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Catecholamine Uptake by Synaptosomes from Rat Brain

Structure-Activity Relationships of Drugs with Differential Effects on Dopamine and Norepinephrine Neurons

ALAN S. HORN,[1] JOSEPH T. COYLE, AND SOLOMON H. SNYDER[2]

Departments of Pharmacology and Experimental Therapeutics and Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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SUMMARY

A number of centrally active drugs, including antiparkinsonian agents, antihistamines, tricyclic antidepressants, and phenothiazines, inhibit catecholamine uptake into synaptosomes prepared from various areas of rat brain. Many of these drugs have markedly differing affinities for the dopamine neurons of the corpus striatum as compared to the norepinephrine neurons in other brain regions. All drugs examined inhibit catecholamine uptake into hypothalamic synaptosomes competitively, but are noncompetitive inhibitors in the corpus striatum. In both brain areas, uptake inhibition appears to be reversible. In a series of drugs, certain structural features are related to the relative selectivity toward norepinephrine or dopamine neurons. Replacement of an alkylamino side chain by a tropine ring system enhances affinity for the dopamine neurons, as does a relative lack of constraint of the aromatic ring. The antidepressant drugs imipramine and amitriptyline are affected oppositely by *N*-demethylation. While *N*-demethylation of imipramine increases its affinity for hypothalamic catecholamine synaptosomes 20-fold, *N*-demethylation of amitriptyline reduces the inhibition of hypothalamic catecholamine uptake 24-fold. Such structure-activity relationships may facilitate the development of drugs with a high degree of selectivity, respectively, for dopamine and norepinephrine neurons.

INTRODUCTION

The catecholamines norepinephrine and dopamine occur in specific neuronal tracts in the brain, where they have been postulated to be neurotransmitters (1). Catecholamines can be accumulated in brain tissue via a neuronal membrane transport system as well as by a reserpine-sensitive granular storage process. The neuronal membrane transport system appears to account for terminating the effects of catecholamines released at synapses both in the peripheral sympathetic nervous system and in the brain (2). This uptake process can be studied in the brain *in vitro* by the incubation of radioactively labeled amines with brain slices (3–5) or synaptosomes (pinched-off nerve terminals) (6).

Recently we reported differences in the characteristics of catecholamine uptake by dopamine and norepinephrine nerve terminals (6, 7). Synaptosomes prepared from areas of the brain where norepinephrine is the predominant catecholamine showed a 4-fold greater affinity for the physiologically occurring *l*-isomer than for *d*-norepinephrine, while synaptosomes prepared from the corpus striatum, where dopamine is the predominant catecholamine, had equal affinities

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for *d*- and *l*-norepinephrine (7). While the β-carbon of norepinephrine is asymmetrical, amphetamine is asymmetrical at the α-carbon. *d*-Amphetamine was 10 times more potent an inhibitor of catecholamine uptake by norepinephrine nerve terminals than was *l*-amphetamine. In the dopamine terminals of the corpus striatum, on the other hand, *d*- and *l*-amphetamine were equally potent inhibitors of catecholamine uptake (6). Catecholamine uptake after intraventricular administration into brain areas rich in dopamine or norepinephrine was affected by amphetamine isomers in the same way as the synaptosomes (8, 9).

A number of clinically efficacious antiparkinsonian drugs are active inhibitors of catecholamine uptake by brain synaptosomes (9, 10). Some of these drugs are more potent inhibitors of catecholamine uptake by striatal synaptosomes than by synaptosomes from other regions of the brain, while certain antidepressant drugs, such as desipramine, are much more active in norepinephrine-containing brain regions than in the corpus striatum (9, 10).

It is well known that the brains of parkinsonian patients are depleted of their dopamine content (11) and that L-dopa, the amino acid precursor of dopamine, dramatically ameliorates the symptoms of this disease (12, 13). Accordingly, we postulated that antiparkinsonian drugs may act, in part, by inhibiting the further uptake of dopamine by striatal nerve terminals, hence potentiating the synaptic activities of the remaining dopamine nerve terminals in the brains of parkinsonian patients (9, 10).

In the present study we have examined the structure-activity relationships for inhibition of catecholamine uptake into synaptosomes of dopamine and norepinephrine neurons by antiparkinsonian drugs as well as by phenothiazines, tricyclic antidepressants, and related agents. We have also evaluated the kinetics of inhibition of catecholamine uptake by these drugs.

MATERIALS AND METHODS

Sprague-Dawley female rats (150–200 g) received intraperitoneal injections of reserpine (5 mg/kg) 18 hr before being killed by cervical dislocation and immediate decapitation. Their brains were rapidly removed and dissected on dental wax. All procedures prior to incubation were carried out at 4°. Slices of the striatum were weighed and homogenized in 20 volumes of ice-cold 0.25 M sucrose in a glass homogenizer with a Teflon pestle (Kontes K88600). Slices of hypothalamus were weighed and homogenized in 8 volumes of 0.25 M sucrose. The homogenates were centrifuged at 1000 × *g* for 10 min. The precipitate was discarded, and the supernatant fluid was gently stirred to make a uniform suspension. An aliquot of the supernatant fluid was added to a 20-ml beaker containing 3.8 ml of Krebs-Henseleit bicarbonate buffer, pH 7.4 (14), with glucose, in which the calcium concentration was lowered by 50%, and containing ascorbic acid (0.2 mg/ml), disodium ethylenediaminetetraacetic acid (0.2 mg/ml), nialamide (12.5 μM), and varied amounts of drug. The incubation mixture was agitated at 37° for 5 min under an atmosphere of 95% O_2–5% CO_2 in a Dubnoff metabolic shaker. After 5 min of incubation, various amounts of *dl*-[^{3}H]norepinephrine or [^{3}H]dopamine were added to the incubation mixture, and incubation was continued for an additional 5 min. At the end of the incubation period, the incubation mixtures were centrifuged at 48,000 × *g* for 20 min at 4°. An aliquot (0.1 ml) of the supernatant fluid was transferred to a vial, and its radioactivity was measured after the addition of 3 ml of absolute ethanol and 10 ml of toluene phosphor (0.4% 2,5-diphenyloxazole (PPO) and 0.01% *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP) in toluene). The remainder of the supernatant fluid was discarded, and the pellet was rinsed twice with 4 ml of ice-cold 0.9% NaCl. The pellet was resuspended in 2 ml of absolute ethanol, transferred to a glass homogenizer, homogenized with a loosely fitting glass pestle, and centrifuged for 10 min at 900 × *g*. An aliquot (0.5 ml) of the supernatant fluid of this centrifugation was transferred to a vial, and its radioactivity was determined after the addition of 10 ml of the toluene phosphor described above.

An alternative method for the separation of the incubated synaptosomes from the medium was found to be more rapid and con-

venient. At the end of the final 5-min incubation period the mixture was cooled to ice-water temperature and removed by filtration under vacuum on a Gooch crucible containing a membrane filter paper (Bac-T-Flex B-6, 22 mm). Each filter paper disc was washed with 10 ml of ice-cold 0.9% NaCl solution to remove labeled catecholamine adhering to the filter that was not taken up into the synaptosome preparation. The filter paper discs were allowed to dry on absorbent paper and then placed in a counting vial and extracted for 10 min with a mixture of Triton X-100 and toluene (1:4, by volume) containing 2,5-diphenyloxazole (5.5 g/liter) and *p*-bis[2-(5-phenyloxazolyl)]benzene (125 mg/liter) as scintillators. Radioactivity in the samples was then measured in a Packard 3375 liquid scintillation spectrometer. The small amount of radioactive medium adhering to the filter was estimated in each experiment and subtracted from the tritium concentration of each sample. Values for catecholamine uptake and drug effects were the same with the two methods of measuring particulate radioactivity.

Under the incubation conditions employed, we previously showed that 85% or more of the synaptosomal content of [^{3}H]norepinephrine or [^{3}H]dopamine was not metabolized (5–7).

The uptake of ^{3}H-labeled amine was linear for at least 10 min, as reported earlier (6, 7). Accordingly, as a measure of initial velocity, amine uptake (v) was determined as nanomoles per gram per 5 min. Inhibition constants were estimated by three different methods. ID_{50} values were determined by incubating homogenates of the striatum or hypothalamus with a constant concentration of [^{3}H]catecholamine and varying concentrations of drugs, and are expressed as the molar concentration of drugs that produced 50% inhibition of [^{3}H]catecholamine accumulation as determined on logarithmic probability paper. According to the method of Lineweaver and Burk (15), the uptake of varying amounts of [^{3}H]catecholamine was measured in the presence or absence of fixed amounts of drugs, and a plot of the reciprocal of [^{3}H]catecholamine uptake with respect to the reciprocal of [^{3}H]catecholamine concentration in the medium (S) was drawn. As specified by Dixon (16), two concentrations of [^{3}H]catecholamine were incubated in the presence of varied concentrations of drugs, and the reciprocal of [^{3}H]catecholamine uptake was plotted against the concentration of drug. The point of intersection of the two lines gives K_i directly. For competitive inhibitors the intersection occurs at a height equivalent to $1/V_{max}$, whereas for noncompetitive inhibitors the intersection occurs at the abscissa (16).

The names and structures of the drugs used are given in Figs. 6–11, and the sources of these compounds are recorded under ACKNOWLEDGMENTS at the end of the text.

RESULTS

Kinetics of Inhibition of Catecholamine Uptake by Drugs

Earlier we reported that the inhibition of catecholamine uptake into synaptosomes from the corpus striatum by the antiparkinsonian drugs benztropine, diphenhydramine, and diethazine was noncompetitive, while these compounds inhibited catecholamine uptake into hypothalamic synaptosomes competitively (10). In the present study the kinetics of the inhibition of catecholamine uptake in the striatum and hypothalamus was assessed for several other drugs by the methods of Lineweaver and Burk (15) and Dixon (16) (Figs. 1 and 2). By both methods of kinetic analysis the antiparkinsonian agent benztropine, the phenothiazine tranquilizer chlorpromazine, and the antidepressant drug amitriptyline were noncompetitive inhibitors in the striatum and competitive inhibitors in the hypothalamus.

It is conceivable that all these drugs inhibit hypothalamic catecholamine uptake by competing with an amine for uptake sites. Ouabain is thought to inhibit catecholamine accumulation by inactivating the membrane sodium pump, to which the catecholamine transport mechanism presumably is linked (17). Accordingly, it should inhibit catecholamine uptake in a noncompetitive fashion regardless of which brain region is studied. In experiments with the hypothalamus (Fig. 2), as well as in the striatum, ouabain inhibited catecholamine uptake noncompetitively,

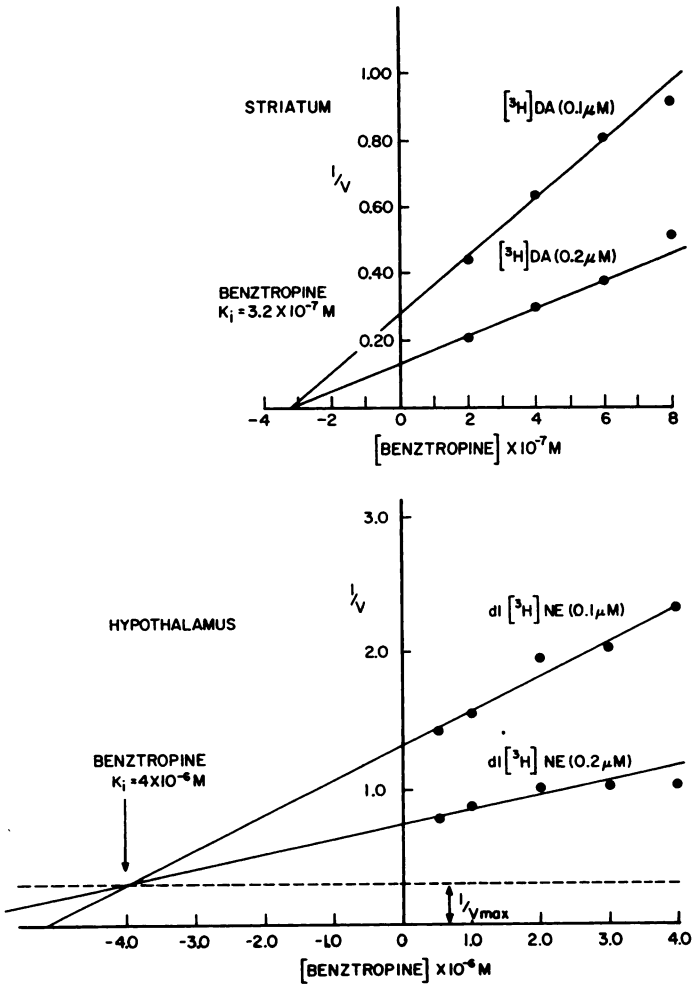


FIG. 1. *Kinetic analysis of [³H]catecholamine uptake inhibition by benztropine*

The upper half of the figure shows a graphic analysis of the kinetics of inhibition of [³H]dopamine ([³H]DA) uptake into striatal synaptosomes by benztropine. The lower portion shows a similar analysis of the kinetics of the inhibition of *dl*-[³H]norepinephrine (*dl*[³H]NE) uptake into hypothalamic synaptosomes by benztropine. The method of graphical analysis in both cases was that of Dixon (16). Homogenates were incubated with 0.1 or 0.2 μM [³H]dopamine or [³H]norepinephrine and varied concentrations of drug. Amine uptake is expressed in millimicromoles accumulated per gram per 5 min.

as has been shown for peripheral sympathetic nerves (18).

The pattern of noncompetitive inhibition in the striatum and competitive inhibition in the hypothalamus was also demonstrated for desipramine, diphenhydramine, and diethazine. Although the antiparkinsonian agents, antihistamines, and tricyclic antidepressants showed different patterns of catecholamine uptake inhibition in striatum and hypothalamus, *d*- and *l*-amphetamine inhibited catecholamine uptake competitively in both brain regions, whether assessed by the method of Dixon (Fig. 3, presentation of data for the corpus striatum) or the method of Lineweaver and Burk.

For all drugs examined except ouabain, the kinetics of uptake inhibition by benztropine, trihexiphenidyl, and imipramine was competitive in the cerebral cortex as well as the hypothalamus, while ouabain displayed noncompetitive kinetics in both regions. This

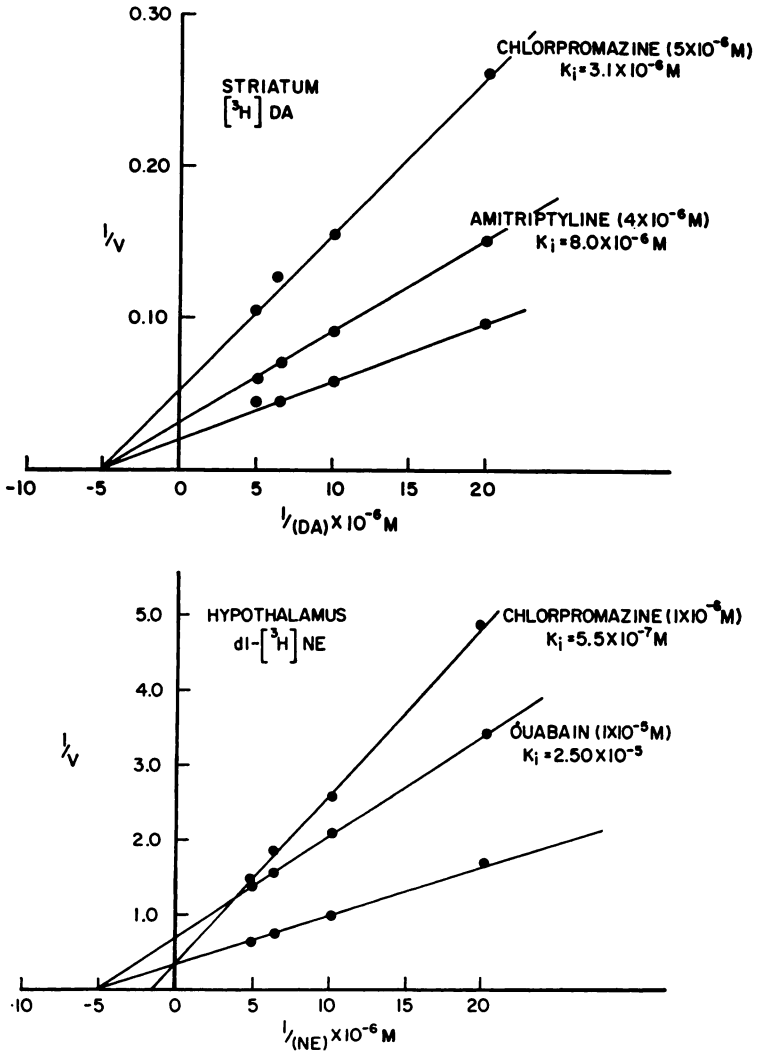


FIG. 2. *Kinetic analysis of [³H]catecholamine uptake inhibition by amitriptyline, chlorpromazine, and ouabain*

The upper half of the figure shows a graphic analysis of the kinetics of inhibition of [³H]dopamine ([³H]DA) uptake into striatal synaptosomes by chlorpromazine and amitriptyline. The lower half of the figure shows a similar analysis of the kinetics of the inhibition of *dl*-[³H]norepinephrine (*dl*-[³H]NE) uptake by chlorpromazine and ouabain in the hypothalamus. The method used in both cases was that of Lineweaver and Burk (15). Homogenates were incubated with concentrations of [³H]dopamine or [³H]norepinephrine ranging from 0.05 to 0.2 μM and a constant concentration of the drug.

suggests that competitive inhibition of catecholamine uptake by these drugs is a characteristic of norepinephrine terminals as opposed to the noncompetitive kinetics in the dopamine nerve terminals of the corpus striatum. The same kinetics of inhibition with all drugs and in all brain regions was observed whether [³H]dopamine or [³H]norepinephrine was utilized as substrate for the uptake system.

In an attempt to discern whether inhibition of striatal catecholamine uptake was reversible or irreversible, experiments were performed by the method of Ackermann and

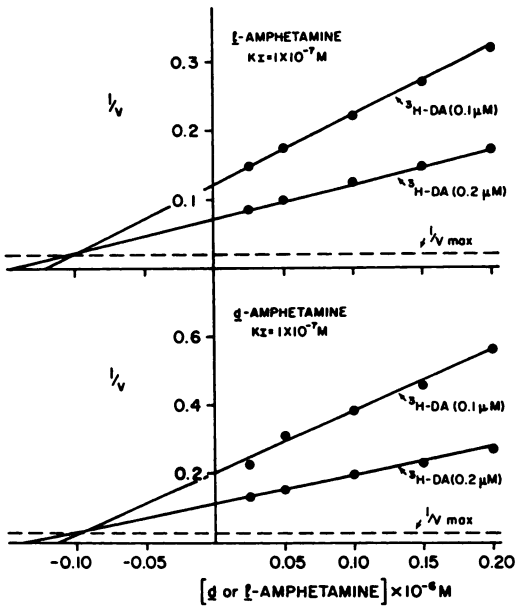


FIG. 3. *Kinetic analysis of [³H]dopamine (³H-DA)uptake inhibition by d- and l-amphetamine in corpus striatum*

The upper half of the figure presents a graphic analysis of the kinetics of inhibition of [³H]dopamine uptake into striatal synaptosomes by *d*-amphetamine; the lower half is the same analysis using *l*-amphetamine. The method of kinetic analysis was that of Dixon (16). Homogenates were incubated with 0.1 or 0.2 μM [³H]dopamine and varied concentrations of the drug.

Potter (19). In this procedure, used predominantly for studies of enzyme inhibition, various concentrations of enzymes are incubated with substrate in the presence or absence of inhibitor. In the presence of a reversible inhibitor, enzyme activity, as in controls, is linear with increasing enzyme concentration, yielding a straight line through the origin, but with a lesser slope than in control incubations. With irreversible inhibitors, the slope of the line relating enzyme activity and tissue concentration is the same as that of the control but intersects the abscissa to the right of the origin by a degree proportional to the concentration of inhibitor (19). Since in our system catecholamine accumulation is proportional to tissue concentration (7), it was possible to investigate the nature of drug inhibition in striatal synaptosomes by the method of Ackermann and Potter (19). Four concentrations of striatal homogenate ranging from 1 to 4 mg were incubated with [³H]dopamine (0.1 μM) in the presence of diphenhydramine, benztropine, *d*-amphetamine, and diethazine (Fig. 4). For all four drugs, catecholamine accumulation was linear with increasing tissue concentration and a straight line intersecting the origin, but with a reduced slope, was obtained. These results suggest that, although the inhibition of striatal catecholamine uptake by several drugs is noncompetitive, it is nonetheless reversible.

Structure-Activity Relationships of Drugs in Inhibition of Striatal and Hypothalamic Catecholamine Uptake

In assessing the potency of inhibition of catecholamine uptake, ID_{50} values were de-

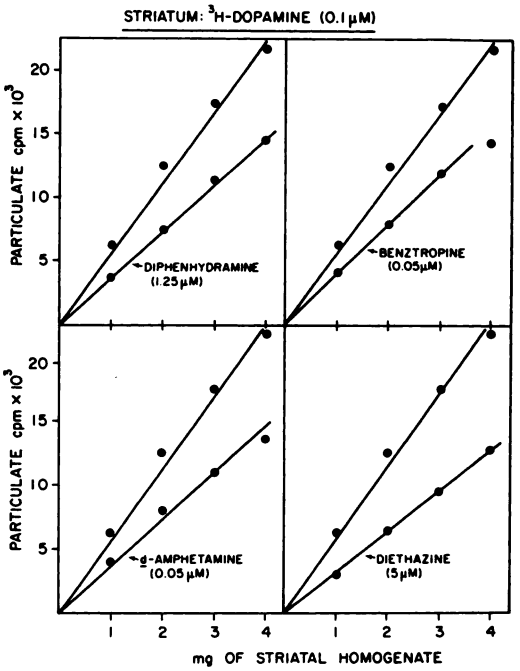


FIG. 4. *Analysis by the Ackermann-Potter (19) method of [³H]dopamine uptake inhibition by diphenhydramine, benztropine, d-amphetamine, and diethazine*

This kinetic analysis, using the method of Ackermann and Potter (19), shows that the mode of inhibitor interaction with the uptake site is reversible. The upper lines in each case give results in the absence of inhibitor.

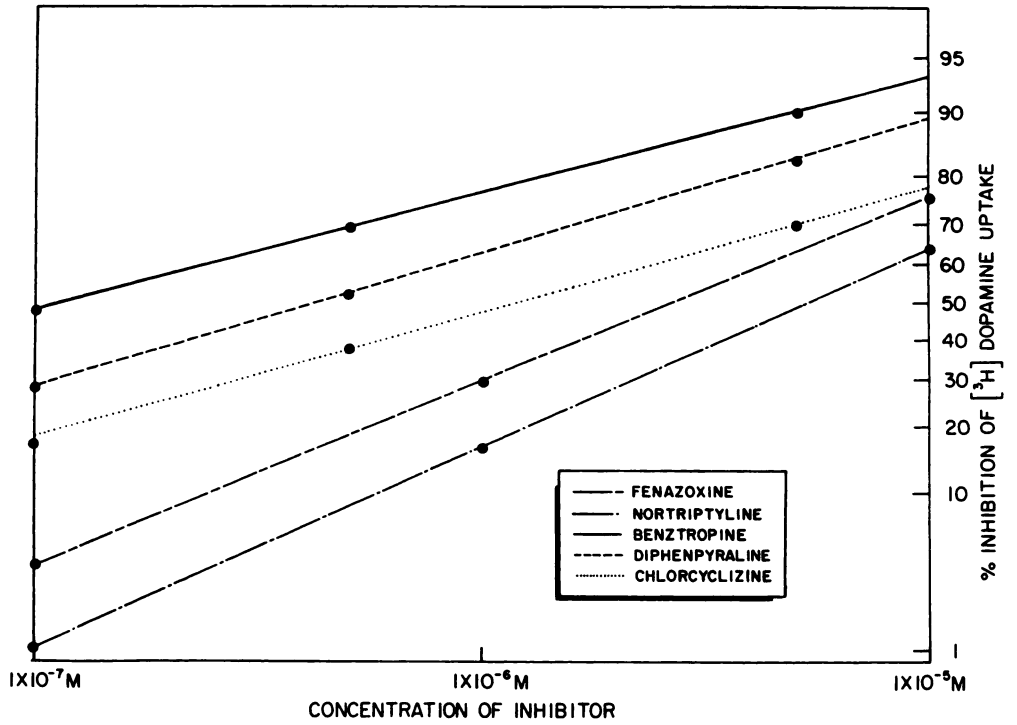


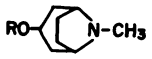
FIG. 5. *Graphic method of obtaining ID_{50} values for inhibition of [^3H]catecholamine uptake by drugs* The figure illustrates the method of determining ID_{50} values, using log probit paper. The percentage inhibition of catecholamine uptake into synaptosomes of the corpus striatum at three concentrations of inhibitor, in quadruplicate, after subtracting 0° uptakes as blanks, were plotted. The [^{3}H]dopamine concentration was 0.1 μM.

termined for synaptosomal preparations from the striatum and hypothalamus by the use of log-probit paper with three drug concentrations. The "relative potency" of the various drugs was expressed as the reciprocal of ID_{50} values $\times$ 10^{-3}. The probit plot method gave satisfactory linear plots in all experiments. In general the results for different drugs yielded probit plots of very similar slopes, some of which are illustrated in Fig. 5.

Tropine ring system. For compounds with comparable aromatic ring systems, replacement of the alkylamine side chain with a tropine ring system markedly enhanced potency in inhibiting striatal, but not hypothalamic, catecholamine uptake. Thus, benztropine (Fig. 6) closely resembles diphenhydramine (Fig. 7) except for the replacement of the dimethylaminoethyl side chain by a tropine ring. Benztropine is 30 times more potent than diphenhydramine in the striatum, but no more active in the hypothalamus. The tropine ring system holds the nitrogen atom of benztropine in a relatively rigid conformation. In diphenpyraline (Fig. 7) the nitrogen atom is part of a piperidine ring, and has relatively more conformational mobility than the nitrogen of benztropine but less than the nitrogen in diphenhydramine. Interestingly, the potency of diphenpyraline in inhibiting striatal catecholamine uptake is intermediate between that of benztropine and diphenhydramine. Similarly, BS 6825 (Fig. 6) differs from orphenadrine (Fig. 7) only by substitution of a tropine for an alkylamine side chain, with a resultant 13-fold increase in potency in the striatum but a 3-fold reduction in potency in the hypothalamus. Accordingly, the ratio of striatal to hypothalamic potency for BS 6825 is 40 times greater than for orphenadrine.

Deptropine (Fig. 6) differs from nortrip-

TROPINES



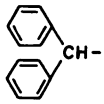
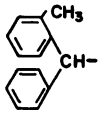
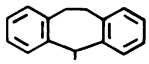
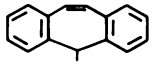
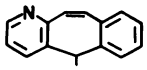
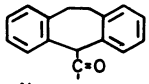
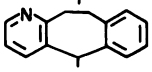
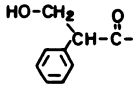
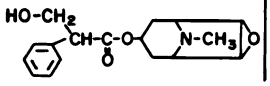
NAME	R	RELATIVE POTENCY STRIATUM (S)	HYPOTHALAMUS (H)	S/H
BENZTROPINE		8,333	370	22.5
BS 6825		2,000	100	20.0
DEPTROPINE		1,333	143	9.3
BS 7039		1,000	33	30.3
BS 7723		167	10	16.7
BS 7042		100	100	1.0
BS 7715		50	15	3.3
ATROPINE		20	12	1.6
SCOPOLAMINE		5	3	1.6

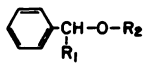
FIG. 6. *Relative potencies for inhibition of [^3H]catecholamine uptake by compounds having a tropine ring system*

The data presented in Figs. 6–11 are the means of two or three independent determinations of ID_{50} values. Each determination was carried out in quadruplicate with three concentrations of inhibitor. The term "relative potency" is defined as the reciprocal of $ID_{50} \times 10^{-3}$, i.e., 1 = 1 mM. In independent determinations, none of the values shown differed by more than 25%. The structure for scopolamine is shown in full and is not attached as a radical (R) to the tropine ring system.

tyline (Fig. 8) principally in the replacement of the alkylamino side chain by a tropine ring, with a resultant 7-fold increase in striatal inhibitory potency but a 5-fold decrease in hypothalamic potency. Deptropine and nortriptyline, however, are not strictly comparable because of the exocyclic double bond in nortriptyline. Replacement of the tropine ring in atropine by the scopine ring in scopolamine (Fig. 6) resulted in a 4-fold decrease in potency in both brain areas.

Aromatic ring system. In general, among the bicyclic compounds (benzyl ether, benzyl alcohols, and phenylmethanes), the presence of two aromatic rings was associated with greater potency. Most of the benzyl ethers (Fig. 7) contain two aromatic rings and were, for the most part, more potent in both the striatum and hypothalamus than the benzyl alcohols, all of which contain only one aromatic ring (Fig. 9). However, these groups are not strictly comparable because of the

BENZYL ETHERS



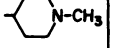
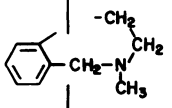
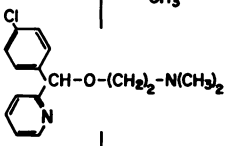
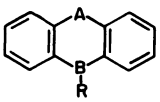
NAME	R_1	R_2	RELATIVE POTENCY STRIATUM (S)	HYPOTHALAMUS (H)	S/H
DIPHENPYRALINE			2,273	1,000	2.2
FENAZOXINE			357	20	17.8
CARBINOXAMINE			300	357	0.8
DIPHENHYDRAMINE		$-(CH_2)_2-N(CH_3)_2$	286	370	0.8
ORPHENADRINE		$-(CH_2)_2-N(CH_3)_2$	161	333	0.5

FIG. 7. *Relative potencies for inhibition of [^3H]catecholamine uptake by compounds having the benzyl ether system*

Fenazoxine has an 8-membered ring fused to a benzene ring.

TRICYCLICS



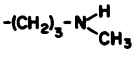
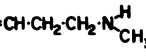
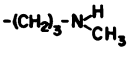
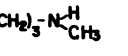
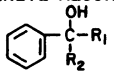
NAME	A	B	R	RELATIVE POTENCY STRIATUM (S)	HYPOTHALAMUS (H)	S/H
60-389a	–	N	$-(CH_2)_3-NH_2$	250	77	3.2
61-425	–	N		250	2.5	100
AMITRIPTYLINE	$-CH_2-CH_2-$	C	$=CH\cdot CH_2\cdot CH_2\cdot N(CH_3)_2$	250	18,181	0.01
NORTRIPTYLINE	$-CH_2-CH_2-$	C		182	769	0.24
PROTRIPTYLINE	$-CH=CH-$	-CH		167	100	1.7
IMIPRAMINE	$-CH_2-CH_2-$	N	$-(CH_2)_3-N(CH_3)_2$	125	1,000	0.1
DOXEPIN	$-O-CH_2-$	C	$=CH\cdot CH_2CH_2-N(CH_3)_2$	40	1,538	0.03
DESIPRAMINE	$-CH_2-CH_2-$	N		20	20,000	0.001

FIG. 8. *Relative potencies for inhibition of [^3H]catecholamine uptake by compounds having a tricyclic ring system*

Compounds 60-389a and 61-425 are carbazoles and have no bridging atoms at "A" between the aromatic rings.

BENZYL ALCOHOLS



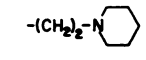
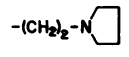
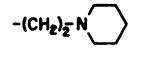
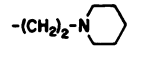
NAME	R_1	R_2	RELATIVE POTENCY STRIATUM (S)	HYPOTHALAMUS (H)	$S/_H$
TRIHEXYPHENIDYL			169	31	5.4
PROCYCLIDINE			109	667	0.2
BIPERIDEN			100	15	6.6
CYCRIMINE			63	26	2.4

FIG. 9. *Relative potencies for inhibition of [^3H]catecholamine uptake by compounds having the benzyl alcohol system*

differences in the phenylmethane, benzyl ether, and alcohol moieties as well as variations in the side chain.

Constraint of the aromatic rings by bridging with a 2-carbon saturated or unsaturated chain was associated with decreased potency in the striatum and generally in the hypothalamus. Thus, in both the hypothalamus and striatum, the tricyclics, deptropine and BS 7039, were considerably weaker than the bicyclic, benztropine.

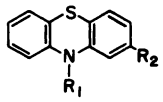
Alteration of one of the benzene rings by *o*-methylation or bioisosteric replacement of one benzene ring with a pyridine ring reduced activity in both the hypothalamus and striatum. Examples of substitution of a pyridine for a benzene ring which reduced activity are the contrasting activities of BS 7715 and deptropine and those of BS 7723 and BS 7039 (Fig. 6). Examples in which *o*-methylation reduced activity include the decreased activities of BS 6825 and orphenadrine, as compared, respectively, with benztropine and diphenhydramine.

Tricyclic compounds. The "antidepressant" activity of tricyclic compounds (Fig. 8) in pharmacological tests has often been reported to be greater for secondary amines, such as desipramine and nortriptyline, than for tertiary amines, such as imipramine and amitriptyline (20, 21). Since antidepressant activity is thought to be related to inhibition of catecholamine uptake, it was of interest to compare the relative potency of the secondary and tertiary amines in inhibiting catecholamine uptake by striatal and hypothalamic synaptosomes. As had been previously reported (10) in the hypothalamus, which reflects norepinephrine neurons, desipramine was 20 times as potent as imipramine. In the striatum, however, desipramine was only one-sixth as potent as imipramine. Thus *N*-demethylation of imipramine produces opposite effects on striatal and hypothalamic catecholamine uptake.

In striking contrast to the increased hypothalamic potency resulting from *N*-demethylation of imipramine, the conversion of the tertiary amine amitriptyline to the secondary amine nortriptyline resulted in a 24-fold decrease in potency in the hypothalamus. Amitriptyline and nortriptyline did not differ markedly in their relative potencies for inhibition of striatal catecholamine uptake.

Maxwell *et al.* (22) found that maximum inhibition of norepinephrine uptake in rabbit aortic strips was produced by nonplanar tricyclic compounds with a large, dihedral angle, while planar tricyclic compounds, such as carbazoles, phenanthridones, and dihydrophenanthridines, were extremely weak inhibitors. We found the carbazole derivatives 60-389a and 61-425 to be quite weak inhibitors of catecholamine uptake in the hypothalamus. In fact, the secondary amine carbazole 61-425 was the least active of all compounds examined in this study. However, in the corpus striatum, the carbazole

PHENOTHIAZINES



NAME	R_1	R_2	RELATIVE POTENCY STRIATUM (S)	HYPOTHALAMUS (H)	S/H
PROPIOMAZINE	$-CH_2-CH(CH_3)-N(CH_3)_2$	$-C(=O)-CH_2-CH_3$	357	182	1.9
TRIFLUOPERAZINE	$-(CH_2)_3-N$ piperazine $-N-CH_3$	$-CF_3$	200	111	1.8
MEPAZINE	$-CH_2-$ piperidine $-N-CH_3$	$-H$	167	435	0.4
CHLORPROMAZINE	$-(CH_2)_3-N(CH_3)_2$	$-Cl$	147	2,000	0.1
DIETHAZINE	$-(CH_2)_2-N(C_2H_5)_2$	$-H$	137	143	0.9
CARPHENAZINE	$-(CH_2)_3-N$ piperazine $-N-(CH_2)_2-OH$	$-C(=O)-(CH_2CH_3$	100	20	5.0
PYRATHIAZINE	$-(CH_2)_2-N$ pyrrolidine	$-H$	71	200	0.4
PROMETHAZINE	$-CH_2-CH(CH_3)-N(CH_3)_2$	$-H$	38	200	0.2
ETHOPROPAZINE	$-CH_2-CH(CH_3)-N(C_2H_5)_2$	$-H$	28	56	0.5

FIG. 10. *Relative potencies for inhibition of [^3H]catecholamine uptake by the phenothiazine group*

derivatives were fairly potent, and one of them, 61-425, was 100 times more active in inhibiting striatal than hypothalamic catecholamine uptake.

Phenothiazines, benzyl alcohols, and phenylmethanes. Among the phenothiazines (Fig. 10), substitutions in position 2 generally were associated with increased potency in the striatum. For example, propiomazine differs from promethazine only in the presence of a ethyl ketonic substituent in position 2, and was 10 times more active than promethazine in the striatum. In the hypothalamus, chlorpromazine was the most active member of this series, being about 5 times as potent as the next most active phenothiazine.

Among the benzyl alcohols (Fig. 9), replacement of the cyclohexane ring of trihexyphenidyl by a cyclopentane or a bicycloheptene ring, as in cycrimine and biperiden, respectively, resulted in decreased inhibition of striatal catecholamine uptake.

The most active inhibitor of striatal catecholamine uptake of the phenylmethanes (Fig. 11) was chlorcyclizine. It is not known whether this enhancement by chlorine substitution was due to an increased lipid solubility and/or a stereoelectronic effect.

DISCUSSION

In the present study we have used hypothalamic synaptosomes as a model for norepinephrine nerve terminals, and those of the corpus striatum as a model of dopamine terminals. This would seem warranted, since the hypothalamic catecholamine content is preponderantly norepinephrine and that of the striatum is dopamine (1). Earlier we reported that kinetic constants and stereospecificity for catecholamine uptake were similar

PHENYLMETHANES

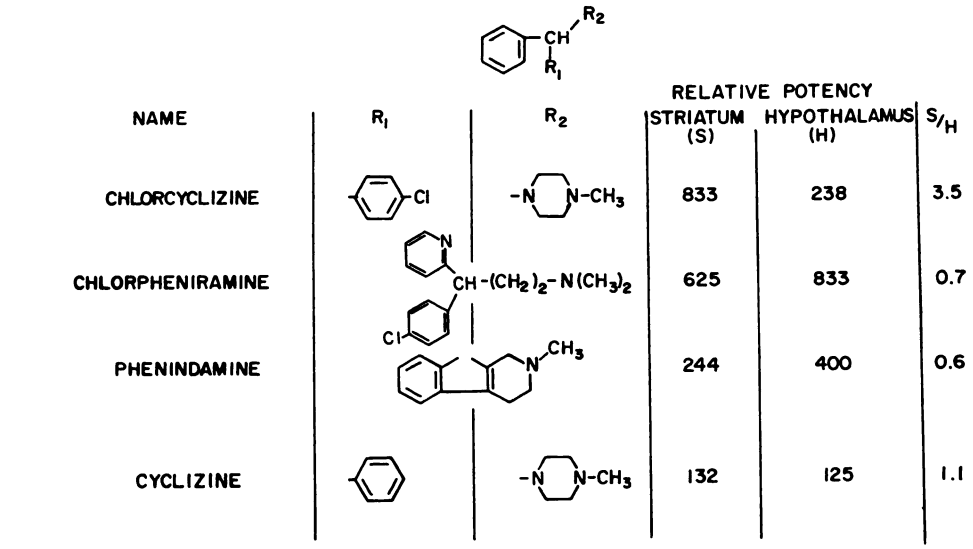


FIG. 11. *Relative potencies for inhibition of [^3H]catecholamine uptake by compounds having the phenylmethane system*

The middle ring of phenindamine is 5-membered.

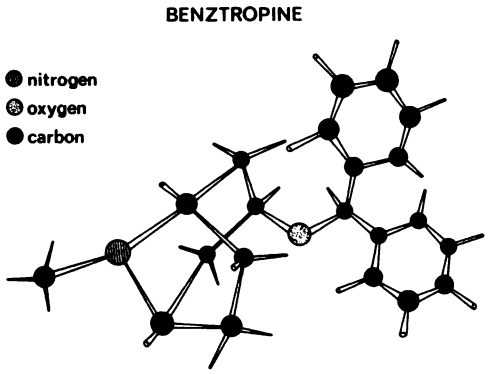


FIG. 12. *Molecular model of benztropine*

The figure is a drawing of a molecular model of benztropine in what is thought might be the preferred conformation in solution. The "piperidine portion" of the tropine ring system is in a chair conformation with the *N*-methyl substituent in an equatorial position. The two benzene rings are inclined at an angle of 140–150 degrees to each other.

in all nonstriatal regions of the rat brain in which norepinephrine is the predominant catecholamine (7). In preliminary experiments we observed that the relative potencies of benztropine, trihexyphenidyl, and diphenhydramine were similar in the hypothalamus, cerebral cortex, and medulla oblongata–pons, suggesting that these drugs have similar effects on norepinephrine neurons in different areas of the brain, which may differ from their effects on the dopamine neurons of the corpus striatum.

Kinetic analysis of the mechanism of inhibition of catecholamine uptake by various drugs indicated that, for both tricyclic and bicyclic compounds, inhibition was competitive in the hypothalamus and noncompetitive in the corpus striatum. It was unclear what features of the norepinephrine and dopamine neurons might account for the differing types of inhibition. Since there are high concentrations of acetylcholine in the corpus striatum and many of the drugs used are also anticholinergics, one is tempted to speculate that such drugs might inhibit striatal catecholamine uptake by interacting with a hypothetical "cholinergic" site on the striatal catecholamine synaptosomes.

A striking finding of the present study was the differential effects of drugs on hypothalamic and striatal catecholamine uptake. For example, replacement of an alkylamino side chain by a tropine ring greatly enhanced the

ability of drugs to inhibit striatal catecholamine uptake while decreasing their potency in the hypothalamus. By contrast, *N*-demethylation of imipramine markedly increased its potency in the hypothalamus while decreasing its activity in the corpus striatum. The planar carbazole compound 61-425 was 100 times more active in the striatum than in the hypothalamus and, hence, might have a highly selective action on dopamine neurons.

The antidepressant activity of tricyclic antidepressants is usually thought to be enhanced by *N*-demethylation, both for imipramine and for amitriptyline. Thus, the secondary amines are frequently more active in reversing reserpine sedation, hypothermia, and ptosis (20, 21). These pharmacological effects are usually assumed to be associated with inhibition of catecholamine uptake by norepinephrine neurons. The greater potency of desipramine and nortriptyline compared to imipramine and amitriptyline, respectively, in inhibiting catecholamine uptake in peripheral sympathetic neurons (23) has often been cited as evidence in favor of this view. As in the peripheral sympathetic nervous system, we found that *N*-demethylation of imipramine to desipramine greatly enhanced potency in inhibiting catecholamine uptake by hypothalamic synaptosomes. However, *N*-demethylation of amitriptyline to give nortriptyline produced a 24-fold decrease in potency in inhibiting catecholamine uptake by hypothalamic synaptosomes. If these effects *in vitro* reflect activity *in vivo*, our results suggest that the greater antidepressant efficacy in animal tests of nortriptyline as compared to amitriptyline is unrelated to more potent inhibition of norepinephrine uptake. Possibly, inhibition of norepinephrine uptake is not a sufficient explanation for antidepressant efficacy.

The structure-activity relationships we have observed for various drugs in inhibiting striatal catecholamine uptake may shed light on steric factors regulating catecholamine uptake by dopamine neurons. Recent work on the conformations of tropane (24) and diphenylmethane (25–27) provides evidence suggesting that the preferred conformation of benztropine in solution might be as shown in Fig. 12. Thus, nuclear magnetic resonance and dipole moment studies indicate that tropanes exist predominantly with the piperidine ring in a chair conformation with the *N*-methyl substituent equatorial. Moreover, dipole moment and other studies (25–27) show that diphenylmethane, in its preferred conformation in solution, has a large dihedral angle between the plane of the two benzene rings of about 140 degrees (experimental values given are 139, 143, and 148 degrees). If the suggested conformation of benztropine (Fig. 12) applies to other bicyclic compounds such as diphenylmethanes and benzyl ethers, the bicyclic derivatives may conformationally resemble the tricyclic compounds.

The relatively lower affinity of the tricyclic compounds for dopamine neurons, associated with aromatic ring constraint and a relatively rigid dihedral angle, suggests that this angle is not optimal for inhibition of catecholamine uptake by dopamine neurons. Presumably, at receptor sites the dihedral angle of the more potent two-ring drugs differs from that of the tricyclics.

The location of the side chain nitrogen in the tropine derivatives appeared to be optimal for inhibiting catecholamine uptake in the striatum. One might predict that compounds would be more potent insofar as their side chains were held rigidly in such a conformation. In support of this argument, comparison of benztropine, diphenpyraline, and diphenhydramine suggests that decreased potency in inhibiting striatal catecholamine uptake parallels increased conformational freedom of the side chain. For these three compounds, the O—N internuclear distance in the preferred conformation is very similar, even though diphenhydramine has a 2-carbon chain between O and N. Since the piperidine ring of diphenpyraline most probably would adopt a chair conformation with its *N*-methyl substituent equatorial, it would be directly superimposable on benztropine. However, loss of the 2-carbon bridge would naturally decrease the rigidity of the ring system, increasing conformational mobility. The simple dimethylaminoethyl side chain of diphenhydramine would, of course, possess the greatest conformational freedom of these three compounds. The increased potency as-

sociated with greater constraint of the side chain may reflect a particularly favorable alignment producing maximal stereoelectronic interaction at the catecholamine uptake site of dopamine neurons. Increased constraint of side chain mobility also enhances the ability of drugs to antagonize the Parkinson-like symptoms produced by tremorine (28), so that tropine derivatives are the most active.

Certain of the features which enhanced potency in inhibiting striatal catecholamine uptake actually decreased potency in the hypothalamus. Thus, in general, drugs with greater conformational side chain mobility and with substituents in the aromatic ring system had increased relative activity in the hypothalamus. Examples of this are diphenhydramine and benztropine, and orphenadrine and BS 6825. The differential steric features required for inhibition of catecholamine uptake by dopamine and norepinephrine neurons may aid in developing new agents with highly selective actions on these two types of neurons.

In general, drugs with greater anticholinergic activity were more potent inhibitors of striatal catecholamine uptake. Exceptions to this include the relatively weak actions of atropine and scopolamine. Moreover, introduction of an *o*-methyl group, as in the transformation of benztropine to BS 6825 or diphenhydramine to orphenadrine, increased anticholinergic activity (28, 29) and enhanced antiparkinsonian activity (29, 30), but decreased effects on striatal catecholamine uptake.

In the iminodibenzyl series, replacement of benzene rings by pyridine rings reduced central stimulant activity (31). In our experiments replacement of a benzene ring by a pyridine ring, as in the transformation of deptropine to BS 7715 or of BS 7039 to BS 7723, also decreased potency in both the striatum and hypothalamus.

Previously we suggested that antiparkinsonian drugs may owe their therapeutic efficacy in part to inhibition of striatal catecholamine uptake, thus potentiating the limited amount of dopamine remaining in the brains of parkinsonian patients (9, 10). In the present study, several centrally active anticholinergic drugs which possess antiparkinsonian activity, such as atropine and scopolamine, were comparatively weak inhibitors of striatal catecholamine uptake. However, atropine as well as benztropine can inhibit striatal catecholamine uptake *in vivo* (32). Perhaps the ideal antiparkinsonian drug will be one that is both a potent central anticholinergic agent as well as a highly selective and potent inhibitor of catecholamine uptake by dopamine neurons.

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REFERENCES

1. N. Å. Hillarp, K. Fuxe and A. Dahlström, *Pharmacol. Rev.* **18**, 727 (1966).
2. L. L. Iversen, "The Uptake and Storage of Noradrenaline in Sympathetic Nerves," Cambridge University Press, New York, 1967.
3. J. J. Dengler, I. A. Michaelson, H. E. Spiegel and E. Titus, *Int. J. Neuropharmacol.* **1**, 23 (1962).
4. B. Hamberger, *Acta. Pharmacol. Scand.* (suppl.) **295**, 7 (1967).
5. S. H. Snyder, A. I. Green, and E. D. Hendley, *J. Pharmacol. Exp. Ther.* **164**, 90 (1968).
6. J. T. Coyle and S. H. Snyder, *J. Pharmacol. Exp. Ther.* **170**, 221 (1969).
7. S. H. Snyder and J. T. Coyle, *J. Pharmacol. Exp. Ther.* **165**, 78 (1969).
8. K. M. Taylor and S. H. Snyder, *Science* **168**, 1487 (1970).
9. S. H. Snyder, K. M. Taylor, J. T. Coyle and J. L. Meyerhoff, *Amer. J. Psychiat.* **127**, 199 (1970).
10. J. T. Coyle and S. H. Snyder, *Science* **166**, 899 (1969).
11. O. Hornykiewicz, *Wien. Klin. Wochenschr.* **75**, 309 (1963).
12. M. D. Yahr, R. C. Duvoisin, M. J. Shear, R. E. Barrett and M. N. Hoehn, *Arch. Neurol.* **21**, 343 (1969).
13. G. C. Cotzias, P. S. Papavasilou and R. Gellene, *N. Engl. J. Med.* **280**, 337 (1969).
14. H. A. Krebs and K. Henseleit, *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33 (1932).
15. H. Lineweaver and D. Burk, *J. Amer. Chem. Soc.* **56**, 685 (1934).
16. M. Dixon, *Biochem. J.* **55**, 170 (1953).
17. A. H. Tissari, P. S. Schönhöfer, D. F. Bogdanski and B. B. Brodie, *Mol. Pharmacol.* **5**, 593 (1969).
18. F. Berti and P. A. Shore, *Biochem. Pharmacol.* **16**, 2091 (1967).
19. N. N. Ackermann and V. R. Potter, *Proc. Soc. Exp. Biol. Med.* **72**, 1 (1949).
20. F. Sulser, J. Watts and B. B. Brodie, *Ann. N. Y. Acad. Sci.* **96**, 279 (1962).
21. M. H. Bickel and B. B. Brodie, *Int. J. Neuropharmacol.* **3**, 611 (1964).
22. R. A. Maxwell, P. D. Keenan, E. Chaplin, B. Roth and S. Batmanglidj Eckhardt, *J. Pharmacol. Exp. Ther.* **166**, 320 (1969).
23. B. Callingham, *in* "Antidepressant Drugs" (S. Garattini and M. N. G. Dukes, eds.), p. 35. Excerpta Medica Foundation, Amsterdam, 1967.
24. R. J. Bishop, G. Fodor, A. R. Katritzky, F. Soti, L. E. Sutton and F. J. Swinbourne, *J. Chem. Soc.* 74 (1966).
25. M. J. Aroney, R. J. W. LeFevre, G. L. D. Ritchie and A. N. Singh, *J. Chem. Soc.* 5810 (1965).
26. A. Unanue and P. Bothorel, *Bull. Soc. Chim. France* 2827 (1965).
27. P. Bothorel and A. Unanue, *C.R. Hebd. Seances Acad. Sci. Paris.* **255**, 901 (1962).
28. M. F. Farquharson and R. G. Johnston, *Brit. J. Pharmacol. Chemother.* **14**, 559 (1959).
29. A. B. H. Funcke, A. F. Harms, M. D. deJonge and W. T. Nauta, *J. Med. Pharm. Chem.* **4**, 215 (1961).
30. M. C. deJonge, H. M. Tersteege, A. F. Harms, A. B. H. Funcke and W. T. Nauta, *J. Med. Pharm. Chem.* **2**, 147 (1960).
31. F. J. Villani and T. A. Mann, *J. Med. Chem.* **11**, 894 (1968).
32. M. Goldstein, K. Fuxe, A. F. Battista, T. Backstrom and S. Nakatani, *Fed. Proc.* **29**, 2445 (1970).