G.H.N. Towers, Experientia, 32, 236 (1976).
9. P. Lindberg, R. Bergman and B. Wickberg, J. Chem. Soc., Chem. Commun., 946 (1975).
10. G.M. Hatfield and J.P. Schaumberg, J. Nat. Products, 38, 489 (1975).
11. N.R. Farnsworth, A.S. Bingel, G.A. Cordell, F.A. Crane and H.H.S. Fong, J. Pharm. Sci., 64, 535 (1975).
12. N.R. Farnsworth, A.S. Bingel, G.A. Cordell, F.A. Crane and H.H.S. Fong, J. Pharm. Sci., 64, 717 (1975).
13. Anon., Chem. Engineering News, 33 (1979); J.D. Edwards et al., J. Am. Chem. Soc., 80, 3798 (1958); S.C. Datta et al., Ind. J. Chem., 10, 263 (1972); T.J. King and L.B. deSilva, Tet. Letters, 261 (1968); and K.N. Campbell et al., J. Am. Chem. Soc., 59, 1723 (1937).
14. A. Pancrashi and C. Shaha, Experientia, 34, 1192 (1978). A. Pancrashi and B. Chakrabarty, ibid., 1977; and S.C. Pakrashi, P.P. Ghosh, D.S. Basu and B. Achari, Phytochem., 16, 1103 (1977).
15. A. Ogiso, E. Kitazawa, M. Kurabayashi, A. Sato, S. Takahashi, H. Noguchi, H. Kuwano, S. Kobayashi and H. Mishima, Chem. and Pharm. Bull., 26, 3117 (1978).
16. E. Kitazawa, A. Ogiso, S. Takahashi, A. Sato, M. Kurabayashi, H. Kuwano, T. Hata and C. Tamura, Tet. Letters, 1117 (1979).
17. S. Kalsner, Brit. J. Pharmacol., 55, 439 (1975).
18. A.J. Weinheimer, C.W.J. Chang, J.A. Matson and P.N. Kaul, J. Nat. Products, 41, 488 (1978).
19. D. H. Aarons, G.V. Rossi and R.F. Orzechkowski, J. Pharm. Sci., 66, 1244 (1977).
20. N. Masaki, H. Iizuka, M. Yokota and A. Ochiai, J. Chem. Soc., Perkin I, 717 (1977).
21. S.V. Bhat, B.S. Bajwa, H. Dornauer, N.J. deSouza and H.W. Fehlhaber, Tet. Letters, 1669 (1977).
22. A. Kato and Y. Hashimoto, J. Nat. Products, 42, 159 (1979).
23. J. Yamahara, T. Sawada, T. Tani, T. Nishino, I. Kitagawa and H. Fujimara, Yakugaku Zasshi, 97, 873 (1977).
24. J. Yamahara, T. Knonshima, T. Sawada and H. Fujimura, Yakugaku Zasshi, 98, 1446 (1978).
25. P. N. Kaul and S. K. Kulkarni, J. Pharm. Sci., 67, 1293 (1978).
26. B.R. Pai, S. Natarajan, M. Suguma, L. Kameswaran, D. Shankaranarayan and G. Gopalakrishnan, J. Nat. Products, 42, 361 (1979).
27. H. Esumi, S. Sato and T. Sugimura, J. Antibiotics, 31, 872 (1978).
28. H. Mischima, H. Kurihara, K. Kobayashi, S. Miyazawa and A. Terahara, Tet. Letters, 537 (1976).
29. T. Hazato, M. Kumagai, H. Naganawa, T. Aoyagi and H. Umezawa, J. Antibiotics, 32, 91 (1979).
30. J.C. Craig, Jr., M.L. Mole, S. Billets and F. ElFeraly, Phytochem., 15, 1178 (1976).
31. W. Liao, J.L. Beal, W.-N. Wu, and R.W. Doskotch, J. Nat. Products, 41, 271 (1978).
32. W. Liao, J.L. Beal, W.-N. Wu, and R.W. Doskotch, J. Nat. Products, 41, 257 (1978).
33. W.-N. Wu, J.L. Beal and R.W. Doskotch, J. Nat. Products, 40, 508 (1977).
34. W.-N. Wu, J.L. Beal, R.-P. Leu and R.W. Doskotch, J. Nat. Products, 40, 281 (1977).
35. A. Furusaki, N. Hashiba, T. Matsumoto, A. Hirano, Y. Iwai and S. Omura, J. Chem. Soc., Chem. Commun., 800 (1978).
36. M. Tamada, K. Endo, H. Hikino and C. Kabuto, Tet.Letters, 873 (1979); Heterocycles, 12, 783 (1979).
37. H. Nakamoto, Y. Iwasaki and H. Kizu, Yakugaku Zasshi, 97, 103 (1977).
38. A. Shoeb, M.D. Manandhar, R.S. Kapil and S.P. Papli, J. Chem. Soc., Chem. Commun., 281 (1978).
39. H. Enomoto, Y. Yoshikuni, Y. Yasutomi, K. Ohata, K. Sempuku, K. Kitaguchi, Y. Fujita and T. Mori, Chem. Pharm. Bull., 25, 507 (1977).
40. H. Koshiyama, M. Hatori, H. Ohkuma, F. Sakai, H. Imanishi, M. Ohbayashi and H. Kawaguchi, Chem. Pharm. Bull, 24, 169 (1976).
41. A. Endo and M. Kuroda, J. Antibiotics, 29, 847 (1976).
42. A. Endo, M. Kuroda and Y. Tsujita, Ibid, 1346 (1976).
43. J.W. Daly, B. Witkop, T. Tokuyama, T. Nishikawa and I.L. Karle, Helv. Chim. Acta, 60, 1128 (1977).

44. H. Kaise, M. Shinohara, W. Miyazaki, T. Izawa, Y. Nakano, M. Sugawara, K. Sugiura and K. Sasaki, J. Chem. Soc., Chem. Commun., 726 (1979).
45. T. Mouri, T. Murahara, H. Kayama, S. Tsutsui, T. Kurokawa, Y. Shibata, N. Ishida, S. Kakimoto, F. Asakura, H. Shirahama and T. Matsumoto, Agr. Biol. Chem., 42, 2179 (1978).
46. N.W. Preston, Phytochem., 16, 143 (1977).
47. R.L. Lyne and L.J. Mulheirn, Tet. Letters, 3127 (1978).
48. M. Takasugi, S. Nagao, S. Ueno, T. Masamune, A. Shirata and K. Takahashi, Chem. Letters, 1239 (1978); M. Takasugi, S. Nagao and T. Masamune, Tet. Letters, 797 (1978).
49. M. Yokota, H. Zenda, T. Kosuge and T. Yamamoto, Yakugaku Zasshi, 98, 1508 (1978).
50. G. Honda and M. Tabata, Planta Med., 36, 85 (1979).
51. F.S. El-Feraly, A.T. McPhail and K.D. Onan, J. Chem. Soc., Chem. Commun., 75 (1978).
52. M. Maruyama and S. Omura, Phytochem. 16, 782 (1977).
53. R. Kazlauskas, R. O. Lidgard and R. J. Wells, Tet. Letters, 3165 (1978).
54. T. Meikle and R. Stevens, J. Chem. Soc. Perkin I, 1303 (1978).
55. S. Tafur, J.D. Nelson, D.C. DeLong, and G.H. Svoboda, J. Nat. Products, 39, 261 (1976).
56. P.H. Dorsett and E.E. Kerstine, J. Pharm. Sci., 64, 1073 (1975).
57. M.C. Wani and M.E.Wall, J. Org. Chem., 34, 1364 (1969).
58. M. Ieven, D.A. Vandenberghe, F. Mertens, A. Vlietinck and E. Lammens, Planta Medica, 36, 311 (1979).
59. J. Yashphe, R. Segal, A. Brener and G. Erdreich-Naftali, J. Pharm. Sci., 68, 924 (1979).
60. V.D. Tripathi, S.K. Agarwal and R.P. Rastogi, Phytochem., 17, 2001 (1978).
61. M. Fukui, K. Koshimizu and M. Egawa, Agr. Biol. Chem. Tokyo, 42, 1419 (1978).
62. K. Boakye-Viadom, N.I.Y. Fiagbe and J.S.K. Ayim, J. Nat. Products, 40, 543 (1977).
63. S. Ghosal and R. K. Chaudhuri, J. Pharm. Sci., 64, 888 (1975); S. Ghosal, K. Biswas and R.K. Chaudhuri, Ibid., 67, 721 (1978).
64. C.D. Hufford and W.L. Lasswell, Jr., J. Nat. Products, 41, 156 (1978).
65. L.A. Mitscher, Y.H. Park, S. Omoto, G.W. Clark, III, and D. Clark, Heterocycles, 9, 1533 (1978).
66. W.-N. Wu, J.L. Beal, R.-P. Leu, and R.W. Doskotch, J. Nat. Products, 40, 384 (1977).
67. L.A. Mitscher, Y.H. Park, D. Clark and G.W. Clark, P.D. Hammesfahr, W.-N. Wu and J.L. Beal, J. Nat. Products, 41, 145 (1978).
68. X.A. Dominguez, C. Martinez, A. Calero, X.A. Dominguez, Jr., M. Hinojosa and A. Zanudio, Tet. Letters, 429 (1978).
69. K. Yamada, Y. Shizuri and Y. Hirata, Tetrahedron, 34, 1915 (1978).
70. R.D. Stipanovic, A.A. Bell, D.H. O'Brien and M.J. Lukefahr, Phytochem., 17, 151 (1978).
71. Y.-L. Chen, Y.-S. Wang, Y.-L. Lin, K. Munakata and K. Ohta, Agr. Biol. Chem., 42, 2431 (1978).
72. D.G. Lynn, K. Nakanishi, S.L. Patt, J.L. Occolowitz, S. Almeida and L.S. Evans, J. Am. Chem. Soc., 100, 7759 (1978).
73. S. Kamiya, F. Konishi and S. Esaki, Agr. and Biol. Chem., 43, 1863 (1979).
74. H. Kohda, R. Kasai, U. Yamasaki, K. Murakami and O. Tanaka, Phytochem., 15, 981 (1976).
75. M. Kobayashi, S. Horikawa, I.H. Degrandi, J. Ueno and H. Mitsuhashi, Phytochem., 16, 1405 (1977).
76. M. Ishii, S. Uemoto, K. Fujieda, M. Nonaka, Y. Shoyama, Y. Miyahara and I. Nishioka, Phytochem., 18, 1211 (1979).
77. S. Marumo and M. Katayama, Abstr. A. C. S. / C. S. J. Chem. Congr., Honolulu, Ha, ORGN 338 (1979).
78. P. Tien-tung, Chinese Med. J., 3, 173 (1977).
79. M. Naruto, K. Ohno, N. Naruse and H. Takeuchi, Tet. Letters, 251 (1979).
80. L. A. Mitscher, G.W. Clark, III and P.B. Hudson, Tet. Letters, 2553 (1978).
81. J. Alexander, A.V. Bhatia, G.W. Clark, III, A. Leutzow, L.A. Mitscher, S. Omoto and T. Suzuki, J. Org. Chem., 45, 24 (1980).

Chapter 28. Pharmacophore Identification and Receptor Mapping

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Introduction - It is generally acknowledged that the unique stereospecificity seen in biological systems is due to a complementary three-dimensional interaction between a drug and an asymmetric receptor site. While the dynamic process of drug-receptor recognition probably involves multiple steps culminating in activation of the receptor, one assumes a unique arrangement of electron density responsible for triggering the coupled response analogous to a transition state, which is common to all the drugs capable of activating that response. The determination of this three-dimensional pattern or pharmacophore (see review by Gund[1]) can ideally best be characterized by the crystal structure of the drug-receptor complex. The general problem facing the medicinal chemist, however, forces an indirect approach due to lack of characterization of the receptor. The concept of a pharmacophore and pharmacological evidence that a set of compounds interact with a unique receptor provide an opportunity to test different pharmacophores for consistency and possible uniqueness.

While in vivo activity can be misleading due to multiple mechanisms besides differences in transport, distribution or metabolism, augmentation by in vitro data and particularly receptor-binding studies can provide sufficient evidence to justify the assumption of a specific receptor interaction. Conformational flexibility of most molecules, providing a mutable three-dimensional arrangement of essential functional groups, has precluded identification of the pharmacophore in most systems. This flexibility has been generally ignored and focus on the energetically most stable conformer has clouded the issue. Whether the methodology is theoretical, crystallographic, or spectroscopic, the studied environment of the molecule (vacuum, crystal, or solution) generally neglects the perturbation to the conformational ensemble by interaction with the receptor itself. Only by determining the manifold of conformers within energetic reach of the perturbations due to receptor interaction can the necessary possible pharmacophores be considered. This necessitates determination of the conformational space available to each molecule and comparison of the multitude of different pharmacophores which each molecule is capable of presenting rather than limitation to an arbitrary conformer. This complexity implies the use of state-of-the-art computational methods and interactive 3D molecular graphics and will be the focus of this review.[2]

Pharmacophore Identification - The traditional approach applied to determine the pharmacophoric groups is a comparative structural analysis of a set of drugs in relation to their biological activity. Once the essential features have been identified, new compounds are synthesized and assayed to test the correlations. Conformationally constrained compounds certainly facilitate this method as they simplify the problem of identifying the 3D patterns required for interaction at the receptor. One should, however, use even such rigid compounds with caution, as the structure-activity relationships can be misleading. For

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example, it has been shown that the "rigid" pentacyclic structure of morphine, the natural opiate taken as the reference molecule in most SAR studies, still has a certain degree of flexibility.[3,4] A pharmacophore deduced from its crystal structure could, therefore, be quite misleading if this degree of freedom was not taken into account.

Crystal structures are widely used in whole or in part in conformational studies applied to drug design.[3,5-8,27] The X-ray diffraction method remains the most accurate and easily accessible approach to describe the spatial molecular organization. One should, however, be aware of the limitations inherent to the crystalline state of the sample studied as they direct considerably the conformation observed.[3] Reviews have been presented which describe the current developments in medicinal chemistry of quantum mechanical approaches.[9-11] A need for generally applicable conformational studies capable of inclusion of larger numbers of geometric degrees of freedom still exists. Most of these methods suffer from a focus on the determination of the most probable conformation(s); the implicit assumption being that the energetically preferred conformer is the biologically active one. Examples are given where the simultaneous use of X-ray diffraction, conformational energy calculations, nuclear magnetic resonance and synthesis led to the establishment of conformation-activity relationships[5,7] and pharmacophore determination.[6] In a conformational analysis of convulsant and anticonvulsant barbiturates,[12] a prescreening of the conformational space used empirical energy calculations. The minima selected in a range of 10 kcal/mole above the global minimum were further refined with a quantum mechanical method. Of two low energy conformations, one is consistent with the SAR data in representing the convulsant conformation.

The use of distance geometry in pharmacophore identification is being explored.[13-17] This methodology has been developed primarily in dealing with protein conformations.[18-19] We have found it useful in limiting the areas of conformational space to be systematically examined for candidate pharmacophores. Determination of the set of possible conformations for the most sterically constrained molecule with the least number of variables places limits which translate into distance constraints limiting the possible pharmacophores for subsequent molecules. These distance constraints can be used to effectively truncate the combinatorial search tree for other more flexible molecules, eliminating much of the computation. Since the object is to determine if overlap in pharmacophoric patterns between a set of molecules can occur, conformations which cannot satisfy all the constraints for the set of molecules are not of interest. The construction of a distance range matrix for each molecule aids in determining possible pharmacophoric groups.[20] A matrix of the maximum and minimum pair-wise distance between atoms is constructed analytically through use of distance geometry. One can limit the search to the range consisting of the smallest maxima and the largest minima which contains all possible distances common to the set of molecules. Intelligent selection of test compounds can maximize the inherent geometrical constraints and reduce large problems to manageable size. Distances are 2D parameters which cannot characterize the stereospecificity of molecules. One additional bit of information is necessary to distinguish the handedness of the molecule (i.e. the sign of the vectorial cross-product of 3 pharmacophoric groups using the center of mass as the origin).

Once a plausible pharmacophore has been proposed then the

conformational properties of all active compounds can be analyzed to investigate their ability to assume this pharmacophore. A constrained minimization would provide the answer in certain cases where restriction of conformational flexibility exists. In most cases, however, more than one conformer can satisfy the pharmacophore and a systematic search of conformational space is required. Such a method of systematically exploring conformational space has been developed in our laboratory.[21] The receptor-bound conformers for all active analogs can be extracted from these analyses. An excellent discussion of the problems of global minimum energy determination, as well as a strategy for determining all low-energy conformations of cyclic peptides, is presented by White and Monroe.[22] Systematic search algorithms have also been incorporated into an internal modeling system at Rohm and Haas by Stuper et al.[23] Nordby and co-workers[24,25] have developed a molecular modeling system which utilizes a combination of systematic search and energy minimization. Determination of the family of possible conformations compatible with the pharmacophore criterion was compared[25] with the total conformational ensemble to determine the probability of occurrence of conformers presenting the phamacophore. This in turn was quantitatively correlated to the biological activity and a distinction was made between analgesic and anti-diarrheal activities for opiate-like compounds.

An elaboration of the distance geometry approach has recently been demonstrated by Crippen to examine binding of inhibitors of chymotrypsin and dihydrofolate reductase.[26] By representation of both a hypothetic receptor site and the ligands as sets of points with conformations given by distance matrices, the rigid body translation and rotation calculations involved with fitting are avoided as a distance matrix is invarient under translation and rotation. Variation of the interaction between the ligands and the site geometry is continued until convergence between the measured binding energies of the ligands and the calculated binding constants. The combinatorial aspects of this approach are also noteworthy in that only 22 of 68 dihydrofolate inhibitors were evaluated due to computational restrictions. In addition, 36 geometric and energetic parameters were used to fit 22 binding energies, while Hansch et al. fit all 68 compounds with 6 parameters with similar accuracy.[27] The advantage of course is the ability to deal with non-congeneric series as well as the physically relevant hypothesis concerning receptor site geometry which is generated as shown in Figure 1.

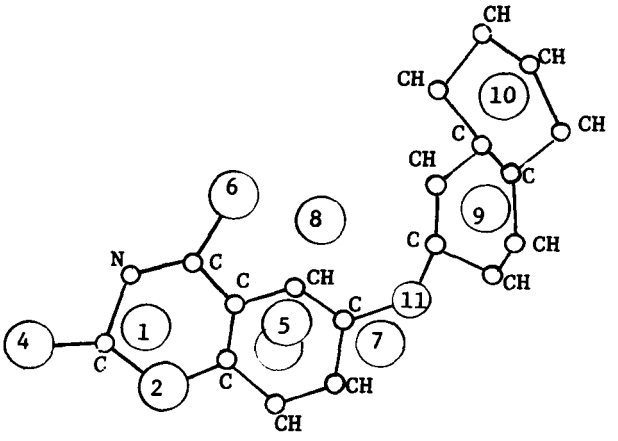


Figure 1. Proposed dihydrofolate reductase binding-site geometry with inhibitor bound.[26] Large spheres are the locations of site points and the small spheres are the nonhydrogen atoms of the ligand connected by bonds.

Besides the distance range methods, manipulation of the data derived from the systematic search for conformational space allows transformation into relative distance space or orientation space where 3D maps of the allowed relative positions of functional groups can be created (Fig. 2).[28]

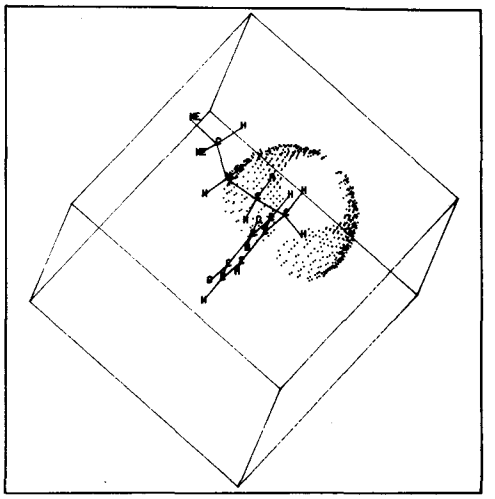


Figure 2. Relative position map of possible positions of Nitrogen of N-isopropyl-dopamine relative to ring. One conformer shown imposed on map to indicate frame of reference.

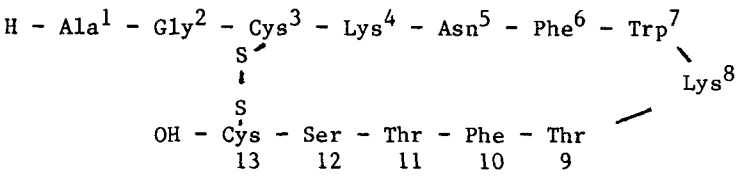
This allows comparison of conformational data from molecules of different molecular structure (non-congeneric series) in which direct comparison of torsional variables is impossible. Intersection of orientation maps derived from active analogs, in which appropriate correspondence between required functional groups has been made, allows determination of the precise 3D arrangement of such groups available in common (i.e. potential pharmacophores). In the case of common molecular fragments which provide an internal molecular coordinate system, the data can be processed to produce relative coordinate maps in which the coordinate position of a group such as a lone pair is plotted relative to the internal frame for each sterically allowed conformer. This procedure can be very useful to evaluate addition of other groups to those already identified as defining the pharmacophore.

When faced with a set of flexible molecules with too large a number of variables, one must turn to chemical modification to reduce the number of variables to a manageable set. Replacement of the α-proton of an amino acid by a methyl group reduces the number of possible combinations of values for the ϕ and ψ torsional rotation to essentially two.[29] Introduction of a cyclic constraint in a linear flexible molecule reduces the degrees of freedom by five.[30] Recent examples of this approach to determining the receptor-bound conformation of peptide hormones can be found with somatostatin,[31-32] LHRH,[33] bradykinin,[34] angiotensin[34] and tuftsin.[34]

The somatostatin example deserves some discussion (Fig. 3). Replacement of residues with proline which restricts the ϕ torsional rotation resulted in two active analogs, Pro^5 and Pro^{13}. Retention of activity by the $D\text{-}Trp^8$ analog can be rationalized by a type I β-turn for residues 7-10 of somatostatin which results in an equatorial relationship of the Lys and Trp sidechains. Substitution of D-Trp would result in the same relationship if a type II' β-turn were now assumed for residues 7-10. Systematic replacement of Asn-5 and Thr-12 and Phe-6 and Phe-11 by cystine disulfide bridges to stabilize the proposed

conformation at residues 7-10 resulted in analogs with activity at least as great as somatostatin. Simplification of the structure has resulted in a bicyclic heptapeptide cyclo-(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys) in which half of the original residues of somatostatin have been eliminated. This is the first analog obtained which shows suppression of growth hormone release when given orally.

Figure 3. Somatostatin amino acid sequence.



Receptor Mapping - The next logical step following pharmacophore identification is to represent spatially the receptor-bound conformers (or essential conformers) with the aim of describing the complementary receptor shape. The unique volume required by each active drug when presenting the pharmacophore can be mapped. A pseudoelectron density map can be constructed for each bound conformer utilizing Gaussian functions calibrated in terms of Van der Waal's radii. Their graphic representation is then obtained by contouring at levels corresponding to space-filling physical models.[35] The union of the maps of the active compounds determines the excluded volume map, i.e., the logical minimum volume that the receptor must allow for the binding of all the active compounds using the pharmacophore as a common frame of reference for orientation (Fig. 4).

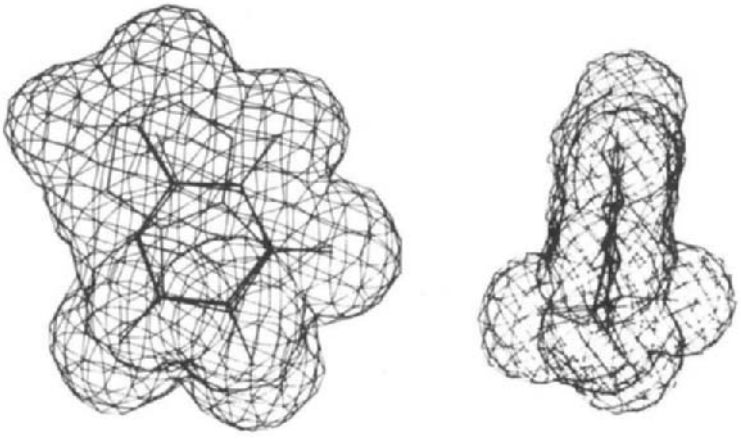


Fig. 4. The receptor-excluded volume in flat (left) or orthogonal projection (right). This volume represents the union of the volume required for alloxan, ninhydrin, D-glucose, D-mannose and their anomers.[51] The darker internal lines represent their interatomic bonds.

This receptor cast can be further refined by using the spatial information contained in the inactive compounds which are capable of presenting the supposed pharmacophore, but which fail to bind to the receptor, presumably because of negative steric interactions. Comparing the electron maps of active and inactive compounds, it is possible to describe the receptor essential volume, i.e., the volumes responsible for preventing binding to the receptor. One can continue to refine this model of receptor shape to be used as a 3D tool for drug design. The

prediction of activity for any new compound can be checked according to the pharmacophore requirements and the receptor-excluded volume model.

Simon et al. have outlined an alternative approach based on the assumption that there exists a general tendency for the affinity to decrease in a linear manner as the volume differences between the receptor site cavity and the molecule increase.[36] A receptor site standard is derived by variation of a starting site based on the most active molecule and correlation with experimental activities. Emphasis on superposition of groups forming strong intermolecular bonds, as well as examination of all conformers within a few kcal/mole of the minimum, are consistent with the claim of correlations for non-congeneric molecules.

In a different approach, the minimization of the total exposed molecular surface area is used as a criterion for detecting the spatial mimesis between several molecules.[37] Limited application of this method to selected areas of molecular correspondence rather than total overlap would appear a more useful approach. Incorporation of molecular flexibility would appear necessary; otherwise, the approach suffers from dependence on some arbitrary choice of conformer.

The description of the electrostatic potential contour maps surrounding the molecules combine 3D structural information with reactivity characteristics of significant parts of the drugs. The electric field created by a molecule in its surroundings defines an "interaction pharmacophore." Drugs sharing the same activity are expected to have similar interaction pharmacophores.[11,38]

Applications:

a) Opiate pharmacophore model - An attempt to use a simplified analgesic pharmacophore to define the receptor-bound conformation of enkephalin has resulted in predictions regarding the activity of enkephalin analogs in which modifications affect backbone conformational possibilities.[39,40] These predictions have been remarkably consistent with the data considering that the criteria were extremely simple.[41] If an analog was capable of assuming the proposed receptor-bound conformation, then the analog was predicted to be active; otherwise, inactivity was predicted. It is somewhat incredulous that this simplified approach has been so successful as it stresses the backbone conformation and not the pharmacophore itself. Recent synthetic studies have yielded active analogs capable of presenting the pharmacophore,[42] but incapable of assuming the backbone conformation. In combination with the increasing evidence for multiple types of opiate and enkephalin receptors,[43] a reevaluation of the analgesic pharmacophore seemed necessary. A extensive study of the SAR applied to the 4-phenylpiperidine (PP) agonist analgesics has been undertaken in order to redesign a pharmacophore model. The PP derivatives can be distinguished according to their conformational properties. The "rigid" compounds are characterized by a polycyclic nucleus and include the following structural families: oripavine, thebaine (hexacyclic), morphine (pentacyclic), morphinan (tetracyclic) and benzomorphan (tricyclic). The "flexible" PP derivatives include the most simplified structures related to meperidine and prodine. The rigid PP present an axial phenyl ring and only the (-)- isomers are active. These chiral properties allow us to characterize an unsubstituted front piperidine edge (Figure 5). The flexible PP on the other hand, usually bear an equatorial

phenyl ring and have shown a stereoselectivity associated with the piperidine edge.[44]

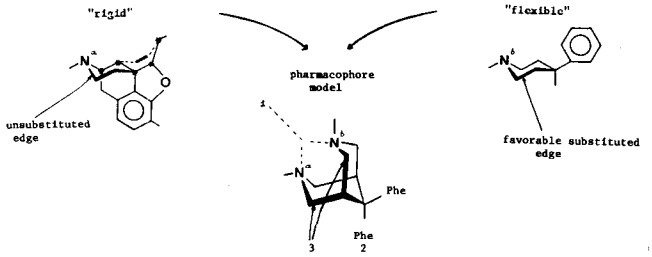


Figure 5: Configurational properties of "rigid" and "flexible" PP used in the description of the pharmacophore model.

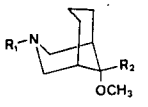
The activity is favored by substitutions introduced on the front piperidine edge. The existence of "hybrid" structures mixing the properties observed in the rigid and flexible PP (figure 6) lead us to suggest a pharmacophore model[45] (figure 5) similar to that of Fries and Portoghese[44] which includes:

1. different spatial orientations of the nitrogen lone pair which interacts with a common site
2. a phenyl ring
3. stereospecific unsubstituted front piperidine edges.

(a) (b)

Figure 6. "Hybrid" PP structures.
(a) phenylmorphan[46,47]
(b) GPA 1657[48,49]

The good activities observed in some azabicyclononane[50] derivatives (figure 7) corroborate the pharmacophore model. A systematic search is being applied to the other flexible opiates (3,3-diphenylpropylamine and 1:2, 1:3 diamines), in order to describe the receptor excluded volume.



R_1	R_2
CH_3	C_6H_5
Phenethyl	C_6H_5
CH_3	phenol
CH_3	methoxyphenyl
phenethyl	phenol
phenethyl	methoxyphenyl
CH_3	furyl

Figure 7. Azabicyclo (3.3.1) nonane derivatives.[50]

b) Pancreatic glucoreceptor - D-glucose is the primary physiological stimulus for insulin release from the islets of Langerhans. This ability is shared with D-mannose, but not other hexoses. Alloxan and ninhydrin both cause an initial release of insulin, followed by irreversible inhibitions of subsequent glucose stimulation. The structures of the two active hexoses were analyzed[51] and found to share common molecular properties with alloxan and ninhydrin suggesting common receptor recognition. This interaction initiates the first phase of insulin release which is followed by covalent binding and irreversible inhibition by alloxan and ninhydrin. A recent report[52] of non-metabolizable analogs of glucose which stimulate insulin release support the glucoreceptor hypothesis.

c) Dopamine receptor - A multiplicity of data suggests different dopamine receptors and the presence in most systems of both pre- and

postsynaptic dopamine sites of actions. This discussion is limited to the action of dopamine on the anterior pituitary where its actions are clearly postsynaptic and inhibit prolactin release. Apomorphine is a full agonist in this assay system. Based on the observation of a potential binding pharmacophore between apomorphine and the dopamine antagonist butaclamol,[53] this hypothetical pharmacophore has been used to examine octoclothepin, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (6,7-ADTN), as well as flexible neuroleptics such as chlorpromazine.[54] The pharmacophore for binding consists of an aromatic ring whose center is approximately 5.1Å from the projection of a nitrogen lying approximately 1Å above the plane of the aromatic ring.[55] All of the semirigid agonists and antagonists examined can be oriented to present this pharmacophore. Agonism appears to require an additional hydroxyl function corresponding to the 11 position of apomorphine when bound. An excluded volume map has been constructed to check the inactive isomers of apomorphine, octoclothepin and butaclamol which are capable of presenting the binding pharmacophore. If any of these inactive isomers fit entirely within the excluded volume map when oriented at the pharmacophore, then either the underlying hypotheses would have to be revised or the pharmacological observations questioned. Each of the three, however, requires novel volume.

These procedures have suggested binding activity for a substituted trans-hexahydrocarbazole, whose crystal structure was available.[56] Activity in both neuroleptic screens and dopamine binding assays confirms this analysis. In addition, suspicions aroused during modeling regarding the optical antipode responsible for the activity of 6,7-ADTN were confirmed by the resolution and characterization of the optical isomers by McDermed *et al.*,[57] who have shown the isomer responsible for activity to have an opposite configuration from apomorphine at the carbon bearing the nitrogen. This allows an orientation of 6,7-ADTN, in which the 7-hydroxyl approximates the position of the 11-hydroxyl of apomorphine when bound, thus explaining the agonist activity.

d) Other Applications - Preliminary studies on the methionine binding site of methionine adenosyltransferase which rationalize the inhibitory activity of rigid bicyclic amino acids and the lack of binding of certain stereoisomers have been presented.[58] The pharmacophore for the glutamic acid binding site, which competes with glutathione to inhibit feeding in *Hydra*, has also been determined and a receptor-bound conformation for glutamic acid proposed.[59] Topographical mapping of the volume of space available for steroid binding at the 3α,20β-hydroxysteroid dehydrogenase (a single enzyme with two activities) from *Streptomyces hydrogenans* was used to help design a suicide substrate for this enzyme.[60]

Conclusions - These studies underline the importance of the 3D nature of the molecular recognition processes. The inclusion of steric volume in the description of the drug-receptor interaction is a realistic improvement resulting from identification of the pharmacophore as a common frame of reference. Identification of the receptor-bound conformation for a series of drugs activating the same receptor enables one to model the receptor cavity. The topographical maps can be useful in predicting the activity of prospective analogs, as well as in directing synthetic work. The combinatorial nature of conformational possibilities implies computer-based molecular modeling as a necessary addition to the arsenal of multidisciplinary methods which the medicinal chemist has to apply in structure-activity studies and drug design. It

is clear, however, that this approach is dependent upon the validity of its assumptions and the quality of the pharmacological data available for analysis and will be most effective when closely coupled with prediction, synthesis and testing.

References

1. P. Gund, Ann. Repts. Med. Chem., 14, 299 (1979).
2. G.R. Marshall, C.D. Barry, H.E. Bosshard, R.A. Dammkoehler, D.A. Dunn, in Computer-Assisted Drug Design, R.C. Olson, R.E. Christofferson, eds. ACS Symposium Series, Vol. 112 (1979).
3. D.J. Duchamp, ibid., 79-102.
4. B.V. Cheney, D.J. Duchamp, R.E. Christofferson, in Qua SAR research monograph 22, G. Barnett, M. Trsie, R. Willette eds., NIDA, 218-249 (1978).
5. G.L. Grunewald, M.W. Creese, D.W. Walters, op cit., E.C. Olson, R.E. Christoffersen, eds. 439-489.
6. D.C. Rohrer, D.S. Fullerton, K. Yoshioka, A.H.L. From, K. Ahmed, ibid., 259-281.
7. V. Cody, ibid., 281-301.
8. P.A.J. Janssen, J.P. Tollenaere, in Neurochemical mechanisms of opiates and endorphins, (Adv. Biochem. Psychopharmacd., Vol. 20). H.H. Loh, D.H. Ross eds., Raven Press, New York, 1979.
9. R.C. Christoffersen, op cit., E.C. Olson, R.E. Christoffersen, eds., 3-21.
10. J.J. Kaufman, H.E. Popkie, P.C. Hariharan, ibid., 415-439.
11. H. Weinstein, R. Osman, J.P. Green, ibid., 161-189.
12. P.R. Andrews, G.P. Jones, Int. J. Quantum Chem., in press (1980).
13. L.N.M. Carnot, Geometrie de position, Paris (1803).
14. L.M. Blumenthal, Theory and application of distance geometry, Chelsea Publishing Co., New York (1970).
15. A.L. Mackay, Acta Cryst., A30, 440-447 (1974).
16. G.M. Crippen, J. Comput. Phys., 24, 96-107 (1977).
17. G.M. Crippen and T.F. Havel, Acta Cryst., A34, 282-284 (1978).
18. I.D. Kuntz, G.M. Crippen, P.A. Kollman, Biopolymers, 18, 939-957 (1979).
19. T.F. Havel, G.M. Crippens, I.D. Kuntz, ibid., 73-81.
20. C.D. Barry and G.R. Marshall, Abst. 6th Int. Symp. Med. Chem., 33 (1978). G. R. Marshall in Medicinal Chemistry VI, A. Simkin ed., Cotswald Press Ltd., Oxford (1979), 225-236.
21. H.E. Bosshard, C.D. Barry, J.M. Fritsch, R.A. Ellis, G.R. Marshall, Proc. 1972 Summer Simulation Conf., 1, 581 (1972).
22. D.N.J. White and C. Morrow, Comput. & Chem., 3, 33-48 (1979).
23. A.T. Stuper, T.M. Dyott and G.S. Zander, op cit., R.C. Olson, R.E. Christoffersen, eds., 383-414.
24. D. Hodges, D.H. Nordby, G.R. Marshall, Abst. 169th Natl. ACS Meeting, Comp-7 (1975).
25. D.H. Nordby, D. Hodges, J.L. Hilton, Mol. Pharmacol., in press (1980).
26. G.M. Crippen, J. Med. Chem., 22, 988-998 (1979).
27. P. Pauling, N. Datta, Proc. Natl. Acad. Sci. USA, 77, 708-712 (1980).
28. G.R. Marshall, M. Zyda, C. Humblet, C.D. Barry, R.A. Dammkoehler, Abst. 179th ACS Natl. Meeting, Phys- (1980).
29. G.R. Marshall and H.E. Bosshard, Circulation Res., 32, II-143 (1972).
30. N. Go and H.A. Scheraga, Macromolecules, 3, 178 (1970).
31. D.F. Veber, F.W. Holley, R.F. Nutt, S.J. Bergstrand, S.F. Brady, R. Hirschmann, M.S. Glitzer and R. Saperstein, Nature, 280, 512 (1979).
32. D.F. Veber in Peptides, structure and biological function, E. Gross and J. Meienhofer, eds., Pierce Chemical Co., Rockford, Illinois (1979).
33. J. Seprodi, D.H. Coy, J.A. Vilchez-Martinez, E. Pedronza, W.Y. Huang and A.V. Schally, J. Med. Chem., 21, 993 (1978).
34. G. Chipens, G. Nikiforovich, F. Mutulis, N. Veretennikora, I. Vosekolna, A. Sosnon, L. Polevaya, J. Ancans, N. Mishlyakova, E. Licpinsh, I. Seracis, M. Breslav, op cit. E. Gross and J. Meienhofer, eds.
35. G.R. Marshall and C.D. Barry, Abst. Amer. Crystallography Assoc., Honolulu (1979).
36. Z. Simon, I. Badilescu, and T. Racovitan, J. Theor. Biol., 66, 485-495 (1977).
37. N.C. Cohen, op cit., R.C. Olson and R.E. Christensen, eds., 371-383.
38. R.P. Sheridan, S.L. Brantley, L.C. Allen, in Drug action and design mechanism-based enzyme inhibitors, Kalman, ed., Elsevier North Holland (1979).
39. F.A. Gorin and G.R. Marshall, Proc. Natl. Acad. Sci. USA, 74, 5179 (1977).
40. F.A. Gorin and G.R. Marshall in Peptides, M. Goodman and J. Meienhofer, eds., John Wiley and Sons, Inc., New York, 277 (1977).
41. F.A. Gorin, T.M. Balasubramanian, C.D. Barry and G.R. Marhsall, J. Supramol. Struc., 9, 27 (1978).
42. F.A. Gorin, Dissertation, Washington University, St. Louis (1978).
43. J.A.H. Lord, A.A. Waterfield, J. Hughes and H.W. Kosterlitz, Nature, 267, 495-499 (1977).

44. D.S. Fries, P.S. Portoghese, J. Med. Chem., 19, 1155 (1976).
45. C. Humblet and G.R. Marshall, 179th ACS Natl. Meetings (1980).
46. H.H. Ong, T. Oh-Ishi, E.L. May, J. Med. Chem., 17, 133 (1974).
47. T.G. Cochran, J. Med. Chem., 17, 987 (1974).
48. F.H. Clarke, R.T. Hill, J.K. Saelens, N. Yokoyama in Narcotic antagonists, M.C. Braude, L.S. Harris, E.L. May, J.P. Smith, J.E. Villarreal, eds., Adv. Biochem. Psychopharmacol., 8, Raven Press, New York (1974).
49. F.H. Clarke, H. Jaggi, R.A. Lovell, J. Med. Chem., 21, 600-606 (1978).
50. E.E. Smissman, P.C. Ruenitz, J. Med. Chem., 19, 184-186 (1976).
51. D.C. Weaver, C.D. Barry and P.E. Lacy, Diabetes, 27, 456 (1978). D.C. Weaver, C.D. Barry, M.L. McDaniel, G.R. Marshall and P.E. Lacy, Mol. Pharmacol, 16, 361-368 (1979).
52. D.G. Johnson and C. DeHaen, Mol. Pharmacol., 15, 287 (1979).
53. L.G. Humber, F.T. Bruderlein and K. Voith, Mol. Pharmacol., 11, 833 (1975).
54. G.R. Marshall, C.D. Barry and L.G. Humber, Abst. Metrochem., '78 Reg. ACS Meeting, 7 (1978).
55. L.G. Humber, A.H. Philipp, F.T. Bruderlein, M. Gotz and K. Voitz, op cit., R.C. Olson and R.E. Christoffersen, eds., 227-242.
56. J.G. Berger, S.R. Teller, C.D. Adams and L.J. Guggenberger, Tetrahedron Lett., 1807 (1975).
57. J.D. McDermed, H.S. Freeman and R.M. Ferris in Catecholamines: Basic and clinical frontiers, E. Usdin, ed., Pergamon Press, New York, 568.
58. J.R. Sufrin and G.R. Marshall, Federation Proc., 38, 562 (1979).
59. M.H. Cobb, G.R. Marshall, W. Heagy, J. Danner and H.M. Lenhoff, Federation Proc., 37, 1822 (1978).
60. D.F. Covey, R.C. Strickler and B. Tobias, 179th ACS Natl. Meetings, Medi-9 (1980).

Chapter 29. Altered Drug Disposition in Disease States

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Introduction - In recent years steadily mounting evidence has shown that disease states can significantly affect both absorption and elimination of various drugs. On the basis of empirical observations of the effects of disease states on drug pharmacokinetics, a few generally accepted models for drug disposition have evolved.[1] These models have become valuable tools in assessing a priori the kinetics of a particular drug in various disease states and in explaining why observed changes have taken place. The physiological parameters that appear to be important in assessing the disposition are: 1) blood flow to the eliminating organ, 2) the intrinsic ability of the eliminating organs to metabolize or excrete drugs (often a function of enzyme concentration, affinity of the enzyme for the substrate, availability of cofactors, etc., 3) the degree of drug protein binding and 4) renal function.

Physiologic Models for Drug Elimination by Metabolism - Physiologic models of drug elimination or clearance define rate of drug removel in terms of various physiologic parameters. The clearance (CL) relates the amount of drug removed per unit time (R) by the eliminating organ to the blood concentration entering the organ (C_{in})

$$CL = R/C_{in} \qquad \text{(Eq. 1)}$$

The rate of elimination is equal to the difference in the concentration entering and leaving the organ ($C_{in} - C_{out}$) multiplied by the organ blood flow (Q)

$$CL = Q(C_{in} - C_{out})/C_{in} = Q \cdot ER \qquad \text{(Eq. 2)}$$

where ER is the extraction ratio. At least two models have been proposed[2] to define the intrinsic eliminating capacity of the liver in terms of a clearance parameter. The most straightforward and most commonly employed model relating the extraction ratio to physiological parameters is the so-called "Well-Stirred" model[3-5] which assumes the unbound drug concentration leaving the organ is equal to the unbound concentration inside the organ,[6] and the intrinsic ability to metabolize or clear drug (CL_{int}) is equal to the rate of elimination divided by the unbound concentration in the organ. The clearance (with respect to blood concentration) then becomes:[7]

$$CL = Q \frac{f \cdot CL_{int}}{Q + f \cdot CL_{int}} \qquad \text{(Eq. 3)}$$

where f is the unbound fraction of drug in blood.

This model indicates that when the capability of the eliminating organ to metabolize the drug is large in comparison to the rate of drug

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presentation to the organ ($f \cdot CL_{int} >> Q$), the clearance will approximate the organ blood flow:

$$CL \simeq Q \qquad \text{(Eq. 4)}$$

i.e., drug elimination is blood-flow-rate limited and the compound is called a high extraction ratio drug. On the other hand, when the metabolic capability is small in comparison to the rate of drug presentation ($Q >> f \cdot CL_{int}$), the clearance will be proportional to the unbound fraction of drug in blood and the intrinsic clearance, i.e.,

$$CL \simeq f \cdot CL_{int} \qquad \text{(Eq. 5)}$$

The drug is then called a low extraction ratio drug. When the capability of elimination is of the same order of magnitude as the blood flow, clearance would be expected to be dependent upon the blood flow as well as the intrinsic clearance and plasma protein binding (Eq. 3). Most drugs can fortunately be characterized either as high or low extraction ratio compounds, thus simplifying the interpretation of the model.

An alternative to the "Well-Stirred" model has been discussed in the literature, the so-called "Parallel Tube" model.[3-5] This model also utilizes the parameters of blood flow, intrinsic clearance and unbound fraction of drug in blood to describe the organ clearance but also takes into account the effect of the blood concentration gradient in the hepatic sinusoids.[2,8-11] The relationship is somewhat different from the "Well-Stirred" model, but for drugs for which the eliminating organ has a high and low metabolic capacity, clearance can again be expressed by Eqs. 4 and 5. It is difficult to prove the validity of either one of these models. However, *in vitro* liver perfusion data in addition to *in vivo* data for drugs principally eliminated via the hepatic route are consistent with the predictions of the models. The models have been especially useful in predicting clearance changes in cardiovascular and hepatic diseases, where the disease causes various parameters in the model to change, as will be discussed.

Model for Drug Excretion by the Kidney - Because the net renal excretion of a drug is determined by filtration, active secretion and reabsorption, the model for renal clearance is more complicated than that described above. Renal clearance can be described by the following equation:

$$CL_R = (CL_{RF} + CL_{RS})\ (1 - FR) \qquad \text{(Eq. 6)}$$

where CL_{RF} is renal filtration clearance, CL_{RS} is renal secretion clearance and FR is the fraction of drug filtered and secreted that is reabsorbed. The rate of filtration depends upon the volume of fluid that is filtered in the glomerulus and the unbound concentration of drug in plasma, because proteins and drugs bound to proteins are not filtered. The volume filtered is usually estimated by inulin or creatinine clearance. The renal filtration is therefore usually expressed as

$$CL_{RF} = f_p \cdot CL_{cr} \qquad \text{(Eq. 7)}$$

where CL_{cr} is the creatinine clearance and f_p the unbound fraction of drug in plasma.

The secretion of drug in the kidney will depend on: the relative binding of drug to the active transport carriers in relation to the binding to plasma proteins, the degree of saturation of these carriers, trans-

fer of the drug across the tubular membrane and the rate of delivery of the drug to the secretory site. A model that combines these factors can be set up in a manner similar to Eq. 3:

$$CL_{RS} = \frac{f \cdot CL_{intK}}{Q_K + f \cdot CL_{intK}} \qquad \text{(Eq. 8)}$$

where Q_K is the blood flow to the transport site, and CL_{intK} the intrinsic ability to transport drug across the tubular membrane, relating the rate of secretion to the unbound concentration at the transport site. In a manner analogous to the hepatic clearance equations, the model predicts that for low kidney extraction drugs ($f \cdot CL_{intK} << Q_K$) secretion will be proportional to the unbound fraction of drug in blood and the intrinsic transport clearance:

$$CL_{RS} = f \cdot CL_{intK} \qquad \text{(Eq. 9)}$$

When the ability to secrete the drug is high ($f \cdot CL_{intK} >> Q_K$) the secretion is limited by the rate of delivery of drug to the secretory site, and the renal secretory clearance approaches the blood flow to the organ:

$$CL_{RS} \simeq Q_K \qquad \text{(Eq. 10)}$$

Protein Binding - 1. Proteins affected. A number of plasma proteins are known to bind drugs, e.g., albumin, α_1-acid glycoprotein, various lipoproteins, transcortin, prealbumin, thyroxin-binding globulin. The most important of these proteins is albumin because it is present in the highest concentration, approximately 600 μM/L in normal plasma. Albumin is known to bind a large number of acidic drugs[12-14] as well as many basic drugs.[12,15] In the past, little focus was directed toward other potential binders in plasma. However, recent reports have presented evidence to suggest that the unbound fraction for a number of basic drugs often is inversely related to the plasma concentrations of α_1-acid glycoprotein (orosomucoid) and/or lipoproteins and cholesterol.[16-23] The α_1-acid glycoprotein and the lipoproteins are usually present at a relatively low concentration in normal plasma (∿20 μM/L and ∿3 μM/L, respectively) and may therefore, play a less important role than albumin at high basic drug concentrations. Other proteins such as transcortin, thyroxin binding globulin, prealbumin and various α-globulins have also been implicated in protein binding. However, to date there is little information to support including them as general binders of drugs in plasma.

2. Documented disease effect on protein binding. A number of conditions are known to affect the concentrations of albumin, α_1-acid glycoprotein and lipoproteins. Albumin is known to be decreased in compromised health states such as: burns, acute febrile infections, surgery, myocardial infarction, acute injury, prolonged bedrest, liver cirrhosis, viral hepatitis, gastrointestinal diseases, cystic fibrosis, gastrointestinal bacterial overgrowth, aging, pregnancy, renal failure, severe malnutrition (Kwashiorkor), and cutaneous hepatic porphyria.[24-29] Observation of an increase in albumin concentration is unusual but has been suggested to occur in various neurologic diseases and disturbances.[24] The α_1-acid glycoprotein is a stress protein which is released during times of body stress, trauma, and inflammatory diseases. Increases in α_1-acid glycoprotein plasma concentrations have been observed in patients having arthritis, complicated renal disease, Crohn's disease, coronary artery disease and in patients undergoing surgery.[30-33] Although levels also appear to be increased in the elderly, variability is much larger than that found in

young healthy subjects (six-fold range vs. two-fold).[34] It is unknown whether this is truly an age-effect or whether it is secondary to various ailments. The α_1-acid glycoproteins have been reported to be decreased in the nephrotic syndrome[35] and probably are also reduced in other glomerulopathies. Lipoproteins have been reported to be decreased in several inherited disorders and in hyperthyroidism, acute liver disease, trauma and malabsorption.[36] Increases occur in hypothyroidism, diabetes mellitus, nephrotic syndrome, obstructive liver disease, as well as in several inherited disease states.[36] Differences in drug binding among patients are not just limited to differences in protein concentrations. In fact, a large number of these differences cannot be associated with changes in protein concentrations.[34,37-43] Endogenous compounds such as free fatty acids, bilirubin and various unknown substance factors accumulate in the course of various disease states and are thought to alter the binding of various compounds.[38,42-44] For example, when renal failure patients undergo hemodialysis and/or renal transplantation, binding increases which is consistent with the removal of endogenous displacers.[42-48] Other factors possibly responsible for the observed differences in binding among patients include different binding protein phenotypes[49] and sex differences.[50]

3. Effect of protein binding on drug kinetics. Changes in plasma protein binding have been found to have a profound effect on various pharmacokinetic parameters. The total clearance for a number of drugs has been found to correlate with the unbound fraction of drug in plasma[45,51-53] as predicted from Eqs. 3 and 5. All of these drugs are low extraction ratio compounds, thus testing the relationships only at the boundary condition. The renal clearance for low extraction ratio drugs is also proportional to the unbound fraction of drug in plasma[54] which is consistent with Eqs. 7 and 9. Drugs that are highly extracted also appear to conform to the model by demonstrating a total clearance that is dependent upon blood flow.[4,5,55] Thyroxin[56] and salicylate,[57] low extraction ratio compounds, appear to be exceptions to the model where the expected inverse relation between total clearance and binding does not necessarily result. For these two compounds total clearance remains constant while unbound clearance increases, a result expected for high extraction ratio compounds. The apparent volume of distribution has also been found to correlate with the unbound fraction in plasma[53,58] confirming the theoretical prediction.[59] Half-life, which depends on both changes in the clearance and the apparent volume of distribution, is sometimes also affected by changes in protein binding.[44] These changes can in turn have profound effects on the duration and intensity of pharmacologic response.

Hepatic Disease - On the assumption that a diseased organ removes drug less effectively than a healthy organ, clinicians are generally advised that if the liver is impaired the elimination of drugs that are removed from the body primarily by hepatic biotransformation or excretion is retarded.[60,61] Although much data supports this concept, it is now apparent that the influence of hepatic disease on drug absorption and disposition parameters may be highly variable. Diminished, unchanged, and even accelerated drug elimination has been reported in patients with liver impairment.

1. High extraction ratio drugs. Because of the complexity of the influence of hepatic disease on drug disposition, physiologic models of hepatic drug elimination as defined in Eq. 3 have been especially useful. These models have been used to develop noninvasive methods and have even formed the basis for estimating intrahepatic shunting of blood in the liver.[63,64] Although it is generally thought that hepatic blood flow is

reduced in patients with hepatic disease, the data available suggest that the influence of acute and chronic hepatic disease on hepatic blood flow may be highly variable[65-69] and may, in turn, account for the high variability in clearance of drugs that are efficiently removed from the blood by the liver.

The reduction in clearance of drugs that are highly extracted by the liver may be attributed not only to alterations in hepatic blood flow, but to changes in CL_{int}. Drugs and other compounds that are highly extracted by the liver in healthy individuals may be poorly extracted in individuals with reduced hepatic function. This has been demonstrated for both indocyanine green and for the model drug d-propranolol.[69,70] One explanation for these observations, termed the "intact hepatocyte hypothesis," is that hepatic disease reduces the total mass of functioning hepatocytes, but that these hepatocytes are normally perfused and function normally.[71,72] According to this hypothesis, the reduction in clearance of highly extracted drugs may be attributed to blood being shunted past normally functioning hepatocytes, while the reduction in clearance of poorly extracted drugs is attributable to the reduced mass of functioning hepatocytes. Because there may be a relationship between the degree of shunting and the reduction in functioning hepatocyte mass,[63,64] a correlation may be observed between clearances of drugs that are highly and poorly extracted by the liver.[71,73] Pessayre et al.[69] suggest that the change in hepatic clearance of d-propranolol in individuals with cirrhosis can be attributed primarily to a reduction in CL_{int} of the drug rather than to a reduction in hepatic blood flow. Since Eq. 4 indicates that changes in intrinsic eliminating capacity for drugs that are highly extracted by the liver must be substantial to be reflected in changes in systemic clearance, the results suggest that hepatic disease may affect the intrinsic eliminating capacity of the liver for highly extracted drugs more profoundly than for those drugs which are poorly extracted.[71,72-74]

Although numerous investigations have defined the influence of hepatic disease on drug disposition, studies of three high extraction ratio drugs, lidocaine,[75,76] meperidine[70,77] and d-propranolol[69] provide estimates of clearance, volume of distribution, and half-life in comparison to values in healthy controls. Williams and Benet[78] compared the ratio of mean pharmacokinetic parameters (patient/normal) for these three drugs. In each instance total drug clearance was reduced by approximately 50% (38-65% range) while drug half-life increased two to three times. No consistent or statistically significant changes in the volume of distribution of these highly cleared drugs were observed in these studies.

For drugs that are highly extracted by the liver, the position of the organ relative to drug absorption is crucial: entry into the systemic circulation may be negligible for drugs that are highly extracted by the liver. Furthermore, minor alterations in the ability of the liver to extract a drug can have a major influence on the bioavailability of a drug because of the relationship between the extraction ratio (Eq. 2) and the fraction of a drug (F) that traverses the liver ($F = 1 - ER$). The relationship between extraction ratio and bioavailability is thought to account for the highly variable plasma or blood drug concentrations that are observed following the oral administration of a drug that is highly extracted by the liver.[79] This variability may be accentuated in patients with hepatic impairment because the blood is shunted past functioning hepatocytes.[63,64,80]

2. Low extraction ratio drugs. Both acute and chronic hepatic disease may alter significantly the disposition of drugs that are poorly

extracted by the liver. For diazepam,[81] chlordiazepoxide,[82] hexobarbital,[83,84] amobarbital,[85] antipyrine,[85] and theophylline,[87,88] the presence of acute and chronic hepatic disease appears to reduce plasma clearance by approximately 50% (29-68% range) without significantly altering volume of distribution.[78] The change in clearance without apparent change in volume of distribution results in a prolongation of half-life for these drugs. For oxazepam,[89] lorazepam (in acute viral hepatitis),[90] warfarin[91] and prednisolone,[92] hepatic disease did not produce alterations in drug disposition. When lorazepam[90] and ampicillin[93] were administered to individuals with chronic liver disease, drug volume of distribution increased without a corresponding change in drug clearance, producing a prolongation in drug half-life. Tolbutamide clearance increased and half-life diminished without change in volume of distribution in individuals with acute viral hepatitis[44] due to decreased protein plasma and apparently tissue binding.

The data for poorly extracted drugs are not as consistent as the data for highly extracted drugs. Because plasma clearance for poorly extracted drugs may be used to estimate intrinsic hepatic clearance (Eq. 5), the data suggest that the influence of hepatic disease on the intrinsic capacity of the liver to eliminate a drug may be highly variable.

Cardiovascular Disease - The influence of cardiac disease on drug pharmacokinetics has recently been reviewed;[94] cardiovascular disease could alter any of the three variables which determine the clearance of a drug by an eliminating organ (Eq. 3). Through reduction in cardiac output because of congestive heart failure, a reduction in blood flow to one or more organs that eliminate a drug may occur.[95] Change in cardiac function or in the function of other organs (e.g., the liver) in individuals with congestive heart failure may alter blood and tissue concentrations of drug binding proteins, alter physiologic pH, or result in the production of endogenous displacing substances. Such changes, in turn, may influence binding of drug to blood or tissue components. In addition, cardiac disease may alter patterns of drug absorption through changes in gastrointestinal motility, splanchnic blood flow, intraluminal pH of the gastrointestinal tract, or change in the secretions or bacterial flora of the gastrointestinal tract.[96] The effects of cardiac disease on drug absorption have been recently reviewed.[96]

In studies performed during cardiac catheterization in individuals with varying degrees of cardiac impairment, Stenson et al.[97] demonstrated that hepatic blood flow and cardiac index correlate directly with one another and that each is inversely related to steady-state arterial concentrations of lidocaine. Subsequent studies[76,98,99] confirmed that lidocaine clearance is reduced in patients with congestive heart failure in comparison to healthy control subjects and that clearance of this drug in the patient group correlates significantly with cardiac output. The change in lidocaine clearance occurred in association with a reduction in both the central volume into which the drug appeared to distribute, as well as the volume of distribution at steady state. No change in lidocaine half-life was observed in the subjects with congestive heart failure.

Although cardiac disease might theoretically alter the intrinsic ability of an organ to eliminate a drug, relatively few studies have assessed this possibility. In a study of individuals immediately after acute myocardial infarction, aprindine half-life was markedly extended in comparison to that found in healthy control subjects.[100] A similar prolongation of canrenone half-life was found in patients in congestive

heart failure.[101] Hepner et al.[102] observed that aminopyrine clearance was reduced in patients with congestive heart failure. This change in clearance was associated with an increase in apparent volume of distribution and a prolongation in drug half-life. Minimal elevations in bilirubin or serum glutamic oxalacetic transaminase were noted in six of the nine subjects in this study, suggesting that hepatic impairment, perhaps secondary to chronic passive congestion of the liver, contributed to the observed alteration in aminopyrine disposition parameters. Higher peak plasma concentrations and prolonged half-lives in comparison to values in normals were observed for procainamide[103] and prazosin.[104,105] Andreasen and Mikkelsen[106] reported that furosemide plasma clearance decreased and terminal half-life increased when values in three groups of patients with cardiac decompensation were compared to those found in normal volunteers, while Greither et al.[107] found no changes in compensated heart failure patients.

Additional data suggests that clearance of a poorly extracted drug by the liver may be changed, not through changes in the eliminating capacity of the liver, but through changes in drug volume of distribution. The volume of distribution of quinidine is decreased in patients with congestive heart failure in the absence of change in the elimination half-life of the drug.[108-110] These observations are not strictly in accord with the theoretical assumptions of the physiologic model of organ elimination as defined in Eq. 3, and may be attributed to changes in drug protein binding in the absence of change in metabolic function or organ perfusion.

Only minimal information is available to document the influence of cardiac disease on drug protein binding, although the extensive physiologic changes that may occur in individuals with acute and chronic congestive heart failure suggest that drug binding changes frequently occur in these patients. The protein binding of warfarin varies widely between, but not within, individuals with congestive heart failure.[111,112] As predicted by Eq. 5 for poorly extracted drugs such as warfarin, the difference in individuals was correlated with plasma drug clearance.

Renal Disease - Renal disease is known to affect pharmacokinetic parameters by three different mechanisms: 1) by decreasing the ability of the kidney to eliminate drug, 2) by alteration of plasma protein binding and, 3) by indirectly altering the metabolism of drugs. The most important of these mechanisms is the decrease in the ability to excrete drugs. Investigators focus only on the total decrease in elimination and do not distinguish among the various mechanisms.[113] This is probably due to the general acceptance of the intact single nephron theory which states that whole nephrons deteriorate in renal failure and that renal function tests are a measure of the remaining intact nephrons which can eliminate drug. Consequently, very few reports attempt to differentiate between various renal diseases,[114,115] thus suggesting that such differentiation may be unimportant. Generally, total renal clearance is correlated with various renal function tests which in practice allows one to estimate total clearance using renal function tests. The effect of renal disease on pharmacokinetics has been focused mainly on drugs that are extensively eliminated unchanged in healthy individuals. Many of the antibiotics fall into this category.

The cephalosporins have been investigated extensively and for most of them renal clearance does decrease in parallel to renal function, i.e., cefazolin,[116-117] cefoxitin,[118-119] cefadroxil,[120] cefamandol [121,122] all fall in this category. For other cephalosporins like cephacetrile,[123]

cefaclor[124,125] and cephalexin[126] the total clearance or the total elimination rate constant was found to vary with renal function implying that also for these drugs the renal clearance is decreasing with decreasing renal function.

The aminoglycosides such as gentamicin, kanamycin, tobramycin, netilmicin and amikacin are mainly eliminated via the renal route and show a decrease in the elimination with a decrease in renal function.[127-130] The penicillins also fall into the group of antibiotics that are chiefly eliminated by the renal route, exhibiting clearances that parallel changes in renal function.[131,132]

For other drugs excreted predominantly by the kidney, the clearance or elimination rate constant decreases in parallel to the decrease in renal function. Examples are digoxin,[133] pindolol,[134] atenolol[135] and cimetidine[136] among others. For most of these cases it is reasonable to assume that renal clearance decreases in proportion to renal function while extra-renal clearance remains constant. However, this is a simplification that does not always hold true. In a number of instances extra-renal elimination is also changed in renal disease.[119,131,137-140] Oxidation, reduction, acetylation and hydrolysis pathways are sometimes found to be altered.[138-140] Several reasons for this change in the metabolism may be put forward. A metabolite accumulating in renal failure may act to decrease its own formation (product inhibition[141]), or perhaps even increase its own metabolism (induction). Other endogenous and exogenous compounds that also accumulate in renal failure may act to inhibit or induce various types of metabolism. Alterations in protein and enzyme concentrations secondary to dietary restrictions may occur as well and alter metabolism. This is not the general situation, however, as metabolism is often unaltered in renal failure patients.[138,139,142]

Renal failure is known to alter protein binding and it is not unusual for protein concentrations to change in renal disease. Albumin tends to be decreased while orosomucoid and lipoprotein concentrations are sometimes increased. The increase or decrease is not always predictable and is not necessarily associated with the severity of the disease. Protein levels can be altered due to the type of renal disease (e.g., in various glomerulopathies) or when diet or protein intake is restricted in order to reduce the amount of nitrogenous waste-products in the body. In general, acidic drugs often show a decreased binding in renal failure[143,144] while the average binding for basic drugs is often unaltered.[143,144] However, a larger variability in binding is found in renal failure patients than in patients with normal renal function.

Drugs that are passively reabsorbed from the nephron should, in theory, be urine flow[145] and also urine pH dependent, if the drug has a pKa value close to or in the normal urine pH range. Single nephron urine flow is often altered in renal failure, and urine pH may be significantly altered in renal systemic acidosis and alkalosis. However, little data is available to delineate the precise role of renal disease upon reabsorption of drugs in the kidney.

References

1. "The Effect of Disease States on Drug Pharmacokinetics," L.Z. Benet, ed., American Pharmaceutical Association, Washington, D.C., 1976.
2. S. Keiding and P.B. Andreasen, Pharmacology, 19, 105 (1979).
3. K.S. Pang and M. Rowland, J. Pharmacokinet. Biopharm., 5, 625 (1977).
4. K.S. Pang and M. Rowland, J. Pharmacokinet. Biopharm., 5, 655 (1977).
5. K.S. Pang and M. Rowland, J. Pharmacokinet. Biopharm., 5, 681 (1977).

6. M. Rowland, L.Z. Benet and G.G. Graham, J. Pharmacokinet. Biopharm. 1, 123 (1973).
7. G.R. Wilkinson and D.G. Shand, Clin. Pharmacol. Ther., 18, 377 (1975).
8. K. Winkler, L. Bass, S. Keiding and N. Tygstrup, "6th Alfred Benzon Symp.", Munksgaard, Copenhagen, 1974, pp 797-807.
9. L. Bass, S. Keiding, K. Winkler and N. Tygstrup, J. Theor. Biol., 60, 393 (1976).
10. S. Keiding, Scand. J. Clin. Lab. Invest., 36, 113 (1976).
11. S. Keiding and E. Chiarantini, J. Pharmac. Exp. Ther., 205, 465 (1978).
12. J.J. Vallner, J. Pharm. Sci., 66, 447 (1977).
13. A.K. Miller, J. Adir, and R.E. Vestal, J. Pharm. Sci., 67, 1192 (1978).
14. M.J. Hayes, M. Sprackling and M.J.S. Langman, Gut, 18, 1054(1977).
15. R. Carlos, R. Calvo and S. Erill, Clin. Pharmacokinet, 4, 144 (1979).
16. L. Bertilsson, R. Braithwaite, G. Tybring, M. Garle and O. Borgå, Clin. Pharmacol. Ther., 26, 265 (1979).
17. K.M. Piafsky and O. Borgå, Clin. Pharmacol. Ther., 22, 545 (1977).
18. M. Brinkschulte and U. Breyer-Pfaff, Naun.-Schmid. Arch. Pharmacol., 308, 1 (1979).
19. O.G. Nilsen, P. Leren, I. Aakeross and S. Jacobsen, Biochem. Pharmacol., 27, 871 (1978).
20. K. Subbarao, B. Rucinski, M.A. Rausch, K. Schmid and S. Niewiarowski, J. Clin. Invest., 60, 936 (1977).
21. B.J. Scott, A.R. Bradwell, R.E. Schneider and H. Bishop, Lancet, 1, 930 (1979).
22. R.E. Kates, T.D. Sokoloski and T.J. Comstock, Clin. Pharmacol. Ther., 23, 30 (1978).
23. A. Danon and Z. Chen, Clin. Pharmacol. Ther., 25, 316 (1979).
24. W.J. Jusko and M. Gretch, Drug Metab. Rev., 5, 43 (1976).
25. J.P. Tillement, F. Lhoste and J.F. Giudicelli, Clin. Pharmacokinet., 3, 144 (1978).
26. N. Buchanan, So. Afr. Med. J., 52, 733 (1977).
27. L. Storstein, Clin. Pharmacokinet., 2, 220 (1977).
28. M.J. Brodie and S. Boobis, Eur. J. Clin. Pharmacol., 13, 435 (1978).
29. W.H. Steele, S.W. Boobis, M.R. Moore, A. Goldberg, M.J. Brodie and D.J. Sumner, Eur. J. Clin. Pharmacol., 13, 309 (1978).
30. S. Snyder, E.L. Coodley, B.C. Durham and R.S. Pennock, Circulation, 56, 359 (1977).
31. K.M. Piafsky, O. Borgå, I. Odar-Cederlöf, C. Johansson and F. Sjöqvist, N. Engl. J. Med., 299, 1435 (1978).
32. D. Fremstad, K. Bergerud, J.F.W. Haffner and P.K.M. Lunde, Eur. J. Clin. Pharmacol., 10, 441 (1976).
33. R.E. Schneider, H. Bishop, C.F. Hawkins and G. Kitis, Lancet, 1, 554 (1979).
34. R.A. Braithwaite. R. Heard and A. Snape, Br. J. Clin. Pharmacol., 6, 448P (1978).
35. T. Kawai in "Clinical Aspects of the Plasma Proteins", T.B. Lippincott, Philadelphia 1973, p 18.
36. A.M. Scanu, C. Edelstein and P. Keim, in "The Plasma Proteins", Vol. 1, F.W. Putnam, ed., Academic Press, New York, NY, 1975, p 365.
37. H. Kurz, Klin. Wschr., 56, 1195 (1978).
38. W.A. Craig and B. Suh, Scand. J. Infect. Dis., Suppl., 14, 239 (1978).
39. H. Kurz, H. Michels and H.H. Stickel, Eur. J. Clin. Pharmacol., 11, 469 (1977).
40. H. Kurz, A. Mauser-Ganshorn and H.H. Stickel, Eur. J. Clin. Pharmacol., 11, 463 (1977).
41. R. Gugler and G. Mueller, Br. J. Clin. Pharmacol., 5, 441 (1978).
42. I. Odar-Cederlöf, Clin. Pharmacokinet., 2, 147 (1977).
43. A. Yacobi and G. Levy, J. Pharm. Sci., 66, 1285 (1977).
44. R.L. Williams, T.F. Blaschke, P.J. Meffin, K.L. Melmon and M. Rowland, Clin. Pharmacol. Ther., 21, 301 (1977).
45. A. Kober, I. Sjöholm, O. Borgå and I. Odar-Cederlöf, Biochem. Pharmacol., 28, 1027 (1979).
46. W.H. Steele, J.R. Lawrence, H.L. Elliott and B. Whiting. Eur. J. Clin. Pharmacol., 15, 69 (1979).
47. P.C. Farrell, F.A. Gotch, J.H. Peters, B.J. Berridge, Jr. and M. Lam, Nephron, 20, 40 (1978).
48. G. Levy, Drug. Metab. Rev., 9, 3 (1979).
49. G. Wilding, B.S. Blumberg and E.S. Vessel, Science, 195, 991 (1977).
50. D.J. Greenblatt, J.S. Harmatz and R.I. Shader, Pharmacology, 16, 26 (1978).
51. P.J. Meffin. E.W. Robert, R.A. Winkle, S. Harapat, F.A. Peters and D.C. Harrison, J. Pharmacokinet. Biopharm., 7, 29 (1979).
52. D. Fremstad, O.G. Nilsen, L. Storstein, J. Amlie and S. Jacobsen, Eur. J. Clin. Pharmacol., 15, 187 (1979).
53. F.M. Belpair and M.G. Bogaert, Acta. Clin. Belgica, 33, 151 (1978).
54. J.L. Cunningham, D.D. Shen, I. Shudo and D.L. Azarnoff, Clin. Pharmacokinet., 2, 373 (1977).
55. A.S. Nies, D.G. Shand and G.R. Wilkinson, Clin. Pharmacokinet., 1, 135 (1976).
56. G.C. Schussler, F. Schaffner and F. Korn, N. Engl. J. Med., 299, 510 (1978).
57. D.E. Furst, T.N. Tozer and K.L. Melmon, Clin. Pharmacol. Ther., 26, 380 (1979).
58. F. Andreasen, H.E. Hansen and E. Mikkelsen, Eur. J. Clin. Pharmacol., 13, 41 (1978).
59. S. Øie and T.N. Tozer, J. Pharm. Sci., 68, 1203 (1979).
60. Editor, Brit. Med. J., 2, 193 (1973).
61. S. Sherlock, in "Diseases of the Liver and Biliary System", 4th ed., F.A. Davis Co., Philadelphia, 1968, p 353.
62. D.M. Kornhauser, A.J.J. Wood, R.E. Vestal, G.R. Wilkinson, R.A. Branch and D.G. Shand, Clin. Pharmacol. Ther., 23, 165 (1978).

63. A. McLean, P. du Souich and M. Gibaldi, Clin. Pharmacol. Ther., 25, 161 (1979).
64. D.G. Shand, Gastroenterol., 77, 184 (1979).
65. N. Tygstrup and K. Winkler, Clin. Sci., 17, 1 (1958).
66. R. Preisig, J.G. Rankin, J. Sweeting and S.E. Bradley, Circulation, 34, 188 (1966).
67. J.N. Cohn, I.M. Khatri, R.J. Groszmann and B. Kotelanski, Am. J. Med., 53, 704 (1972).
68. P. Lundbergh and T. Strandell, Acta Med. Scand., 196, 315 (1974).
69. D. Pessayre, D. Lebrec, V. Descatoire, M. Peignoux and J.-P. Benhamou, Gastroenterol., 74, 566 (1978).
70. T.S. McHorse, G.R. Wilkinson, R.J. Johnson and S. Schenker, Gastroenterology, 68, 775 (1975).
71. R.A. Branch and D.G. Shand in "The Effect of Disease States on Drug Pharmacokinetics", L.Z. Benet, ed., Am. Pharm. Assn., Washington, D.C., 1976, p 76.
72. R.A. Branch and D.G. Shand, Clin. Pharmacokinet., 1, 264 (1976).
73. R.A. Branch, J.A. James and A.E. Read, Clin. Pharmacol. Ther., 20, 81 (1976).
74. P.B. Andreasen, L. Ranek, B.E. Statland and N. Tygstrup, Eur. J. Clin. Invest., 4, 129 (1974).
75. P.D. Thomson, M. Rowland and K.L. Melmon, Am. Heart J., 82, 417 (1971).
76. R.L. Williams. T.F. Blaschke, P.J. Meffin, K.L. Melmon and M. Rowland, Clin. Pharmacol. Ther., 20, 290 (1976).
77. U. Klotz, T.S. McHorse, G.R. Wilkinson and S. Schenker, Clin. Pharmacol. Ther., 16, 667 (1974).
78. R.L. Williams and L.Z. Benet, Ann. Rev. Pharmacol. Toxicol., 20, 389 (1980).
79. L.Z. Benet, J. Pharmacokinet. Biopharm., 8, 559 (1978).
80. R. Groszmann, B. Kotelanski, J.N. Cohn and I.M. Khatri, Am. J. Med., 53, 715 (1972).
81. U. Klotz, G.R. Avant, A. Hoyumpa, S. Schenker and G.R. Wilkinson, J. Clin. Invest., 55, 347 (1975).
82. R.K. Roberts, G.R. Wilkinson, R.A. Branch, S. Schenker, Gastroenterol., 75,479 (1978).
83. D.D. Breimer, W. Zilly and E. Richter, Clin. Pharmacol. Ther., 18, 433 (1975).
84. W. Zilly, D.D. Breimer and E. Richter, Clin. Pharmacol. Ther., 23, 525 (1978).
85. G.E. Mawer, N.E. Miller and L.A. Turnberg, Brit. J. Pharmacol., 44, 549 (1972).
86. R.A. Branch, C.M. Herbert and A.E. Read, Gut, 14, 569 (1973).
87. K.M. Piafsky, D. Sitar, R.E. Rangno, R.I. Ogilvie, N. Engl. J. Med., 296, 1495 (1977).
88. A. Mangione, T.E. Imhoff, R.V. Lee, L.Y. Shum and W.J. Jusko, Chest, 73, 616 (1978).
89. H.J. Shull, G.R. Wilkinson, R. Johnson, S. Schenker, Ann. Int. Med., 84, 420 (1976).
90. J.W. Kraus, P.V. Desmond J.P. Marshall, R.F. Johnson, S. Schenker, and G.R. Wilkinson, Clin. Pharmacol. Ther., 24, 411 (1978).
91. R.L. Williams, W.L. Schary, T.F. Blaschke, P.J. Meffin, K.L. Melmon and M. Rowland, Clin. Pharmacol. Ther., 20, 90 (1976).
92. S.W. Schalm, W.H.J. Summerskill and V.L.W. Go, Gastroenterol., 72, 910 (1977).
93. G.P. Lewis and W.J. Jusko, Clin. Pharmacol. Ther., 18, 475 (1975).
94. N.L. Benowitz and W. Meister, Clin. Pharmacokinet., 1, 389 (1976).
95. G.D. Dunn, P. Hayes, K.J. Breen, S. Schenker, Am. J. Med. Sci., 265, 174 (1973).
96. L.Z. Benet, A. Greither and W. Meister, in "The Effect of Disease States on Drug Pharmacokinetics", L.Z. Benet, ed., Am. Pharm. Assn., Washington, D.C., 1976, p 33.
97. R.E. Stenson, R.T. Constantino and D.C. Harrison, Circulation, 43, 205 (1971).
98. P.D. Thomson, K.L. Melmon, J.A. Richardson, K. Cohn, W. Steinbrunn, R. Cudihee and M. Rowland, Ann. Int. Med., 78, 499 (1973).
99. R.A. Zito and P.R. Reid, N. Engl. J. Med., 298, 1160 (1978).
100. F. Hagemeijer, Eur. J. Clin. Pharmacol., 9, 21 (1975).
101. L. Jackson, R. Branch, D. Levine, L. Ramsay, Eur. J. Clin. Pharmacol., 11, 177 (1977).
102. G.W. Hepner, E.S. Vesell and K.R. Tatum, Am.J.Med., 65, 271 (1978).
103. E.-G.V. Giardina, J. Dreyfuss, J.T. Bigger, J.M. Shaw and E.C. Schreiber, Clin. Pharmacol. Ther., 19, 339 (1976).
104. R.A. Baughman, E.T. Lin, R.L. Williams, L.Z. Benet, Clin.Pharmacol.Ther., 25, 213 (1979).
105. P. Jaillon, P. Rubin, Y.-G. Yee, R. Ball, R. Kates, D. Harrison and T. Blaschke, Clin. Pharmacol. Ther., 25, 790 (1979).
106. F. Andreasen and E. Mikkelsen, Eur. J. Clin. Pharmacol., 12, 15 (1977).
107. A. Greither, S. Goldman, J.S. Edelsen, L.Z. Benet, K. Cohn, Pharmacology, 19,121 (1979).
108. W.G. Crouthamel, Am. Heart J., 90, 335 (1975).
109. C.T. Ueda and B.S. Dzindzio, Clin. Pharmacol. Ther., 23, 158 (1978).
110. K.M. Kessler, D.T. Lowenthal, H. Warner, T. Gibson, W. Briggs, and M.M. Reidenberg, N. Engl. J. Med., 290, 706 (1974).
111. A. Yacobi, J.A. Udall and G. Levy, Clin. Pharmacol. Ther., 19, 552 (1976).
112. A. Yacobi, J.A. Udall and G. Levy, Clin. Pharmacol. Ther., 20, 300 (1976).
113. E.K. Brodwall, T. Bergan and O. Ørjavik, J. Antimicr. Chem., 3, 585 (1977).
114. V. Prát, J. Grafnetterová, O. Schück, E. Kotanová, Int. J. Clin. Pharm. Biopharm., 13, 71 (1976).
115. O. Schück, J. Grafnetterová, H. Nadvornikova and V. Prátt, Int. J. Clin. Pharmacol. Biopharm, 14, 149 (1976).
116. J.M. Brogard, M. Pinget, C. Brandt, J. Lavillaureix, J.Clin.Pharmacol., 17, 225 (1977).
117. T. Bergan, E.K. Brodwall and O. Orjavik, J. Antimicr. Chem., 3, 435 (1977).
118. M.J. Garcia. A. Dominquez-Gil, J.M. Tabernero and J.A. Sanchez Tomero, Eur. J. Clin. Pharmacol., 16, 119 (1979).

119. J.P. Fillastre, A. Leroy, M. Godin, G. Oksenhendler and G. Humbert, J. Antimicr. Chem., 4, 79 suppl. B (1978).
120. R.E. Cutler, A.D. Blair and M.R. Kelly, Clin. Pharmacol. Ther., 25, 514 (1979).
121. A.W. Czerwinski and J.A. Pederson, Antimicr. Ag. Chemother., 15, 161 (1979).
122. D. Höffler, D. Moecke and M. Sassmann, Deutsche Med. Wschr., 34, 1334 (1978).
123. A. Dominquez-Gil, M.C. Castiñeiras, J.M. Tabernero, J.L. Rodríquez Colmmes and S. de Castro, Eur. J. Clin. Pharmacol., 13, 445 (1978).
124. J. Santoro, B.N. Agarwal, R. Martinelli, M. Wenger and M.E. Levison, Antimicr. Ag. Chemother., 13, 951 (1978).
125. R. Block, J.J. Szwed, R.S. Sloan and F.C. Luft, Antimicr.Ag.Chemother., 12, 730 (1977).
126. D.A. Spyker, B.L. Thomas, M.A. Sande, W.K. Bolton, Antimicr.Ag.Chemother., 14, 172 (1978).
127. A. Leroy, G. Humbert, G. Oksenhendler, J.P. Fillastre, Antibio.Chemother., 25, 163 (1978).
128. N. Buchanan, M.D. Davis and C. Eyberg, Br. J. Clin. Pharmacol., 8, 451 (1979).
129. F.C. Luft, D.R. Brannon, L.L. Stropes, R.J. Costello, R.S. Sloan and D.R. Maxwell, Antimicr. Ag. Chemother., 14, 403 (1978).
130. J.M. Lanao, A. Dominquez-Gil, J.M. Tabernero and S. de Castro, Int.J.Clin. Pharmacol. Biopharm., 17, 171 (1979).
131. P. Frigel and K. Becker, Antimicr. Ag. Chemother., 14, 288 (1978).
132. G. Humbert, D.A. Spyker, J.P. Fillastre, A. Leroy, Antimicr.Ag.Chemother., 15, 28 (1979).
133. R.D. Okada, W.D. Hagu, P.E. Graves, M. Mayersohn, D.G. Perrier and F.I. Marcus, Circulation, 58, 1196 (1978).
134. M.E. Safar, N. Ph. Chan, J.A. Levinson, A. Ch. Simon and Y.A. Weiss, Clin. Sci. Mol. Med., 55, 275S, suppl. 4 (1978).
135. J. Sassard, N. Pozet. J. McAinsh, J. Legheand, P. Zeck, Eur. J. Clin. Pharmacol., 12, 175 (1977).
136. R. Larsson, G. Bodemar and B. Norlander, Eur. J. Clin. Pharmacol., 15, 153 (1979).
137. H.E. Mellin, P.G. Welling and P.O. Madsen, Antimicr. Ag. Chemother., 11, 262 (1977).
138. M.M. Reidenberg and D.E. Drayer, Drug Metab. Rev., 8, 293 (1978).
139. M.M. Reidenberg, Am. J. Med., 62, 482 (1977).
140. T.P. Gibson, A.J. Atkinson, E. Matusik, L.D. Nelson and W.A. Briggs, Kidney Int., 12, 422 (1977).
141. S. Øie, Int. J. Pharmaceu., 3, 311 (1979).
142. S. Scherrer, B. Haldimann, A. Küpfer, F. Reubi, J. Bircher, Clin. Sci. Mol. Med., 54, 133 (1978).
143. M.M. Reidenberg, Am. J. Med., 62, 466 (1977).
144. F.M. Belpair, M.G. Bogaert, M.M. Musschi, Eur. J. Clin. Pharmacol., 11, 27 (1977).
145. E.J. Cafruny, Am. J. Med., 62, 490 (1977).

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Chapter 30. Vitamin D Metabolites and Their Analogs

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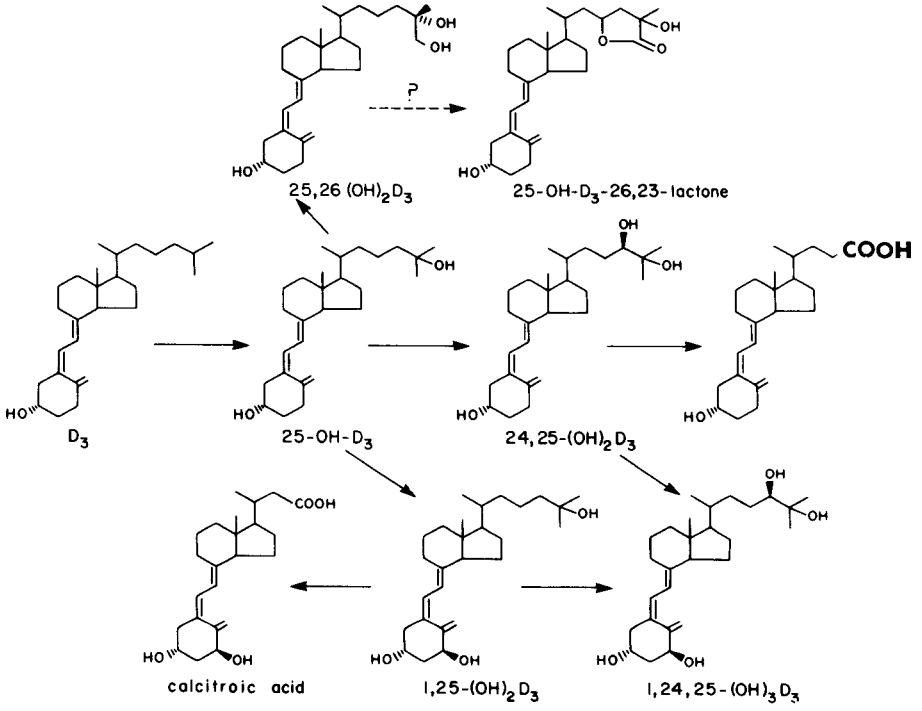
Introduction - A substantial advance has been realized in recent years in our understanding of the basis of disorders of calcium, phosphorus and bone metabolism with the discovery that vitamin D is converted in the body to at least one hormone. This hormone, known as 1α,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) is believed to be responsible for the regulation of calcium and phosphorus metabolism.[1] It has received considerable attention in the treatment of such disorders as hypoparathyroidism, renal osteodystrophy, vitamin D-resistant rickets and its possible application in the treatment of steroid and postmenopausal osteoporosis.[1,2,3]

Figure 1 illustrates the metabolism of vitamin D_3.[1,2,3] Although the metabolism of vitamin D_2 has been briefly studied, much less information is available in this area. Insofar as has been presently elucidated, the metabolism of vitamins D_2 and D_3 is identical at least in mammals and qualitatively similar in birds.[1-4]



Vitamin D undergoes its first obligatory metabolic activation reaction in the liver. Two hepatic vitamin D-25-hydroxylases function. A microsomal enzyme is specifically responsible for the hydroxylation of vitamin D to 25-hydroxyvitamin D (25-OH-D).[5,6] This reaction is a mixed function monooxygenase requiring a flavoprotein and a cytochrome P-450.[7] It is blocked by

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carbon monoxide as might be expected, and it is product-inhibited. Mitochondria possess another 25-hydroxylase that appears to function not only on the vitamin D molecule but also on cholesterol to form the 25-hydroxy derivative.[8] This enzyme appears to have a much higher Michaelis constant for vitamin D and thus probably functions under conditions where large amounts of vitamin D are present.[9] 25-OH-D is then transported to the kidney where it undergoes a further metabolic activation reaction. This reaction occurs in the mitochondria exclusively where 25-OH-D_3 is converted to the vitamin D hormone, 1,25-$(OH)_2D_3$.[10-12] The enzyme involved is a 3-component mixed-function monooxygenase involving a flavoprotein known as renal ferredoxin reductase, an iron/sulfur protein known as renal ferredoxin and a cytochrome P-450 which is the specific 1α-hydroxylase.[13-15] The 1,25-$(OH)_2D_3$ is transported to the target organs of intestine, bone and perhaps kidney where it carries out the known functions of the vitamin. Recent work suggests that there are additional target sites for the vitamin D hormone.[16] For example, it specifically localizes in the nuclei of parathyroid glands,[17] endocrine cells of the stomach, certain cells of the pituitary believed to be the TSH-producing cells, in addition to the well known sites of the small and large intestine, osteoblasts, chondrocytes and osteoclasts of bone (Stumpf, unpublished).[16] Since this 1,25-$(OH)_2D_3$ is made exclusively in the kidney and has its function elsewhere, it can be considered a hormone.

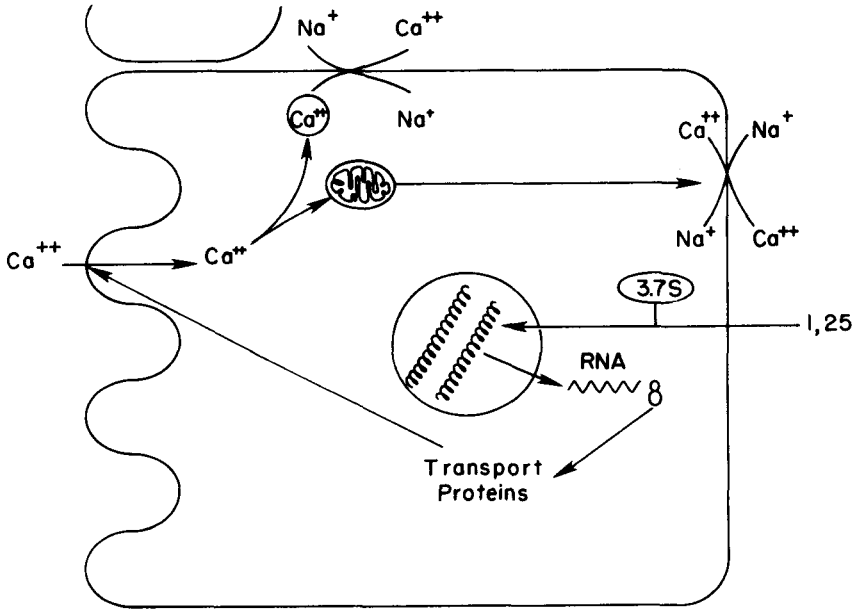
In addition to 1,25-$(OH)_2D_3$, another major metabolite of vitamin D is synthesized in the kidney. This compound has been chemically identified as 24R,25-dihydroxyvitamin D_3 (24R,25-$(OH)_2D_3$).[18] Its role remains controversial, but it is synthesized under conditions where the 1,25-$(OH)_2D_3$ synthesis is repressed.[19,20] Other metabolites in Figure 2 will be discussed in appropriate sections dealing with the metabolites.

The production of 1,25-$(OH)_2D_3$ by the kidney is markedly regulated by the plasma calcium concentration,[20] parathyroid hormone[20,21] and by blood phosphorus concentration.[22] Thus, under conditions of low blood calcium, normal animals produce large amounts of 1,25-$(OH)_2D_3$, a potent calcium mobilizing hormone. Under conditions of sufficient blood calcium or slight hypercalcemia, this hormone is produced in minimal amounts. It is believed that the parathyroid glands monitor serum calcium, and the parathyroid hormone, which is secreted in response to low blood calcium, is responsible for stimulating the 25-OH-D-1α-hydroxylase.[20,21] However, addition of parathyroid hormone to kidney cell cultures producing 1,25-$(OH)_2D_3$ has not yielded convincing results that this is a direct action.[23,24] Nevertheless, from a physiologic point of view, low blood calcium and the parathyroid system is the combined message that stimulates production of 1,25-$(OH)_2D_3$ in response to the need for calcium.

Sites of Action of 1,25-$(OH)_2D_3$ - 1,25-$(OH)_2D_3$ is transported to the intestine, bone and elsewhere in the kidney where it stimulates (A) the active transport of calcium from the lumen of intestine to the extracellular fluid compartment,[25] (B) the active transport of phosphate from the lumen of intestine to the extracellular fluid compartment,[1-4] and (C) the mobilization of calcium from bone in a reaction requiring the presence of parathyroid hormone.[26,27] It is generally believed that the mobilization of calcium from bone is primarily a parathyroid stimulated process, although the 1,25-$(OH)_2D_3$ is thought to provide a permissive role for this function. In the kidney, the 1,25-$(OH)_2D_3$ likely stimulates renal reabsorption of calcium although this is not entirely accepted by all investigators in the field.[27] As a result of these actions, blood calcium will rise, suppressing parathyroid hormone secretion and in turn suppressing 1,25-$(OH)_2D_3$ production. For a detailed review of the vitamin D endocrine system, readers are directed to several reviews.[1-4,28-30]

As might be expected for a steroid hormone, a macromolecule considered a receptor for 1,25-$(OH)_2D_3$ has been found in the intestinal mucosa of chicks,[31,32] rats[33] and man;[34] in the bone of rats and chicks;[36] in the kidneys of chicks; in skin epithelium, parathyroid glands,[37] and functional mammary glands (Simpson and DeLuca, unpublished). Concentration of this receptor is highest in the intestine, bone and the parathyroid gland. This receptor in the chick has a molecular weight of about 72,000 as determined by gel filtration methods and has a K_d of 5-7 X 10^{-11} M.[38] It has been partially purified but is far from homogeneous.[39] It is believed to bind to nuclear chromatin although this has not been clearly established in a definitive manner.[40] However, frozen section autoradiography supports the idea that a cytosolic receptor together with the 1,25-$(OH)_2D_3$ specifically localizes and binds to nuclear components.[16] Of interest to the present discussion, the cytosolic receptor from the chick has been used to assess the molecular structural requirements for binding in preparation for activation of the intestinal calcium transport system.

The intestinal nuclei are believed to respond by producing specific mRNAs that code for calcium and phosphorus transport proteins. These in turn stimulate calcium transport across the micro villus membrane. Calcium is then either sequestered by mitochondria or packaged into vesicles and transported to the basal-lateral membrane where it is expelled by a sodium gradient anti-port system. The proposed mechanism is illustrated in Fig. 2. Most of the mechanism is speculation with only a few facts known. It however comprises an adequate working hypothesis. Other mechanisms have been proposed, however.[41,42]



Assessment of Activity of Analogs and Metabolites - To assess the biological activity of the metabolites of vitamin D and its analogs, the following systems have been used: (A) stimulation of intestinal calcium transport in vivo, (B) stimulation of calcium mobilization from bone in vivo, (C) mineralization of rachitic bone in vivo, (D) in vitro binding to chick intestinal cytosol receptor, (E) in vitro resorption of embryonic bone, (F) stimulation of intestinal calcium binding protein synthesis in embryonic intestine in culture.

The Metabolites of Vitamin D

25-Hydroxyvitamin D_2 and 25-Hydroxyvitamin D_3 - 25-OH-D_3 is 2-5 times more active than vitamin D_3 in the stimulation of intestinal calcium transport, mobilization of calcium from bone and mineralization of rachitic bone.[43] It is 100 times more active than vitamin D_3 in the stimulation of intestinal calcium binding protein synthesis in vitro in intestinal organ cultures.[44] In all of the in vivo systems, it is approximately one-half to one-third as active as 1,25-$(OH)_2D_3$.[45] It is therefore reasonable that this compound is 1/5000 as active as 1,25-$(OH)_2D_3$ in the binding to intestinal cytosol receptor,[46] as well as in the stimulation of bone resorption in culture[47] and confirms the idea that 25-OH-D_3 must be further activated in vivo to 1,25-$(OH)_2D_3$ before function. 25-OH-D_3 is used therapeutically in France and will shortly become available through the Upjohn Company of the United States. It has been applied to a variety of bone diseases, especially renal osteodystrophy, vitamin D-resistant rickets and disease secondary to hepatic dysfunction and in some cases of osteoporosis, especially steroid-induced. In all of the medical treatments, the 25-OH-D_3 is utilized at a level of about 50-100 μg per day which is super physiologic. The 25-OH-D_3 is believed to act primarily as an analog of 1,25-$(OH)_2D_3$ when supplied in large amounts. The blood levels of this metabolite rise to approximately 800-1000 ng/ml at which level it may begin to bind to the 1,25-$(OH)_2D_3$ receptor.

25-OH-D_2 was isolated and identified in 1969,[48] but the chemical synthesis of this compound has not yet been reported although it has been achieved at the Upjohn Company. As expected, it is equal in activity to 25-OH-D_3 in the rat in mineralization of bone and stimulation of intestinal calcium transport. In the bird, however, 25-OH-D_2 is about 1/10 as active as 25-OH-D_3.[49] This reflects the well-known discrimination against the vitamin D_2 compounds by birds. It has now been demonstrated that 25-OH-D_2 in birds is rapidly converted to the 25-OH-D_2 25-β-glucuronide and rapidly excreted in the bile.[50] This likely represents the mechanism whereby birds discriminate against the vitamin D_2 compounds. 25-OH-D_2 has, therefore, not been studied extensively and is not, at the present time, considered significant from the medical point of view.

1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$ - 1,25-$(OH)_2D_3$ is approximately ten times more active than vitamin D_3 in mineralization of bone, stimulation of intestinal calcium transport and stimulation of the mobilization of calcium from bone in vivo in rats.[43,51] Boris has provided strong evidence of the high potency of this compound in the mineralization of bone in the rachitic chicken.[54] On the other hand, it has been claimed by Goodwin et al[53] and Bordier et al[54] that 1,25-$(OH)_2D_3$ does not mineralize bone. However, these investigators have either neglected to indicate the amount of the vitamin D metabolite administered and have furthermore not administered it frequently in accordance with its known physiologic turnover. Thus, Tanaka et al[55] had shown that 1,25-$(OH)_2D_3$ must be given at least once each day and preferably more often to elicit marked bone mineralization responses. The primary reason for this is that the phosphate systems responsive to 1,25-$(OH)_2D_3$ demonstrate quick responses and rapid decreases in activity as a function of time following dose. In any case, when 1,25-$(OH)_2D_3$ is given frequently in small doses to rats,[55] children[56] and birds,[57] it induces extensive mineralization of bone.

In the in vitro cytosol receptor system, 1,25-$(OH)_2D_3$ is the most biologically active naturally occurring compound known showing a K_d of $5\text{-}7 \times 10^{-11}$ M.[38] In this system and in resorption of embryonic bone in culture, it is 5,000 times more active than 25-OH-D.[47] It is about 1000

times more active than vitamin D in stimulating intestinal CaBP production in embryonic organ cultures.[44] Additionally, 1,25-$(OH)_2D_3$ is extremely important medically, being used for the treatment of renal osteodystrophy, hypoparathyroidism, pseudohypoparathyroidism and certain types of vitamin D-resistant rickets. The dosage level used is of the order of 0.25 to 1.0 μg daily. Preliminary results suggest that this compound could be useful in the prevention of renal osteodystrophy in renal failure patients, in the treatment of calcium loss in postmenopausal osteoporosis and in the treatment of calcium loss brought about by steroid therapy.

1,25-$(OH)_2D_2$ was isolated and identified in 1974.[55] At the present time, it has not been chemically synthesized, and thus, material has not been generally available for testing. Limited testing has revealed it to be equally active to 1,25-$(OH)_2D_3$ in the binding to chick intestinal cytosol receptor,[59] in the mineralization of rachitic rat bone *in vivo*,[58] and in the stimulation of intestinal calcium transport *in vivo* in rats. It also appears to be approximately equal to 1,25-$(OH)_2D_3$ in the stimulation of bone resorption in embryonic cultures[47] and in stimulation of intestinal calcium binding protein synthesis *in vitro* in embryonic chick intestine.[60] On the other hand, when it is given to rachitic chickens, it is about 1/10 as active as 1,25-$(OH)_2D_3$.[61] Thus, the side chain discrimination by birds is carried through to the 1,25-$(OH)_2D$ compounds. The practical importance, however, of 1,25-$(OH)_2D_2$ remains uncertain primarily because the compound has not been prepared synthetically in quantity, and its biological value has not been tested.

24,25-$(OH)_2D_3$ and 24,25-$(OH)_2D_2$ - 24,25-$(OH)_2D_3$ was isolated and identified by Holick *et al* in 1972.[18] The compound has been isolated earlier by Suda *et al*, but its structure has been tentatively identified as 21,25-$(OH)_2D_3$. This compound has been synthesized by two groups in which both the *S* and *R* isomers have been prepared.[63,64] Using these isomers, it has been possible to demonstrate that the naturally produced compound is 24*R*,25-$(OH)_2D_3$.[63] The enzymes responsible for biosynthesizing 24*R*,25-$(OH)_2D_3$ can be found in kidney,[65] intestine[66] and cartilage.[67] Furthermore, it seems likely that the 24-hydroxylase is widely distributed probably among all the target organs of 1,25-$(OH)_2D_3$ function. The 24*R*-hydroxylase is a mitochondrial enzyme and appears to be induced by 1,25-$(OH)_2D_3$.[68] The importance of the 24*R*,25-$(OH)_2D_3$ compound is a matter of considerable debate. When tested biologically, this compound is equally active to 25-OH-D_3, its precursor, in the stimulation of intestinal calcium transport, the mineralization of bone and the mobilization of calcium from bone in the rat.[63] It is extremely important to note that it is *not* more active but approximately equal and perhaps somewhat less active than its precursor in this species. In the bird, 24*R*,25-$(OH)_2D_3$ compound is, however, only about 1/10 as active as 25-OH-D_3.[59,69] Its further metabolism has not been extensively studied, although in vitamin D-deficient animals, it is rapidly converted to the 1,24*R*,25-trihydroxyvitamin D_3 (1,24*R*,25-$(OH)_3D_3$) compound.[70] There is some question whether this occurs under normal circumstances *in vivo*.[71] Of considerable importance is that 24*R*,25-$(OH)_2D_3$ like 25-OH-D_3 is inactive in the stimulation of intestinal calcium transport and in the mobilization of calcium from bone when given in physiologic doses to nephrectomized animals.[72] This illustrates that 1-hydroxylation is essential for demonstrable biological activity.

24*S*,25-$(OH)_2D_3$, a synthetic isomer, is much less active in the rat than is the R-isomer;[63] i.e., 1/10 to 1/100 as active. The primary reason for this lack of activity is that the 1-hydroxylase of the kidney discriminates against the *S*-hydroxyl configuration, hence, the 24*S*-epimer does not become activated to the corresponding 1,24*S*,25-$(OH)_3D_3$ compound.[73]

There has been considerable interest in assigning specific functions to the 24R,25-$(OH)_2D_3$ compound. It has been reported that this compound is markedly effective together with 1,25-$(OH)_2D_3$ in reducing parathyroid gland size.[74] However, it must be kept in mind that 1,25-$(OH)_2D_3$ is rapidly turned over and must be administered frequently or by steady infusion before conclusions can be made regarding comparative biological activity. It is likely that large amounts of 24,25-$(OH)_2D_3$ used in the studies remained in the circulation and slowly converted to the corresponding trihydroxy compound and might account for the activity observed. In any event, experiments such as this involving administration of two metabolites differing in pharmacological properties carry with them considerable opportunities for misinterpretation. Similar, although less well defined, experiments have been carried out by Goodwin et al who claim that 24,25-$(OH)_2D_3$ has a specific function in the mineralization of bone.[53] Again, these authors claim that either 1α-OH-D_3 or 1,25-$(OH)_2D_3$ is ineffective in mineralizing bone. This conclusion is inconsistent with the reports of others.[43,51,57] Furthermore, 24,24-difluoro,25-hydroxyvitamin D_3, (24,24-F_2-25-$(OH)_2D_3$) has been chemically synthesized by two groups[75,76] and been tested to determine if specific functions of 25-OH-D_3 are absent in animals given this compound. The 24,24-difluoro compound cannot be 24-hydroxylated and, furthermore, the difluoro groups do not act as hydroxyls in any of the known vitamin D systems. Finally, the fluoro groups remain stable during the experiments. The administration of the difluoro derivative of 25-OH-D_3 brings about responses at least equal in magnitude to the 25-OH-D_3.[77] In fact, in the mineralization of bone, there is no doubt that the difluoro derivative is equal if not slightly more active than 25-OH-D_3. These results make untenable the conclusions of Goodwin et al.[53] Garabedian and her colleagues have shown that cartilage organ cultures will convert 25-OH-D to 24,25-$(OH)_2D_3$.[67] Increased biological activity by 24R-hydroxylation has been shown in the stimulation of chondromucoprotein synthesis.[67] However, in the 24,24-difluoro-25-OH-D_3-treated animals, no evidence has been obtained for improper mineralization or growth of cartilage when it is given instead of 25-OH-D_3. Much reliable work remains to be done on the further metabolism and excretion of the 24,25-$(OH)_2D_3$ compound, although current evidence appears to support the idea that it may be an intermediate in an excretory pathway.

24,25-$(OH)_2D_2$ has recently been isolated and identified by Jones et al.[78,79] Unfortunately, no information is available regarding its biological activity in any of the systems described above. Likely, it is no more active, and is probably less active, than the corresponding 24R,25-$(OH)_2D_3$ compound.

25,26-$(OH)_2D_3$ - This metabolite was isolated and identified in 1970 by Suda et al.[80] Its biological activity appears to be restricted to intestinal calcium transport where it is less active than 25-OH-D_3 by a factor of 10. Chemical synthesis of the 25,26-$(OH)_2D_3$ has been completed, and more recently the two possible isomers have been separated. The naturally occurring 25,26-$(OH)_2D_3$ appears to comigrate with the 25S,26-$(OH)_2D_3$ compound.[81] However, an error was made in assigning the structure of the synthetic S and R isomers resulting in the incorrect statement that the natural product comigrates with 25R,26-$(OH)_2D_3$ (Redel, unpublished). The nature of the 26-hydroxylation reaction remains unknown, and the significance of this metabolite remains to be determined.

25-Hydroxyvitamin D_3-26,23-lactone (25-OH-D_3-26,25-lactone) - This metabolite was discovered as a consequence of the attempts at measurement of 24R,25-$(OH)_2D_3$ in the plasma of animals and patients.[82] A metabolite which comigrates on silica gel high-performance liquid chromatography (HPLC) with

24,25-$(OH)_2D_3$ can be separated by reverse-phase HPLC. This compound has been isolated in pure form from the blood of chickens given adequate amounts of vitamin D or large amounts of vitamin D.[83] Its structure has been unequivocally established. The metabolite appears in substantial amounts under conditions where large amounts of vitamin D are administered. It does not appear normally in the plasma of either cattle or the rat. Whether it occurs in man has yet to be established. This unusual compound appears to have little biological activity in stimulating intestinal calcium transport or bone calcium mobilization. It is more active than all of the other vitamin D metabolites in binding to the plasma transport protein, but is approximately equal to 25-OH-D_3 in its capacity to bind to the chick intestinal cytosol receptor. Thus, its physiologic significance remains unknown.

1α-Hydroxyvitamin D-23-carboxylic Acid - When radioactive 1,25-$(OH)_2$-[26,27-^{3}H]D_3 is given to rats, large (40-50%) losses of radioactivity are experienced.[3] To examine further the nature of this loss, 25-OH-[26,27-^{14}C]D_3 was prepared and administered to vitamin D-deficient animals. Seven percent of the administered ^{14}C appeared in expired $^{14}CO_2$.[84] When the 1,25-$(OH)_2$[26,27-^{14}C]D_3 was prepared and administered, as much as 30% of the radioactivity appeared in expired CO_2. This indicated the presence of a metabolite that had lost a portion of its side chain. Tritium-labeled 1,25-$(OH)_2D_3$ (labeled at C-3) was prepared and administered together with the 1,25-$(OH)_2$[26,27-^{14}C]D_3 to rats.[85] A metabolite appeared in the aqueous soluble fraction of the extract that had lost the ^{14}C and retained the tritium.[85] This compound was isolated in pure form and chemically identified as 1α-OH-D-23-carboxylic acid, named, calcitroic acid. Metabolism to this compound accounts for as much as 40-50% of administered 1,25-$(OH)_2D_3$. It is a major form of 1,25-$(OH)_2D_3$ in the intestine at the time it responds to 1,25-$(OH)_2D_3$. The chemical structure of this compound has been confirmed by synthesis. However, it has little or no biological activity in stimulating intestinal calcium transport or the mobilization of calcium from bone being less than of 1/100 as active as vitamin D_3. In chick intestinal cytosol receptor binding, its activity is approximately 1/10,000 that of 1,25-$(OH)_2D_3$. It is likely that this compound represents an inactivation product, although it is also possible that it is synthesized within the intestinal cells where it might carry out a function in calcium transport.

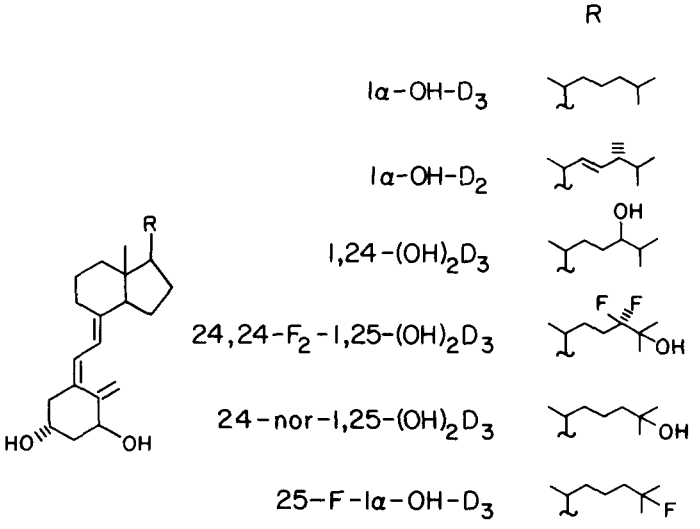
1α,24R,25-$(OH)_2D_3$ - As discussed above, this compound was discovered as a result of the observation that 24R,25-$(OH)_2D_3$ requires processing by kidneys before inducing a response. This, therefore, indicated that 24R,25-$(OH)_2D_3$ must be converted to the corresponding 1α-hydroxylated metabolite.[70] It was isolated in pure form from homogenates and identified as 1,24,25-$(OH)_2D_3$.[70] This compound is approximately 1/2 as active as 1,25-$(OH)_2D_3$ in mineralization of bone, stimulation of intestinal calcium transport and the mobilization of calcium from bone in the rat.[70,86] It is 1/10 as active as 1,25-$(OH)_2D_3$ in the binding to chick intestinal cytosol receptors. However, in the chick, 1,24R,25-$(OH)_3D_3$ is 50-80 times less active than 1,25-$(OH)_2D_3$ in stimulating intestinal calcium transport.

The structure of the 1,24,25-$(OH)_3D_3$ has been confirmed by chemical synthesis.[87,88] Both S and R isomers have been prepared, and it has been clearly demonstrated that the natural product is 1α,24R,25-$(OH)_3D_3$.[89] The isomers are equally biologically active in the rat supporting the idea that the S and R isomers of 24,25-$(OH)_2D_3$ differ in activity only because of discrimination by the 1α-hydroxylase. It is not known whether 24R-hydroxylation of 1,25-$(OH)_2D_3$ has functional significance. In the bird, 1,24R-$(OH)_3D_3$ is about 50 to 80 times less active than 1,25-$(OH)_2D_3$.[51,69]

Analogs of $1,25\text{-}(OH)_2D_3$

This review will not attempt a comprehensive list of many of the analogs prepared. Instead, the most important ones will be discussed, and a table will be provided listing biological activity of the most important and more recently discovered compounds in this series. The synthesis of these compounds has been reviewed recently.[90]

Side Chain Analogs - The most important side chain analogs of $1,25\text{-}(OH)_2D_3$ are 1α-hydroxyvitamin D_3 ($1\alpha\text{-OH-}D_3$), 1α-hydroxyvitamin D_2 ($1\alpha\text{-OH-}D_2$), 1,24-dihydroxyvitamin D_3 ($1,24\text{-}(OH)_2D_3$), 24,24-difluoro-1,25-dihydroxyvitamin D_3 ($24,24\text{-}F_2\text{-}1,25\text{-}(OH)_2D_3$), 24-nor-1,25-dihydroxyvitamin D_3 (24-nor-$1,25\text{-}(OH)_2D_3$) and 25-fluoro-1α-hydroxyvitamin D_3 ($25\text{-F-}1\alpha\text{-OH-}D_3$) (Fig. 3).



$1\alpha\text{-OH-}D_3$, synthesized in 1973, must be 25-hydroxylated before function in both rats and man.[91,92] Its biological activity is approximately equal to that of $25\text{-OH-}D_3$, approaching 1/2 to 1/3 the biological activity of $1,25\text{-}(OH)_2D_3$ in both the rat and the chick in the stimulation of intestinal calcium transport, bone calcium mobilization and the mineralization of rachitic bone.[45,93] It is 1/500 as active as $1,25\text{-}(OH)_2D_3$ in binding to the intestinal cytosol receptor and in the resorption of embryonic bone in culture. It is also 100 times less active than $1,25\text{-}(OH)_2D_3$ in stimulating intestinal calcium binding protein synthesis in culture.[44] It is commercially important being used throughout most of Europe in the treatment of such metabolic bone diseases as renal osteodystrophy, hypoparathyroidism, osteoporosis and vitamin D-resistant rickets.[94]

$1\alpha\text{-OH-}D_2$ has only recently been synthesized in quantity[95] and is an interesting compound inasmuch as it is equally active as $1\alpha\text{-OH-}D_3$ in the mineralization of bone and intestinal calcium transport but is only 1/5 as active in the mobilization of calcium from bone.[96] In the chick, it is 1/10 as active as $1\alpha\text{-OH-}D_3$ simply because the chick discriminates against the vitamin D_2 side chain regardless of the other substituents.[61]

Both $1,24\underline{R}\text{-}(OH)_2D_3$ and $1,24\underline{S}\text{-}(OH)_2D_3$ have been synthesized.[97] The $1,24R\text{-}(OH)_2D_3$ is approximately 1/5 as active as $1,25\text{-}(OH)_2D_3$ *in vivo* in the rat (Tanaka and DeLuca, unpublished) but is equally active in the binding to chick intestinal cytosol receptor. *In vivo*, it is likely that this compound undergoes 25-hydroxylation, forming the $1,24\underline{R}$-25-trihydroxyvitamin D_3

(1,24R-25-(OH)$_3$D$_3$) that is much less active than 1,25-(OH)$_2$D$_3$. This compound has not yet achieved commercial importance, although it is a possible therapeutic analog of 1,25-(OH)$_2$D$_3$.

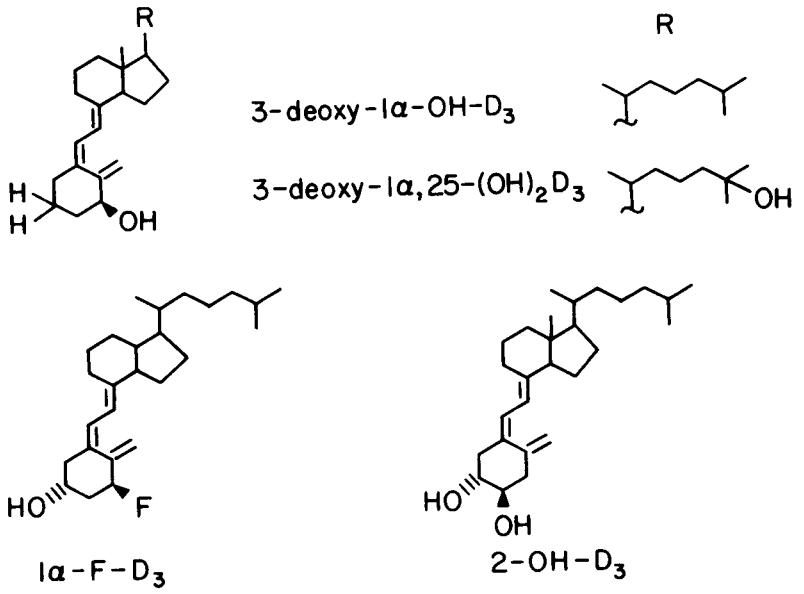
The most active of the known vitamin D analogs is 24,24-F$_2$-1,25-(OH)$_2$D$_3$.[98] This compound has been prepared in an attempt to examine the importance of 24-hydroxylation in the function of vitamin D. It has proved to be more active than 1,25-(OH)$_2$D$_3$, being 5 times more active in the stimulation of intestinal calcium binding protein synthesis in vitro in embryonic cultures (Corradino and DeLuca, unpublished). It is also more active than 1,25-(OH)$_2$D$_3$ in stimulating intestinal calcium transport, bone calcium mobilization and the mineralization of bone in the rat (Tanaka and DeLuca, unpublished). This compound to date represents the most biologically active form of vitamin D known.

Another analog of considerable interest is 24-nor-1,25-(OH)$_2$D$_3$.[90,99] This compound is about 1/50 as active as 1,25-(OH)$_2$D$_3$ in the binding to chick intestinal cytosol receptor. Its biological activity is of the order of 1/100 that of 1,25-(OH)$_2$D$_3$, illustrating that the length of side chain is of great importance to biological activity.[90]

The 25-fluoro analog, 1α-OH-25-F-D$_3$, is equal to 1α-OH-D$_3$ in the receptor binding assay, indicating that fluorine mimics hydrogen in this system.[100] The compound exhibits quite remarkable in vivo activity - 1/50 that of 1α,25-(OH)$_2$D$_3$ - which implies metabolism to a more active form, very likely the 24-hydroxylated product, 1α,24-(OH)$_2$-25-F-D$_3$.

Ring A Analogs (Fig. 4) - Perhaps the most important of the A-ring analogs are 3-deoxy-1α-hydroxyvitamin D$_3$ (3-deoxy-1α-OH-D$_3$)[101,102] and 3-deoxy-1α-25-dihydroxyvitamin D$_3$ (3-deoxy-1α-25-(OH)$_2$D$_3$).[103] These compounds were prepared to examine the function of importance of the 3-hydroxyl group once the 1α-hydroxyl group had been chemically inserted into the molecule. The possibility existed that the 3-hydroxyl serves only to provide a recognition point for the 25- and 1-hydroxylase enzymes, thereafter losing its biological importance. Furthermore, Okamura and Norman[101] have suggested that these compounds should be extraordinarily active based on conformational arguments and purely hypothetical considerations of preferred modes of ligand/receptor interaction. However, the 3-deoxy-1α-OH-D$_3$ proved to have only ca. 1/50 the activity of 1α-OH-D$_3$ in vivo.[101] In organ cultures of embryonic bone, it proved to be devoid of activity.[105] 3-Deoxy-1,25-(OH)$_2$D$_3$ proved to be 1/10 as active as 1,25-(OH)$_2$D$_3$ in binding to chick intestinal cytosol receptor and in the resorption of embryonic bone in organ culture and in the stimulation of intestinal calcium transport.[90] Based on these data, a "super activity" hypothesis must be abandoned.

1α-F-D$_3$ has been prepared and shown to be approximately 1/10 as active as vitamin D$_3$.[106] Likely this compound undergoes 25-hydroxylation and can then in large doses bind to the intestinal cytosol receptor much as 25-OH-D binds to the cytosol receptor when present in very large quantities. 2-Hydroxyvitamin D$_3$ (2-OH-D$_3$) has been shown to be devoid of biological activity illustrating the necessity for a C-1 hydroxy function.[107] That the stereochemistry of the C-1-hydroxy groups is of great importance is demonstrated by the finding that 1β,25-(OH)$_2$D$_3$ is ca. 3,000 times less effective than 1α,25-(OH)$_2$D$_3$ in competing for receptor sites.[108]



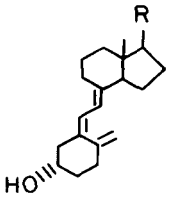
Antivitamin D Compounds - Figure 5 illustrates the structures of the two known antivitamin D compounds. Onisko et al[109] attempted to prepare an antivitamin D compound by synthesizing compounds that could not be 25-hydroxylated. Four compounds were prepared: the 24- and 25-dehydrovitamin D_3 compounds, 25-fluorovitamin D_3 and 25-aza-vitamin D_3. All of these compounds showed biological activity in inhibiting the in vivo conversion of vitamin D_3 to 25-OH-D_3.[110] However, 24- and 25-dehydro compounds, as well as the 25-fluoro compound, possessed intrinsic biological activity. The 25-fluoro compound possessed biological activity presumably by virtue of its conversion to small amounts to 1,24-dihydroxy-25-fluorovitamin D_3.[11C] The 24- and 25-dehydro compounds likely underwent reduction to the saturated compounds to be then hydroxylated in the appropriate manner to form 1,25-$(OH)_2D_3$ in small amounts. These compounds with intrinsic biological activity could not be used as antivitamins since the amount required to serve as an antivitamin provided sufficient vitamin D activity in their own right. On the other hand, the 25-aza-vitamin D proved to be a clear antivitamin D substance.[111] At 1400-fold excess, it can prevent the intestinal calcium transport and bone calcium mobilization responses to vitamin D_3 and 25-OH-D_3 but not to 1,25-$(OH)_2D_3$. Because of the quantities required, it is not considered to be a highly favorable antivitamin.

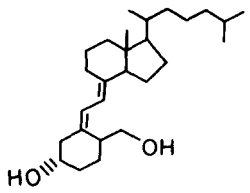
Two groups have synthesized 19-hydroxy-10(19)-dihydro(10S) and (10R) hydroxyvitamin D_3 isomers. Of the two isomers, the 10S derivative possessed unexpected antivitamin D activity.[112,113] It is active at 25-fold excess in blocking the conversion of vitamin D to 25-OH-D_3 in vivo. At the same concentration, it can prevent the bone calcium mobilization response to vitamin D, but it is unable to block the intestinal calcium transport response totally. Although it is more potent than the 25-aza-vitamin D compound, its failure to block entirely the intestinal calcium transport detracts from its potential use as an antivitamin D compound. The search therefore will continue for the preparation of an antivitamin D compound because of its potential use as an experimental tool and as a therapeutic agent in the treatment of hypercalcemias.

Table 1 Biological Activity of Analogs of $1,25\text{-}(OH)_2D_3$

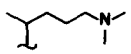
Compound	Bone Mineralization	Intestinal Calcium Transport	Bone Calcium Mobilization	In Vitro Bone Resorption	Receptor Binding
$1,25\text{-}(OH)_2D_3$	100	100	100	100	100
$1\alpha\text{-OH-}D_3$	50	50	50	0.5	0.5
$1\alpha\text{-OH-}D_3$	50	50	10	0.5	0.5
$1,24R\text{-}(OH)_2D_3$	20	20	20	-	100
24-nor-$1,25\text{-}(OH)_2D_3$	-	1.0	1.0	2.0	2.0
$24,24\text{-}F_2\text{-}1,25\text{-}(OH)_2D_3$	>100	>100	>100	-	100
$25\text{-F-}1\alpha\text{-OH-}D_3$	2.5	2.0	2.0	2.0	3.0
3-deoxy-$1\alpha\text{-OH-}D_3$	1.0	2.5	1.5	0.0	0.1
3-deoxy-$1\alpha,25\text{-}(OH)_2D_3$	-	50	50	10	10
$1\alpha\text{-F-}D_3$	3.0	2.0	10	-	10^{-3}
$2\text{-OH-}D_3$	0.0	0.0	0.0	-	-

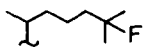
Biological activity of $1\alpha,25\text{-}(OH)_2D_3$ is set at 100% and the activity of the analogs are expressed as % of the activity of $1\alpha,25\text{-}(OH)_2D_3$.

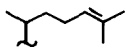


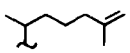


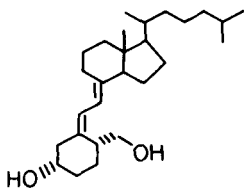
19-OH-10S(19)-dihydro D_3

25-aza-D_3 

25-F-D_3 

24-ene-D_3 

25-ene-D_3 



19-OH-10R(19)-dihydro D_3

Summary

There has been an explosion of new vitamin D compounds, primarily analogs of the active hormonal form of vitamin D_3 1,25-$(OH)_2D_3$. One analog of superior biological activity has been prepared, namely, the 24,24-F_2-1,25-$(OH)_2D_3$. Other than this compound, the most important analogs are 1α-OH-D_3, 1α-OH-D_2 and 1,24R-$(OH)_2D_3$. Of the compounds described, the important therapeutic compounds are 1,25-$(OH)_2D_3$, 25-OH-D_3, its immediate biological precursor and 1α-OH-D_3. These compounds are already in use in the treatment of metabolic bone diseases.

In the area of metabolism, several new metabolites of vitamin D have been described, although the only known activation pathway is the conversion of vitamin D to 25-OH-D_3 in the liver and to 1,25-$(OH)_2D_3$ in the kidney. The metabolism of 25-OH-D_3 to 24R,25-$(OH)_2D_3$ has received considerable interest, although current evidence suggests that it is not an activation pathway. This is supported by experiments carried out with 24,24-F_2-25-OH-D_3, which possesses a potency at least equal, if not superior, to 25-OH-D_3 based on all activity parameters tested, including mineralization of bone.

References

1. H.F. DeLuca, IN: "Monographs on Endocrinolgoy" Vol. 13, Springer-Verlag, New York, pp. 1-78 (1979).
2. H.F. DeLuca, Nutr.Rev. 37, 161 (1979).
3. H.F. DeLuca, IN: Proc. Ann. Meeting Royal College of Physicians & Surgeons of Canada, pp. 216225 (1977).
4. G. Jones, H.K. Schnoes, and H.F. DeLuca, J.Biol.Chem. 251, 24 (1976).
5. T.C. Madhok and H.F. DeLuca, Biochem.J. 184, 491 (1979).
6. M. Bhattacharyya and H.F. DeLuca, Arch.Biochem.Biophys. 160, 58 (1974).
7. P.S. Yoon and H.F. DeLuca, Arch.Biochem.Biophys. (1980) in press.
8. I. Björkhem and I. Holmberg, J.Biol.Chem. 253, 842 (1978).
9. J.I., Pedersen I. Holmberg, and I. Björkhem, FEBS Lett., 98, 394 (1979).
10. M.F. Holick, H.K. Schnoes, H.F. DeLuca, T. Suda, and R.J. Cousins, Biochemistry 10, 2799 (1971).
11. D.R. Fraser and E. Kodicek, Nature 228, 764 (1970).
12. R.W. Gray, J.L. Omdahl, J.G. Ghazarian, and H.F. DeLuca, J.Biol.Chem. 247, 7528 (1972).
13. J.G. Ghazarian, C.R. Jefcoate, J.C. Knutson, W.H. Orme-Johnson and H.F. DeLuca, J.Biol.Chem. 249, 3026 (1974).
14. J.I. Pedersen, J.G. Ghazarian, N.R. Orme-Johnson and H.F. DeLuca, J.Biol.Chem. 251, 3933 (1976).
15. P.S Yoon and H.F. DeLuca, Biochemistry (1980) in press.
16. W.E. Stumpf, M. Sar, F.A. Reid, Y. Tanaka, and H.F. DeLuca, Science 206, 1188 (1979).
17. H.L. Henry and A.W. Norman, Biochem.Biophys.Res.Commun. 62 781 (1975).
18. M.F. Holick, H.K. Schnoes, H.F. DeLuca, R.W. Gray, I.T. Boyle, and T. Suda, Biochemistry 11, 4251 (1972).
19. I.T. Boyle, R.W. Gray, and H.F. DeLuca, Proc.Natl.Acad.Sci., 68, 2131 (1971).
20. M. Garabedian, M.F. Holick, H.F. DeLuca, and I.T. Boyle, Proc.Natl.Acad.Sci. 69, 1673 (1972).
21. D.R. Fraser and E. Kodicek, Nature New Biol. 241, 163 (1973).
22. Y. Tanaka and H.F. DeLuca, Arch.Biochem.Biophys. 154, 566 (1973).
23. H.L. Henry, IN: "Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism" Walter de Gruyter, Inc., Berlin, pp. 125-133 (1977).
24. U. Trechsel, J.A. Eisman, J.P. Bonjour and H. Fleisch, IN: "Vitamin D: Basic Research and Its Clinical Application" Walter de Gruyter & Co., Berlin, pp. 511-513 (1979).
25. H.F. DeLuca, IN: "Calcium Transport and Cell Function" Vol. 307, Ann. New York Acad. Sci. pp. 356-376 (1978).
26. M. Garabedian, Y. Tanaka, M.F. Holick, and H.F. DeLuca, Endocrinology 94, 1022 (1974).
27. R.A.L. Sutton, C.A. Harris, N.L.M. Wong, and J. Dirks, IN: "Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism" Walter de Gruyter, Inc., Berlin, pp. 451-453 (1977).
28. M.R. Haussler and T.A. McCain, New Eng.J.Med. 297, 974 (1977).
29. M.R. Haussler and T.A. McCain, New Eng.J.Med. 297, 1041 (1977).
30. A.W. Norman, and H.L. Henry, Rec.Progr.Hormone Res., 30, 431 (1974).
31. P.F. Brumbaugh and M.R. Haussler, Life Sci., 16, 353 (1975).
32. B.E. Kream, R.D. Reynolds, J.C. Knutson, J.A. Eisman, and H.F. DeLuca, Arch.Biochem. Biophys. 176, 779 (1976).

33. B.E. Kream, S. Yamada, H.K. Schnoes, and H.F. DeLuca, J.Biol.Chem. 252, 4501 (1977).
34. W.R. Wecksler, R.S. Mason and A.W. Norman, J. lin. Endocrinol.Metab. 48, 715 (1979).
35. B.E. Kream, M. Jose, S. Yamada, and H.F. DeLuca, Science 197, 1086 (1977).
36. W.S. Mellon and H.F. DeLuca, J.Biol.Chem. (1980) in press.
37. P.F. Brumbaugh, M.R. Hughes, and M.R. Haussler, Proc.Natl.Acad.Sci. 72, 4871, (1975).
38. W.S. Mellon and H.F. DeLuca, Arch.Biochem.Biophys. 197, 90, (1979).
39. J.W. Pike and M.R. Haussler, Proc.Natl.Acad.Sci., 76, 5485 (1979).
40. P.F. Brumbaugh and M.R. Haussler, J.Biol.Chem. 249, 1258 (1974).
41. H. Rasmussen, E.E. Max, and D.B.P. Goodman, IN: Vitamin D: Biochemical, Chemical and Clinical Aspects Related to Calcium Metabolism" Walter de Gruyter, Inc. Berlin, pp. 913-925 (1977).
42. D.D. Bikle, D.T. Zolock, R.L. Morrissey, and R.H. Herman, J.Biol.Chem. 253, 484 (1978).
43. Y. Tanaka, H. Frank, and H.F. DeLuca, Endocrinology 92, 417 (1973).
44. R.A. Corradino, Science 179, 402 (1973).
45. M.F. Holick, P. Kasten-Schraufrogel, T. Tavela, and H.F. DeLuca, Arch.Biochem.Biophys. 166, 63 (1975).
46. B.E. Kream, M.J.L. Jose, and H.F. DeLuca, Arch.Biochem.Biophys., 179, 462 (1977).
47. P.H. Stern, T. Mavreas, C.L. Trummel, H.K. Schnoes, and H.F. DeLuca, Molec. Pharm. 12, 879 (1977).
48. T. Suda, H.F. DeLuca, and Y. Tanaka, J.Nutr. 100, 1049 (1970).
49. D. Drescher, H.F. DeLuca, and M.H. Imrie, Arch.Biochem.Biophys. 130, 657 (1969).
50. L.W. LeVan and H.F. DeLuca, Fed.Proc.Abst. (1980) in press.
51. A. Boris, J.F. Hurley, and T. Trmal, J.Nutr. 107, 194 (1977).
52. J.J. Partridge, S.-J. Shiuey, A. Boris, J.P. Mallon, and M.R. Uskokovic, IN: "Vitamin D: Basic Research and Its Clinical Application" Walter de Gruyter & Co., Berlin, pp. 37-44 (1979).
53. D. Goodwin, D. Noff and S. Edelstein, Nature 276, 517 (1978).
54. P. Bordier, H. Rasmussen, P. Marie, L. Miravet, J. Gueris, and A. Ryckwaert, J.Clin. Endocrinol.Metab., 46, 284 (1978).
55. Y. Tanaka, and H.F. DeLuca, Proc.Natl.Acad.Sci., 71, 1040 (1974).
56. R.W. Chesney, A.V. Moorthy, J.E. Eisman, D.K. Jax, R.B. Mazess, and H.F. DeLuca New England J.Med., 298, 238 (1978).
57. K.W. McNutt and M.R. Haussler, J.Nutr. 103, 681 (1973).
58. G. Jones, H.K. Schnoes, and H.F. DeLuca, Biochemistry 14, 1250 (1975).
59. J.A. Eisman and H.F. DeLuca, Steroids 30, 245 (1977).
60. C.O. Parkes, and H.F. DeLuca, Arch.Biochem.Biophys. 194, 271 (1979).
61. G. Jones, L.A. Baxter, H.F. DeLuca and H.K. Schnoes, Biochemistry, 15, 713 (1976).
62. T. Suda, H.F. DeLuca, H.K. Schnoes, G. Ponchon, Y. Tanaka, and M.F. Holick, Biochemistry, 9, 2917 (1970).
63. Y. Tanaka, H.F. DeLuca, N. Ikekawa, M. Morisaki, and N. Koizumi, Arch.Biochem.Biophys. 170, 620 (1975).
64. J.J. Partridge, V. Toome, and M.R. Uskokovic, J.Am.Chem.Soc., 98, 3739 (1976).
65. J.C. Knutson and H.F. DeLuca, Biochemistry, 13, 1543 (1974).
66. R. Kumar, H.K. Schnoes, and H.F. DeLuca, J.Biol.Chem., 253, 3804 (1978).
67. M. Garabedian, M.T. Corvol, M. Baily du Bois, M. Lieberherr and S. Balsan, 6th Parathyroid Conf. Vancouver, B.C, June 1977, Abs. P. 160.
68. Y. Tanaka, R.S. Lorenc, and H.F. DeLuca, Arch.Biochem.Biophys., 171, 521 (1975).
69. M.F. Holick, L.A. Baxter, P.K. Schraufrogel, T.E. Tavela, and H.F. DeLuca, J.Biol.Chem. 251, 397 (1976).
70. M.F. Holick, A. Kleiner-Bossaller, H.K. Schnoes, P.M. Kasten, I.T. Boyle, and H.F. DeLuca, J.Biol.Chem. 248, 6691 (1973).
71. E.J. Friedlander and A.W. Norman, Arch.Biochem.Biophys., 170, 731 (1975).
72. I.T. Boyle, J.L. Omdahl, R.W. Gray, and H.F. DeLuca, J.Biol.Chem., 248, 4174 (1973).
73. Y. Tanaka, H.F. DeLuca, A. Akaiwa, M. Morisaki, and N. Ikekawa, Arch.Biochem.Biophys., 177, 615 (1976).
74. H.L. Henry, A.N. Taylor, and A.W. Norman, J.Nutr., 107, 1918 (1977).
75. Y. Kobayashi, T. Taguchi, T. Terada, J. Oshida, M. Morisaki, and N. Ikekewa, Tetra. Lett., 22, 2023 (1979).
76. S. Yamada, M. Ohmori, and H. Takayama, Tetr.Lett., 21, 1859 (1979).
77. Y. Tanaka, H.F. DeLuca, Y. Kobayashi, T. Taguchi, N. Ikekawa, and M. Morisaki, J.Biol.Chem., 254, 7163 (1979).
78. G. Jones, H.K. Schnoes, L. LeVan, and H.F. DeLuca, Arch.Biochem.Biophys., (1980) in press.
79. G. Jones, A. Rosenthal, D. Segev, Y. Mazur, F. Frolow, Y. Halfon, D. Rabinovich, and Z. Shakked, Biochemistry, 18, 1094 (1979).
80. T. Suda, H.F. DeLuca, H.K. Schnoes, Y. Tanaka, and M.F. Holick, Biochemistry, 9, 4776 (1970).
81. J. Redel, N. Bazely, Y. Tanaka, and H.F. DeLuca, FEBS Letters 94, 228 (1978).
82. R.M. Shepard, R.L. Horst, A.J. Hamstra, and H.F. DeLuca, Biochem.J., 182, 55 (1979).
83. J.K. Wichmann, H.F. DeLuca, H.K. Schnoes, R.H. Horst, R.M. Shepard, and N.A. Jorgensen, Biochemistry, 18, 4775 (1979).
84. R. Kumar, D. Harnden, and H.F. DeLuca, Biochemistry, 15, 2420 (1976).
85. R.P. Esvelt, H.K. Schnoes, and H.F. DeLuca, Biochemistry, 18, 3977 (1979).

86. L. Castillo, Y. Tanaka, H.F. DeLuca, and N. Ikekawa, Min. Elec. Metab., 1, 198 (1978).
87. J.J. Partridge, S.-J. Shiuey, E.G. Baggiolini, B. Hennessy and M.R. Uskokovic, IN: "Vitamin D: Biochemical, Chemical, and Clinical Aspects Related to Calcium Metabolism" Walter de Gruyter, Inc., Berlin, pp. 47-55 (1977).
88. N. Ikekawa, M. Morisaki, N. Koizumi, Y. Kato, and T. Takeshita, Chem.Pharm.Bull., 23, 695 (1975).
89. Y. Tanaka, L. Castillo, H.F. DeLuca, and N. Ikekawa, J.Biol.Chem. 252, 1421 (1977).
90. H.F. DeLuca, H.E. Paaren, and H.K. Schnoes, IN: Topics in Current Chemistry" Springer-Verlag, Berlin, pp. 1-65 (1979).
91. M.F. Holick, T.E. Tavela, S.A. Holick, H.K. Schnoes, H.F. DeLuca, and B.M. Gallagher, J.Biol.Chem. 251, 1020 (1976).
92. M.F. Holick, M.C. deBlanco, M.B. Clark, J.W. Henley, R.M. Neer, H.F. DeLuca, and J.T. Potts, J.Clin.Endocrinol.Metab., 44, 595 (1977).
93. M.R. Haussler, J.E. Zerwekh, R.H. Hesse, E. Rizzardo, M.M. Pechet, Proc.Natl.Acad. Sci., 70, 2248 (1973).
94. H.F. DeLuca, Clin. Endocrinol. 7, 1S (1977).
95. H.E. Paaren, D.E. Hamer, H.K. Schnoes, and H.F. DeLuca, Proc.Natl.Acad.Sci., 75, 2080 (1978).
96. L.E. Reeve, H.K. Schnoes, and H.F. DeLuca, Arch.Biochem.Biophys. 186, 164 (1978).
97. H. Kawashima, K. Hosina, Y. Hashimoto, T. Takeshita, S. Ishimoto, T. Noguchi, N. Ikekawa, M. Morisaki, and H. Orimo, FEBS Lett. 76, 177 (1977).
98. Y. Tanaka, H.F. DeLuca, H.K. Schnoes, N. Ikekawa, and Y. Kobayashi, Arch.Biochem. Biophys. 199, 473 (1980).
99. R.L. Johnson, W.H. Okamura, and A.W. Norman, Biochem.Biophys.Res.Commun. 67, 797 (1975).
100. J.L. Napoli, M.A. Fivizzani, H.K. Schnoes, and H.F. DeLuca, Biochemistry 17, 2387 (1978).
101. H.Y. Lam, B.L. Onisko, H.K. Schnoes, and H.F. DeLuca, Biochem.Biophys.Res.Commun. 59, 845 (1974).
102. W.H. Okamura, M.N. Mitra, R.M. Wing, and A.W. Norman, Biochem.Biophys.Res.Commun. 60, 179 (1974).
103. W.H. Okamura, M.N. Mitra, D.A. Procsal, and A.W. Norman, Biochem.Biophys.Res. Commun. 65, 24 (1975).
104. W.H. Okamura, A.W. Norman, and R.M. Wing, Proc.Natl.Acad.Sci. 71, 4194 (1974).
105. P.H. Stern, C.L. Trummel, H.K. Schnoes, and H.F. DeLuca, Endocrinology 97, 1552 (1975).
106. J.L. Napoli, M.A. Fivizzani, H.K. Schnoes, and H.F. DeLuca, Biochemistry, 18, 1641 (1979).
107. C. Kaneko, S. Yamada, A. Sugimoto, M. Ishikawa, T. Suda, M. Suzuki, and S. Sasaki, J.Chem.Soc.Perkin Trans. I, 1104 (1975).
108. H.E. Paaren, H.K. Schnoes, and H.F. DeLuca, JCS Chem.Commun. 890, (1977).
109. B.L. Onisko, H.K. Schnoes, and H.F. DeLuca, Tetra.Lett. 13, 1107 (1977).
110. B.L. Onisko, H.K. Schnoes, H.F. DeLuca, and R.S. Glover, Biochem.J. 182, 1 (1979).
111. B.L. Onisko, H.K. Schnoes, and H.F. DeLuca, J.Biol.Chem. 254, 3493 (1979).
112. H.E. Paaren, Ph.D. Thesis, University of Illinois, Chicago Circle, (1976).
113. A.W. Norman, M.L. Hammond, and W.H. Okamura, Fed.Proc. 36, 914 (1977).

Chapter 31. Drug Delivery Systems

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Introduction - The search for new drugs has been the major path of pharmaceutical innovation over the past decades, with selectivity of drug action and duration of effect being the prime criteria for pursuit of product development.[1-4]

Most drugs, for patient convenience, have been formulated in oral dosage forms. All these dosage forms, including tablets, capsules, slow-release formulations, exhibit first-order kinetics and share an inability to hold constant the drug concentration in body tissues.[5-9] Such dosage forms, as well as injectables and eyedrop solutions, give up their drug rapidly at first to surrounding tissues and then at continually declining rates over several hours.[7-12] This pattern has several disadvantages, including the requirement for frequent drug administration, which is inconvenient and often results in non-compliance.[5,9,11,13]

Most drugs are believed to have concentration-related actions, indicating that receptors show an increasing response with increasing concentration;[10,13] furthermore, in most instances, the effect of a drug will be maintained if the plasma concentration is maintained.

Thus, during the last decade interest has arisen in development of dosage forms specified by rate and duration of drug delivery rather than by drug content.[1-6,9,13-15] To develop the optimum drug program for such a dosage form, it is necessary to have some understanding of the pharmacokinetic and pharmacodynamic properties of the drug. It is now accepted that by controlling the rate at which drugs enter the systemic circulation and/or reach their site of action, one can enhance selectivity of action,[10-13] by preventing dose-related peaks in blood or tissue drug concentrations that are inevitable with conventional dosage forms.[2,3,5-7,11-14] (Figure 1) It is now possible to design and manufacture rate-controlled, rate-specified dosage forms which we have called therapeutic systems.

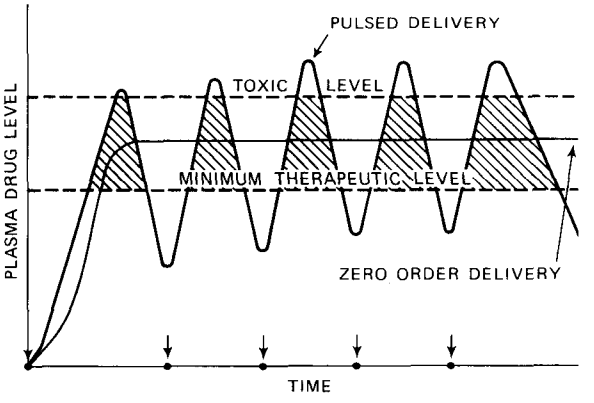

Figure 1. Schematic representation of blood concentration of a drug versus time, following conventional methods of drug administration.

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Some systems have been designed for placement at the required site of therapy; others provide controlled blood levels of a drug which can modify receptor sites not accessible through local placement.[1-8,10-13,15]

Three rate-specified therapeutic systems have received regulatory approval; two for topical therapy (an ocular and an intrauterine system) and one for systemic therapy (a transdermal system). The duration of drug action is a design property of such dosage forms rather than an inherent property of the drug's absorption, metabolism, and excretion properties.

Some of these therapeutic systems will be described, with particular emphasis being placed on the mechanism by which control over drug release rate is provided.

Ocular Therapeutic Systems - Ocular Therapeutic Systems have been designed as alternatives to eyedrops or ointments to provide local therapy at the site of application (the cul-de-sac of the eye).[16] The "OCUSERT"® pilocarpine ocular therapeutic system, following placement in the upper or lower cul-de-sac of the eye delivers pilocarpine at a rate of either 20 μg/hr or 40 μg/hr for one week, for reduction of intraocular pressure. The system comprises a core of pilocarpine base surrounded by a dense membrane of ethylene vinyl acetate copolymer (Figure 2). Pilocarpine diffuses through the membrane at a rate controlled by choice of the appropriate polymeric material.[17,18] The rate remains essentially constant over the 7-day prescribed lifetime of the system.

The release of pilocarpine *in vitro* was determined by following the release of drug into aqueous medium over consecutive prescribed intervals. Release of drug *in vivo* was determined by measuring residual drug content within a system after known times of wearing in the cul-de-sac of the eye. The correlation between drug release *in vitro* and *in vivo*[19] is shown in Figure 3.

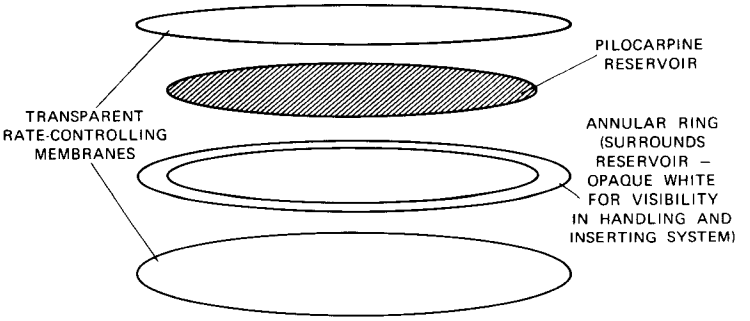

Figure 2. Exploded diagram of multilaminate structure of the OCUSERT® system.

As can be deduced from this Figure, the amount of pilocarpine released from the 20 μg/hr system in one week is on the order of 3.5 mg; one weeks dosage of pilocarpine given in 2 per cent eyedrops four times daily is about 28 mg.

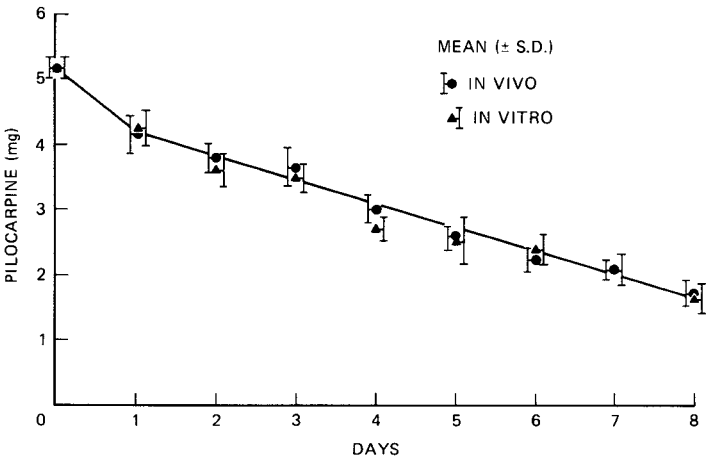

Figure 3. The pilocarpine ocular therapeutic system releases drug at an essentially constant rate of 20 µg or 40 µg per hour for at least a week, following an initial rise above the rated release during the first few hours. Plotted above are the amounts of pilocarpine remaining in 20 µg per hour systems incubated in vitro (saline) for 8-days or used by human subjects for the same amount of time.

The peaks and valleys of drug concentration in the fluids surrounding the eye associated with eyedrop therapy, are associated with fluctuations in intraocular pressure and observed transient miosis and myopia. Controlled clinical studies, and extensive clinical experience have demonstrated that the controlled delivery of pilocarpine from the ocular therapeutic system maintains the drugs hypotensive efficacy[20,21] while reducing the incidence and severity of unwanted pharmacological effects,[22,23] without loss of efficacy after prolonged use.[24] Thus, by controlling the rate of drug delivery we effect selectivity of pilocarpine action; the desired action (ocular hypotension) is significant and maintained, while the incidence and severity of unwanted pharmacological effects is reduced. In addition, use of an ocular therapeutic system eliminates topical delivery of the usual preservative and acid-buffering excipients of eyedrop formulations.[25]

Thus, innovative dosage form design has made pilocarpine, prescribed for over 100 years, a much more readily tolerated drug and one that may actually gain in efficacy through more effective administration. The data indicate that therapeutic innovation can be provided without resorting to chemical synthesis of a new drug, and usual medication can be effective at lower sustained concentrations. This technology can be expanded to include additional medication for treatment of ocular hypotension or other ocular disorders.

Intrauterine Therapeutic Systems - The PROGESTASERT® Intrauterine Therapeutic System the second therapeutic system to reach the market place, is a polymeric, T-shaped unit (Figure 4) designed to provide a contraceptive effect through precisely controlled release of progesterone within the uterus.[26-29] The release rate (65 µg/day[30,31]) remains virtually constant throughout the one-year lifetime of the product, and the total

amount of progesterone delivered is minute. This system also provides concomitant reduction of menstrual blood loss (MBL)[32-34] and cramps.[32,35,36] The design of the system permits its modification so that it can function over more extended time periods.[37] Intrauterine release of progesterone by the contraceptive is essential to its high efficacy (approximately 2 pregnancies per 100 women years of use),[38,39] but does not exert any extrauterine effects;[40,41] suppression of the endometrium is the only detectable pharmacologic action of the exogenous hormone released.[41-43] This suppression has been suggested as the mechanism for the relief of menstrual complaints by the PROGESTASERT® system.[32-36] This therapeutic action is unique among IUDs; ordinarily they exacerbate menstrual problems.[32,33,36,44] Quantitative studies have shown that, on average, MBL among PROGESTASERT® system users has declined about 50% from pre-insertion levels.[33] Menstrual cramps of moderate or severe intensity, which 15% of women reported on admission to the clincial trials, affected only about 9% of users a few months after insertion, and approximately 7% during second year use.[45] Among women having the system removed to become pregnant, recovery of fertility has been rapid.[26,38] About 80% conceived within 12 months.[26]

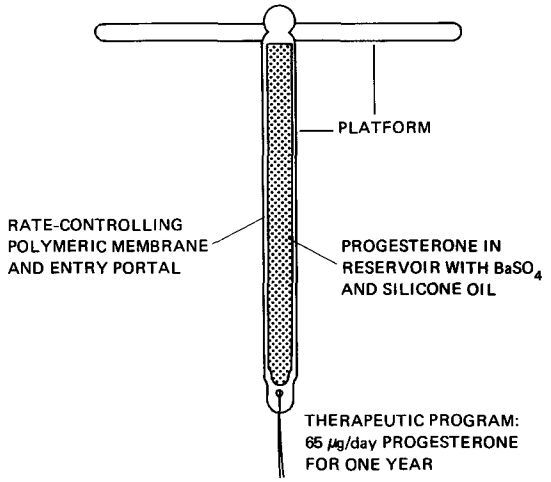

Figure 4. Diagram of PROGESTASERT® - The stem of the T contains 39 mg progesterone. The membrane controls diffusion of hormone. During one year, 24 mg of progesterone are released. Excess, unreleased progesterone acts as the thermodynamic diffusional energy source to effect sustained release.

This therapeutic system permits deployment of a natural hormone to the required site of action, where it elicits both a contraceptive effect and therapeutic effects unattainable with conventional dosage forms of the drug.

Transdermal Therapeutic Systems - Transdermal Therapeutic Systems have been designed to provide controlled continuous delivery of drugs via intact skin to the systemic circulation.[46-50] The relative impermeability of skin is well known, and is associated with its functions as a dual protective barrier against invasion by micro-organisms and loss of physiologically essential substances such as water.[51-55] Elucidation

of factors that contribute to this impermeability, has made possible the use of skin as a route for controlled systemic drug delivery.[56-58]

Transdermal drug delivery has, in the past, been attended by a number of difficulties, the most serious being its inherent unpredictability. The use of ointment or cream preparations of potent drugs can result in adequate systemic therapeutic effects.[59,60] However, such effects are unpredictable for at least three reasons: (1) variation in area and thickness of ointment or cream formulation applied by the patient; (2) the inability of such first-order dosage forms to maintain control over the rate of drug release to the skin surface; and (3) wide differences in skin permeability that affect the amount of drug entering the circulation.[61,62] In development of a rate-specified transdermal dosage form, compensation for individual and regional differences in skin permeability is a necessity, to attain predictable blood levels of drug.

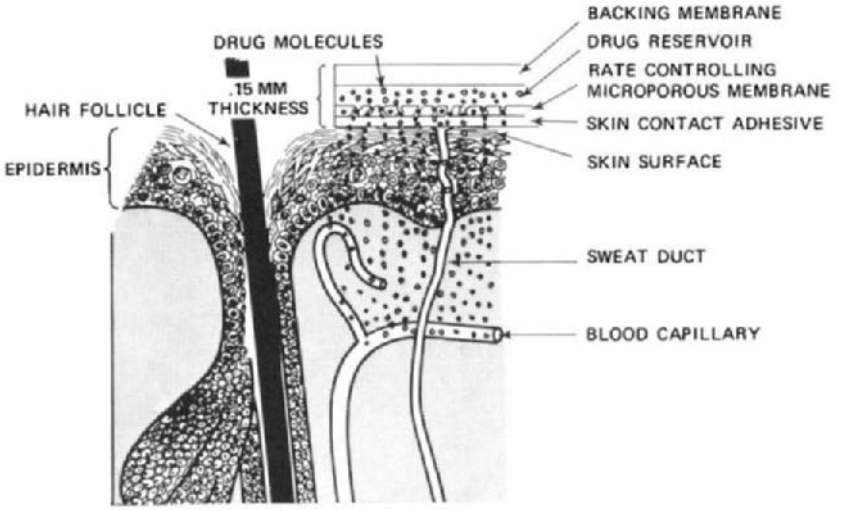

Figure 5. Schematic drawing of the TTS-scopolamine in place on surface of intact skin.

The first transdermal therapeutic system was developed for delivery of scopolamine to prevent motion-induced nausea.[49,50] The system (TTS-scopolamine) is a multilayer laminate (Figure 5), comprising a steady-state reservoir of scopolamine in a gel, sandwiched between an impermeable backing layer and a rate-controlling microporous membrane. On the dermal side of this membrane is an adhesive gel, also containing scopolamine; this layer serves both to secure the system to the skin surface and to provide a priming dose drug reservoir. The priming dose is administered by a relatively rapid initial rate of scopolamine release, which saturates skin sites for the drug, prior to establishment of steady-state input of drug from the steady-state reservoir. The drug diffuses through intact skin to the capillaries within the dermis, whence it is carried into the general circulation. The system is 2.5 cm^2 in area. Worn on the post-auricular skin, the TTS-scopolamine delivers 0.5 mg of the drug in programmed fashion over three days; 0.2 mg every 6 hours is the intramuscular dose recommended for prevention of nausea and vomiting.[63]

The microporous membrane is chosen to ensure that the delivery rate of scopolamine to the skin surface is much less than the rate at which even the most impermeable skin can absorb the drug.[61,62] Hence the system, and not the skin, controls entry of drug into the systemic circulation. This negates differences in skin permeability among different subjects; all receive scopolamine into the circulation at the same rate, predetermined by the system's delivery characteristics.

Scopolamine is a powerful anti-emetic drug which causes troublesome side effects when administered several times daily. These side effects result from wide fluctuations in scopolamine plasma concentrations that occur between doses, and are reflected in urinary excretion levels (Figure 6). The TTS-scopolamine, 2.5 cm^2 in area, applied once every three days, prevents motion sickness in adults over that interval.[64-67] It reduces side effects compared with those elicited by conventional dosage forms. Thus, this dosage form prevents imprecise self-administration and maintains predictable blood levels of scopolamine that selectively evoke the therapeutic response. Again we observe that controlled delivery enhances the therapeutic value of a long-existing drug.

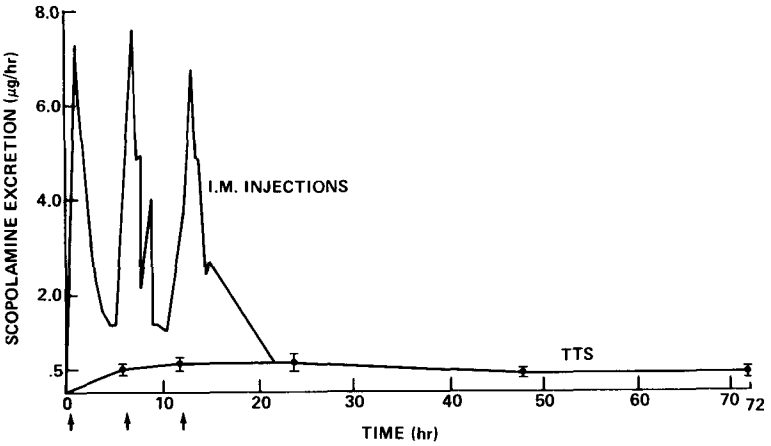

Figure 6. Excretion of scopolamine following a single application of the TTS-scopolamine or repeated intramuscular, injections (arrows) of scopolamine in humans. The drug's excretion pattern correlates with plasma concentrations.

Transdermal delivery of additional drugs is under investigation by a number of companies. It offers advantages for controlled delivery of potent drugs whose effects are mediated via the systemic circulation.

Oral Therapeutic Systems - Oral therapeutic systems (OROS®) are minute osmotic pumps that have the size and shape of ordinary tablets,[68] but comprise a core of solid drug coated with an appropriate water permeable polymer membrane, containing a single small orifice (Figure 7). In the gastrointestinal tract the membrane selectively admits water, which gradually dissolves the drug. The internal pressure forces the drug solution out of the orifice at a rate that remains constant as long as excess solid osmotic agent remains in the core. Thereafter, release rate decreases parabolically in a predictable manner. The empty membrane capsule is excreted intact. A typical release rate profile is shown in

Figure 8 for a system delivering potassium chloride *in vitro* in water, and *in vivo* in the gastrointestinal tract of dogs.

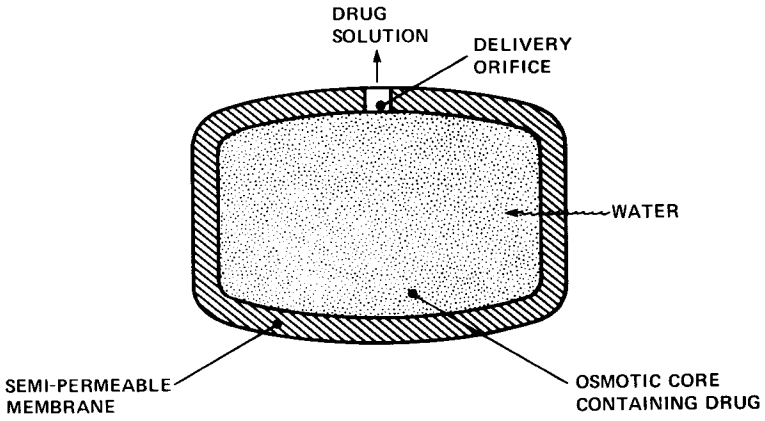

Figure 7. Cross-sectional diagram of an OROS® system.

In vitro data were obtained by transferring each system at regular intervals from one test tube to the next in sequence. In each test tube, the systems resided, for the time indicated, in water maintained at 37°C, and were agitated at a frequency of 0.5 strokes/sec over an amplitude of 2.5 cm. In the three dogs, systems were administered at regular intervals. One hour after the last system was administered, the dogs were sacrificed, and all systems were recovered from the tract.

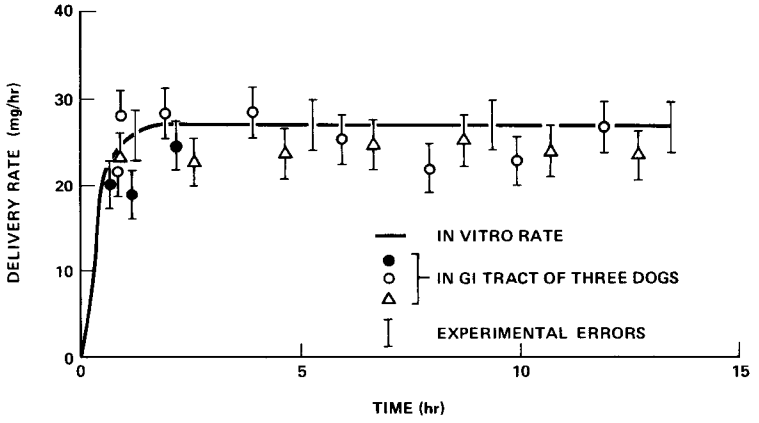

Figure 8. *In vitro* and *in vivo* delivery rate of potassium chloride from OROS® systems.

This osmotic technology permits controlled oral administration of drugs that minimizes the sharp fluctuations in plasma concentration observed with capsules and tablets.[69-71] The OROS® system releases medication at a controlled rate for up to 24 hours, the average emptying time of the gastrointestinal tract. In addition, this techology permits

predictability of drug release rate in vivo from simple in vitro tests and is applicable to drugs of a wide range of solubilities and desired delivery rates. The performance of the OROS® is independent of pH and motility variations in the gastrointestinal tract and isolates solid drug from gut contents and mucosa.

Formulation of an OROS® system for a specific drug requires identification of the drug delivery rate needed to produce therapeutic plasma concentrations, and the duration for which a single dosage form can deliver drug that will be absorbed. In principle, the system can deliver any solid drug, and some combinations of drugs. Since release rate is in part a function of the drug's solubility, techniques have been developed for modifying the osmotic pressure of the core and/or the hydraulic conductivity of the membrane, when it is desirable either to reduce or optimize the drug delivery rate associated with a particular drug formulation.

Membrane-controlled, osmotic technology has been applied to the development of two additional types of rate-controlled delivery systems, -- ALZET® minipumps, and OSMET™ modules. These research systems are for conducting pharmacologic studies respectively in animals and humans.

ALZET Osmotic Minipump, for Use in Animals - Each osmotic pump[72-74] consists of an inert, impermeable, flexible reservoir with a single orifice. A thin wall of osmotic agent (energy source), enclosed in a rigid, semipermeable membrane, surrounds the reservoir (Figure 9). The user fills the reservoir with liquid formulation through the orifice (using a special filling unit attached to a 1 cc syringe). When the pump is placed in an aqueous environment, the osmotic agent imbibes water at a rate controlled by the rigid membrane. The imbibed water swells the osmotic compartment, causing it to squeeze the reservoir volumetrically, displacing the fluid drug formulation which results in continuous flow through the orifice at a specified rate.

To restrict diffusional and convective losses from the pumps and assure control by the osmotic process, the user can insert a flow moderator through the portal after filling the pump. If the transparent overcap is removed from the flow moderator, a catheter (PE 50 or 60 size) can be attached for administration of drugs to remote areas or target organs.[75-82]

Two models of osmotically driven pumps are available for use in animal pharmacology. Each has a capsular shape, fill volume of 200 µl, a length of 2.5 and diameter of 0.6 cm. ALZET® Model 2001 delivers its contents at 1.0 µl/hr for one week; Model 2002 delivers at 0.5 µl/hr for two weeks. Generally, the minipumps are implanted by a simple surgical procedure, either subcutaneously or intraperitoneally.

OSMET™ Drug Delivery Modules for Human Biopharmaceutics - These osmotically driven pumps have the same dimensions, internal volume, and operational principles and procedures as minipumps.[83] OSMET™ modules can be administered to humans,[84] and deliver their contents at a rate of either 15 µl/hr over 12 hr, or 8 µl/hr over 24 hr. In studies in dogs and humans, they have delivered anti-inflammatory agents, antihypertensives, vitamins, and receptor blocking agents.

Use of these pumps has facilitated collection of pharmacokinetic and pharmacodynamic data on drugs and elucidation of specifications of

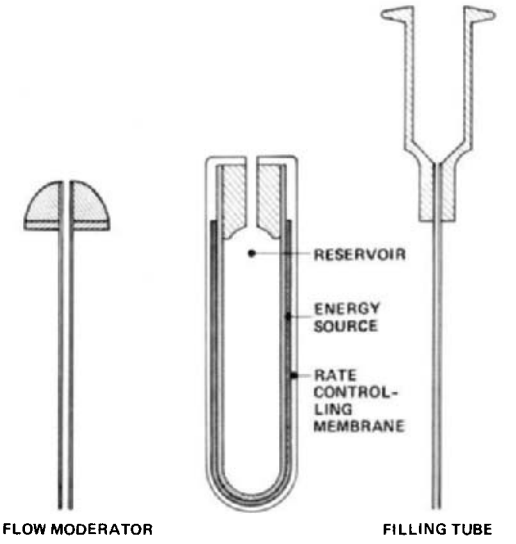

Figure 9. ALZET^{R} osmotic mini-pump with flow moderator at left, and filling tube.

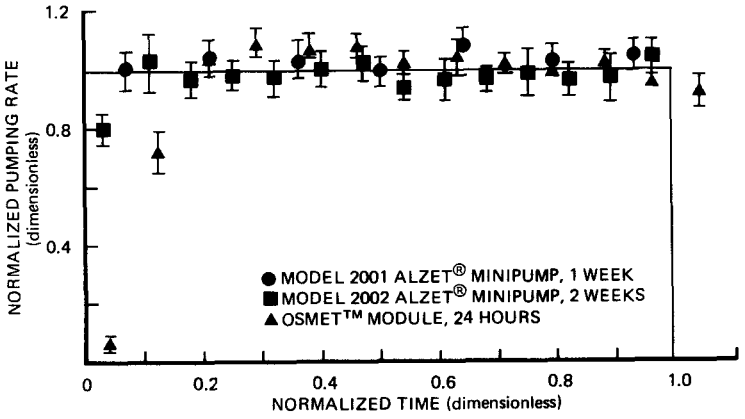

Figure 10. In vitro performance of osmotic minipumps OSMET drug delivery module, summarized by plotting pumping rate, normalized to the pumping rate specified on the label, versus time, normalized to the label duration. The theoretical and actual pumping rates in isotonic saline for the three sets of pumps are:

Model 2001 ALZET® Minipump:
Theoretical Pumping Rate, 1.0 µl/hr
Actual Pumping Rate, 1.03 ± 0.04 µl/hr

Model 2002 ALZET® Osmotic Minipump: 0.5 µl/hr
Theoretical Pumping Rate, 0.5 µl/hr
Actual Pumping Rate, 0.49 ± 0.02 µl/hr

24 Hour OSMET™ Module
Theoretical Pumping Rate, 8 µl/hr
Actual Pumping Rate, 8.17 ± 0.49 µl/hr

rate and duration of drug delivery, for OROS® systems, prior to initiating full-scale product development.

In Vitro/In Vivo Performance of ALZET® and OSMET™ Systems - Figure 10 shows in vitro performance of the two osmotic minipumps and the 24 hour OSMET™ modules,[85] delivering a dye solution in isotonic saline at 37°C. Delivery rate is essentially constant from 0.1 of delivery duration specified, after an initial lag. Thereafter, the steady-state precision is ±5% for individual systems. Figure 11 shows a comparison between performance in vitro and in vivo (rats or dogs) for the same three sets of pumps. The in vivo and in vitro precision of the pumps is practically identical but average pumping rates over time for minipumps and the 24-hour OSMET™ modules are systematically different, due to minor differences in temperatures of the media.

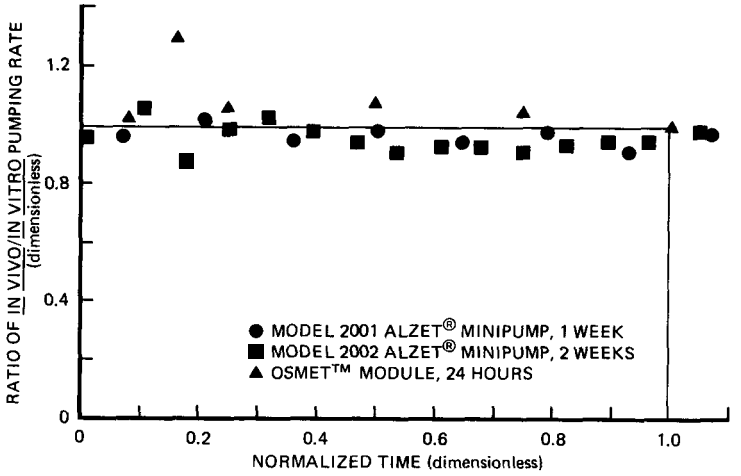

Figure 11. Comparison of in vitro and in vivo performance of ALZET® osmotic minipumps and 24-hour OSMET™ drug delivery module, plotted as in vivo/in vitro ratio versus label duration. The in vivo performance of both minipumps was examined in the subcutaneous tissue of rats at 24 hour intervals. OSMET™ modules were tested in the alimentary tract of dogs. The modules were orally administered, 2, 4, 6, 12, 18 and 24 hours prior to sacrifice.

The minipumps have found a wide range of consumers in academic and industrial laboratories. Their use provides a means for generating pharmacologic data from animal studies that we can apply to development of drug systems for human therapy.

AR/MED® Infusor, for human use - A further development that embodies technology which permits accurate and precise rate and duration of drug delivery is the AR/MED® infusor. Several drugs require parenteral therapy, and various forms of energy have been utilized to power portable pumps for medication infusion.[86-88]

The AR/MED® infusor is a lightweight disposable infusion pump which utilizes elastomeric energy for parenteral administration of drug solutions. The system functions without external connections and can be worn comfortably on the arm, thus allowing a patient to remain ambulatory while receiving medication. The infusor comprises an elastomeric

reservoir, a pre-set flow-control element containing particle filter and a capillary flow-control tube, and a flexible collapse-resistant delivery tube. These elements are integrated into a tubular housing. The completely filled system weighs approximately 90 grams and holds a maximum of 60 ml of solution, thus providing at least 24 hours of continuous infusion. The rate of delivery of normal saline is 2 ml/hr, and testing under controlled laboratory conditions has demonstrated a standard deviation of ±5%.

The infusor is especially useful when ambulation and normal activity patterns are desirable for the patient, when large volumes of parenteral fluids are unnecessary or contraindicated, and when continuous intravenous drug administration over several days is the treatment of choice. Previous models of this infusor have been used for intravenous administration of insulin[89] and cancer chemotherapeutic agents.[90]

Summary - The ocular, intrauterine, transdermal and oral therapeutic systems in human use have validated the concept that controlled continuous drug release can minimize the daily dose of drug required to maintain the required therapeutic effect, while minimizing unwanted pharmacological effects. By minimizing patient intervention, a design feature of therapeutic systems, compliance is automatically enhanced.

Development of drug delivery systems has required innovation in materials science to provide materials biocompatible during prolonged contact with body tissues, bioengineering to develop drug delivery modules, and clinical pharmacology for elucidation of drug action under conditions of continuous controlled drug administration.

There is now great awareness of the merits of drug delivery by use of therapeutic systems, within the medical profession. We look forward to the next decade when therapeutic systems should become a significant entity in the marketing of pharmaceutical dosage forms. During this time we hopefully will see justification for the effort expended to bring these advanced drug delivery systems to the point of extensive human use.

References

1. A. Zaffaroni, "Proceedings of the Sixth International Congress of Pharmacology," Vol. 5, J. Tuomisto and M.K. Paasonen, Eds., Forssan Kirjapaino Oy, Helsinki, 1975, p 53.

2. K. Heilmann, "Therapeutic Systems - Pattern-specific drug delivery: concept and development," G. Thieme, Stuttgart, 1978.

3. S.K. Chandrasekaran, F. Theeuwes and S.I. Yum, "Drug Design," Vol. 8, E.J. Ariens, Ed., Academic Press, New York, N.Y., p 133.

4. A. Zaffaroni, Pharm.Int., 1, 3 (1979).

5. F.E. Yates, H. Benson, R. Buckles, J. Urquhart, and A. Zaffaroni, "Advances in Biomedical Engineering," Vol. 5, J.H.U. Brown and J.F. Dickson III, Eds., Academic Press, New York, N.Y., 1975, p 1.

6. J. Urquhart, Proceedings of the Sixth International Congress of Pharmacology," Vol. 5, J. Tuomisto and M.K. Paasonen, Eds., Forsaan Kirjapaino Oy, Helsinki, 1975, p 63.

7. A. Zaffaroni, Chemtech, 6, 756 (1976).

8. S. Kent, Geriatrics, 146 (Sept. 1977).

9. A. Zaffaroni, Chemtech, 10, 82 (1980).

10. J.E. Shaw and F. Theeuwes, Aust.J.Pharm.Sci., 7, 49 (1978).

11. A. Zaffaroni in "Drug Metabolism Review, Vol. 8, F. DiCarlo, Ed., Marcel Dekker, New York, N.Y., 1978, p 191.

12. A. Zaffaroni, in "Organic Coatings and Plastics Chemistry," Vol. 42, preprints of papers presented by the Division of Organic Coatings and Plastics Chemistry at the American Chemical Society Meeting, Houston, March 23-28, American Chemical Society, Washington, D.C. 1980, p 441.

13. J. Urquhart in "Institute of Medicine Conference Proceedings: Pharmaceuticals for Developing Countries," National Academy of Sciences, Washington, D.C., 1979, p 329.

14. A. Zaffaroni in "Future Trends in Therapeutics," F.G. McMahon, Ed., Futura Pub. Co., Mt. Kisco, N.Y., 1978, p 143.

15. N. Damani and F. Theeuwes, Indian.J.Pharm., 39, 1 (1977).

16. J. Urquhart in "Ophthalmic Drug Delivery Systems," Academy of Pharmaceutical Sciences, Washington, D.C., 1980 (in press).

17. J.W. Shell and R.W. Baker, Ann.Ophthalmol., 6, 1037 (1974).

18. K.T. Richardson, Arch.Ophthalmol., 93, 74 (1975).

19. ALZA Corporation in "The OCUSERT® (pilocarpine) pilo-20/pilo-40 ocular therapeutic system: a new approach to treating ocular hypertension. A monograph," 1974, p 13.

20. ALZA Corporation in "The OCUSERT® (pilocarpine) pilo-20/pilo-40 ocular therapeutic system: a new approach to treating ocular hypertension. A monograph," 1974, p 32.

21. F.T. Fraunfelder, J.W. Shell, and S.F. Herbst, Ann.Ophthalmol., 8, 1031 (1976).

22. H.S. Brown, G. Meltzer, R.C. Merrill, M. Fisher, C. Ferre and V.A. Place, Arch.Ophthalmol., 94, 1716 (1976).

23. V.A. Place, M. Fisher, S. Herbst, L. Gordon and R.C. Merrill, Am.J. Ophthalmol., 80, 706 (1975).

24. K. Heilmann, Long-term use of OCUSERT®. "Documenta ophthalmol. proc. series," Vol. 12, E.L. Greve, Ed., W. Junk b.v. Pub., The Hague, 1977, p 61.

25. J. Urquhart, "Proceedings of the Sixth International Congress of Pharmacology," Vol. 5, J. Tuomisto and M.K. Paasonen, Eds., Forssan Kirjapaino Oy, Helsinki, 1975, p 63.

26. R. Erickson, C. Mitchell, B. Pharriss and V. Place, "Advances in Planned Parenthood," Excerpta Medica, Princeton, N.J., 1976, p 167.

27. J. Martinez-Manautou, IPPFMed.Bull., 10, 3 (1976).

28. B.B. Pharriss, R. Erickson, J. Bashaw, S. Hoff, V.A. Place and A. Zaffaroni, Fertil.Steril., 25, 915 (1974).

29. J. Martinez-Manautou, R. Aznar, M. Maqueo and B.B. Pharriss, Fertil. Steril., 25, 922 (1974).

30. J. Martinez-Manautou, J.Steroid.Biochem., 6, 889 (1975).

31. B.B. Pharriss, V.A. Place, L. Sendelbeck and E. Schmitt, J.Reprod.Med., 17, 91 (1976).

32. S. Kent, Contemp.O/G, 10, 33 (1977).

33. G. Rybo, J.Reprod.Med., 20, 175 (1978).

34. B.B. Pharriss, J.Reprod.Med., 20, 155 (1978).

35. G. Trobough, A.M. Guderian, R.E. Erickson, S.A. Tillson, P. Leong, D.A. Swisher and B.B. Pharriss, J.Reprod.Med., 21(3), 153 (1978).

36. G.E. Trobough, J.Reprod.Med., 20(3), 167 (1978).

37. J. Martinez-Manautou, "Proceedings of the Symposium on Clinical Experience with the Progesterone Uterine Therapeutic System," D.R. Mishell and J. Martinez-Manautou, Eds., Acapulco, 15-16 October 1976, Excerpta Medica Amsterdam, 1978, p 129.

38. Anonymous, DerDeutscheApotheker, 28(6), (1976).

39. Anonymous, Fam.Plann.Perspect., 8, 132 (1976).

40. S.A. Tillson, M. Marian, R. Hudson, P. Wong, B.B. Pharriss, R. Aznar and J. Martinez-Manautou, Contraception, 11, 179 (1975).

41. L.S. Wan, Y.-C. Hsu, M. Ganguly and B. Bigelow, Contraception, 16, 417 (1977).

42. G. Dallenbach-Hellweg and S. Sievers, VirchowsArch.Pathol.Anat., 368, 289 (1975).

43. J. Martinez-Manautou, M. Maqueo, R. Aznar, B. Pharriss and A. Zaffaroni, Am.J.Obstet.Gynecol., 121, 175 (1975).

44. P. Ylostalo, P.A.J. Rantakyla and E. Kauppila, ActaObstet.Gynecol.Scand., 58, 279 (1979).

45. Y. Gibor and C. Mitchell, Contraception. (in press).

46. J.E. Shaw, S.K. Chandrasekaran, A.S. Michaels and L. Taskovich, in "Animal Models in Dermatology Relevance to Human Dermatopharmacology and Dematotoxicology," H. Maibach, Ed., Churchill Livingston, San Francisco, CA., 1975, p 138.

47. J.E. Shaw, S.K. Chandrasekaran and P. Campbell, J.Invest.Dermatol., 67, 677 (1976).

48. J.E. Shaw, S.K. Chandrasekaran, P.S. Campbell and L.G. Schmitt, in Cutaneous Toxicity, Academic Press, Inc., New York, N.Y., 1977, p 83.

49. S.K. Chandrasekaran and J.E. Shaw, Contemp.TopicsPolymerSci., Pearce & Schaefgen, Eds., Plenum Press, New York, N.Y., 1978, p 291.

50. J.E. Shaw and S.K. Chandrasekaran, DrugMetab.Rev. 8(2), 233 (1978).

51. R.J. Scheuplein, J.Invest.Derm., 45, 334 (1965).

52. R.J. Scheuplein, J.Invest.Derm., 48, 79 (1967).

53. R.J. Scheuplein, Edgewood Laboratory, Contract Report 18, (1967).

54. R.J. Scheuplein and I.H. Blank, Physiol.Rev., 51, 702 (1971).

55. R.J. Scheuplein and I.H. Blank, J.Invest.Derm., 60, 286 (1973).

56. A.S. Michaels, S.K. Chandrasekaran and J.E. Shaw, Am.Inst.Chem.Eng., 21, 985 (1975).

57. S.K. Chandrasekaran, A.S. Michaels, P.S. Campbell and J.E. Shaw, Am.Instit.Chem.Eng., 22, 828 (1976).

58. S.K. Chandrasekaran, W. Bayne, J.E. Shaw, J.Pharm.Sci., 67(10), 1370 (1978).

59. N. Reichek, R.E. Goldstein, D.R. Redwood and S.E. Epstein, Circulation, 50, 348 (1974).

60. H.H. Wayne, Angiology, 28, 203 (1977).

61. J.E. Shaw and S.K. Chandrasekaran, "Proceedings of International Conference on Drug Absorption, L. Prescott, Ed., ADIS Press (1980) (in press).

62. J.E. Shaw and J. Urquhart, TrendsPharmacol.Sci., 1, 208 (1980).

63. J.J. Brand, W.P. Colquhoun, A.H. Gould and W.L.M. Perry, Br.J.Pharmacol.Chemother., 30, 463 (1967).

64. A. Graybiel, J. Knepton and J.E. Shaw, Aviat.SpaceEnviron.Med., 47, 1096 (1976).

65. J.E. Shaw, L.G. Schmitt, M.E. McCauley and J.W. Royal, Clin.Pharmacol.Ther., 21, 117 (1977).

66. N. Price, L.G. Schmitt, and J.E. Shaw, Clin.Thera., 2(4), 258 (1979).

67. M.E. McCauley, J.W. Royal, J.E. Shaw and L.G. Schmitt, Aviat.SpaceEnviron.Med., 50(11), 1108, (1979).

68. F. Theeuwes, J.Pharm.Sci., 64, 1987 (1975).

69. F. Theeuwes and W. Bayne, J.Pharm.Sci., 66, 1388 (1977).

70. F. Theeuwes, W. Bayne and J. McGuire, Arch.Ophthalmol., 96, 2219 (1978).

71. A. Zaffaroni, "Drug Metabolism Reviews," Vol. 8, F. Di Carlo, Ed., Marcel Dekker, New York, N.Y., 1978, p 191.

72. F. Theeuwes and S.I. Yum, Ann.Biomed.Eng., 4, 343 (1976).

73. R. Capozza, B. Eckenhoff and S. I. Yum, J. Med. Eng. Tech., 1, 281 (1977).

74. R. Capozza, "Polymeric Delivery Systems," Midland Macromolecular Monographs No. 5, R.J. Kostelnick, Ed., Gordon and Breach, New York, N.Y., 1978, p 261.

75. E. Wei and H. Loh, Science, 193, 1262 (1976).

76. M.G. Falcon and B.R. Jones, Trans.Ophthal.Soc. U.K., 97, 330 (1977).

77. C. Siew and D.B. Goldstein, J.Pharm.Exp.Ther., 204, 541 (1978).

78. T. Kasamatsu, J.D. Pettigrew and M. Ary, J.Comp.Neur., 185, 163 (1979).

79. R.J. Gronan and D.H. York, Pharmacol.Biochem,Behav.. 10, 121, (1979).

80. B.R. Pratt, R.L. Butcher and E.K. Inskeep, J.Anim.Sci., 48, 1441 (1979).

81. S.K. Shen, S. Williams, C. Onkelinx, Jr. and F.W. Sunderman, Toxicol.Appl.Pharamcol., 51, 209 (1979).

82. J.A. Eliason and D.M. Maurice, Invest.Ophthalmol.Vis.Sci., 19, 102 (1980).

83. B. Eckenhoff, F. Theeuwes and J. Urquhart, Pharm.Technol. (in press).

84. A. Zaffaroni, Pharm.Int., 1, 3 (1980).

85. B. Eckenhoff, in "Proceedings of the American Institute of Chemical Engineers Meeting on Controlled and Topical Release" (in press).

86. J.C. Wright, R.G. Buckles, J.T. Dunn, H.M. Leeper and S.I. Yum, Rubber Chemistry and Technology, 50(5), 959 (1977).

87. H.M. Leeper, R.G. Buckles, G.V. Guittard, M.A. Lorberbaum, E.R. Sevilla and S.I. Yum, Rubber Chemistry and Technology, 50(5) 969 (1977).

88. S.I. Yum, R.G. Buckles and H.M. Leeper, Elastomer and Plastics, 10, 340 (1978).

89. E.P. Paulsen, J.W. Courtney and W.C. Duckworth, Diabetes, 28(7), 640 (1979).

90. J. Bottino, K.B. McCredie, D.W.H. Ho, E.J. Freireich, R. Buckles, S. Herbst and M. Lawson, Cancer, 43, 2197 (1979).

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