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1P NERVE GROWTH FACTOR SUPPRESSES TUMORIGENICITY OF HUMAN PROLACTINOMAS

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Prolactin (PRL)-secreting tumors are the most frequently occurring neoplasms in the human pituitary and are associated with varying degrees of hyperprolactinemia. The first line therapy of patients with prolactinomas was neurosurgical intervention until the breakthrough discovery that dopamine (DA) D-2 receptor agonists, such as bromocriptine, are effective both in lowering PRL levels and in reducing the tumor size. This pharmacological therapy, however, does not invariably reduce PRL secretion in prolactinoma patients and in fact there is a population of non-responders requiring the surgical approach. The mechanisms underlying such a resistance to DA drugs are not known.

In the present work tumor specimens from 8 patients refractory to bromocriptine and 5 sensitive subjects who, however, did not tolerate the drug were studied. The results have shown that prolactinomas may be divided into two subtypes: one sensitive to the DA therapy which has a very low degree of malignancy and expresses D-2 receptors for DA and one refractory to bromocriptine which is characterized by a high potential of growing in soft agar and forming tumors in nude mice and does not express the D-2 receptor protein.

These tumors express the receptors for Nerve Growth Factor (NGF) and are sensitive to its differentiating activity. NGF dictates in fact the permanent conversion of the more transformed DA-resistant prolactinomas into a more differentiated, less malignant lactotroph-like phenotype re-expressing the D-2 receptor protein. Following exposure to NGF for 4 days, the more malignant, DA-resistant prolactinoma cells decreased their proliferation rate, lost their capability of forming colonies in soft agar, lost their tumorigenic activity in nude mice and re-expressed the lactotroph-specific D-2 receptor protein inhibiting prolactin release. These effects were permanent after NGF withdrawal and were reproducible also in vivo in nude mice transplanted with the tumors. NGF in fact remarkably and lastingly depressed the tumors growth and induced the permanent expression of D-2 receptors when injected intravenously once a day for 5 days into prolactinoma-bearing nude mice.

These data suggest that NGF may induce a permanent switch of gene expression in human prolactinomas modifying their transforming phenotype and reverting them to more differentiated, less malingnant, dopamine-sensitive lactotroph-like cells. The possibility thus arises that a short term treatment with NGF may restore the refractory patients to the conventional pharmacological therapy with D-2 agonists.

2P DOPAMINE D_1 AND D_2 RECEPTOR BINDING SITES IN DEPRESSED SUICIDE VICTIMS

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The possible involvement of dopamine receptors in the aetiology of depression and antidepressant drug action was studied. Dopamine receptors were measured in brain tissue obtained at post-mortem from suicides with a firm retrospective diagnosis of depression and controls matched with suicides for age, sex and post-mortem delay. Antidepressant drug-free (ADF) and antidepressant drug-treated (ADT) suicides were studied. [^{3}H]SCH23390 [^{3}H](R)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine, (D_1) and [^{3}H]raclopride, (D_2) binding was performed at 8 ligand concentrations (0.06-8nM). Specific binding was defined by 10 µM cis-flupenthixol (D_1) or 10 µM (-) sulpiride (D_2). Binding parameters were determined using weighted non-linear regression analysis.

Table 1. The number of D_1 and D_2 binding sites in caudate, putamen and nucleus accumbens (NA).

	D_1			D_2		
	Caudate	Putamen	NA	Caudate	Putamen	NA
Control n=21	370 ± 13	361 ± 11	353 ± 10	221 ± 10	269 ± 10	191 ± 7
Suicide (ADF) n=24	373 ± 10	393 ± 16	356 ± 13	234 ± 12	282 ± 14	192 ± 5
Control n=16	408 ± 22	415 ± 14	363 ± 13	188 ± 7	231 ± 7	183 ± 9
Suicide (ADT) n=17	430 ± 18	404 ± 18	428 ± 17*	249 ± 16**	301 ± 22**	272 ± 18**

B_{max} fmol/mg protein; means ± SEM; * $p < 0.05$; ** $p < 0.01$, Wilcoxon test.

The number (B_{max}, Table 1) and K_d of D_1 and D_2 binding sites did not differ significantly between antidepressant-free suicides and controls in the regions studied. The number of D_2 binding sites was significantly higher in each brain region of antidepressant-treated suicides than controls whereas the number of D_1 binding sites was only increased in nucleus accumbens (Table 1). K_d values for D_2 binding were also higher in each brain region in antidepressant-treated suicides (2.35 - 2.73 nM) than controls (1.12 - 1.40 nM) whereas the K_d values of D_1 binding were not significantly altered. The results suggest that antidepressant drugs increase D_2 receptors in human brain.

3P LACK OF ALTERNATIVE mRNA SPLICING IN MMQ PROLACTIN-SECRETING CELL LINE: EXPRESSION OF A NATIVE DOPAMINE D_{2L} RECEPTOR ISOFORM

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The application of recombinant DNA technology to dopamine (DA) receptor biology allows the transfection of expression vectors containing a reporter gene into either primary or stable cell cultures.This technique, although commonly accepted and used, presents many factors of error, such as, in the case of transfected receptors, the presence into receptive cells of coupling mechanisms other than those serving native receptors. To further characterize the dopamine D_2 receptor expressed in the prolactin secreting pituitary MMQ cell clone (Judd et al., 1988), we used the polymerase chain reaction (PCR) amplification technique, using oligonucleotide (22 bases) primers flanking the 87 base pairs alternatively spliced region. We found that the mRNA encoding the D_2 receptor does not undergo the process of alternative splicing. Although the pharmacology of the D2 receptor has been well established in many studies both on native and on transfected receptor isoforms, MMQ cells represent the first example of cells expressing only one D_2 receptor, the D_{2L} isoform, conceivably coupled to its native transduction mechanisms. Thus, we evaluated the binding characteristics and the second messenger systems known to be modulated by DA. The Bmax for [^{3}H]-Spiperone was 27±3.5 fmoles/mg prot, with a Kd of 0.055±0.002 nM. In the presence of 100 μM GTP, the competition curve of DA for [^{3}H]-Spiperone was shifted to the right and was best fit by a one site model. DA inhibited basal and forskolin (F)-stimulated adenylate cyclase (AC) activity (Basal=14±0,7; DA 10μM=11±0.5; F=120±1.2; DA+F=99±1 pmoles/mg prot); it inhibited basal and K+-induced $[Ca^{++}]_i$ rise (Basal=185±10 nM; DA10μM=140±8; K40mM= 720±35; K+DA=516±38), and K+-stimulated PRL release (Basal PRL secretion=4.4±0.4; K40mM=14.58±1.4; DA10μM=3.8±0.2; DA+K=9.54±0.8 ng/well). DA was also able to reduce the percentage of PRL-secreting cells upon F stimulation (Basal=25%+0.15; DA=23%+3.05; F=78%±3.9; DA+F=50%±9). The selective D2 receptor agonist LY171555 repeated the inhibitory effects of DA on basal and forskolin-stimulated AC activity, basal and K+-activated $[Ca^{++}]_i$ and on K+- and forskolin-stimulated prolactin secretion. Conversely, the D_2 receptor antagonist L-sulpiride (10 μM) and the pretreatment of cells with pertussis toxin (100 ng/ml,16 hr) reverted the effects of DA and quinpirole. Since the D_2 receptor has been demonstrated to preferentially couple to Gi2 protein, we evaluated by PCR and found the expression in MMQ cells of mRNA encoding this G-protein. Thus, MMQ cells represent a unique tool to study new D_2 selective ligands which could be helpful in the pharmacological therapy of neurological disorders characterized by deficits of dopaminergic neurotransmission.

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4P THE IRON CHELATOR 1,2-DIETHYL-3-HYDROXY-PYRIDYN-4-ONE (CP94) DOES NOT PROTECT AGAINST MPP+ TOXICITY TO SUBSTANTIA NIGRA

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Oxidative stress in Parkinson's disease (PD) may contribute to nigral cell death. One reason may relate to increased iron and decreased ferritin levels found in the substantia nigra (SN) in PD (Dexter *et al*, 1991). Indeed Ben-Shachar *et al* (1991) showed that the iron chelator desferrioxamine retarded biochemical and behavioural deficits produced by intracerebroventricular injection of 6-OHDA in rats. Therefore we investigated the ability of the novel 1,2-diethyl-3-hydroxypyridin-4-one iron chelator (CP94) to prevent nigral cell loss induced by intranigral administration of 1-methyl-4-phenylpyridinium (MPP+).

Male Wistar rats (180-220g, n=11) received CP94 (100mg/kg, ip) or vehicle 45 min prior to an injection of MPP+ (4μg in 1μl 0.9% saline) into the left SN and a vehicle injection into the right. The same dose of CP94 or vehicle was given twice daily for a total of eight days post-lesion. Levels of dopamine (DA) and its metabolites were determined by HPLC. Striatal sections (20μm) were used to determine levels of [^{3}H]-mazindol binding. Nigral slices (30μm) were subjected to tyrosine hydroxylase (TH)-immunohistochemistry and cells counted.

Intranigral injection of MPP+ reduced DA levels to the same extent in the lesioned striatum (STR) of vehicle-treated (71.9%) and CP94-treated (70.3%) animals compared to the sham-lesioned side (Figure 1). However, in the sham-operated STR, CP94 administration also reduced DA (52.6%) and HVA (9.1%) levels and reduced DA turnover (44.4%) compared to vehicle-treated controls. MPP+ administration decreased both [^{3}H]-mazindol binding in the STR and the number of TH-positive cells in the SN compared to the sham-operated hemisphere. These effects were not altered by CP94 treatment.

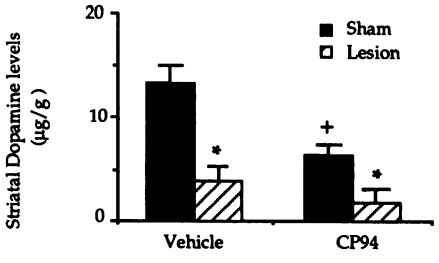


Figure 1 . The effect of CP94 (n=6) and vehicle (n=5) on DA levels after a sham lesion and MPP+ lesion. *p<0.05 compared to sham-lesioned side; +p<0.05 compared to vehicle sham, Dunn's test.

In summary CP94 did not protect the SN against MPP+-induced toxicity but potentiated striatal DA loss and reduced DA turnover. This action may be related to the ability of CP94 to chelate iron required by tyrosine hydroxylase.

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5P CORRELATION BETWEEN 1-METHYL-4-PHENYL-PYRIDINIUM ION (MPP+) LEVELS AND MPTP-INDUCED NEUROCHEMICAL CHANGES IN THE RAT STRIATUM

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The mechanism of MPP+ block of the mitochondrial oxidation seems to be related to oxidative stress. Cleeter *et al.* (1992) demonstrated that irreversible MPP+-induced inhibition of mitochondrial complex I is prevented by free radical scavengers glutathione (GSH) and ascorbic acid (AA), thus suggesting a possible involvement of free radicals. In a previous paper (Desole et al., 1993), evidence was given of a mechanism of MPTP toxicity involving oxidative stress produced by xanthine oxidase.

Levels of dopamine (DA), DOPAC, AA, dehydroascorbic acid (DHAA), GSH and MPP+ were determined by HPLC in the crude striatal synaptosomal fraction (taken as a model of neuronal terminals) and in the whole striatum of 6 month old male Wistar rats, after single injections of MPTP 35 mg/kg i.p., according to the method previously described (Desole *et al.*, 1993) (Table 1).

Table 1 - Levels of MPP+, DHAA, GSH (nmol/mg protein, mean ± S.D.) and DOPAC (pmol/mg protein, mean±S.D.) in the rat striatum (STR) and striatal synaptosomes (SYN)

		Controls (n=10)	Sacrifice after MPTP				ANOVA (P)
			1h (n=8)	3h (n=8)	8h (n=6)	24h (n=5)	
MPP+	(SYN)	--	0.38±0.06	0.50±0.06	0.16± 0.01	0.01±0.001	
	(STR)	--	1.32±0.19	0.79±0.03	0.33± 0.02	--	
DOPAC	(SYN)	88.1±3.5	37.4±7.8*	28.9±14*	41.5±7.3*	116.5±38	<0.00001
	(STR)	152.1±22	20.9±22*	19.4±8*	27.3±8*	136.8±21	<0.00001
DHAA	(SYN)	0.79±0.16	1.37±0.15*	1.20±0.31	1.00±0.40	0.99±0.17*	<0.002
	(STR)	1.41±0.23	1.49±0.31	1.85±0.22*	1.91±0.33*	1.89±0.43*	<0.005
GSH	(SYN)	6.78±1.79	4.68±1.76*	4.67±1.17*	5.62±1.18	6.44±0.64	<0.05
	(STR)	14.4±1.36	12.6±2.02*	14.7±1.40	13.2±1.44	13.7±1.10	<0.05

(*) P<0.05 vs controls. Changes in AA and DA levels were of minor importance. Pearson's correlation coefficient between individual levels of:a) MPP+ SYN vs: DOPAC, r=-0.932, P<0.005; DHAA r= +0.383, P=0.053; GSH r=-0.496, P<0.02; b) MPP+ STR vs: DOPAC r=-0.687 P<0.0001; DHAA r= -0.392, P<0.05.

We conclude that the response of the striatal antioxidant system (increase in AA oxidation, decrease in GSH levels) well correlates with MPP+ levels. *(Supported by MURST 40% and 60%).*

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6P REGIONAL HETEROGENEITY IN THE INTERACTION OF MPP+ AND La^{3+}-IONS WITH THE [^{3}H]-TYRAMINE-LABELLED VESICULAR CARRIER OF MONOAMINES IN THE CNS

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The interaction of the neurotoxin MPP^+ with the [^{3}H]-Tyramine (TY)-labelled vesicular transporter (VT) for monoamines in brain membranes displays region-specific kinetics, which is consistent with the unorthodox heterogeneity of the VT in organelles obtained from differently-innervated (dopaminergic or noradrenergic) brain regions (Vaccari *et al.*, 1993).
A similar heterogeneity has been also found with two Ca^{2+}-channel antagonists. La^{3+}-ions potently enhanced (by 90 ± 9%, n=4, at 5.10^{-8}M) TY binding in the striatum, while not affecting that in the cerebellum. Similarly, ω-conotoxin increased striatal binding of TY, starting from 10^{-10}M (+10%, n=2). Additional organic and inorganic Ca^{2+}-antagonists, on the contrary, inhibited striatal TY binding, with K_i values ranging from 0.1 to >100 μM (Vaccari, 1992). The plateau of TY association in striatal membranes with La^{3+}-ions lasted almost twice more than in controls, with K_{-1} (dissociation) constants of 14.2 and 9.6 min^{-1}, respectively (n=2). Two saturation curves for TY performed without or with $LaCl_3$ or ω-conotoxin revealed a non-competitive interaction, with no changes in K_d,s, and increased B_{max} values (from 10.6 and 11.1 pmol mg^{-1} protein in controls, to 17.4 and 16.9 pmol mg^{-1} protein in the presence of 10^{-7}M $LaCl_3$ and 0.5 μM ω-conotoxin, respectively). Thus, the vesicle membrane in the striatum, and not in the cerebellum, seemed to be endowed with "cryptic" sites for TY which can be unmasked by both drugs. Since TY labels the VT for dopamine in the striatum (Vaccari, 1986), more abundant sites were expected to facilitate the vesicular uptake of TY. Preliminary experiments indicated that TY, in the presence of ATP/Mg^{2+}, though entering abundantly the synaptic vesicles obtained from the rat striatum, was poorly taken up in those from the cerebellum (3.1% than in the striatum, n=2), with K_m values (0.27 and 1.15 μM, respectively) again denoting regional heterogeneity. La^{3+}-ions up to 10^{-4}M did not affect either TY uptake, or the recently demonstrated (Del Zompo *et al.*, 1993) [^{3}H]-MPP^+ uptake in striatal vesicles, nor TY-evoked in vivo release of dopamine from the ventral striatum of rats. Furthermore, 10^{-7}M La^{3+} incubated with increasing concentrations of MPP^+ did not prevent the toxin-provoked inhibition of TY binding in striatal membranes, thus suggesting that La^{3+} does not act directly on the VT for dopamine. On the other hand, region-specific differences in the MPP^+-provoked inhibition of TY *binding* were reflected in the ability of MPP^+ to inhibit TY *uptake* (K_i = 257 nM, n=2) in striatal and not cerebellar vesicles. Thus, La^{3+}-ions seem to promote allosterically the association of TY with functionally non-relevant sites, located in the domain of the striatal VT for dopamine, an event which is not shared by the VT for noradrenaline in the cerebellum. Heterogeneity for MPP^+ interactions may, on the contrary, bear functional and neurotoxic significance.

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7P SELECTIVE ANTAGONISM OF THE ANTIPARKINSONIAN EFFECTS OF BENZAZEPINE D_1 DOPAMINE AGONISTS IN THE MPTP-TREATED MARMOSET

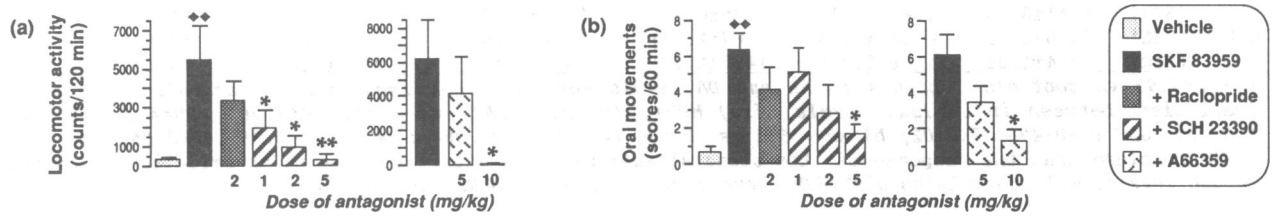
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We have previously reported the lack of correlation between the behavioural effects of benzazepine D-1 dopamine (DA) agonists in the MPTP-treated marmoset and their efficacies in stimulating adenylate cyclase (Gnanalingham et al., 1993). We now report on the effects of pretreatment with the selective D-1 DA antagonists, SCH 23390 (a benzazepine) and A66359 (an isoquinoline) and the D-2 DA antagonist raclopride, on the antiparkinsonian actions of full (ie SKF 80723) and partial (ie SKF 83959) efficacy benzazepine D-1 DA agonists, in the MPTP-treated marmoset.

Four to eight weeks following MPTP treatment (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; total dose 8-12 mg/kg over 5-7 days; sc), common marmosets were utilised for behavioural studies using automated activity cages. Locomotor activity was measured for 120 mins. Motor disability, grooming and oral activity were observer rated for 60 mins. The animals were pretreated with raclopride (2 mg/kg; ip), SCH 23390 (1-5 mg/kg; ip) or A66359 (1-[2-bromo-4,5-dimethoxybenzyl]-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline; 5 and 10 mg/kg; sc) and 20 mins later were challenged with either 2.5 mg/kg (ip) of SKF 80723 (6-bromo-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine) or SKF 83959 (6-Cl, 3-CH_3, 3'-CH_3 analogue).

Administration of SKF 83959 (figure 1) and SKF 80723 increased locomotor activity and reversed motor disability. Oral activity (tongue protrusions and vacuous chews) and grooming were also increased. Pretreatment with SCH 23390 and A66359, but not raclopride, inhibited the behavioural effects of SKF 83959 (figure 1) and SKF 80723. Doses of SCH 23390 needed to antagonise SKF 83959 induced changes in oral and grooming activities (5 mg/kg) were higher than that needed to inhibit its effects on locomotor activity and motor disability (1 mg/kg). However, A66359 appeared to be equally effective in antagonising SKF 83959 induced changes in locomotion, disability, oral and grooming activities (figure 1).

Figure 1: Effects of DA antagonist pretreatment on the (a) locomotor and (b) oral effects of SKF 83959 in MPTP-treated marmosets. Values are expressed as mean ± s.e.mean; (n = 4-6); ◆p<0.05; ◆◆p<0.01 compared to vehicle treatment; *p<0.05; **p<0.01 compared to the effect of SKF 83959 alone (2.5 mg/kg) (ANOVA and Duncan's multiple range test or Kruskal Wallis and Mann-Whitney U-test).



The behavioural effects of both benzazepine D-1 DA agonists appear to be mediated via the D-1 DA receptor. However, the locomotor, motor disability, oral and grooming behaviours induced by SKF 83959 may also exhibit differential sensitivities to antagonism by SCH 23390.

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8P THE EFFECT OF SCH-23390 ON FOOD AND COCAINE SELF-ADMINISTRATION IN THE RAT

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Recently, a role for the D_1 receptor in the mediation of addict behaviour has been hypothesized (Di Chiara & North,1992). In particular, the selective D1 antagonist SCH-23390 has been shown to be effective in blocking psychomotorstimulant -induced hypermotility (Arnt, 1985), cocaine self-administration (Koob *et al*, 1985) and reward-related learning (Beninger, 1992). It is not clear, yet, whether the effect of SCH-23390 in these tests was due to a reduction of reinforcing properties of psychomotorstimulants or, less specifically, to a depression of the dopaminergic transmission in the mesolimbic and mesostriatal pathaways. Therefore, the purpose of the present study was to evaluate whether the effect of SCH-23390 on drug self-administration was due to a generic blockade of "natural reward" processes or to a specific interaction with the psychomotorstimulant -induced dopaminergic modulation.

Initial experiments were performed to establish the doses of SCH-23390 devoid of an inhibitory effect on locomotor activity. Locomotor parameters were monitored every 15 min up to 3 h, by using Digiscan Activity monitors (Omnitech). Below the dose of 10 μg/kg s.c., SCH-23390 did not affect locomotor activity and, therefore, doses below this threshold were tested in two models of self-administration in rat:

a) Cocaine i.v. self-administration. Sprague-Dawley rats were trained to lever press for food (FR2, TO 30s, 2-h session). After acquisition of the task, animals were anaesthetized and a silastic catheter was inserted in the jugular vein. After recovery, training sessions were performed using cocaine as a reinforcer (FR2, TO 30s, 2-h session). Cocaine (0.25, 0.5, 1, or 2 mg/kg/infusion i.v.) decreased the rate of responding in a dose-related manner. Cocaine 0.5 mg/kg/infusion was chosen as the baseline dose. SCH-23390, 5 μg/kg s.c., 30 min pretreatment, increased rate of responding for the baseline dose of cocaine, and the effect is interpreted as a decreasing of the reinforcing properties of the self-administered drug. Vehicle 24.4±1.00 (mean±s.e.mean) infusions/session; SCH-23390 33.1±2.86 (mean±s.e.mean) infusions/session (P=0.02, Student's paired t-test, n=7).

b) Food self-administration. Food was presented with the same schedule of reinforcement used in the cocaine model to different animals. SCH-23390, 1 or 5 μg/kg s.c. 30 min pretreatment, did not modify the responding for food pellets in comparison to vehicle-administration.

In conclusion, it appears that SCH-23390 at doses which do not reduce spontaneous locomotor activity in rat, is able to antagonize the reinforcing properties of cocaine i.v. without interfering, at the same doses, with the responding for a "natural reward", i.e. food.

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9P EFFECTS OF 6-HYDROXYDOPAMINE LESIONS OF THE PREFRONTAL CORTEX ON THE ATTENTIONAL SET-SHIFTING ABILITY IN MONKEYS

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The ability to shift the attentional set from one perceptual dimension of a complex stimulus to another (e.g. from color to shape) is commonly attributed to the frontal lobes. Deficits in attentional set-shifting have been reported in patients with frontal lobe damage as well as in patients with Parkinson's disease. The aim of the present study was to investigate the effects of 6-hydroxydopamine lesions of the prefrontal cortex on both attentional set-shifting and spatial delayed response in marmosets. The former test provides a componential analysis of the Wisconsin Card Sort Test, a commonly used clinical test of frontal lobe function in man. Acquisition of a visual compound discrimination requiring a shift of attention from one dimension to another (extradimensional shift), e.g. shapes to lines, was significantly [$F(1,16)=6.33$, $p=0.02$] improved in lesioned animals (mean errors to criterion $\pm$ S.E.M.: control 346.4$\pm$137, lesion 153.8$\pm$65). This enhancement was behaviorally specific in that there were no effects on acquisition of a discrimination that required the continued maintenance of attention towards one particular dimension (intradimensional shift), nor any effect on a series of visual or spatial discrimination reversals which involved the repeated shifting of responding between two exemplars from the same dimension. In contrast, spatial delayed response performance was impaired [$2i=4.57$, $df=1$, $p<0.05$], in agreement with previous results *(Brozoski et al., 1979)*. In particular, whilst 10 out of 12 sham-operated monkeys completed all stages of the spatial response task within 400 trials, only 4 out of 10 lesioned monkeys were successful. Neurochemical measures showed a marked depletion of dopamine (ranging between 56 and 81%) limited to the prefrontal cortex and a smaller loss of prefrontal noradrenaline (ranging between 58 and 67%). This was accompanied by a long term adaptive change in the striatum such that extracellular dopamine in the caudate nucleus, as measured by in vivo microdialysis, was elevated in response to potassium stimulation as long as 18 months post-surgery [$F(11,66)=2.197$, $p=0.025$]. It is proposed that attentional set-shifting is mediated by a balanced interaction between prefrontal and striatal dopamine and that elevated dopamine contributes to the improvement in attentional set-shifting ability. This interpretation is consistent with the impairment in attentional set-shifting ability observed in patients with Parkinson's disease or with damage to the frontal lobes using the same test as used here for infra-human primates *(Owen et al., 1991; Owen et al., 1992)*.

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10P THE EFFECT OF CLOZAPINE AND MIANSERIN ON THE DEFENCE RESPONSE IN THE RAT

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Specific stimulation of cell bodies using glutamate analogues such as D, L-Homocysteic acid (DLH) has revealed the dorsal periaqueductal grey (PAG) as the site of integration of the defence response (Hilton & Redfern 1986). In contrast to studies employing less specific electrical stimulation methods (Jenk *et al.* 1989) PAG administration of the $5HT_1$ agonist 1-(3-chlorophenyl)piperazine (mCPP) has been shown to potentiate the DLH induced response (Beckett *et al.* 1993). The present study has examined the effect of systemic administration of the 5-HT antagonists mianserin (mian) and clozapine (clz) on DLH induced defence in the presence and absence of prior PAG administration of mCPP.

Male Lister hooded rats (200-250g), anaesthetised with a halothane N_2O/O_2 mixture, had guide cannulae implanted into the dorsal PAG (AP -6.7mm, ML +1.7mm, DV -5.1mm relative to bregma at an angle of 20°). On day 1, one week after surgery, rats were placed in a circular arena and DLH (5nmols in 250nl) injected through the cannula. On day 3 animals received an i.p. injection of either saline (0.9% 1mlkg^{-1}), mian (1 or 5mgkg^{-1}) or clz (0.5, 2 or 8mgkg^{-1}) 30 mins prior to a PAG injection of DLH (as above). On day 5 systemic drug treatments were repeated and followed 20 mins later by a PAG injection of mCPP (20nmols in 250nl). Ten mins later, rats received a final PAG injection of DLH (as above). After each DLH administration behaviour was recorded on video tape for 5 mins for later analysis.

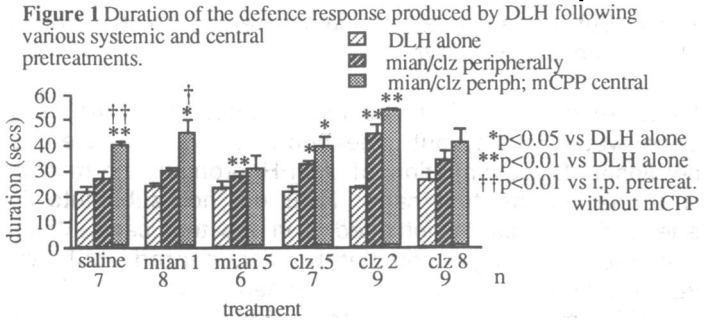


Figure 1 Duration of the defence response produced by DLH following various systemic and central pretreatments.

DLH administered into the dorsal PAG produced the characteristic overt pattern of defence behaviour. The duration of this response was significantly increased by pretreatment with 0.5 and 2 mgkg^{-1} clz but not by 8mgkg^{-1} (fig 1). 1mgkg^{-1} mian had no effect on the duration while 5mgkg^{-1} produced a significant increase compared to saline controls (fig 1). PAG pretreatment with mCPP significantly enhanced the duration of the response in saline animals but caused no further significant increase in clz pretreated rats (fig 1). In 1mgkg^{-1} mian treated animals mCPP significantly augmented the duration of the response but this effect was not seen in the group given the higher dose (5mgkg^{-1}, fig1).

This study agrees with previous findings that PAG administration of mCPP potentiates the duration of the DLH induced defence response and that this is reversed by systemic pretreatment with mian (Beckett *et al.* 1993). In addition, pretreatment with 0.5 and 2, but not 8 mgkg^{-1} clz alone also enhanced the response duration, however, central mCPP pretreatment caused no further increase. These data support the findings that decreased 5-HT transmission within the dorsal PAG is proaversive.

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11P STIMULATION OF 5-HT_{1A} RECEPTORS CAN PREVENT HALOPERIDOL-INDUCED CATALEPSY WITHOUT INFLUENCING THE CONCURRENTLY ENHANCED DOPAMINE UTILISATION IN THE RAT BRAIN

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It is not known how systemic administration of the 5-HT_{1A} agonist, 8-OHDPAT, prior to haloperidol (HAL) suppresses the induction of catalepsy associated with HAL treatment (Invernizzi *et al.*, 1988). In the present study, we asked whether any interaction between the two drugs on brain dopamine (DA) metabolism could be discerned.

Groups of male Wistar rats (215-300 g) were injected with 154 mM saline s.c. or 0.25 mg/kg 8-OHDPAT s.c. After 30 min, each group received 0.5 mg/kg i.p. HAL or 154 mM saline. Two further groups received 154 mM saline i.p. or 5 mg/kg i.p. of the selective D_2 antagonist, remoxipride. In each group, catalepsy was assessed at 10 min intervals using a vertical grid and 2 hr after the last injection, the caudate-putamen and nucleus accumbens of each animal was removed and analysed for DA and its two major metabolites, DOPAC and HVA, using HPLC with electrochemical detection as previously detailed (Kilpatrick *et al.*, 1986).

Table 1 Concentrations of DOPAC and HVA in rat nucleus accumbens and caudate-putamen

	Nucleus Accumbens		*Caudate-Putamen*	
	DOPAC	HVA	DOPAC	HVA
Saline + Saline (5)	7.81 ± 0.52	2.76 ± 0.28	8.35 ± 0.86	5.07 ± 0.77
Saline + HAL (4)	+124 % P<0.005	+155 % P<0.005	+177 % P<0.005	+202% P<0.005
8-OHDPAT + HAL (3)	+161 % P<0.001	+279 % P<0.02	+186 % P<0.005	+213 % P<0.02

Values on the top row are the mean ± s.e.mean in *pmol* mg^{-1} with the number of determinations shown in parentheses. Other data are the % changes with respect to the appropriate control group and the probability level from Student's *t*-test.

HAL caused up to a 3-fold increase in the concentrations of DA metabolites in both regions and simultaneously produced a maximum cataleptic response. In agreement with published data, the cataleptic response to HAL was entirely prevented by pretreatment with 8-OHDPAT. After 120 min, however, such treatment did not alter the HAL-induced elevations in DA metabolism although this may occur at earlier survival times.

Since remoxipride alone (n = 6) caused similar increases in regional DA metabolism but failed to induce catalepsy, it is unlikely that the mechanism responsible for the anti-cataleptic action of 8-OHDPAT involves a direct alteration in DA metabolism and by inference, DA release, in either the nucleus accumbens and/or caudate-putamen. It is also apparent that increases in DA metabolism *per se* are an unreliable index of drug-induced catalepsy.

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12P AMPHETAMINE-MEDIATED HYPERDIPSIA IS PREVENTED BUT ALSO REINSTATED BY (-)-NORPSEUDOEPHEDRINE

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Chronic intermittent administration of amphetamine or cathinone in rats produces hyperdipsia (Nencini, 1988; Nencini et al, 1988). Because this effect is not produced by the weak sympathomimetic agent (-)-norpseudoephedrine (NPE) (Nencini & Valeri, 1991), its development probably requires full psychomotor stimulant properties. Once established, however, hyperdipsia could be maintained by weaker agents such as NPE. In experiments designed to test this hypothesis, rats were injected with *d,l*-amphetamine (AMPH; 4 mg/kg ip) alone or in combination with NPE (10 mg/kg ip) and then returned to the home cage or transferred to a test cage. Water intake was measured for 3 hr, in the absence of food. AMPH treatment lasted for 10 days and was followed by a 6-day extinction period during which AMPH, but not NPE, administration was discontinued. Subsequently, all animals received an injection of NPE (10 mg/kg ip), or morphine (1 mg/kg ip) on 2 non-consecutive days.

Chronic AMPH stimulated drinking and this effect had an early onset in the home cage. When the small dose of NPE was given alone, it did not influence water intake, but, unexpectedly, was sufficient to prevent the development of AMPH-mediated hyperdipsia. Discontinuation of AMPH promptly normalized water intake in the home cage but increased it further in the test cage. NPE did not substitute for AMPH in maintaining hyperdipsia in the home cage and significantly inhibited it in the test cage. Within 6 days of AMPH discontinuation hyperdipsia in the test cage completely disappeared, but was reinstated by a challenge injection of either NPE or morphine. In conclusion, when it was paired with specific environmental stimuli NPE reinstated AMPH-mediated hyperdipsia. Since morphine also produced this reinstatement, AMPH-like properties were not necessary. The inhibition of AMPH-mediated hyperdipsia by NPE was unexpected and suggests that NPE had a primary antidipsic effect.

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13P ANGIOTENSIN II FACILITATES RELEASE OF ENDOGENOUS NORADRENALINE FROM SLICES OF RAT PARAVENTRICULAR NUCLEUS

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Angiotensin II (A-II) can enhance the secretion of vasopressin and there is evidence indicating that this effect of A-II may involve the release of noradrenaline (NA) within the hypothalamus (e.g. Stadler *et al.*, 1992). The present experiments should test whether A-II may directly modulate the release of NA within the paraventricular nucleus (PVN).

Rats were killed by decapitation and slices of the PVN were prepared. The slices were placed in a teflon basket and incubated in modified Krebs solution which contained 1 µM desipramine, 10 µM tyrosine and in some experiments 1 µM yohimbine. Endogenous NA released into the medium was determined by HPLC with electrochemical detection. Two periods of electrical field stimulation (S1, S2 after 60 and 120 min of incubation) were carried out, during each, 540 pulses were applied at a frequency of 3 Hz.

Tissue NA determined at the end of incubation was 44 ± 2.4 pmol/slice (n=60). The spontaneous outflow of NA amounted to 0.63 ± 0.04 % of the respective tissue NA. Electrical stimulation in the absence of test drugs evoked the release of 1.09 ± 0.10 % of tissue the NA (n=24). The evoked NA release was abolished in the presence of 1 µM tetrodotoxin (n=4) and enhanced to 3.20 ± 0.33 % of the tissue NA, when the α_2-adrenoceptor antagonist yohimbine was present from the onset of incubation (n=24). In control experiments S2/S1 was 0.95 ± 0.08 and 0.95 ± 0.09 in the absence and presence of yohimbine, respectively (each n=8). In the absence of yohimbine, A-II (100 nM), added 1 min before S2, enhanced the evoked NA release by 77 % (S2/S1 1.68 ± 0.20, n=7), but not significant effect was observed when A-II (100 nM) was present already 10 min before S2 (S2/S1, 1.11 ± 0.17, n=7). The effect of 100 nM A-II, was prevented by the AT1 receptor antagonist losartan (300 nM), but not significantly affected by the AT2 antagonist CGP 42112B (300 nM). A stimulatory effect of A-II (added 1 min before S2) on NA release was also observed in the presence of yohimbine (S2/S1 was increased to 1.15 ± 0.05 (n=4), 1.49 ± 0.05 (n=8) and 1.30 ± 0.07 (n=5) in the presence of 10, 100 and 1000 nM A-II, respectively).

In conclusion, A-II, most likely via AT1 receptors, can facilitate NA release from noradrenergic nerves terminating in the PVN, but *in vitro* this effect appears to desensitize very rapidly.

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14P CLONIDINE AND YOHIMBINE MODULATE THE ANTINOCICEPTIVE EFFECT OF CHOLECYSTOKININ IN THE RAT

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One well-described action of CCK-8 when given centrally or peripherally to experimental animals is the production of antinociception (Pittaway,Hill,1987),and in some cases this analgesia is reported to be antagonized by naloxone. The actual mechanism whereby CCK-8 exerts its antinociceptive effect does not well known at present.The observation that CCK-8 is active in tests where noxious thermal,mechanical or chemical stimuli have been used,suggests that it may interact with different neurotrasmitter systems in moduling nociceptive response. Current evidences suggest that Noradrenaline has been implicated in nonopioid stress-induced hypoalgesia (Bodnar et al.,1985). On the basis of this consideration the present study was undertaken in order to determine if descending noradrenergic system was involved in the antinociceptive action of CCK-8 in rats using the rat Tail-Flick Test,as spinal reflex. To investigate if endogenous noradrenaline was involved in spinal CCK-8 nociception,rats were pretreated with α_2 receptor antagonist Yohimbine (1.5 mg/kg s.c.) and α_2 receptor agonist Clonidine (0.04 mg/kg s.c.).These selective ligands were selected on the basis of results suggesting that α_2 receptor ligands modulate pain perception more potently than ligands selective for other noradrenergic receptor subtypes(Haman et al.,1991). All drugs dissolved in 0.9 % saline , were injected to rats 30 min prior intracerebroventricular injection of CCK-8(10 µg/rat). Analgesia was expressed as % MPE (% Maximum Possible Effect). All data were analyzed by Student's t test, followed by ANOVA.
Rats which received CCK-8 displayed a longer latency of pain thresholds than saline-treated rats: 21 ± 3 vs 6 ± .5 ($p<.05$). The pretreatment with Yohimbine significantly reduced the increased latency CCK-8-induced:7 ± .8 vs 21 ± 3($p<.01$). On the contrary,the pretreatment with Clonidine significantly potentiated the increased layency CCK - 8 - induced: 45 ± 2 vs 21 ± 3 ($p<.01$).
The results of the present experiments could suggest that noradrenergic substrates whould mediate antinociception CCk-8-induced,which occurs through α_2 noradrenergic receptors. A selective action of Yohimbine on presynaptic receptor whould implicate reduced spinal cord noradrenergic trasmission in the mediation of CCK-8 increased latency , suggesting that this effect is mediated via reduction noradrenergic trasmission. The resolution of the issue , if enhanced or reduced noradrenaline release subserves CCK-8-induced analgesia,will require other experiments.

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15P HETEROGENEITY OF ENDOTHELIN/SARAFOTOXIN RECEPTORS MEDIATING CONTRACTIONS OF THE HUMAN ISOLATED CORONARY ARTERY

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We previously found that [^{125}I]ET-1 (30 pM) labeled a homogeneous receptor population in the human coronary artery. [^{125}I]Sarafotoxin S6b (30 pM) labeled a similar receptor, but apparently also labeled a non-ET_A, non-ET_B receptor with relatively high affinity for both BQ-123 (cyclo[D-Asp-L-Pro-D-Val-L-Leu-D-Trp-]) and [Ala^{1,3,11,15}]ET-1 (Bax et al., 1993). In the present experiments, we further investigated the nature of endothelin/sarafotoxin receptors mediating vasoconstriction. Human coronary artery segments, obtained from 11 organ donors (non-cardiac cause of death, 4 male, 7 female, age 12-53 years; heart valves used for transplantation purposes, endothelium intact) were mounted in organ baths (oxygenated Kreb's bicarbonate solution, 37 °C) for isometric tension measurement. Cumulative concentration response curves (CRC) were obtained in a paired, parallel manner. ET-1, Sarafotoxin S6b, Big-ET-1 and ET-3, but not [Ala^{1,3,11,15}]ET-1 and Big-ET-3, caused concentration dependent contractions. The order of potency was: Sarafotoxin S6b = ET-1 > Big ET-1 ≥ ET-3 (Table 1). BQ-123 (an ET_A receptor antagonist: Ihara et al., 1992; 0.1 μM and 1 μM), antagonized sarafotoxin S6b-induced contractions (approximate pK_B: 7.86±0.08), but did not attenuate ET-1-induced contractile responses. Only 10 μM BQ-123 caused a rightward shift of the ET-1-induced CRC (approximate pK_B: 5.56±0.01). [Ala^{1,3,11,15}]ET-1 (ET_B receptor selective: Saeki et al, 1991; 1 μM) did not affect the ET-1-induced CRC. Contractions to Big-ET-1 were attenuated after pretreatment of the vessel segment with phosphoramidon (100 μM), indicating a phosphoramidon-sensitive endothelin converting enzyme located in the vessel wall. The present results indicate that a heterogeneous population of endothelin / sarafotoxin receptors mediates contraction of the human coronary artery. These receptors can be differentiated by a relatively high or low affinity for BQ-123. [Ala^{1,3,11,15}]ET-1 was inactive, both as agonist and antagonist against ET-1, which virtually rules out the ET_B receptor. Supported by the Netherlands Heart Foundation, grant 89.252.

Table 1. Effects of endothelin analogues (± s.e.mean). The maximal contractile effect (E_{MAX}) is expressed as a percentage of the contractile response to 100 mM potassium.

Analogue	E_{MAX}	pD_2	Concentration Range Studied	n
Sarafotoxin S6b	104.4±4.3	7.95±0.08	30 pM - 0.3 μM	7
Endothelin-1	107.3±6.7	7.68±0.08	30 pM - 0.3 μM	10
Big-ET-1	124.3±12.4	7.12±0.02	0.1 nM 1 μM	4
Endothelin-3*	84.4±9.6	<6.93±0.14	0.1 nM - 1 μM	4
[Ala^{1,3,11,15}]ET-1	No Effect		0.1 nM - 1 μM	3
Big-ET-3	No Effect		0.1 nM - 0.3 μM	3

*, maximum not reached at 1 μM; n, number of segments tested.

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16P DYNORPHIN GENE EXPRESSION AND RELEASE IN THE MYOCARDIAL CELL

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Opioid peptides cleaved from the prodynorphin precursor have been detected in the mammalian cardiac tissue (Spampinato & Goldstein, 1983; Spampinato *et al* ,1990). In a previous study we observed that prodynorphin mRNA occurs both in rat atria and ventricular tissue, as well as in adult cultured rat ventricular cardiac myocytes (Canossa *et al.*, 1983). This last preparation has an advantage over the isolate heart, since it is devoid of nerve fibres and other cells types which may contribute to the cardiac pool of prodynorphin mRNA. In the present study we investigated the effects of KCl-induced cell calcium loading on the expression of prodynorphin gene (evaluated by a sensitive solution hybridization RNAse protection assay) as well as on the release of ir-dynorphin B (determined by RIA), a biologically active end product which acts as a selective k agonist, in cultured rat ventricular myocytes. Exposure to KCl (60 mM) resulted after 4 h in a marked increase in cellular prodynorphin mRNA (3.15 ± 0.36 vs 11± 0.7 pg mRNA/μg total RNA, p< 0.001; n=6). On the contrary, a more prolonged exposure to KCl for 12 or 24 h progressively down-regulated the levels of prodynorphin mRNA below the control value (1.8 ± 0.4 vs 3.45 ± 0.66 or 0.8 ± 0.3 vs 3.25 ± 0.6 pg mRNA/μg total RNA, p< 0.001; n=6). Immunoreactive dynorphin B was detected in the culture medium in significantly higher amounts than in cardiac myocytes (1.9 ± 0.2 vs 0.4 ± 0.06 pmol/10^6 cells , p< 0.001; n=6).The levels of this peptide were significantly increased after 4 h of KCl treatment either in the medium or in the cells (8.4 ± 0.6 and 2.2 ± 0.2 pmol/10^6 cells, respectively) and markedly reduced following a 24 h exposure (0.1 ± 0.02 and 0.3 ± 0.02 pmol/10^6 cells , respectively). The effects induced by 60 mM KCl were abolished by the calcium channel blocker verapamil (2 μM). Moreover, the selective k agonist U-50,488H and dynorphin A induced an increase of intracellular calcium on a single isolated cardiac myocyte when "puffed" from a micropipette positioned above a single cell (Ventura *et al.* 1991); an effect abolished by the k opioid antagonist MR-1452 (1 μM).This study supports the hypothesis of a possible autocrine or paracrine role for the dynorphin-related peptides in the regulation of the myocardial function.

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17P FUNCTIONING β_1-AND β_2-ADRENOCEPTORS COEXIST IN RAT CARDIOMYOCYTES AND ARE MODIFIED BY AGING

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Available evidence from functional and radioligand binding studies indicates the coexistence of both ß1- and ß2-adrenoceptors (ß1-AR and ß2-AR) in the heart of various mammalian species (Borea *et al.*, 1992). Age-related decrease in myocardial ß-AR responsiveness has been described in humans and in experimental animals; changes in ß-AR density may occurr in the senescent myocardium. Since the presence of functional ß2-AR in rat myocytes is controversial (Buxton & Brunton, 1985; Mauz & Pelzer, 1990), we first assessed their presence by means of electrophysiological and binding measurements; then, we evaluated if the ratio ß1-AR/ß2-AR is affected by aging. Left ventricular cardiomyocytes were obtained by enzymatic dissociation from the heart of 2- or 22-month old Wistar Kyoto rats. Competition binding experiments were performed by using the hydrophilic non selective ß-AR antagonist [^{3}H] CGP 12177 in the presence of subtype-saturating concentrations of the selective ß1-AR antagonist CGP 20712A or of the selective ß2-AR antagonist ICI 118,551 (Cerbai *et al.*, 1990). The effect of ß2-AR stimulation on calcium current (I_{Ca}) was studied using the whole-cell configuration of the patch-clamp technique. In order to block potassium currents, KCl was replaced by CsCl in both pipette and Tyrode's solution. Displacement curves with CGP 20712A demonstrated the presence of two ß-AR subtypes in cardiomyocytes from 2-month old rats (n=9). ß2-AR subtype represented 19.6±2.2% (IC_{50}=43.8±14.2 μM) and ß1-AR subtype represented 80.4±2.2% (IC_{50}=41.3±8.0 nM) of the total ß-AR population. Zinterol (10 μM), a selective ß2-AR agonist, increased the peak amplitude of I_{Ca} by 25.3±4.4% (n=8); this effect was prevented by pretreatment with the ß2-AR antagonist ICI 118,551 (50 nM) (n=3). A similar increase of I_{Ca} (28.9±5.1% of the control value, n=4) was observed with selective ß2-AR stimulation by isoprenaline (1 μM) in the presence of CGP 20712A (0.1 μM). The ratio between ß1-AR and ß2-AR subtypes varied with aging. In fact, myocytes from 22-month old rats contained 66.1±1.2% (IC_{50}=60.0±8.0 nM) ß1-AR and 33.9±1.2% (IC_{50}=37.5±4.1 μM) ß2-AR, a figure which was significantly different from that obtained in 2-month old rats ($P<0.01$). Qualitatively similar results were obtained by using as a displacer the selective ß2-AR antagonist ICI 118,551. In conclusion, our results indicate that functioning ß1-AR and ß2-AR are present in rat cardiomyocytes; their relative proportion changes with aging.

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18P ANGIOTENSIN II MEDIATES CELLULAR HYPERTROPHY IN CULTURED RAT NEONATE CARDIAC FIBROBLASTS BUT NOT IN CARDIOMYOCYTES

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Cardiac hypertrophy results from both increase in mechanical overload and/or direct trophic effect through humoral factors. Evidence suggests that angiotensin II (AII) in addition to its vasoconstrictor effect can regulate cardiac cell growth (Weber and Brilla, 1991). We have therefore developed a model of rat heart cells in culture to test the hypothesis of a trophic effect of AII at the cellular level. Total RNA accumulation in response to AII or the hypertrophic agent phenylephrine (Phe) (Bishopric *et al.*, 1987) was measured as an index of cell hypertrophy.

Primary cultures of rat ventricular cells were obtained from 1 to 3 day-old rats. Myocytes (M) and fibroblasts (F) were isolated and grown separately in culture medium supplemented with fetal calf serum (10%) for 24 hours. Culture medium was then replaced by serum-free medium supplemented with insulin and transferrin (10 µg/ml each) for 3 days. Increase in total RNA synthesis in response to AII or Phe was measured in M and F cells by [^{3}H]uridine incorporation into acid-insoluble material. Collagen synthesis was also measured in F cells by the mean of [^{3}H]proline incorporation.

A 24-hour exposure of M cells to either AII (1 nM - 100 µM) or Phe (1 nM - 100 µM) resulted in a concentration-dependent increase in [^{3}H]uridine incorporation with EC_{50} higher than 1 µM. At 10 µM, the effects averaged 20 ± 3 % stimulation over basal value for AII and 19 ± 3 % for Phe. In contrast, in cultured F cells, AII (1 nM - 100 µM) but not Phe (1 nM - 100 µM) increased [^{3}H]uridine uptake with an EC_{50} of 15.8 ± 5.5 nM. Maximum effect of AII was reached at 1 µM and corresponded to a 23 ± 2 % increase over basal incorporation. Furthermore, in F cells, AII (1 nM - 10 µM) increased [^{3}H]proline incorporation in a concentration-dependent manner, with an EC_{50} of 24 ± 10 nM. Maximum effect of AII was reached at 1 µM and corresponded to a 27 ± 6 % fold increase over basal incorporation. This AII-induced increase in [^{3}H]proline incorporation was completely antagonized by the selective AT_1 receptor antagonist losartan (Chiu *et al.*, 1990) whereas the selective AT_2 receptor antagonist CGP42112A (Whitebread *et al.*, 1989) did not antagonize AII-induced effect.

Taken together, these results support the view that AII is more potent in F than in M cells at stimulating total RNA synthesis. Moreover, the ability of AII to increase collagen synthesis in F cells suggests that AII can play a determinant role in the development of the fibrosis observed in some forms of cardiac hypertrophy. In F cells, the AII-induced increase of collagen synthesis is apparently mediated through an AT_1-like receptor subtype.

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19P TRANSCELLULAR BIOSYNTHESIS OF SULFIDOPEPTIDE-LEUKOTRIENES REGULATES CORONARY FUNCTION IN ISOLATED, PMNL-PERFUSED RABBIT HEART

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The generation of LTs exhibits remarkable cellular specificity; however the cooperation of different cell types (PMNL, platelets, endothelial cells, erythrocytes) in the metabolism of unstable intermediates (LTA_4) suggests that cell-cell interaction is an important control of LT production. This process has been termed transcellular biosythesis (TB).
We have studied the TB of LTC_4-LTD_4 through PMNL-vessel wall interaction, and its functional consequences, in a spontaneously beating, isolated rabbit heart, perfused under recirculating conditions (50 ml volume) according to Langendorff. Purified human PMNL (10^5 cells/ml) were added and challenged with A-23187 (0.5 μM); coronary perfusion pressure (CPP), left ventricular end-diastolic pressure (LVEDP), left ventricular pressure (LVP), heart rate (HR) were monitored. LTs were identified positively and quantitated by a combined HPLC-EIA assay. Addition of PMNL "per se" did not cause mechanical obstruction of the vasculature; challenge with A-23187 "per se" did not alter CPP (45±4 mm Hg) nor any of the other heart functions; however when recirculating PMNL were stimulated, a marked increase in CPP was observed (165±18 mm Hg), which was slow in onset, very persistent, and was rapidly reversed by a bolus injection of Nipride (100 μg); such increase was fully antagonized by two structurally unrelated LTD_4 receptor antagonists (LY-171883 and SKF-104353, 10μM). The rise in CPP correlated significantly with the amount of LTC_4-D_4 quantitated by EIA in the incubating medium after positive identification by HPLC. LVEDP did also increase during ischemia from basal values of 9.8±0.5 to 23.4±6 mmHg). When PMNL were pretreated with an inhibitor of LT synthesis (MK886, 1 μM), washed extensively, perfused through the coronary vascular bed and challenged, a negligible increase in CPP was observed (76.7±9 mm Hg); no rise in LVEDP was observed. Lactate dehydrogenase (LDH) levels in recirculating medium were below the sensitivity of the assay (<25 B-B units) in basal conditions, but rose to 165±41 B-B units at 30' after ionophore challenge in presence of PMNL. This increase was also prevented by pretreatment with MK-886 or with LTD_4 receptor antagonists.
The concentration of LTC_4-D_4 present in the recirculating buffer at 30', in presence of challenged PMNL (4.3±1 ng/ml) is significantly higher than the amount released by heart alone (1.1±0.2) and cannot be contributed by PMNL only; it is therefore plausible that challenged PMNL function as LTA_4 donors to the adhering endothelial cells, where the unstable intermediate is metabolized to sulfidopeptide LT and act on the proximal smooth muscle cells to cause coronary spasm. These results suggest a key role for peptide leukotrienes in the genesis of ischemic damage and indicate a therapeutic potential for LT synthetase inhibitors and receptor antagonists.

20P PLATELET-NEUTROPHIL INTERACTION AND GENERATION OF SUPEROXIDE ANION

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Neutrophils contribute to tissue injury during inflammation and myocardial ischemia by the release of oxygenated species with cytotoxic activity. On the other hand, the participation of platelets to the inflammatory reaction has been widely demonstrated, starting from the observation that platelets accumulate at the inflammatory site. In addition, it has been shown that neutrophils and platelets interact and that this interaction affects many aspects of platelets and neutrophil function. However, if one considers the available knowledge on the effect of platelets on neutrophil production of oxygenated species, both stimulatory and inhibitory effects are described (McGarrity et al., 1988; Ward et al., 1988). In the present study, we have evaluated the effects of platelets or of platelet-derived products on superoxide formation by neutrophils in an "in vitro" model of interaction between platelets and neutrophils isolated from human blood. Various entity of cell-cell cooperation were observed according to the stimulus used in the system. Maximal cooperation between neutrophils and platelets to generate superoxide anion, determined by spectrophotometric technique, was observed with the calcium ionophore A23187 (2.41± 0.22 nmoles O_2^- for neutrophils alone and 5.96± 0.40 nmoles O_2^- for neutrophils+platelets, n=30, $p<0.001$) and with sodium arachidonate, stimuli directed to both cell types, indicating that the simultaneous activation of platelets and neutrophils resulted in more than a doubling of the levels of superoxide anion measured in the system. The effect of cooperation was dependent on the number of platelets present in the system and did not depend on metabolites of the arachidonic acid cascade. Platelet lysates mimicked the effect exerted by intact cells, thus suggesting that the product(s) responsible for the observed effect is (are) stored, preformed, in platelets. Among platelet products tested in our system, ATP and to a lesser extent ADP were able to reproduce, at least partially, the effect of intact platelets (2.30± 0.69 nmoles O_2^- for neutrophils alone and 4.10± 1.25 nmoles O_2^- for neutrophils in the presence of 10 μM ATP, n=8, $p<0.01$). The involvement of these purine nucleotides seems very likely because neutrophils possess specific receptors for ATP and ADP, whose occupancy causes enhancement of superoxide formation (Dubyak et al., 1988; Axtell et al., 1990). On the other hand, platelets can release other products that are able to inhibit superoxide formation during cell-cell interaction, among these adenosine and P-selectin. From this study it emerges that: 1) platelets modulate neutrophil superoxide formation by a great array of mechanisms which act with opposite effects; 2) the multiplicity of the effects exerted by platelets on superoxide anion formation by neutrophils should be taken into account in approaching this issue under a pharmacologic point of view.

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21P LACIDIPINE: *IN VIVO* EFFECTS ON INTIMAL CAROTID THICKENING IN HYPERCHOLESTEROLAEMIC RABBITS

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Targets for actual or potential pharmacological intervention in atherogenic processes are multiple and all aimed at causing existing lesions to regress, become stable, or progress more slowly, and also at preventing the formation of new lesions. Until recently, in antihypertensive trials little efforts was made to evaluate either the arterial changes or the effect of drug therapy on the arterial wall. Agents such as calcium antagonists have received increasing attention as pharmacological tools with antiatherogenic potentials. The in vivo direct antiatherogenic activity of lacidipine has been investigated on carotid rabbit intimal hyperplasia induced by perivascular stimulation of one carotid artery in hypercholesterolemic normotensive rabbits by assessing neointimal formation. The contralateral carotid served as sham. The hypercholesterolemic diet (1.5% cholesterol) and lacidipine (1,3,10 mg/kg), mixed with food, were given daily for 8 weeks. Six weeks after dietary and drug treatment started, intimal hyperplasia was acutely induced in one carotid artery of each rabbit as described (Soma MR et al 1993). The drug treatment, at all doses, did not alter either rabbit blood pressure or plasma lipid levels. The neointimal formation was followed by measuring cross-sectional thickness of intimal (I) and medial (M) tissue of fixed arteries with light microscopy. In control animals, by 14 days after collar placement, the process of intimal myocyte proliferation was pronounced: the arteries with no collar showed an I/M tissue ratio of 0.03 ± 0.02, whereas in the carotids with collar the ratio was 20 fold higher (0.62 ± 0.04). The intimal thickening was mostly cellular but abundant extracellular matrix and lipid depositions were present. Light microscopic observation of the sham tissue confirmed that it had no intimal thickening or cholesterol deposits. In the animals that received lacidipine neointimal formation was significantly decreased at all doses (I/M tissue ratios were 0.47 ± 0.02, 0.40 ± 0.09 and 0.32 ± 0.02, for the 1, 3, 10 mg/kg doses, respectively). Thus, the effect of the drug appeared to be dose-dependent, inhibiting by about 50% the intima media ratio at higher doses. These observations suggest a direct effect of lacidipine on smooth muscle cell migration and/or proliferation. The antiatherosclerotic activity of lacidipine in this experimental situation is of scientific and therapeutic significance and suggests that this calcium antagonist act at an early stage of atherosclerosis. This direct antiatherosclerotic activity of lacidipine offers new therapeutic direction for such a calcium antagonist.

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22P EFFECT OF INCREASING CONCENTRATIONS OF ANGIOTENSIN II INFUSED INTO UNRESTRAINED CONSCIOUS RATS ON LDL AND FIBRINOGEN UPTAKE BY AORTA

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The atherogenic uptake of low-density lipoprotein (LDL) by arterial walls is accelerated by adrenaline in anaesthetized rabbits (Cardona-Sanclemente & Born, 1992a) and in conscious, unrestrained rats (Cardona-Sanclemente, Gorog & Born, 1992). Angiotensin II (Ang II) increases the uptake of fibrinogen by rat aorta, while increasing diastolic blood pressure (Cardona-Sanclemente & Born, 1992b).

The latest experiments were to determine the effect of Ang II at increasing concentrations on the uptake of LDL and on that of fibrinogen. Ang II was infused at 0.5μl/h by osmotic minipump containing 9.6, 19.2 and 38.4 mg/ml, or saline, implanted subcutaneously, for 6 days. Blood pressure was determined via a cannula in the carotid artery. Five days after implanting the minipumps, LDL or fibrinogen labelled with ^{125}I or ^{131}-tyramine cellobiose (^{125}I-TC-LDL or ^{131}I-TC-fibrinogen) was injected i.v. The radioactivity of thoracic aorta was determined after 24h. when it provided a measure of intact plus degraded LDL or fibrinogen (Pittman et al., 1983).

The lowest dose of Ang II increased diastolic blood pressure for 7 days (123$\pm$3 vs 139$\pm$3 mmHg, mean$\pm$sem,n=7). The two higher doses increased blood pressure for 4 days (123$\pm$3 vs 152$\pm$5 and 151$\pm$7 respectively). Some body weight was lost with the two higher Ang II concentrations. The uptakes of both LDL and fibrinogen were increased most by the lowest Ang II dose (0.11$\pm$0.01 vs 0.22$\pm$ 0.02 ng protein/mg dry weight,n=7,$p<0.001$; and 0.61$\pm$0.07 vs 1.05$\pm$0.10, n=7, $p<0.0025$); less by the intermediate dose (0.17$\pm$0.01 and 0.93$\pm$0.04,n=7,$p<0.0025$); and not increased by the highest dose (0.12$\pm$0.03 and 0.63$\pm$0.15,n=7,n.s. respectively). The clearance of the two atherogenic proteins was similar to that of another plasma protein viz. albumin.

Thus, the lowest pressor dose of Ang II, like adrenaline, increased the uptakes of LDL and fibrinogen by artery walls. This effect was less with the higher Ang II concentrations which decreased body weight.

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23P INCREASED MEMBRANE MICROVISCOSITY AFTER CHRONIC EXPOSURE OF CULTURED ENDOTHELIAL CELLS TO OXIDISED LOW DENSITY LIPOPROTEIN

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Oxidatively modified low density lipoprotein (Ox-LDL), which is thought to be implicated in primary events of atherosclerosis, modifies many intracellular processes, including phospholipase C activity and prostacyclin release from endothelial cells (Thorin *et al.*, 1993). One hypothesis linking these events is an Ox-LDL-dependent change in plasma membrane physical properties. In order to investigate this possibility, we studied the effects of repeated Ox-LDL exposure (3 days) on membrane cholesterol content and microviscosity in cultured bovine aortic endothelial cells prepared by enzymatic digestion.

Cells were plated in 3 cm diameter multiwells plates for cholesterol determination at a density of 10^5 cells per well and grown to confluence (24 hr) in the presence of LDL or Ox-LDL (20 $\mu g\ ml^{-1}$), or vehicle. Growth medium was then replaced by medium 199 for 48 hours. Cells were washed with phosphate buffer (pH 7.4) and lipids extracted with chloroform and methanol. The method for free cholesterol employed cholesterol oxidase to generate H_2O_2 and peroxidase to catalyse the reaction of H_2O_2 with *p*-hydroxyphenylacetic acid to yield a stable fluorescent product. Cholesterol esterase was included for determination of total cholesterol. For microviscosity determination (Dominiczak *et al.*, 1991), cells were grown to confluence in the absence or in the presence of lipoproteins in 175 cm^2 culture flask. Medium 199 was added 48 h before crude membrane preparation. Fluorescence anisotropy of 2 fluorophores were measured: diphenylhexatriene (DPH), localized in the hydrocarbon core of the membrane and trimethylammonium derivative of DPH (TMA-DPH), localized close to the polar heads of the phospholipids, were incubated for 30 min at 37°C with membrane (0.2 mM per mg of protein ml^{-1}) before fluorescence measurements (Perkin Elmer LS50, ex: 350 nm; em: 430 nm). LDL was prepared from human plasma by sequential ultra centrifugation. Oxidation of LDL was carried out in the presence of $CuCl_2$ (4 μM).

Chronic exposure of endothelial cells to Ox-LDL increased the amount of free and esterified cholesterol ($[cholesterol]_{total}$ - $[cholesterol]_{free}$) per unit of protein by 103% and 112% compared to control, respectively (table 1). The rise in cholesterol concentration was found to be accompanied by an increased TMA-DPH anisotropy, but without any change in DPH anisotropy (table 1). Native LDL affected neither membrane cholesterol concentration nor TMA-DPH and DPH anisotropy.

Table 1: Effects of LDL and Ox-LDL (20 $\mu g\ ml^{-1}$) on membrane cholesterol concentration (ng $\mu g protein^{-1}$) and crude membrane microviscosity (anisotropy units) at 37°C.

Groups	n	Cholesterol		n	Microviscosity	
		Free	Esterified		TMA-DPH	DPH
control	16	15.3±1.3	1.7±0.3	8	0.219±0.002	0.161±0.004
LDL	16	20.6±1.5	2.5±0.4	8	0.220±0.003	0.162±0.006
Ox-LDL	16	31.0±1.9¶	3.6±0.7*	8	0.238±0.003¶	0.171±0.006

Values are means ± sem. (¶) significantly different from all groups, and (*) from control (ANOVA, $P < 0.05$).

These results suggest that chronic exposure to Ox-LDL alters cholesterol homeostasis and microviscosity at the level of the lipid-water interface of the plasma membrane. We suggest that these changes could influence signal transduction and contribute to the development of the atherosclerotic lesion.

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24P EFFECTS OF SIMVASTATIN ON THE PROGRESSION OF ATHEROSCLEROSIS IN AORTAE FROM WATANABE HERITABLE HYPERLIPIDAEMIC RABBITS

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Watanabe heritable hyperlipidaemic rabbits (WHHL) are an animal model of familial hypercholesterolaemia and atherosclerosis. Both homozygous (hh) and heterozygous (hH) WHHL have be shown to have significantly impaired endothelium dependant relaxation (Dowell et al, 1993). We have examined the effects of the HMG CoA reductase inhibitor, simvastatin, on this loss of function. Four age groups were studied (3, 6, 9, 12 months), with two treatment periods (3-6 months and 9-12 months). Simvastatin was administered orally at a dose of 10mg/Kg/day. Upper thoracic aortic rings (2 mm) were suspended in an organ bath filled with Krebs bicarbonate buffer at 37°C, aerated with 95% O_2 and 5% CO_2. Cumulative concentration response curves to phenylephrine (PE) and carbachol (C) (10^{-9} M - 10^{-5} M) were obtained, the C was followed by 10^{-4} M sodium nitroprusside (SNP). Responses to PE were expressed as grams tension per mg wet weight tissue. Responses to C were expressed as a percentage of the PE induced contraction. The adjacent aorta was stained with sudan red dye to show fatty lesions in the arterial intima. Using image analysis the % area stained (% sudan) was calculated. Blood was sampled for cholesterol (mM) (CHOL) measurement (CHOD-PAP) periodically.

In hh and hH both a progressive loss of PE induced contraction and endothelium dependent relaxation were observed with increasing age. Simvastatin significantly reduced the loss of vasoactive function in 6 month hH. Similar trends were observed in the 6 month hh, however this did not reach significance. Simvastatin significantly decreased serum cholesterol levels in th 6 month hH group, but not in other groups. These effects were not paralleled by a decrease in sudan red staining. All rings relaxed completely when exposed to 10^{-4}M SNP.

TABLE 1.

GROUP	n	TREATMENT	CHOL(8w)	CHOL(study)	PE	C	%SUDAN
hh12	7	control	20±4.9	9.0±3.5	0.37±0.17	20±26	85±26
hh12	6	simvastatin	22±5.8	10±4.4	0.38±0.15	15±18	79±12
hh9	6	control	23±4.2	12±4.1	0.52±0.13	27±21	71±27
hh6	5	control	20±3.8	17±3.1	0.70±0.13	58±22	32±23
hh6	5	simvastatin	18±1.1	15±2.1	0.74±0.18	66±38	30±40
hh3	4	control	17±5.6	11±6.2	0.85±0.12	77±4.6	12±20
hH12	7	control	3.6±2.3	1.8±1.3	0.59±0.13	77±23	0±0
hH12	5	simvastatin	2.8±0.7	0.8±0.4	0.67±0.10	80±15	0±0
hH6	7	control	2.5±0.72	1.5±0.4	0.54±0.07	77±8.1	0±0
hH6	5	simvastatin	2.9±0.46	0.7±0.3*	0.78±0.14*	89±4.3*	0±0
hH3	7	control	2.7±0.90	1.9±0.8	0.74±0.11	88±6.8	1.5±3.4

Values given are means ± SD. * denotes simvastatin treatment significantly different from untreated control (ANOVA, $p<0.05$).

In conclusion, treatment with simvastatin significantly retards the loss of function associated with the progression of atherosclerosis in younger hH WHHL, but not in hh or older hH. Higher doses of simvastatin or a longer treatment schedule may be effective in hh and older hH with more severe lesions.

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25P 5-HT-INDUCED TACHYCARDIA IN PIGS IS ANTAGONIZED BY DAU 6285 AND CISAPRIDE, TWO COMPOUNDS INTERACTING WITH 5-HT$_4$ RECEPTORS

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Some excitatory effects of 5-hydroxytryptamine (5-HT) at cardiac level are reportedly due to stimulation of 5-HT4 receptors: the positive chronotropic effect in porcine heart *in vitro* (Kaumann, 1990) and *in vivo* (Villalón *et al.*, 1991) and the inotropic action in the human atrium (Kaumann *et al.*, 1990). Among these experimental models, 5-HT-induced tachycardia in the anaesthesized pig represents the better established paradigm for the evaluation of the antagonism at 5-HT4 receptors *in vivo*. In this test, we have compared the action of two compounds which interact with 5-HT4 receptors: the antagonist DAU 6285 (Dumuis et al, 1992) and the agonist cisapride (Dumuis et al, 1989).

The method is the one described by Villalón *et al.* (1991). In anaesthetized domestic pigs, ECG and heart rate were recorded by subcutaneously implanted electrodes. Basal rate was 76 ± 6 beats per min (bpm, n=4). The i.v. injection of 5-HT (1-30 μg/kg) caused a dose-dependent increase in heart rate with a maximum of 40 ± 3 bpm at 30 μg/kg. A second dose-response curve was constructed 5 min after the i.v. administration of DAU 6285 or cisapride. This procedure was repeated for each dose of the two compounds separately. Both DAU 6285 (0.3-3 mg/kg i.v.) and cisapride (0.1-1 mg/kg i.v.) caused a dose-dependent rightward shift of 5-HT dose-response curve, with a comparable efficacy. The increase in heart rate caused by 30 μg/kg 5-HT, consisting of 41 ± 5 bpm over the basal values, was reduced to 23 ± 3 bpm by 1 mg/kg of DAU 6285 (n=4). The same dose of cisapride depressed 5-HT-induced tachycardia (48 ± 6 bpm at 30 μg/kg) to 23 ± 9 bpm (n=4). Neither DAU 6285 nor cisapride had any significant effects *per se*.

The response to a testing dose of 5-HT (30 μg/kg) was evaluated up to 480 min after the injection of saline or DAU 6285 (3 mg/kg i.v.), in order to assess the time-course of its action. The protective effect of DAU 6285 against tachycardia (inhibition after 5 min pre-treatment: 59.7 ± 6.0 %) remained unaltered up to at least 300 min from the administration (inhibition: 55.6 ± 11.6 %, n=4).

These results suggest that cisapride, which is an efficacious 5-HT4 receptor agonist in some tissues (Dumuis *et al.*, 1989), but a very weak partial agonist in the heart (Kaumann, 1990; Villalón *et al.*, 1991), may behave as an antagonist at cardiac 5-HT4 receptors *in vivo*. DAU 6285, already known as a blocker at such receptors (Dumuis *et al.*, 1992), is shown to be an antagonist with a long duration of action in this *in vivo* model.

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26P 5-HT$_4$ RECEPTOR-MEDIATED ARRHYTHMIAS IN ISOLATED HUMAN ATRIUM

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We have reported that 5-hydroxytryptamine (5-HT) induces occasional arrhythmias in human atrial strips paced at 0.5 Hz (Kaumann & Sanders, 1993) and have suggested that these arrhythmias may be caused by 5-HT-induced cell Ca^{2+} overload resulting from the ability of 5-HT markedly to enhance inward Ca^{2+} current through stimulation of 5-HT$_4$ receptors (Kaumann & Sanders, 1993). We have now investigated the dependence of 5-HT-evoked arrhythmias on heart rate and their mediation through 5-HT$_4$ receptors. Right atrial appendages were obtained from 9 patients undergoing coronary artery bypass surgery. All patients but one received chronic β-adrenoceptor blocker treatment. Atrial strips were paced to contract isometrically at 1 Hz at 37°C. An interval-force relationship (0.1, 0.2, 0.5, 1, 2 Hz) (staircase) was determined for each strip. Enhanced $[Ca^{2+}]_i$ can lead to delayed after-depolarisations that can cause triggered action potentials that may be associated with or cause triggered contractions. To enable detection of these triggered contractions the staircase was repeated in the backward direction with each pacing rate being followed by a 2 min rest period. 5-HT caused or enhanced arrhythmias and triggered contractions in 23 out of 24 strips. The arrhythmias and triggered contractions persisted for the period of observation (≥1 h). The potent and selective 5-HT$_4$ receptor antagonist SB 203186 ((1-piperidynyl)ethyl 1H-indole 3-carboxylate) 100 nM (Parker *et al.*, 1993) blocked both the 5-HT-evoked arrythmias and the 5-HT-evoked triggered contractions in all strips tested (n = 11) with variable half-times (range 0.5–15 min, mean ± s.e. mean = 4.9 ± 1 min, 13 strips from 9 patients). The blockade by SB 203186 was surmounted by high 5-HT concentrations (400–1800 μM), which caused the arrhythmias and triggered contractions to reappear.

We conclude that 5-HT elicits rate-dependent (up to 1 Hz) atrial arrhythmias and triggered contractions through 5-HT$_4$ receptors. Our findings support our earlier proposal that 5-HT released from aggregating platelets may cause arrhythmias such as atrial fibrillation and that these could be prevented by 5-HT$_4$ receptor antagonists.

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27P BENEFICIAL EFFECTS OF DEFIBROTIDE ON CARDIAC PERFORMANCE IN ISCHAEMIC RABBIT HEARTS

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We report on "in vivo" cardioprotection by defibrotide (DFT), a single-stranded polydeoxyribonucleotide shown to prevent ischaemic and reperfusion damage in isolated hearts (Bianchi & Berti, 1992).

Conscious rabbits received i.v. saline infusion, containing or not DFT (32 mg/Kg/h), for 6 h before surgery. The left anterior descending coronary artery was then irreversibly ligated and animals were allowed to recover from anaesthesia. Three days after, the hearts were excised from survived animals (sham operated = 4/4; DFT = 6/6; saline = 8/17) and mounted in a Langendorff apparatus for measuring performance parameters. Heart rate, left ventricular systolyc pressure and dP/dTmax in sham hearts were 180±2 bpm, 120±1 mmHg and 1025 ± 25 mmHg/sec, respectively. Control hearts were severely impaired (144±6 bpm, 94±3 mmHg and 712±44 mmHg/sec) ($P < 0.05$), whereas DFT-pretreated hearts were normal (P=ns vs sham and $P < 0.05$ vs saline): 196±7 bpm, 118±2 mmHg and 1033±49 mmHg/sec. Acetylcholine-induced coronary dilatation and isoproterenol-induced positive inotropism were impaired (roughly by 50 and 75% vs sham) in control hearts but were normal in DFT-treated hearts. Plasma creatinine phosphokinase concentration at time of sacrifice was greatly enhanced (2.6-fold) in saline- but not in DFT-treated rabbits (1.1-fold), compared to sham operated controls.
DFT effectively protected rabbit hearts also from reperfusion injury. The circumflex branch of the left coronary artery was ligated and released (5 min ischaemia + 30 min reperfusion) repeatedly, until death occurred. Among 10 saline- and 10 DFT-treated rabbits, 50% died within 78 or 150 min, respectively.

To explain these results, we investigated the effects of DFT on rabbit polymorphonuclear leukocytes (PMN) "in vitro". These were either quiescent or activated by autologous serum incubated with zymosan. We observed that PMN were less reactive (about - 40%), when incubated in the presence of DFT (500 μg/ml) than without.

We conclude that DFT effectively prevents cardiac impairment due to coronary artery ligation in rabbit hearts and that blunting of PMN activation by DFT may be involved in this effect.

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28P SR 33589B, A NEW AMIODARONE-LIKE AGENT: EFFECT ON ISCHAEMIA-INDUCED VENTRICULAR ARRHYTHMIAS IN ANAESTHETIZED PIGS

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Amiodarone is established to be one of the most effective antiarrhythmic agents currently available for clinical use (Cairns et al., 1991) and is known to be effective following intravenous administration for the short-term control of ventricular arrhythmias (Hohnloser et al., 1992). The aim of the present study was to compare the effect of SR 33589B (2-n-butyl 3-[4-(3-di-n-butylamino-propoxy)benzoyl] 5-methylsulphonamido benzofuran hydrochloride) , a new amiodarone-like agent (Bruyninckx et al, 1992), and amiodarone in a model of ischaemia-induced ventricular arrhythmias in anaesthetized domestic pigs. The incidence of ventricular fibrillation (VF), ventricular tachycardia (VT) and the number of premature ventricular complexes per minute PVC's/min were evaluated following a 30 min occlusion period of the left descending coronary artery (n=10). SR 33589B, amiodarone and vehicle (PEG/$H_2$0, 2/1, 0.2 ml/kg) were administered intravenously 15 min before coronary occlusion. SR 33589B at 1.25, 2.50 and 5 mg/kg iv markedly reduced the incidence of ventricular arrhythmias during coronary occlusion. SR 33589B especially at the two higher doses caused a sustained reduction in both the number of PVC'/min and the incidence of VT. In addition, the incidence of VF was reduced from 90 % in the control group to 30% ($P<0.05$) at 1.25 mg/kg, to 10% ($P<0.001$) at 2.50 mg/kg and to 20 % ($P<0.01$) at 5 mg/kg. In comparison, amiodarone at 10 and 20 mg/kg iv decreased significantly the number of PVC's/min and the incidence of VT. The incidence of VF was also decreased (from 90 to 40 % and from 90 to 50 % respectively) but this protection never became statistically significant.

drug	dose (mg/kg)	n	PVC's/min	%VT	% VF
vehicle	-	10	4.61 ± 1.13	60	90
SR 33589B	1.25	10	4.48 ± 1.36	30	30*
SR 33589B	2.50	10	2.05 ± 0.56	20*	10***
SR 33589B	5	10	1.88 ± 0.44	20*	20**
Amiodarone	10	10	1.77 ± 0.44*	20*	40
Amiodarone	20	10	1.40 ± 0.31*	40	50

Results are shown as mean ± sem or incidence , *P<0.05, **P<0.01, ***P<0.001 (Student's *t* test or chi-square with Yates correction)

Thus, in this model of sudden cardiac death, SR 33589B demonstrates significant antifibrillatory and antiarrhythmic actions at lower doses than amiodarone.

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29P THE ANTIARRHYTHMIC EFFECT OF PRECONDITIONING, IN ANAESTHETISED RATS, DOES NOT DEPEND ON PERTUSSIN TOXIN-SENSITIVE G_i PROTEINS

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Conflicting evidence surrounds the involvement of functional G_i proteins in the antiarrhythmic effect of ischaemic preconditioning in *in vitro* rat models of regional ischaemia (Piacentini et al, 1993, Lawson et al, 1993). This study examines whether pertussis toxin pretreatment modifies the protective effect of preconditioning in an *in vivo* model of coronary artery ligation. Pertussis toxin treatment results in ADP-ribosylation of certain G proteins, including G_i, rendering the affected G proteins nonfunctional.

Male Sprague - Dawley rats were anaesthetised with sodium pentobarbitone (60 mg kg^{-1} i.p.) and prepared for occlusion of the left main coronary artery. All animals were subjected to 30 min of regional ischaemia with or without preconditioning. A preconditioning cycle of 3 min ischaemia and 10 reperfusion, before the 30 min occlusion, was employed. The rats were divided into the following groups: vehicle (10% glycerol in saline) treated control (1) and preconditioned (2); pertussis toxin (25 μg kg^{-1}, i.p.) treated control (3) and preconditioned (4). Pertussis toxin or it's vehicle were administered 48 h prior to the experimental procedure. The incidences of ventricular tachycardia (VT), ventricular fibrillation (VF) and mortality during the sustained 30 min occlusion are shown in Table 1. To ascertain the effectiveness of pertussis toxin pretreatment, hearts taken from either vehicle (n = 5) or pertussis toxin (n = 4) treated rats were Langendorff perfused and given acetylcholine (a 5 min infusion of 10mM). Pertussis toxin pretreatment attenuated the bradycardic response to acetylcholine (heart rate reduced by 14 ± 9 compared to 209 ± 16 in the vehicle treated group).

Table 1.

Group	n	MABP (mm Hg)	HR (beats min^{-1})	%VT	%VF	%MORTALITY
Vehicle treated						
(1) control	9	90 ± 7	420 ± 12	100	78	67
(2) preconditioned	9	93 ± 6	434 ± 17	44*	0**	0**
Pertussis toxin treated						
(3) control	9	82 ± 5	393 ± 17	100	89	56
(4) preconditioned	9	81 ± 4	433 ± 13	33**	13**	0*

Values are mean ± s.e.mean. *P < 0.05, **P < 0.01 compared to appropriate control group

Preconditioning resulted in a significant reduction in VT, VF and mortality in both vehicle and pertussis toxin treated rats. Pertussis toxin pretreatment had no significant effect on heart rate (HR) or mean arterial blood pressure (MABP) throughout the experimental protocols. Since the protective effect of preconditioning is not modified by pertussis toxin pretreatment, these results suggest that functional pertussis toxin sensitive G proteins are not necessary for the antiarrhythmic effect of preconditioning in anaesthetised rats.

L. Piacentini holds a British Heart Foundation studentship.

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30P CAN TOLERANCE ENSUE AFTER LONG-TERM QUINIDINE TREATMENT?

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Taouis et al. (1991) have recently demostrated that long-term treatment with the class-I antiarrhythmic drug mexiletine induces sodium channel up-regulation. The aim of our study was to ascertain whether prolonged in vivo treatment with another Na channel blocker, quinidine, could alter the in vitro responsiveness of the heart.

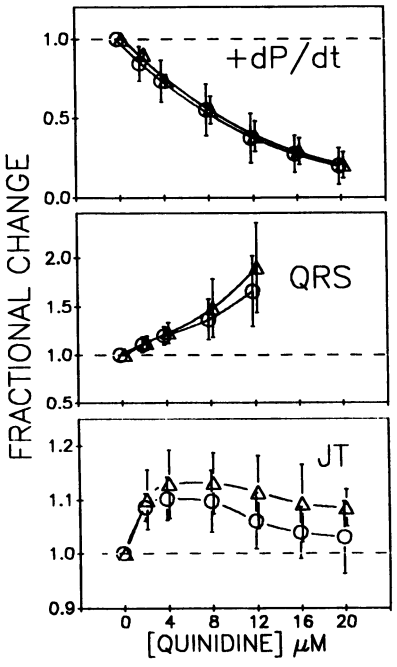
Guinea pigs of both sexes (200-300 g weight) were treated intraperitoneally (once a day) for 6 days: one group (n= 7) received normal saline solution and the other (n= 7) was treated with 75 mg/Kg quinidine sulphate (Q). Preliminary experiments indicated that this Q dose could reach plasma concentrations of about 2 ug/ml, during the 6 hours following drug administration. At the end of treatment, hearts were removed and retrogradely perfused at constant flow rate, as previously described (Padrini et al., 1992). The pacing frequency was 2.5 Hz. The following parameters were measured: coronary perfusion pressure (Cp); intraventricular pressure (P) and maximum P derivative (dP/dt max); PR, QRS and JT intervals (surface bipolar recordings). Figure 1 shows the concentration-effect curves for the main parameters, in the 2-20 uM Q concentration range (circles: controls; triangles: pretreated). Maximum effect for the QRS interval could not be achieved because of the onset of conduction block at the highest concentrations tested.

Our results show that the inotropic and the electrophysiological effects of Q, in a range of "therapeutic" concentrations, are not decreased by a 6-day pretreatment. At variance with the results obtained with mexiletine, our observations argue against the occurence of drug tolerance to quinidine.

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FIGURE 1



31P IDENTIFICATION OF ACTIVE SITES OF INTERLEUKIN-1β BY LOOP SUBSTITUTION AND SITE-DIRECTED MUTAGENESIS

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Interleukin-1β (IL-1β) is an important mediator of inflammatory and immune responses which binds to two different receptors (IL-1Rs I and II) on target cells (Dinarello & Wolff, 1993). In search of putative active sites of the cytokine, we have mutagenised a region of the molecule which can be involved in receptor binding.

By computer analysis of the aminoacid sequence of human IL-1β and of IL-1R I two hydropathically complementary peptides which bind to each other have been identified. The sequence of the cytokine peptide corresponds to the loop 7 of the cytokine crystal structure (residues 88-99), one of the exposed and highly charged regions of the molecule. Consequentely, a number of mutant proteins have been generated by both loop substitution and site-directed mutagenesis of loop 7. The binding of mutants to IL-1R I and II has been measured on murine thymoma EL4-6.1 and human B-cell lymphoma Raji 1H-7 cell lines respectively. Moreover the biological activity of the mutants has been tested on the PGE_2 release and IL-6 mRNA accumulation in human fibroblasts and on the proliferation of murine T-cells.

The binding affinity constants of wild type IL-1β were $2.1x10^9 \pm 8.2x10^7$ M and $5.3x10^8 \pm 5.2x10^7$ M for IL-1R I and II respectively. The substitution of loop 7 with a sequence of same length, but different hydropathic profile caused a dramatic decrease in the affinity to IL-1R I ($3.9x10^6 \pm 5.8x10^5$ M), but not to IL-1R II ($3.2x10^8 \pm 2.0x10^7$ M). This mutant was still able to stimulate PGE_2 release, IL-6 mRNA accumulation and T-cell proliferation. However the protein concentration required was 100 to 200-fold higher than that of the wild type. Site-directed mutagenesis of the single aminoacids of loop 7 generated mutants with affinity constants slightly lower than wild type. All these mutants exerted biological effects with similar potency compared to mature IL-1β. These results indicate that (i) loop 7 is part of the binding site of IL-1β to IL-1R I, but not to IL-1R II. (ii) The substitution of loop 7 causes a local change without grossly altering the protein structure. (iii) The activation of IL-1R I mediates the immunostimulatory and inflammatory effects of IL-1β.

We propose a binding site in which the cooperation of several low energy bonds results in a high affinity complex between IL-1 and IL-1R I. Moreover the lack of correspondence between the decrease in receptor affinity and the decrease of biological activity suggests that the binding step is not the only limiting factor in receptor activation and triggering of signal transduction.

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32P DISTRIBUTION OF CYTOKINES DURING THE DEVELOPMENT OF THE CHRONIC GRANULOMATOUS TISSUE OF THE MURINE CROTON OIL-INDUCED AIR POUCH

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Cytokines have profound effects on various aspects of granulomatous tissue formation. However, there is little information regarding their distribution during tissue development. This study investigated the temporal and spatial distribution of a variety of cytokines including, transforming growth factor beta (TGFß), platelet derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), tumour necrosis factor alpha (TNFα), interleukin-1α (IL-1) and IL-1ß in developing chronic granulomatous tissue.

Female TO mice (30±2g) were anaesthetized and 3ml of air injected into the dorsal subcutaneous tissue, followed on day 1 by injection of 0.5ml of 0.1% v/v croton oil in Freund's complete adjuvant (Kimura *et al.*, 1985). The pouch was left for 3,5,7,14,21, and 28 days, animals were then sacrificed by terminal anaesthesia. Samples were prepared for immunohistochemistry and labelled using antibodies against TGFß, bFGF, PDGF, EGF, TNFα, IL-1α and IL-1ß using a standard peroxidase anti-peroxidase technique.

Immunoreactivity to TGFß, PDGF, TNFα, IL-1α and IL-1ß was localized to a proportion of macrophages within the granulomatous tissue. Immunopositive macrophage numbers increased with time, and with the exception of PDGF were associated with areas of active fibrogenesis between days 14 to 28. Heterogeneous labelling of capillaries for EGF was observed within the granulomatous tissue juxtaposed to dermal musculature. Diffuse labelling of bFGF, associated with extracellular matrix (ECM), was always observed. After day 14, bFGF immunoreactivity was discretely localized to endothelial cells and the basement membrane of vessels within the granulomatous tissue. TGFß immunoreactivity was also associated with ECM components, being most intense in the area of fibrogenesis between 14 and 28 days. Occasional fibroblasts were also labelled with TGFß in this region.

The spatial and temporal confinement of the individual cytokines suggests that a sequential, coordinated process of repair and fibrosis is occurring. It is hoped that these observations will provide a more effective therapeutic approach for the sequential application of cytokines in abnormalities of wound healing.

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33P LIPOPOLYSACCHARIDE INDUCES BOVINE AORTIC ENDOTHELIAL CELLS TO SYNTHESIZE CYCLO-OXYGENASE BUT NOT NITRIC OXIDE SYNTHASE: A COMPARISON WITH MACROPHAGES

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Under physiological conditions, the release of nitric oxide (NO) and prostacyclin by the endothelium is regulated by the constitutive isoforms of NO synthase (NOS) and cyclo-oxygenase (COX-1), respectively. Bacterial lipopolysaccharide (LPS serotype: 0111 B4) induces the biosynthesis of different isoforms of NOS and cyclo-oxygenase (COX-2) in macrophages and vascular smooth muscle. Although LPS induces NO synthase in endothelial cells (Radomski et al., 1990), the relative degree of this induction compared to that occuring in LPS-activated macrophages is not known. Here we investigate whether NOS and COX-2 can be induced by LPS in bovine aortic endothelial cells (BAE) and compare the degree of induction of NOS and COX-2 in BAE and J774 macrophages. BAE and J774 macrophages were cultured in 96-well plates as previously described (Mitchell et al., 1993). All drugs were sterilized by filtration through a 0.2 μ filter before addition to the cells under sterile conditions. The induction of NOS was measured by the appearance of nitrite in the culture medium using the Griess reaction (Gross et al., 1992). The induction of COX-2 in BAE was measured by the amount of 6-keto prostaglandin $F_{1\alpha}$ (6-keto PGF1α) and prostaglandin (PG) E2 accumulating in the culture medium of BAE. The induction of COX-2 in J774 macrophages was measured by the formation of 6-keto PGF1α as an indicator of COX-2 activity in these cells (Mitchell et al., 1993). The induction of COX-2 protein was determined by western blot analysis using a specific COX-2 antibody (Cayman Chemical Company; MI, USA).

Table I: *Effect of LPS (1 μg ml) on the release of NOS and COX metabolites*

		0	3	6	12	24
BAE	6-kctoPGF1α (ng/ml)	2.3±0.3	8.72±2.7	11.4±3.0	23.3±5.8	94.3±19.0
	PGE2 (ng/ml)	0.04±0.01	0.2±0.1	0.2±0.1	3.3±0.4	15.9±5.0
	nitrite (μM)	1.4±0.1	0.9±0.1	0.7±0.04	0.7±0.1	0.8±0.04
J774	6-keto PGF1α(ng/ml)	0.1±0.02	0.1±0.02	0.3±0.04	1.6±0.2	2.1±0.3
	nitrite (μM)	3.9±0.8	3.1±0.6	4.4±0.6	49.1±1.9	83.2±2.8

Incubation of either BAE or J774 macrophages with LPS caused an induction of COX-2 protein. Activation of BAE with LPS (1 μ g/ml) caused a substantial elevation in the release of 6-keto PGF1α and PGE2 (Table I). In J774 macrophages the release of 6-keto PGF1α was also significantly elevated (Table I) when cells were incubated with LPS (1 μg/ml) for the same time course. Prostanoid release in response to LPS was inhibited by >80% when the protein synthesis inhibitor cyclohexamide (10 μg/ml) was added 30 min prior to LPS (n=12). LPS-activated BAE differed from LPS-activated J774 macrophages in the levels of induction of NO synthase. J774 macrophages released significant levels of nitrite (Table I) following exposure time to LPS (1 μg/ml) while LPS-activated BAE released undetectable (less than 1 μM) nitrite at any time point after LPS (1 μg/ml) administration (Table I). These results show that LPS induces the synthesis of COX-2 in both BAE and J774 (41 and 21 fold increase respectively). However, only J774 macrophages display a significant induction of NO synthase.

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34P DIFFERENTIAL EFFECTS OF DETERGENTS ON INTERLEUKIN-1 PRODUCTION IN MURINE KERATINOCYTE CELL LINE

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Many environmental chemicals produce contact hypersensitivity or local inflammatory responses in the skin. Keratinocytes (kc) play a key role in these events, producing several immunoregulatory factors (Barker *et al.*, 1991). Between these immunoregulatory factors, it has been shown that many cytokines are produced by the kc, mainly interleukin-1 α and β, IL-3, IL-6, TNF-α, etc. (McKenzie *et al.*, 1990). Using a murine keratinocyte cell line (HEL30) we characterized changes in interleukin-1α (IL-1) profile in response to three non-ionic detergents (Dodoxinol-9, Δ-9; Polyglyceryl-4-luryl ether, PEL; Polyoxyethyl-20-glyceryl ricinoleate + diethyl-ricinoleamide, PEG), all widely used in the cosmetic industry. Confluent cells were treated with different concentrations of the detergents (1-3270μg/ml). After 1 or 2 hours the IL-1 intracellular and extracellular levels were evaluated by Western blot analysis and by ELISA (representative results are shown in the table-1).

Table-1. Effect of non-ionic surfactants on *in vitro* IL-1 release after 1 hour of treatment and in vivo ocular irritation.

TREATMENT	IL-1α (pg/ml)	Draize test classification
Control	19.7±6.1	
Δ-9 : 30μg/ml	46.4±11.7**	Severe
PEL: 30μg/ml	20.4±5.1	Moderate
180μg/ml	39.0±7.2*	
PEG: 30μg/ml	27.4±2.9	Non irritant
500μg/ml	46.5±4.1**	

Each value represents the mean ± standard deviation of 3-6 determinations. Statistical analysis was performed by Student's t test, with *p<0.01 and **p<0.001 versus control values.

All the detergents analysed show a dose response in IL-1 production. However, in order to achieve a similar IL-1 production different doses of detergent were necessary: Δ-9 was approximately 6 times more potent of PEL and 15 times more of PEG. It was also possible to evidentiate a strong difference between the different detergents in their ability to induce IL-1 release: Δ-9>PEL>PEG. The surfactants were tested in vivo using the Draize test showing a good parallelism with the ocular toxicity (Kay *et al.*, 1962). Considering the preminent role of IL-1 in the inflammatory response and the ability of kc to costitutively produce IL-1, the release of this cytokine could be used as an in vitro substitute for in vivo tests to determine the potential of detergents to produce dermatotoxicity.

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35P CONTROL OF PROLIFERATION OF HTLV-I-INFECTED MONONUCLEAR CELLS BY CYCLO-PENTENONE PGs IS INDEPENDENT OF INTERLEUKIN-2 AUTOCRINE REGULATION AND IS ASSOCIATED WITH HSP70 INDUCTION

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Cyclopentenone prostaglandins PGA_1 (4 μg/ml) and PGJ_2 (2 μg/ml) can inhibit the growth of cord blood-derived human mononuclear cells (CBMC) infected with HTLV-I (Human T-cell Leukemia Virus type I), both after acute infection (D'Onofrio et al., 1990) and in chronically infected, immortalized cells (D'Onofrio et al., 1992). In acutely infected CBMC multiple treatments with PGAs (every 4 days) prevented the selection of immortalized and potentially transformed clone in long-term cultures. High expression levels of a cellular heat shock-related protein of 72/74 kDa (HSP70) were found in these long-term cultures after multiple treatments with PGA_1 (D'Onofrio et al., 1993). The antiproliferative effect of PGAs was a late effect and it was not clear how they could interfere with the antiviral immune response of CBMC during the early phase of infection. In fact, binding of HTLV-I particles to T lymphocytes induces production of interleukin-2 (IL-2) and expression of the IL-2 receptor, and triggers T-cell proliferation (Gazzolo & Duc Dudon, 1987). On the other hand, PGEs, the precursor molecules of PGAs, are known to down-regulate the IL-2-dependent proliferation of T lymphocytes. To investigate how cyclopentenone PGs could interfere with the activation and proliferation of CBMC during the early phase of infection with HTLV-I, cell proliferation and HSP70 synthesis were studied during the first week after cell-to-cell virus transmission, i.e. after coculturing with lethally irradiated, virus-donor allogeneic MT-2 cells. Cocultured CBMC underwent a transient proliferative response, that peaked within 6 to 8 days and then declined (n=6). Increasing concentrations (20, 40, 80 IU/ml) of recombinant IL-2 did not affect this proliferative response. Moreover, exogenous IL-2 did not revert the antiproliferative effect of both PGs. The pharmacological effects of PGs were reversible and therefore multiple treatments (every 4 days) were required to maintain their antiproliferative activity. PG treatment at the onset of the coculture did not inhibit the initial proliferation (day 4) of CBMC, while multiple treatments completely suppressed the late cell proliferation (from day 8 onward) (n=6). Arrest of proliferation in cocultured CBMC occurred concomitantly with expression of high levels of HSP70 in the cells (SDS-PAGE and Western blot analysis). In fact, though HSP70 expression was induced early (day 5) in cocultured CBMC, its expression was further increased after multiple PG treatments and high HSP70 levels were found when the antiproliferative effect of PGs became manifest. Since HSP70 protein family is involved in the control of cell cycle as well as in antigen processing and presentation during the immune response against tumor cells and pathogens, the persistent expression of this protein in PG-treated cocultures suggested that, beside inhibiting the growth of virus-infected cells, HSP70 expression might play a role in modulating the immune function of CBMC. However, unlike in most DNA and RNA virus infection models, in which cyclopentenone PGs exert clear antiviral effects, no antiviral activity was found in this model of retroviral infection. Hence the main effect of these PGs against HTLV-I remains the arrest of proliferation of virus-infected cells, an effect that is mediated at the cellular level by factors that are apparently independent of the IL-2 autocrine regulation.

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36P INTERLEUKIN-2 INDUCES AIRWAY HYPERRESPONSIVENESS AND CELL ACCUMULATION IN NAIVE BUT NOT OVALBUMIN-SENSITISED GUINEA PIGS

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Chronic asthma may be characterised by airway hyperresponsiveness to spasmogens which is correlated with increased numbers of eosinophils in bronchoalveolar lavage fluid (BALF) (Wardlaw et al. 1988). The T-lymphocyte derived cytokine, interleukin-2 (IL-2) is a potent chemoattractant for eosinophils in vitro (Rand et al. 1991). We have therefore assessed the effects of IL-2 given by aerosol to guinea-pigs, on leukocyte accumulation in BALF, and on airway reactivity to acetylcholine (Ach). In addition, ovalbumin-sensitised guinea-pigs were used in some experiments to test whether the immunological state of the animal could affect responses to IL-2.
Guinea-pigs were sensitised to ovalbumin over 2 weeks. Naive or sensitised animals were exposed to an aerosol of IL-2 (30μg) or vehicle (0.1% BSA) delivered over 10min. 24h later, the animals were anaesthetised and the increase in airway resistance (R) in response to Ach (10μg kg^{-1}) was measured. Lungs were lavaged (40ml buffer) and a total and differential count made of cells in BALF. Results were compared using a Student's t-test.
In naive animals exposure to IL-2 induced airway hyperresponsiveness to Ach relative to BSA (IL-2, 557±52; BSA, 275±36 $cmH_2Ol^{-1}s^{-1}$; mean±s.e.mean, n=6) and increased total leukocytes (IL-2, 14.7±1.5; BSA, 9.3±0.7 $X10^6$ cells) and eosinophils (IL-2, 2.0±0.4; BSA, 0.7±0.1 $X10^6$ cells). In animals sensitised to ovalbumin, the airway resistance to Ach was not different to naive animals (sensitised, 335±58; naive, 232±55 $cmH_2Ol^{-1}s^{-1}$) and BSA had no effect (BSA, 269±42 $cmH_2Ol^{-1}s^{-1}$). Furthermore, the effect of IL-2 aerosol was identical to that of BSA in terms of the airway response to Ach (286±69 $cmH_2Ol^{-1}s^{-1}$) and eosinophil number (IL-2, 2.0±0.4; BSA, 2.5±0.4 $X10^6$ cells), although leukocyte numbers were still increased (IL-2, 19.2±1.1; BSA, 15.1±1.4 $X10^6$ cells).
Our results have confirmed in vivo the chemotactic activity of IL-2 for eosinophils and have further shown this cytokine to be capable of inducing airway hyperresponsiveness. However, both these activities are lost in sensitised animals; the mechanism of this change in the activity of IL-2 is not clear at present.

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37P EFFECT OF L-CARNITINE AND SOME OF ITS ACYL-DERIVATIVES ON SERUM TUMOUR NECROSIS FACTOR AND SURVIVAL IN A MURINE ENDOTOXIN (LPS) SHOCK MODEL

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It is generally accepted that a component of the bacterial outer membrane, referred to as LPS or endotoxin, causes septic shock by interacting with various components of the host's immune system, primarily macrophages, which in turn release large amounts of cytokines and other mediators responsible for the immuno-physiological dysregulations occurring in this disease. A number of investigations indicate that an important role may be played by a cytokine termed Tumour Necrosis Factor (TNF). A role for L-Carnitine in the treatment of sepsis was first suggested by Border et al. [1]. Afterwards, a few groups have shown a significant improvement in survival of LPS-challenged animals following treatment with L-Carnitine [2,3] as well as ameliorated health conditions in septic patients [4,5]. The present study was set up to investigate the effect of L-Carnitine and some of its acyl-derivatives on serum TNF production and survival in a murine endotoxin shock model. In our experimental model, C57BL/6 mice received 30 mg kg^{-1} LPS (*E. coli* 055:B5) injected intraperitoneally (i.p.) while the compounds under investigation were administered first i.p. 60 minutes prior to LPS injection, and then intravenously 10 minutes following the LPS challenge. Animals were bled 1 hour later and their sera were assayed for TNF activity. In other experiments, the treated animals were also monitored daily for differences in health and survival for 10 days after LPS injection. The results (Table 1) show that although all of the tested substances were able to significantly affect TNF production, the marked decrease in serum TNF levels was not paralleled by a significant increase in survival, with the only exception of the compound ST 722. The general picture that this study conveys to us is that in our LPS shock model TNF itself cannot be viewed as the only crucial mediator of lethality. Moreover, we observed that even though treatment with Carnitines was not always correlated with a significant reduction of lethality, all the same it did ameliorate health conditions of treated animals.

TABLE 1 Effect of L-Carnitine and some acyl-derivatives on serum TNF release and survival of mice challenged with LPS.

Compound	Dose (mg kg^{-1})	TNF			Survival[a]	
		Number of mice	% variation	Pb	Survivors/Total	Pc
ST 198	50 i.p. and 50 i.v.	16	-62	0.05	10/24 (8/24)	n.s.
ST 784	50 i.p. and 50 i.v.	24	-36	0.02	7/16 (5/16)	n.s.
ST 1025	15 i.p. and 15 i.v.	16	-60	0.001	6/16 (6/16)	n.s.
ST 687	50 i.p. and 50 i.v.	8	-44	0.05	3/8 (1/8)	n.s.
ST 1037	5 i.p. and 1 i.v.	8	-44	0.05	3/8 (3/8)	n.s.
ST 722	2.5 i.p. and 1.25 i.v.	9	-75	0.01	8/10 (2/10)	0.01

a = Survival of LPS control group is indicated in brackets; b = Student "t" test; c = Fisher exact test.
ST 198 : L-Carnitine chloride (M.W. 161.20); ST 784 : L-Carnitine chloride ester with β-hydroxybutyric acid (M.W. 283.75); ST 1025 : Acetyl L-Carnitine amide with citrulline (M.W. 360.41); ST 687 : Isovaleryl L-Carnitine chloride ester with β-hydroxybutyric acid (M.W. 367.87); ST 1037 : Isovaleryl L-Carnitine chloride ester with dodecanol (M.W. 450.09); ST 722 : Isovaleryl L-Carnitine chloride ester with undecanol (M.W. 436.08).

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38P VOLTAGE-OPERATED CALCIUM CHANNELS CONTROL HORMONE RELEASE FROM HUMAN SMALL CELL LUNG CARCINOMA CELL LINES

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Small-cell lung carcinoma is an aggressive neuroendocrine tumor accounting for 25% of all lung cancers (Weynants *et al.*, 1990). The proliferation of small cell lung carcinoma (SCLC) cells is under the control of several hormones (Sorensen *et al.*, 1981; Woll, 1991), some of which act as autocrine growth factors. Serotonin is one of these hormones.

A key step in the process of serotonin-induced cell proliferation is the stimulated release of serotonin (5-HT). We here report that [^{3}H]-5-HT release from SCLC cells is a Ca^{2+}-dependent event and that voltage-operated calcium channels play a crucial role in its control. We found that, when added in a Ca^{2+} containing buffer, the calcium ionophore ionomycin induced a dose-dependent Ca^{2+} influx, and that both ionomycin and the Ca^{2+}-ATPase antagonist thapsigargin induced a dose-dependent release of Ca^{2+} from intracellular stores when applied in a Ca^{2+}-free buffer. Both agents also induced a dose-dependent release of [^{3}H]-5-HT, previously taken up by the cells. Depolarization of the cells with KCl induced a dose- and Ca^{2+}- dependent [^{3}H]-5-HT release that was enhanced by the L-type Ca^{2+} channel agonist BayK8644, antagonized by the inorganic calcium channel blocker Cd^{2+} and by both nitrendipine and verapamil (targeting L-type calcium channels), ω-conotoxin (targeting N type calcium channels) and ω-agatoxin IVA (targeting P type calcium channels). Autoantibodies against calcium channels present in the sera of Lambert-Eaton myasthenic patients antagonized KCl- but not ionomycin-induced [^{3}H]-5-HT release. Polymerase chain reaction analysis of SCLC mRNA indicates that both L-, N- and P-type neuronal calcium channel α_1 subunits are expressed by these cells. Furthermore, two types of calcium channel β subunits were identified. Using the patch clamp technique we have evidence for the existence of three HVA-VOCC subtypes, identified as dihydropyridine-(Ltype), ω-conotoxin- (N-type) and ω-agatoxin IVA-sensitive (P-type).

Our data demonstrate that small cell lung carcinoma cells are able to release [^{3}H]-5-HT in a Ca^{2+}-dependent manner; confirm (Sher *et al.*, 1990) that heterologous calcium channel subtypes are expressed by these cells and suggest that both L-, N- and P-type voltage-operated calcium channels might have an important role in its control. The modulation of hormone secretion might be an alternative approach to the control of SCLC proliferation.

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39P METABOLISM OF ω-CONOTOXIN SENSITIVE VOLTAGE-OPERATED CALCIUM CHANNELS IN CULTURED NEURONAL CELLS

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In many vertebrate cells it is possible to define two main families of voltage-operated calcium channels (VOCCs): low-threshold, rapidly inactivating, slowly deactivating VOCCs (also referred to as T-type or LVA), and high-threshold, slowly inactivating, fast deactivating VOCCs (often subdivided into L, N, P, etc.) (Bean, 1989).

The pharmacology of high-threshold VOCCs has been studied more extensively, and the use of ω-conotoxin (ωctx) represents an important step toward a better definition of VOCC subtype (Sher & Clementi, 1991). ωctx binds and blocks selectively one particular subtype of VOCC present in both neuronal and endocrine cells (Sher *et al.*, 1991). By using this specific ligand (^{125}I-ωtx) we have characterized the turnover of VOCCs in different neuronal cell lines (Passafaro, 1992). Neuronal cells internalize and degrade ^{125}I-ωtx-VOCC complexes in a time- and temperature-dependent manner. Drug-induced differentiation of human neuroblastoma cells is characterized by an increased expression of plasmamembrane calcium channels, and we have found that VOCC turnover is slowed down in these differentiated cells. Both human (from Lambert-Eaton myasthenic patients) and murine (from immunized mice) anti-VOCC antibodies increased channel internalization and degradation rates.

We now describe a new modulatory event: a rapid recruitment of (ωctx binding sites to the cell surface, induced by VOCC antagonists. In the presence of ^{125}I-ωtx at 37°C, the binding to living cells continuously increased, reaching a plateau only after 6-8 hours. The binding was 400-500% (S.E. + 23) of that normally expressed on the cell surface. A short pulse with (ωctx was also sufficient to stimulate a subsequent recruitment of binding sites to the plasmamembrane. The recruited ωctx binding was specific (i.e. displaceable by cold ωctx), on the plasma membrane (as revealed by acidic washes), and prevented by incubating the cells at 20° or 4°C. The recruitment was not affected by protein synthesis inhibitors but was blocked in a dose-dependent manner by Brefeldin A (BFA) and Nocodazole, two drugs affecting different steps of the secretory pathway. A similar recruitment of ωctx binding sites was achieved with the inorganic HVA VOCC antagonist Cd^{2+}. The presence of an intracellular pool of ωctx binding sites, four times larger than the amount present on the cell surface, was directly demonstrated in permeabilized cells; the recruitment to the plasmamembrane occurred in parallel to a reduction in this intracellular pool.

Current studies are addressed to the characterization of the functional properties of the recruited channels and to the definition of the intracellular "messages" which stimulate the transport of this protein to the neuron.

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40P THE MUSCARINIC MODULATION OF D-[^{3}H]ASPARTATE RELEASE AND $[Ca^{2+}]_i$TRANSIENTS IN RAT CEREBELLAR GRANULE CELLS

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The stimulus-triggered transmitter release depends on $[Ca^{2+}]_i$ availability (Smith & Augustine, 1988). Thus, the parallel measure of release and $[Ca^{2+}]_i$ in a homogeneous neuronal population can help in understanding the mechanism of the presynaptic control exerted by agonists and drugs. To this aim, the effect of acetylcholine on D-[^{3}H]aspartate (D-[^{3}H]asp) release and on $[Ca^{2+}]_i$ in primary cultures of cerebellar granule cells was investigated.

The recently described method of electrical field stimulation of cultured cerebellar granule cells, preloaded with D-[^{3}H]asp was used (Bianchi et al., 1992). The same kind of stimulation, applied to neurons grown on glass coverslips and preloaded with FURA-2, in Mg^{2+}-free medium, elicited evident transient peaks of flurescence, easily quantified with double beam spectrofluorimeter (Tomasini et al., this Meeting). Acetylcholine (ACh) was tested on 7-10 DIV cells submitted to both measurements.

ACh caused a concentration related increase in electrically- (10 Hz, 2 min) evoked D-[^{3}H]asp release. The threshold concentration was 0.3 nmol/l, while ACh 1 μmol/l increased the release by a factor of 4-5. This enhancing effect was antagonized in a competitive manner by pirenzepine and by DAMP-4. The pA_2 of these M antagonists were 6.90 and 8.77 respectively. ACh also increased the electrically-evoked peaks of $[Ca^{2+}]_i$ and this effect was antagonized by pirenzepine and DAMP-4. However, the threshold concentration was 0.01 μmol/l, while ACh 1 μmol/l increased the peaks by only 65%.

Clearly, muscarinic (M_3) stimulation simultaneously enhances both electrically-evoked release and $[Ca^{2+}]_i$ peaks in cerebellar granule cells (Ciardo & Meldolesi, 1991). The observed differences in ACh threshold concentrations and in the maximal responses of the two parameters investigated can be accounted for by i) the limited resolution power of $[Ca^{2+}]_i$ measurement technique and/or ii) the campartmentation of the M_3-stimulated $[Ca^{2+}]_i$ transients confined to the axon terminals.

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41P DIFFERENTIAL RESPONSE OF IP_3-SENSITIVE INTRACELLULAR Ca^{2+} STORES TO MULTIPLE POLYPHOSPHOINOSITIDE HYDROLYSIS-COUPLED RECEPTORS IN SINGLE HUMAN NEUROBLASTOMA CELLS

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In LAN-1 human neuroblastoma cells, a cell line which possess M_1 and M_3 subtypes (Fatatis et al., 1992a) and endothelin-1 (ET-1) receptors (Fatatis et al., 1992b), 1 minute pulse of carbachol (CCh), bradykinin (BK), adenosine-trisphosphate (ATP) and ET-1 were all able to elicit Ca^{2+} release from IP_3 dependent intracellular stores, monitored by fura-2 single-cell microfluorimetry. The dose-response curve of the 4 agonists showed that CCh elicited the highest $[Ca^{2+}]_i$ increase. When ET-1, BK and ATP were superfused consecutively at 2 minute intervals in a random sequence and in absence of extracellular Ca^{2+}, a condition which prevents the refilling of IP_3 stores from extracellular Ca^{2+} source, the 2nd and the 3th agonist application still evoked a $[Ca^{2+}]_i$ increase (Fig.1), although progressively reduced, suggesting that Ca^{2+} released by IP_3 is in a certain amount reloaded into the stores and in part is extruded by plasmamembrane efflux pathways. However, if CCh was added consecutively as a 4th stimulus, a marked elevation of $[Ca^{2+}]_i$ occurred (Fig.2). On the other hand, when CCh was

Fig.1

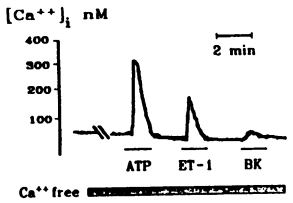


Fig.2

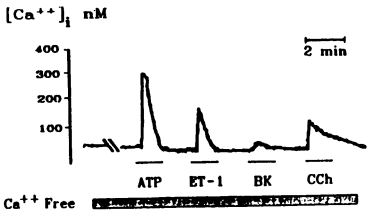
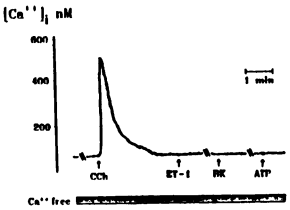


Fig.3



applied as 1st stimulus, all the 3 other polyphosphoinositide hydrolysis coupled receptor agonists, subsequently applied, were unable to elicit $[Ca^{2+}]_i$ elevation (Fig.3). The same phenomenon happened when CCh was applied as second or third stimulus. All together these results are compatible with the hypothesis that when ET-1, BK and ATP have exhausted their Ca^{2+} releasing actions from intracellular stores, the activation of M_1 and M_3 subtype receptors was still able to elicit an additional $[Ca^{2+}]_i$ increase from a distinct IP_3-dependent internal store. (Supported by 40% and 60% MURST funds, CNR grants Progetto Finalizzato Biotecnologie to L.A. and G.F.D.R)

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42P INS(1,3,4,5)P_4 MOBILISES Ca^{2+} STORES IN HUMAN SH-SY5Y NEUROBLASTOMA CELLS: EVIDENCE FOR AN INTERACTION WITH INS(1,4,5)P_3 RECEPTORS

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It is now well established that inositol 1,4,5-trisphosphate ($InsP_3$) is a fundamental second messenger which mobilises organellar Ca^{2+}-stores via a specific $InsP_3$-gated Ca^{2+}-channel. However, whether its phosphorylated product inositol 1,3,4,5-tetrakisphosphate ($InsP_4$) fulfills a distinct second messenger role is much less clear. Using the D- and L-enantiomers of $InsP_4$, we have provided some evidence to suggest that $InsP_4$ may release Ca^{2+} by interacting with the $InsP_3$-receptor (Wilcox *et.al.*, 1993): here we provide further strong evidence to support this hypothesis.

SH-SY5Y cells were cultured in monolayer, harvested, saponin-permeabilised and pre-loaded with $^{45}Ca^{2+}$ in a 'cytosol-like' buffer at 25° as described previously (Strupish *et.al.*, 1988; Wilcox *et.al.*, 1993). The magnitude of $^{45}Ca^{2+}$ release stimulated by various inositol polyphosphates was quantified by comparison to the total $^{45}Ca^{2+}$ organellar pool released by ionomycin (10 μM). Parallel radioligand binding studies were performed using a cerebellar microsomal fraction as a source of $InsP_4$ binding sites (Wilcox *et.al.*, 1993).

A maximally effective concentration of Ins(1,3,4,5)P_4 mobilised the entire $InsP_3$-sensitive Ca^{2+} store. $InsP_4$ caused a concentration-dependent release (EC_{50} 2.54 ± 0.30 μM), and was about 50-fold less potent than $InsP_3$ (EC_{50} 52 ± 2 nM). Inclusion of the competitive $InsP_3$-receptor antagonist heparin (M.Wt. 4000-6000; 50 μg/ml) caused the concentration-response curves for $InsP_3$- and $InsP_4$-evoked $^{45}Ca^{2+}$-release to be similarly rightward shifted (to give apparent EC_{50}s of 11.6 ± 1.0 and 0.35 ± 0.06 μM respectively). Addition of L-chiro-inositol 2,3,5-trisphosphorothioate (L-ch-$InsPS_3$; 30 μM), a partial agonist at the $InsP_3$-receptor (Safrany *et.al.*, 1993), also, caused the $InsP_3$- and $InsP_4$-induced $^{45}Ca^{2+}$ release curves to be rightward shifted to a similar extent (by 16.4 fold and 12.1-fold respectively). Like $InsP_3$, L-ch-$InsP_3$ possesses a relatively high affinity for the $InsP_3$-receptor (K_i 0.50 ± 0.02 μM), but interacts only very weakly with the cerebellar $InsP_4$ binding site (IC_{50} 29.1 ± 6.0 μM). Consequently, the shift in $InsP_4$ (and $InsP_3$)-induced Ca^{2+} mobilisation concentration-response curves produced by L-ch-$InsPS_3$ are likely to derive solely from a competitive interaction at the $InsP_3$-receptor.

These experiments provide strong evidence that $InsP_4$ interacts specifically with $InsP_3$ receptors to evoke mobilisation of Ca^{2+} from intracellular stores in permeabilised SH-SY5Y cells: the physiological relevance of this finding remains to be established.

We thank the S.E.R.C. for financial support. B.V.L.P. is a Lister Institute Fellow.

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43P PLATELET-DERIVED GROWTH FACTOR INDUCES AN INCREASE IN INTRACELLULAR CALCIUM CONCENTRATION IN INDIVIDUAL HUMAN LIVER FAT-STORING CELLS

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Platelet-derived growth factor (PDGF) is a key mitogen for different cell types. In particular, it has been demonstrated that PDGF induced proliferation of activated rat fat-storing cells (Pinzani et al., 1991). This effect seems to be mediated by an increase in intracellular calcium concentration ($[Ca^{2+}]i$) and inositoltrisphosphate production. The aim of the present work is a) to investigate the effect of PDGF isoforms on human fat-storing cells (HFSC) and b) to characterise spatial and temporal dynamic of the calcium increase. Angiotensin II (Angio II), known to induce rapid $[Ca^{2+}]i$ increase in HFSC (Pinzani et al., 1992), was employed as control.
HFSC were isolated from normal human liver unsuitable for transplantation and cultured as described. $[Ca^{2+}]i$ was measured in fura-2 loaded HFSC using an image analysis system to measure spatial dynamic characteristics of calcium signal (Pinzani et al., 1992).
PDGF-bb (10 ng/ml) induced a significant increase in $[Ca^{2+}]i$ in fura-2 loaded cells. This increase began in the different cells with a lag time ranging from 1 min to 7 min and then declined to the basal value. In some cells, calcium oscillations were also observed (Figure 1). In addition, $[Ca^{2+}]i$ increase induced by PDGF-bb was still observed in the absence of extracellular calcium and was abolished by pretreatment of 100 nM thapsigargin, an effective inhibitor of sarco-endoplasmic calcium APT-ase pump suggesting that internal pools were mainly responsible for the increase. Spatial analysis of $[Ca^{2+}]i$ increase showed that perinuclear area contributed to the general increase in $[Ca^{2+}]i$. Angio II induced a rapid $[Ca^{2+}]i$ increase with a lag time of few seconds. Furthermore, no calcium oscillations were observed after AngioII. These preliminary data suggest that calcium signalling is probably a part of the complex system related to PDGF-induced mitogenesis.

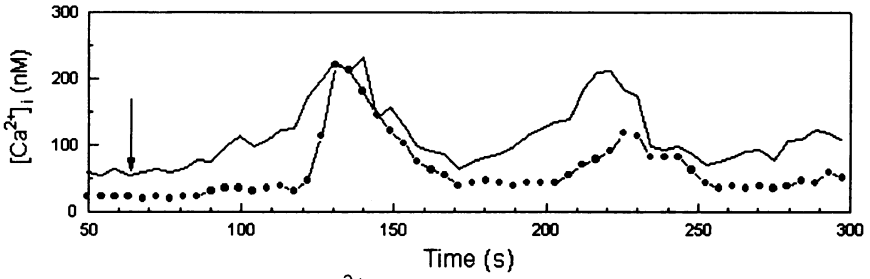


Figure 1. Oscillations of $[Ca^{2+}]i$ in two fura-2-loaded HFSC. 10 ng/ml PDGF-bb were added at the time point indicated by the black arrow.

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44P ANTAGONISM OF CALCIUM FLUXES IN ILEUM AND PLATELETS BY N,N-DIETHYL-2-[-4(PHENYLMETHYL) PHENOXY]ETHANAMINE (DPPE)

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Intracellular histamine (H_{ic}) has been proposed to act as a novel second messenger, promoting responses such as cellular proliferation and platelet aggregation. DPPE was used as a specific antagonist of an H_{ic} receptor (Brandes et al, 1990). Here, actions of DPPE on established signal transduction pathways in human platelets (HP) and guinea-pig ileum (GPI) have been examined. HP aggregation was measured using a microtitre-plate assay and contraction of GPI using a standard technique.

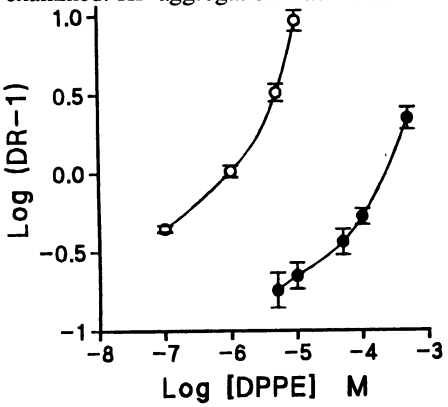


Figure 1. *Schild analysis of DPPE antagonism of HP aggregation with T(●), and GPI contraction by Ca^{2+} in 60mM K^+ (o), (n=4, ±s.d.)*

DPPE inhibited aggregation of HP (1mM Ca^{2+}) stimulated with thrombin (T), phorbol-12-myristyl-13-acetate (PMA) and ionomycin (I) with pA_2 values of 3.44 (Fig.1), 5.0 and 3.70 respectively. Inhibition by DPPE was surmountable (at <100μM DPPE) by increasing [T] or [I], but not PMA. Lowering extracellular $[Ca^{2+}]$ to 10μM reduced the pA_2 for DPPE antagonism of T to that of PMA. Contraction of GPI in response to Ca^{2+} in K^+-depolarized muscle was surmountably inhibited by DPPE (at <10μM), with a pA_2 value 360 times less than required to inhibit responses in platelets (Fig.1). Responses to ACh, 5-HT and histamine showed typical non-competititive inhibition by DPPE. Two hours incubation with 50μM (S)-α-fluroromethylhistidine (an irreversible inhibitor of histidine decarboxylase) had no effect on any of the GPI responses. DPPE appears to act through multiple mechanisms apart from its putative role as an H_{ic} receptor antagonist (Salmon, 1993), but the most sensitive action observed here is inhibition of Ca^{2+}-dependent contraction in depolarized ileum. This suggests that DPPE may interfere with voltage-operated Ca^{2+} channel function. Such channels are not found in platelets, which could account for the lower sensitivity of these cells to DPPE.

I am grateful to Dr. L. Brandes, University of Manitoba, Canada for the sample of DPPE, M.S.D. Research, U.S.A. for (S)-α-fluoromethylhistidine and The Wellcome Trust for a Travel Grant.

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45P HISTAMINE-AND CARBACHOL-INDUCED INOSITOL PHOSPHATE FORMATION IN HUMAN U373 MG ASTROCYTOMA CELLS: RECEPTOR CHARACTERISTICS AND Ca^{2+}-DEPENDENCE

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Histamine-induced inositol phosphate (IP) formation in brain slices is strongly dependent on the extracellular Ca^{2+} concentration (Alexander et al., 1990), whereas in HeLa cells IP accumulation induced by H_1-receptor activation is unaffected by an increase in the extracellular Ca^{2+} from 0.3 to 4.0 mM (Arias-Montaño & Young, 1992). Carbachol-stimulated IP formation in both brain and HeLa cells is independent of extracellular Ca^{2+} above 0.3 mM. There is a need for a simple model system on which to study the Ca^{2+}-dependent component of the response to histamine in brain and we report here the possible utility of the U373 MG human astrocytoma cell line for this purpose.

U373 MG cells were grown in DMEM/F-12 nutrient mixture supplemented with 10% foetal calf serum, 2 mM L-glutamine, penicillin and streptomycin. For the last 20-24 hrs of growth the cells were labelled with [^{3}H]-inositol in inositol-free medium containing 10 μM inositol. After dissociation cells were incubated for 15 min at 37°C in Krebs-Henseleit medium with 30 mM Li^+ and then exposed to agonists for a further 30 min in the presence of different Ca^{2+} concentrations (0-4.0 mM; 2.5 mM for standard experiments). Incubations were terminated by addition of 10% perchloric acid and [^{3}H]-inositol phosphates separated by anion-exchange chromatography.

[^{3}H]-IP_1 accumulation in response to either 1 mM histamine or 1 mM carbachol was linear with time. At 30 min [^{3}H]-IP_1 represented the dominant fraction of the inositol phosphates formed (84 ± 1%, n=4, and 88 ± 1%, n=7, for histamine and carbachol, respectively) and the mean formation (fold of basal accumulation) was 9.1 ± 0.3 (12) for histamine and 2.8 ± 0.1 (17) for carbachol. The concentration-response curve for histamine (EC_{50} 4.3 ± 0.2 μM) was shifted to the right in a parallel manner by increasing concentrations of mepyramine (pK_d, 8.5 ± 0.1) indicating the involvement of H_1-receptors. The degree of antagonism of the response to carbachol (EC_{50} 48 ± 6 μM) by pirenzepine (pK_d, 7.1 ± 0.1) was consistent with a muscarinic M_3-receptor-mediated effect. The response to histamine was markedly augmented when the calcium concentration was raised from 0 (nominal) to 0.3 mM with a further linear increase up to 4.0 mM. In contrast, after a marked increase on going from no added Ca^{2+} to 0.3 mM and a modest augmentation from 0.3 to 1.3 mM, the response to carbachol showed no further increase on raising the extracellular Ca^{2+} concentration to 4.0 mM.

The pattern of Ca^{2+} dependence of histamine H_1-receptor-induced IP formation in U373 MG astrocytoma cells is similar to that observed in guinea-pig cerebral cortex (though less marked than that in rat cerebral cortex) whereas the pattern of Ca^{2+} dependence for the response to carbachol resembles that observed in both HeLa cells and brain slices. Thus U373 MG astrocytoma cells show, at least to a degree, the pattern of Ca^{2+}-dependence of histamine H_1-receptor-induced inositol phosphate accumulation observed in brain tissues.

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46P T CELL ACTIVATION IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IS ASSOCIATED WITH INCREASE IN SIZE OF THE ER-ASSOCIATED, BUT NOT OF THE IP_3-SENSITIVE, Ca^{2+} POOL

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Experimental allergic encephalomyelitis (EAE) is an immunologically-mediated disease of the central nervous system (CNS) that can be induced in rats by immunization with purified bovine myelin. Lesions of CNS from animals affected by EAE contain antigen-specific activated T cells. In the present study we have focused on the Ca^{2+} trasduction pathway of T cells isolated from spleen of rats with acute EAE. Proteins involved in controlling Ca^{2+}-homeostasis and variations in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were investigated. 15 affected rats showed increase in the total protein content of the cells (from 23 ± 0.18 to 38 ± 0.24 μg/10^6cells) compared to 15 control animals; western blot analysis, performed as described (Villa *et al.*, 1992), showed a 2 to 3 fold increase in the expression of the following Ca^{2+}-related proteins of the endoplasmic reticulum (ER): i) calreticulin, the major high capacity-low affinity Ca^{2+}-binding protein of the ER; ii) protein dysulfide-isomerase, a chaperone molecule that is also able to bind Ca^{2+}; iii) endoplasmic/sarcoplasmic reticulum Ca^{2+}-ATPases. These data are consistent with an increase in the ER size and of its Ca^{2+} buffering and storing/releasing capacity. Fluorimetric analysis and $^{45}Ca^{2+}$ measurements were then performed as previously described (Fasolato *et al.*, 1991). Cells treated with the Ca^{2+}-ATPase blocker thapsigargin revealed an increase of 125 ± 13% of the ER-associated Ca^{2+} pool in EAE-derived activated T cells. Moreover, after treatment with the Ca^{2+} ionophore, ionomycin, a similar enlargement in the total mobilizable Ca^{2+} pool of these cells was recorded, in parallel with increase in their total Ca^{2+} content (3.7 ± 0.15 vs. 1.8 ± 0.09 mmol/liter in the control cells). In contrast, the $[Ca^{2+}]_i$ responses induced by phytohemagglutinin via inositol trisphosphate (IP_3) generation were unchanged, while the size of the IP_3-sensitive Ca^{2+} store was only moderately increased (70 ± 11%), as assessed by direct investigation in permeabilized cells. Our study, revealing complex and discrete modifications in Ca^{2+} homeostasis of activated T cells in rats affected by acute EAE, supports a role for Ca^{2+}-related signal trasduction pathway(s) in the pathogenesis of this experimental disease.

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47P NK_1 RECEPTOR MEDIATED EXTRAVASATION IN THE GUINEA-PIG

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Recent findings with new non-peptide tachykinin antagonists suggest the existence of species variants of the NK_1 receptor. Thus CP-96,345 has high affinity at most species tested including man, but has lower affinity in the rat, while RP-67580 shows the reverse selectivity (Fardin *et al.* 1992). The use of CP-96,345 to test the involvement of NK_1 receptors in nociception in models of pain in the rat has been attempted, but interpretation of results is complicated by non-specific effects (Nagahisa *et al.* 1992). It is clear that for the possible development of NK_1 antagonist as therapeutic agents, the rat may not be the most appropriate species. We describe here a model of inflammatory, plasma protein extravasation in the guinea-pig, and the effects of the new tachykinin antagonists on the response.

Extravasation was evoked in the anaesthetised guinea-pig (4 animals *per* treatment group) by the NK_1 selective agonist substance P-methyl ester (SPOME) 0.1-30nmol kg^{-1} i.v., following administration of Evans Blue (30mg kg^{-1} i.v.). A dose-related effect was obtained in many tissues, but was most reproducible in the urinary bladder (ED_{50} 1.6nmol kg^{-1}, range 1.2-2.1). [β-Ala^8]NKA(4-10) and senktide, agonists selective for the NK_2 and NK_3 receptors respectively, were active in the same dose range, but produced only 25-45% of the maximum effect of SPOME.

The actions of antagonists were measured against the response in the bladder to a 3nmol kg^{-1} dose of SPOME. (±)-CP-96,345 blocked the response (ID_{50} 102μg kg^{-1} i.v., 95% c.l. 50-252) producing complete inhibition of extravasation at 0.3-1.0mg kg^{-1}, whereas the inactive enantiomer CP-96,344 was ineffective at 1mg kg^{-1}. The dipeptide antagonist FK888 (Fujii *et al.*, 1992), that also shows marked preference for the NK_1 receptor in the guinea-pig, was powerfully active in our hands producing complete block of the extravasation response to SPOME in guinea-pig bladder at 0.3mg kg^{-1} (ID_{50} 17μg kg^{-1} i.v., 95% c.l. 14.2-19.7). The rat-preferring antagonist RP-67580 was the least active blocker of the extravasation response (ID_{50} 277μg kg^{-1} i.v., 95% c.l. 151-512).

In conclusion the extravasation response in the guinea-pig to the NK_1 agonist SPOME shows the appropriate sensitivity to species-specific NK_1 antagonists, and points to the possibility of developing tests in this species that will be of greater relevance than those in rat.

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48P TACHYKININ NK_1 RECEPTOR LIGANDS SHOW TWO DISTINCT PHARMACOLOGICAL PROFILES IN RABBIT IRIS SPHINCTER

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Tachykinins contract the rabbit isolated iris sphincter directly through an interaction with NK_1 and NK_3, but not NK_2, receptors (Hall *et al.*, 1991, 1993). In this study, we have compared a number of NK_1-preferring agonists with respect to their sensitivity to NK_1-selective antagonists, and the effect of phenoxybenzamine-pretreatment on washout kinetics, since both approaches have suggested heterogeneity between agonists' actions.

Table 1	Apparent pK_B (s.e.m.; n) (n.d. = not determined)		
	(±)CP-96,345	RP67580	GR82334
GROUP 1			
Substance P methyl ester	8.9 (0.3; 6)	7.0 (0.1; 6)	7.5 (0.1; 12)
Septide	9.3 (0.3; 6)	7.4 (0.2; 6)	7.7 (0.2; 9)
[Apa^{9-10}]-SP	9.5 (0.5; 6)	n.d.	7.1 (0.1; 7)
[Glp^6]-SP(6-11)	n.d.	n.d.	8.2 (0.3; 7)
GR73632	n.d.	n.d.	7.5 (0.2; 15)
GROUP 2			
Substance P	< 7.0 (3)	< 6.0 (3)	< 5.5 (6)
[Pro^9]-SP	< 7.0 (3)	< 6.0 (3)	< 6.0 (5)
Physalaemin	n.d.	n.d.	< 6.0 (4)
Ranamargarin	n.d.	n.d.	< 6.0 (8)

Methods have been described previously (Hall *et al.*, 1991, 1993); phenoxybenzamine (20 μM) was applied for 10 min (Too *et al.*, 1988). Responses to the NK_1-preferring agonists collected into GROUP 1 (shown in Table 1), reversed rapidly (8 - 16 min) on washout and were antagonised by NK_1-selective antagonists. In contrast, responses to GROUP 2 NK_1-preferring agonists were very prolonged in offset (40 - 70 min); and moreover, were insensitive to antagonism by NK_1-selective antagonists. However, following phenoxybenzamine pretreatment, responses to GROUP 2 agonists now reversed with kinetics similar to GROUP 1 agonists (13 - 26 min), and were antagonised by GR82334 (e.g. pK_B 6.8 ± 0.3; n = 7 *vs.* [Pro^9]-SP). For GROUP 1 agonists the affinity of antagonists was not significantly affected (e.g. for GR82334 against septide pK_B of 7.7 ± 0.2 & 7.9 ± 0.2; n=7), before and after treatment, respectively) ($P > 0.05$). The atypical pharmacology of GROUP 2 agonists is unlikely to be a consequence of simultaneous NK_1/NK_3 receptor interaction since [Pro^9]-SP is highly selective for the NK_1 receptor, and responses to NK_3 selective agonists reverse relatively rapidly on washout: further, co-administration of NK_1 and NK_3 agonists does not result in prolonged washout kinetics.

In conclusion, assuming simple competition, these data could be explained by the existence of two NK_1-receptor isoforms or subtypes (as proposed for the guinea-pig ileum; Petitet *et al.*, 1992), or interaction with multiple binding domains on a single NK_1-receptor.

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49P FUNCTIONAL ACTIVITY AT NK_1 RECEPTORS OF NOVEL PEPTOID ANTAGONISTS

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The rational design of the peptoid [4-Me]Z-(R)-αMe-Trp-NH-(S)-CH-Me-Ph (Cam-2445) as a ligand with high affinity at the NK_1 site in guinea-pig cortex (pK_i against [^{125}I]-BH-substance P binding 7.82±0.10) has been described (Guard *et al.*, 1993). We report here on the functional activity of this compound and two analogues Cam-2709 and Cam-2819, where the N-terminal protecting groups are 2-Naphthyl-methoxycarbonyl- or [2-Fl]Z-, in place of the [4-Me]Z- of Cam-2445, in non-rodent *in vitro* and *in vivo* tests.

Affinity was measured *in vitro* in terms of inhibition of the binding of [^{125}I]-BH-substance P (0.1nM) in IM9 cells, antagonism of responses to substance P in U373MG cells using calcium-fluorescence measurements, or antagonism of contractions by substance P-methyl ester (SPOME) in guinea-pig ileum (GPI), guinea-pig trachea (GPT) and rabbit jugular vein (RJV). Activity *in vivo* was measured (4 animals *per* treatment group) against extravasation of plasma protein by 3nmol kg^{-1} SPOME i.v. in the urinary bladder of the guinea-pig (PPE, Boyle *et al.* this meeting).

Table 1. Functional activity of peptoid antagonists at tachykinin NK_1 receptors

	IM9 pIC_{50} (±s.e.mean)	GPI apparent pK_B (±s.e.mean)	GPT apparent pK_B (±s.e.mean)	RJV apparent pK_B (±s.e.mean)	PPE ID_{50} (μg kg^{-1}) (95% c.l.)
Cam-2445	7.7 (0.25, n=5)	7.9 (0.03, n=3)	7.1 (0.20, n=4)	8.1 (0.80, n=4)	588 (40-891)
Cam-2709	8.0 (0.17, n=4)	8.3 (0.14, n=14)	7.2 (0.39, n=3)	8.7 (0.08, n=6)	409 (342-489)
Cam-2819	8.3 (0.10, n=3)	8.4 (0.05, n=3)	7.3 (0.41, n=3)	8.9 (n=2)	111 (96-130)

All three compounds were competitive antagonists in the ileum and trachea of the guinea-pig, and the rabbit jugular vein, but were non-competitive antagonists in the U373 cell. Although the measures of affinity in ileum and jugular vein agreed well with the estimates of binding affinity in the IM9 human cell line, the estimates of affinity in guinea-pig trachea were consistently lower. The order of affinities of the compounds was followed by the order of activities *in vivo* for antagonism of SPOME-induced extravasation.

In conclusion the peptoid ligands Cam-2445, -2709 and -2819 are antagonists of high affinity at non-rodent NK_1 receptors *in vitro*, and exhibit good activity in the whole animal.

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50P THE ROLE OF BRADYKININ AND SENSORY NERVES IN THE VASCULAR EXTRAVASATION EVOKED BY ANTIGEN IN GUINEA-PIG TRACHEA

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The role of bradykinin (BK) and sensory nerves on vascular extravasation evoked by aerosolized ovalbumin (OVA) was studied by measuring the extravasation of the Evans blue dye in the trachea of sensitized guinea-pigs. Administration of OVA (aerosol, 2 min) into the trachea of anaesthetized guinea-pigs produced a dose-dependent (0.5-10%) increase in vascular extravasation. Vascular extravasation evoked by OVA was also time-dependent, being higher at 10 min than at 5 min after the beginning of the aerosol. The inhibitor of neutral endopeptidase (NEP), phosphoramidon (2.5 mg/kg), and the selective non-peptide antagonist of NK_1 tachykinin receptor, CP-96345 (Snider et al., 1991) (1 mg/kg) did not affect the response to OVA (5%) at 5 min. However, phosphoramidon increased (40%) and CP-96345 decreased (70%) vascular extravasation evoked by OVA at 10 min. The inhibitory effect of CP-96,345 was dose-dependent. At the highest dose (4 mg/kg) CP-96,345 did not reduce the vascular extravasation evoked by platelet activating factor (100 nmol/kg, i.v.), which exerts this effect without the involvement of sensory nerves.

BK aerosol (100 μM, 2 min) increased vascular extravasation in guinea-pig trachea. The peptide B_2 BK antagonist HOE 140 (Hock et al., 1991) abolished and the NK_1 receptor antagonist, CP-96,345 (1 mg/kg), reduced by 60% the response to BK. Aerosol administration of substance P (SP) (10 μM, 2 min) caused an increase in vascular extravasation that was abolished by CP-96,345 and unaffected by HOE 140. The increase in vascular extravasation evoked by OVA challenge (5%) in the presence of phosphoramidon (2.5 mg/kg), was similarly inhibited by HOE 140 (57%), CP-96,345 (72%) or by the combination of HOE 140 and CP-96,345 (75%).

We conclude that: i) tachykinins released from peripheral endings of sensory nerves play a relevant role in the vascular extravasation evoked by antigen challenge in sensitized guinea-pigs; ii) this contribution of tachykinins is evident only after an early phase of extravasation had taken place; iii) inhibition of NEP exaggerates the contribution of sensory nerves in the response to OVA; iii) the ability of an NK_1 receptor antagonist to reduce the vascular extravasation evoked by BK, and the lack of additive effect of the NK_1 antagonist and the B_2 antagonist in reducing the response to OVA suggest that BK release is an intermediary step in the antigen-antibody reaction and the activator of sensory nerves to increase this inflammatory response to antigen. If a similar mechanism exists in humans, tachykinin NK_1 and BK B_2 receptor antagonists may play a potential therapeutic role in allergic airway diseases.

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51P [D-Arg^0,Hyp^3, Thi^5,D-Tic^7,Oic^8]-BRADYKININ IS AN EFFECTIVE BRADYKININ ANTAGONIST IN THE HUMAN NASAL AIRWAY

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Evidence suggests that kinins may be mediators of allergic rhinitis, since bradykinin and kallidin are found in the nasal secretions of patients with naturally occuring allergic rhinitis (Svensson et al., 1990), and application of bradykinin to the nasal airway has been shown to induce symptoms of rhinitis (Proud et al., 1988). We have studied the ability of 3 bradykinin (BK) antagonists to attenuate the nasal response to BK, and we have investigated the effect of the potent and long acting BK_2 receptor antagonist, [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK, on the response of the human nasal airway to allergen challenge.

The BK antagonists, [D-Arg^0,Hyp^3,D-Phe^7]-BK (BKA1), [1-Adamantaneacetyl-D-Arg^0,Hyp^3,Thi^{5,8},D-Phe^7]-BK (BKA2) and [D-Arg^0,Hyp^3,Thi^5,D-Tic^7,Oic^8]-BK (BKA3), were used in this study. Nasal blockage was determined objectively by measuring nasal airway resistance (NAR) using active posterior rhinomanometry, and changes in vascular permeability were studied by measuring albumin in nasal lavage, using immmunodiffusion. Each antagonist was given by aerosol, to each nostril, in 5 non-atopic subjects, 2 minutes before an intranasal challenge with BK, 100µg. On another occasion, subjects underwent nasal lavages with 5ml saline, 10 minutes after identical kinin challenges.
8 patients taking no medication, and allergic to the house dust mite (HDM), D. *pteronyssinus*, with a positive skin prick test, were challenged with either vehicle or bradykinin antagonist (BKA3), 200µg to each nostril, followed 2 minutes later by HDM, 500 U intranasally. Each subject participated in both challenges in a random order, separated by at least one week. Responses were assessed by measuring the minimum nasal cross-sectional area (A.min) using acoustic rhinometry. Responses were quantified as the maximal decrease in A.min during the post-challenge measurements. Results were expressed as % decrease from saline control value. Patients also recorded nasal symptoms on visual analogue scales.

Intranasal administration of BK, 100µg induced an increase in NAR of 85.9±9.2% (n=15) and an increase in albumin content of nasal lavage from 1.9±0.4mg/dl to 24.3±6.6mg/dl. BKA1, 1000µg, was not effective in reducing BK-induced nasal responses. Both BKA2 and BKA3, 100µg, produced a significant inhibition of NAR in response to BK ($p<0.05$, n=5 for both), and a decrease in the albumin content of the lavage to 6.9±3.1mg/dl and 1.85±1.3mg/dl respectively.
Nasal challenge with HDM, 500 U, in atopic subjects resulted in a decrease in Amin. of 22±5.8% (n=8), and an increase in symptom scores. Administration of BKA3, 200µg prior to HDM challenge blocked the HDM-induced decrease in Amin. which decreased by 1.7±4.1%, (n=8, $p=0.018$) at this time. In addition, there was a marked effect on symptom scores: rhinorrhoea, 84% reduction; nasal blockage, 74% reduction; itching, 67% reduction, and sneezing, 28% reduction, (n=8).

In conclusion, these data indicate that the BK_2 receptor antagonist, BKA3, is an effective antagonist of the BK-induced response in the human nasal airway, and of the nasal response to allergen challenge.

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52P DIFFERENTIAL VASCULAR AND BRONCHIAL ACTIONS OF VASOACTIVE SUBSTANCES IN THE PERFUSED RAT LUNG

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The isolated perfused lung is a widely utilised model which has been used to assess the production and catabolism of many vasoactive substances. In such studies the direct pulmonary effects of the infused agents have often been ignored despite the fact that such changes may affect the lung's metabolic capability. Therefore in isolated perfused rat lungs we have monitored changes in three parameters: pulmonary perfusion pressure (PPP), pulmonary inflation pressure (PIP) and lung weight in response to various vasoactive agents.

Male Wistar rats (300-350g) were anaesthetized (Sagatal,60mg/kg i.p.). Heparin (500iu) was injected via the tail vein. The chest was opened and the pulmonary artery(PA) cannulated via the right ventricle. The trachea was also cannulated. Lungs were dissected free and transferred in to a heated chamber (37°C). The lungs were perfused(5ml/min) via the PA with oxygenated(95% O_2/ 5% CO_2) Krebs solution and ventilated with room air(stroke vol 1ml;28 strokes/min). Lung weight was recorded continuously via an isometric transducer. Lungs were allowed to stabilise for 30 min before adding bolus doses (10-100µl) via the PA.

The effects of perfusion time on lung weight were initially determined. Initial lung weight 2.8±0.2g (mean±sem;n=5) was increased by 6.6±3.7%, 41±6% and 96±20% after 2h,3h and 4h of perfusion respectively. Therefore in all subsequent experiments a maximum perfusion time of 2h was employed. In separate experiments following the initial stabilisation, the effects of three drugs were studied. Phenylepherine(PE) 3.12-25nmol dose dependently increased PPP (ED_{50} 4.1±0.3nmol n=3). However PE did not affect lung weight or airway tone. Carbachol(CCh) 3-24nmol selectively increased PIP(ED_{50} 5.16±0.72nmol n=3) without affecting PPP or lung weight. In contrast bolus doses of bradykinin(BK) 6.25-200nmol dose dependently increased PPP (ED_{50} 23.7±4.1nmol n=3), PIP (ED_{50} 51.33±10.97nmol n=3) and lung weight(ED_{50} 31.7±9.3nmol n=3). Studies after 90 min of perfusion showed that dose-response curves (DRC) to PE and BK with regard to PPP and lung weight were not different from the initial DRC. However the effect of CCh and BK on PIP were attenuated.

These studies reveal that of the three agonists used PE produces selective pulmonary vasoconstriction and CCh a selective bronchoconstriction. BK however produces pulmonary vasoconstriction, an increase in lung weight and bronchoconstriction.

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53P ACTIVITY OF THE NEW HISTAMINE H_2-RECEPTOR ANTAGONIST ZOLANTIDINE AT CARDIAC AND GASTRIC H_2 RECEPTORS

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The effect of the new histamine H2-receptor antagonist zolantidine (ZOL) was studied in different cardiac and gastric H2-receptor assays in comparison with ranitidine (RAN). ZOL (0.1-10 μM) competitively antagonized the positive effects of histamine in the spontaneously beating guinea pig atria and in the electrically-stimulated papillary muscle (Table 1). Conversely from RAN, ZOL at the highest concentrations reduced also basal heart rate and cardiac contractility. In the isolated rat gastric fundus ZOL did not modify histamine-induced acid secretion up to 100 μM; it was also ineffective against dimaprit-induced acid secretion in conscious gastric fistula cats (up to 3 μmol/kg i.v.) and in the anaesthetized rat with lumen-perfused stomach (up to 10 μmol/kg i.v.). In all these gastric secretory models RAN antagonized H2-mediated responses, showing a potency comparable to that found in cardiac preparations (Table 1).

Table 1. Activity of zolantidine and ranitidine in different H2-receptor assays

	Guinea pig atria	Guinea pig papillary muscle	Rat gastric secretion "in vitro"	Rat gastric secretion "in vivo"	Gastric fistula cat
ZOLANTIDINE	6.98±0.28	6.78±0.27	inactive up to 100 μM	inactive up to 10 μmol/kg	inactive up to 3 μmol/kg
RANITIDINE	6.84±0.11	6.38±0.19	6.78±0.38	0.036±0.01 μmol/kg	0.30±0.025 μmol/kg

Data (±SEM) are reported as pA_2 ("in vitro" studies) and as ID_{50} (intact animals).

These data clearly showed that ZOL is a very peculiar histamine H2-receptor antagonist, capable of distinguishing between cardiac and gastric H2-receptors; however, it is still to be elucidated whether this depends on a true receptor heterogeneity or on the high lipophilicity of the drug.

54P POSSIBLE INVOLVEMENT OF HISTAMINE H_3 RECEPTORS IN GASTRIC MUCOSAL PROTECTION IN THE RAT

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Histamine has been demonstrated to be present in high concentration in the gastric mucosa and it has been hypothesized to be a mediator both in the development of acute gastric mucosal injury and in cytoprotection. Recently H3 receptors have been demonstrated to be involved in the regulation of gastric functions (Coruzzi et al., 1991). In this study the involvement of histamine H3 receptors in acute gastric lesions in the rat has been examined by testing the activity of the H3 agonist (R)α-methylhistamine (MHA) and of the H3 antagonist thioperamide (THIO).
Different treatments were used to induce gastric mucosal lesions: absolute ethanol (administered intragastrically in 1 ml volume); aspirin (200 mg/kg in 1% methylcellulose in 0.15 N HCl administred intragastrically in a 5 ml/kg volume); stress (rats were placed in restraint cages and immersed in cold water, 18 °C, to the xiphoid process for 2 h). Rats were killed 1 h after ethanol and 2 h after aspirin or stress-induction. THIO was administered 60 min and MHA 30 min before the treatments, both intragastrically. Control rats received vehicle alone. At the end of the experiments, the stomachs were removed and examined for the presence of gastric mucosal lesions. Each individual lesion was measured along its greatest lenght under a microscope and the overall total was designed as "lesion index".
MHA dose-dependently reduced the incidence and the severity of hemorrhagic gastric lesions produced by ethanol, in the dose range 1-100 mg/kg, an almost complete protection being reached with 100 mg/kg (97% decrease in lesion index, $P < 0.01$, when compared to controls). ED50 was 10.72 mg/kg (5.24-21.87). Pretreatment with THIO at doses up to 3 mg/kg did not influence the MHA protective activity. MHA was similarly active in preventing the development of gastric mucosal lesions induced by aspirin or by stress, its effect being dose-dependent in the dose range 1-100 mg/kg. The largest dose, 100 mg/kg, produced a 62% and 78% decrease in lesion index in aspirin-treated and stressed rats respectively. Intragastric administration of MHA at 1-100 mg/kg doses had no effect or slightly increased gastric acid and pepsin output in 2h pylorus-ligated rats. Present findings provide evidence that MHA is effective in protecting the gastric mucosa. The question whether this effect could be ascribed to the activation of H3 receptors or to the molecule of MHA is open. Further experiments using other agonists and antagonists of H3 receptors will clarify this problem.

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55P NMDA RECEPTORS MODULATE NEUROTRANSMISSION AND PERISTALSIS IN THE GUINEA-PIG COLON

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Excitatory amino acid (EAA) receptors are widely distributed in the central nervous system, where they are involved in the control of the release of several neurotransmitters. Indeed, activation of EAA receptors by specific agonists can potently enhance the release of acetylcholine and noradrenaline in different brain regions (Scatton & Lehmann, 1982; Fink *et al.*, 1992). There is also evidence for the presence of EAA receptors in peripheral organs (Erdö, 1991), where they are putative mediators of a number of functions. In the gastrointestinal tract, several lines of evidence support the notion that L-glutamate may serve as an excitatory neurotransmitter in the myenteric plexus by an action on receptors of the N-methyl-D-aspartate (NMDA) subtype (Moroni *et al.*, 1986; Wiley *et al.*, 1991).

Since only fragmentary information is available on the possible role of NMDA receptors in the modulation of the enteric nervous system, we have examined the effects of NMDA on electrically evoked noradrenaline release in isolated neuromuscular preparations of the guinea-pig distal colon. The functional effects of NMDA on the efficiency of propulsion were also studied.

In Mg^{++}-free Tyrode's solution, NMDA concentration-dependently increased electrically evoked noradrenaline release (1-30 nM) and decreased the efficiency of propulsion (5-1,000 nM). All these effects of NMDA were antagonized by (±)AP5 (10 μM) and were virtually suppressed in the presence of 1.2 mM Mg^{++}.

The present data are consistent with a modulatory role played by NMDA receptors at the level of the enteric nervous system. Such a modulation could be exerted through the activation of specific neural pathways, which, in turn, determine the final effect on intestinal propulsion.

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56P EVIDENCE FOR A ROLE OF ATYPICAL β-ADRENOCEPTORS IN THE REGULATION OF COLONIC MOTILITY IN THE CONSCIOUS DOG

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Several lines of evidence suggest the involvement of atypical β-adrenoceptors in the regulation of colonic motility in the rat and guinea-pig (Bianchetti & Manara, 1990; Bond & Clarke, 1988). The aim of the present study was to assess the effects of SR 58611A (an agonist at atypical β-adrenoceptors, Bianchetti & Manara, 1990) on canine colonic motility in vivo, comparing its effects to those of isoproterenol (non-specific β-adrenoceptor agonist) and atropine (reference compound).

In four conscious dogs, chronically fitted with electrodes along the colon, colonic spike activity was monitored by a computerized system. The colonic motor response to a standard meal was assessed in control experiments and after administration of SR 58611A (10-300 μg/kg) or isoproterenol (1.5 μg/kg) injected as intravenous boluses 15 min before the meal. Heart rate was also monitored. In some experiments, β-adrenoceptor antagonists (see below) were administered (300 μg/kg i.v. bolus followed by 300 μg/kg/h infusion for 1 h, starting 30 min before agonist administration). The colonic motor response to the standard meal was quantified by calculating the number of spikes in the 10-min period immediately following the end of the meal. The colonic and chronotropic effects of β-adrenoceptor agonists were expressed as a percentage of the response to a supramaximal dose of atropine.

Colonic spike activity was increased after the standard meal (mean number of spikes at each recording site = 757±107). Atropine (300 μg/kg i.v.) profoundly inhibited the colonic motor response to the standard meal (Δ number of spikes -553±49) and induced marked tachycardia (Δ heart rate +187±15 beats/min). A dose-dependent inhibition of the colonic motor response to the meal was observed after SR58611A. The dose of 30 μg/kg significantly reduced the colonic motor response, without significant chronotropic effect. The highest dose tested (300 μg/kg) showed a maximal colonic inhibitory effect (95% with respect to atropine), but induced a positive chronotropic response that was only 45% of that of atropine. The reverse was true for isoproterenol, which displayed a maximal chronotropic effect (97% with respect to atropine) at a dose (1.5 μg/kg) that inhibited the colonic response by only 45% with respect to atropine. Colonic inhibition by SR 58611A was antagonized by alprenolol, but not by CGP 20712A or ICI 118,551 (β_1- and β_2-adrenoceptor antagonists, respectively).

These data strongly suggest a role for atypical β-adrenoceptors in the regulation of canine colonic motility. SR 58611A and isoproterenol show an opposite pharmacological profile when their colonic inhibitory and chronotropic effects are compared in vivo.

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57P CYCLIC AMP ENHANCES NMDA RECEPTOR-MEDIATED STIMULATION OF RAT CEREBELLAR NITRIC OXIDE FORMATION

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Activation of NMDA receptor-operated Ca^{2+} channels in rat cerebellum results in the stimulation of nitric oxide (NO) synthesis (Bredt & Snyder, 1989; Garthwaite *et al.*, 1989). This is believed to occur primarily within the granule cells and involves the constitutive Ca^{2+}-calmodulin-dependent enzyme, nitric oxide synthase (NOS). Glutamate and other excitatory amino acids also stimulate cyclic AMP (cAMP) formation in cerebellum, possible indirectly *via* release of other neurotransmitters.Thus, in this study, we have investigated the ability of intracellular cAMP to regulate NMDA-induced stimulation of nitric oxide synthase activity.

Cerebellar slices (0.4 x 0.4mm cross-chopped) from 10 day-old rats were preincubated in oxygenated Mg^{2+}-free Krebs-bicarbonate medium for 60 min at 37° C and then transferred to vials containing either forskolin, 1,9-dideoxyforskolin, dibutyryl cAMP (dbcAMP), or NMDA antagonist and incubated for 15min. L-[^{3}H]-arginine (57 Ci/mmol) was then added together with NMDA and the incubation continued for a further 15min, before termination by addition of ice-cold EDTA (4mM)/arginine (5mM). Slices were pelleted, sonicated in 1M TCA and, after removal of acid with diethyl ether, L-[^{3}H]arginine (formed stoichiometrically with NO) was separated from L-[^{3}H]citrulline by ion exchange chromatography.

Exposure of slices to NMDA resulted in a dose-dependent increase in NO formation, with a concentration of 10μM producing a sub-maximal stimulation of approximately 130% above basal. Addition of either forskolin (60μM) or dbcAMP (1mM) produced a substantial potentiation (203 and 209% respectively over basal) of the response to NMDA. Both forskolin and dbcAMP failed to stimulate NO formation in the absence of NMDA. Similarly, 1,9-dideoxyforskolin (60μM), which lacks the ability to stimulate adenylyl cyclase, neither stimulated NO formation nor enhanced the effects of NMDA receptor activation. Dibutyryl cAMP produced a parallel sinistral shift in the NMDA concentration-effect curve, without affecting the maximal response. The enhancement was fully antagonized by inclusion of the competitive NMDA antagonist, D-AP5 (50μM).

These results indicate that a cAMP-dependent process may modulate NMDA receptor/NO coupling in the rat pup cerebellum, possibly *via* direct phosphorylation of the NMDA receptor by protein kinase A. Interestingly, it has been suggested that cAMP may play a physiological role *via* NMDA receptors in hippocampal neurones (Harada *et al.*, 1991).

NJT is an SERC Research Student.

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58P EVIDENCE OF THE INVOLVEMENT OF NITRIC OXIDE IN THE OXIRACETAM-INDUCED POTENTIATION OF GLUTAMATE RELEASE IN RAT HIPPOCAMPAL SLICES

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Previous works reported that the nootropic drug oxiracetam increased the depolarization-evoked release of glutamate or [^{3}H]D-aspartate ([^{3}H]D-ASP) from slices of rat hippocampus (Marchi et al., 1990; Raiteri et al., 1992). We here investigated the mechanisms involved in the releasing effect of this drug. NG-nitroarginine, an inhibitor of nitric oxide (NO) synthase (30-100 µM), prevented (by 60 and 100%, respectively) the effects of oxiracetam (1 µM) on the release of endogenous glutamate and of [^{3}H]D-ASP evoked by 35 mM K^+ from hippocampus slices. The effect caused by 100 µM NG-nitroarginine was completely reversed by 100 µM L-arginine. In standard hippocampal synaptosomes, both the basal outflow and the K^+(15 mM)-evoked overflow of [^{3}H]D-ASP, known to be unaffected by oxiracetam, were enhanced by the NO donor S-nitroso-N-acetylpenicillamine (SNAP). SNAP, at 0.5, 0.7 and 1 mM increased by 30 ± 2%, 78 ± 3% and 148 ± 6% the basal release of [^{3}H]D-ASP overflow. Oxiracetam remained ineffective also when added in the presence of the NO generator. Experiments of release carried out with "mossy fiber" synaptosomes purified from rat hippocampus provided results similar to those with "standard" synaptosomes. The potentiation of the K^+-evoked [^{3}H]D-ASP overflow caused by 1 µM oxiracetam in slices (+ 38%) was counteracted by dizocilpine (IC_{50} = 0.5 µM), an antagonist at the N-methyl-D-aspartate (NMDA) receptor and by 6-cyano-7-nitroquinoxaline-2-3-dione (CNQX; IC_{50} = 2.0 µM), an antagonist at the α-amino-3-xydroxy-5-methyl-4- isoxazolepropionic acid (AMPA) receptor.

The results suggest that oxiracetam potentiates the release of excitatory amino acids in depolarized rat hippocampus slices by making available to presynaptic axon terminals the releasing agent NO produced following activation of postsynaptic glutamate receptors by glutamate/aspartate released during depolarization.

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59P INHIBITION OF NITRIC OXIDE SYNTHASE ENHANCES NMDA-EVOKED DOPAMINE RELEASE IN RAT STRIATUM *IN VIVO*

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Nitric oxide (NO) may both mediate and modulate N-methyl-D-aspartate (NMDA)-evoked dopamine release in the striatum. Indeed, NO donors increase basal [^{3}H]dopamine release from rat striatal slices and inhibition of NO synthase inhibits NMDA-evoked [^{3}H]dopamine release (Hanbauer et al, 1992). However, NO can also afford protection against NMDA-evoked neurotoxicity *in vitro* possibly by feedback inhibition of the NMDA-receptor (Lei et al, 1992). Nevertheless, the role of NO in NMDA-evoked dopamine release in the striatum *in vivo* is not known. Therefore, we report the effect of inhibition of NO synthase on NMDA-evoked dopamine release in the rat striatum using *in vivo* microdialysis.

Male Wistar rats (280-320g) were anaesthetised with chloral hydrate (500mg/kg ip). Microdialysis probes (3mm membrane length) were implanted in the striatum using standard stereotaxic techniques. The probes were continuously perfused with artificial extracellular fluid (138mM NaCl, 5mM KCl, 1.2mM $CaCl_2$, 1mM NaH_2PO_4, 10mM $NaHCO_3$; aECF) for 1h before collecting 10 min fractions. After perfusion for a further 1h, NMDA (1mM) was incorporated in the aECF for 20min (S1 stimulation) followed by normal aECF for 40min. Probes were then perfused with either L-NAME (nitro-L-arginine methyl ester; 1mM) or normal aECF for 1h before stimulating again with NMDA (1mM) as previously described (S2 stimulation). Dialysate samples were assayed for dopamine, DOPAC and HVA by HPLC.

The initial NMDA-stimulation (S1) increased dopamine efflux (993-1760% of basal values; figure 1A), with concomitant decreases in DOPAC (39%-56%) and HVA (31%-36%). In the presence of L-NAME, the second (S2) NMDA-stimulation greatly increased dopamine efflux compared to control (3466% compared to 481%). The S2/S1 ratio of total dopamine efflux was dramatically increased during L-NAME infusion compared to control (Figure 1B). However, NMDA-mediated decreases in DOPAC and HVA were not enhanced during infusion with L-NAME (DOPAC, 43%; HVA, 38% of control values).

These data suggest that inhibition of NO formation enhances NMDA-evoked dopamine release in the intact rat striatum. Thus, NO may modulate NMDA-evoked dopamine release in the striatum possibly by feedback inhibition of NMDA-receptor function or by modulation via the striato-nigro-striatal loop.

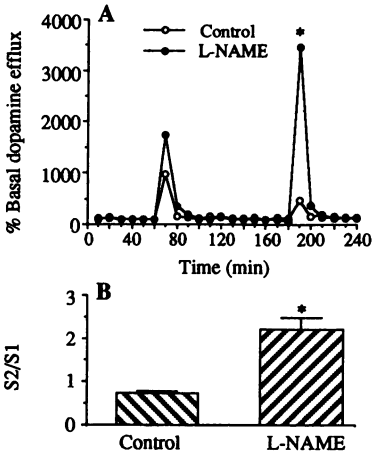


Figure 1 The effect of L-NAME on NMDA-evoked dopamine release in rat striatum expressed **A** as % basal release and **B** as S2/S1 ratio. (n=3-5). *p<0.05 compared to control.

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60P EVIDENCE THAT HIV gp120 GLYCOPROTEIN ACTIVATES THE INDUCIBLE ISOFORM OF NO SYNTHASE IN CULTURED ASTROCYTOMA CELLS

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HIV-related cognitive disorders and cortical neuronal loss have been reported in patients suffering from AIDS, though the pathophysiology remains still unclear. *In vitro* experiments have shown that the HIV coating gp120 protein enhances intraneuronal Ca^{++} and causes neurotoxic effects sensitive to the N-methyl-D-aspartate (NMDA) receptor antagonists, APV and MK801, thus leading to the suggestion of an "excitotoxic" mechanism for HIV-induced brain damage (see Lipton, 1992). However, in the present experiments we report that a single intrahippocampal (1 pmol; n=6) or intracerebroventricular (3 pmol; n=6) injection of gp 120 failed to produce consistent convulsive and/or neurodegenerative effects in rats. On the other hand, intrahippocampal gp 120 (1 pmol; n=6) did not sinergize with NMDA (0.5 μg) given into the same site, in producing neurotoxicity. These data rise some doubts on a glutamate-mediated mechanism for gp 120-induced brain damage. Interestingly, HIV activates glial cells to release cytokines (Merrill, 1992). In addition, cytokines have been shown to enhance, in cultured astrocytoma cells, the production of nitric oxide (NO; Mollace et al., 1993), a cytotoxic free radical species. Therefore, we have performed experiments to verify whether gp 120 was able to stimulate the production of NO from human cultured astrocytoma cells. Incubation of cultured T 67 astrocytoma cells for 48h with gp120 (10, 100 and 500 nM; n=4 for each concentration) significantly increased the levels of nitrite (the breakdown product of NO) in cell supernatant by 22±3, 52±6 and 72±3%, respectively. The increase in nitrite level produced by gp120 (500 nM; n=4), was reduced by 72% following pretreatment of astrocytoma cells with N^{ω}-nitro-L-arginine methyl ester (L-NAME; 100 μM). Furthermore, gp 120 (100 nM; n=4), stimulated the inducible NO synthase activity measured as the rate of [^{3}H]-citrulline formation in astrocytoma cell homogenates. In fact, a Ca^{++}-independent increase of NO synthase activity (+ 45±4%) occurred when cells were incubated for 48h with gp 120 protein. In conclusion, activation of a Ca^{++}-independent inducible isoform of NO synthase in astrocytes may represent a target for the effects of gp 120 protein in the brain and this may contribute to explain some neurological disorders accompanying AIDS.

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61P THE EFFECT OF SYSTEMIC PHENYLEPHRINE ON EXCITATORY AMINO ACID RELEASE IN THE RAT DORSOMEDIAL MEDULLA OBLONGATA

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Using push-pull cannulae Kubo and Kihara (1988) demonstrated release of L-glutamate (GLU) and L-aspartate (ASP) in the nucleus of the solitary tract (NTS), but did not assess the effect of baroreflex manipulations on this release. The present study has employed the technique of in vivo microdialysis to investigate the potential role of excitatory amino acids as neurotransmitters of vagal afferent terminals by measuring their endogenous release in this brain region, and to determine the effect of eliciting a sympathetic component of the baroreflex with systemic phenylephrine on this release.

Male Wistar-Kyoto rats (250-320g) were anaesthetised with urethane (1.5 g/kg i.p.) and concentric dialysis probes were perfused with artificial cerebrospinal fluid (aCSF, 1µl/min) and implanted into the region of the dorsomedial medulla containing the medial NTS (Lawrence & Jarrott, 1993). In some cases the left femoral artery and vein were cannulated for measurement of blood pressure and drug administration respectively. After a 90 min. equilibration period, 20 min. samples were collected. Amino acid content of dialysates were determined both for basal and evoked efflux. In the case of evoked release, two depolarising pulses of aCSF containing 80mM KCl were employed with an hour gap in between (S1 and S2). Drug treatment was after the fourth sample in the case of basal, or during S2 in the case of evoked release. Results are given as mean±s.e.m.

Basal extracellular levels of GLU (7.9±0.8 pmol/20µl, n=55) and ASP (2.2±0.1 pmol/20µl, n=55) were increased following perfusion of aCSF containing 80mM KCl to 266±19% (n=5) and 204±40% (n=4) of basal respectively. This response was reproducible yielding S2/S1 ratios of 0.96±0.07 (GLU) and 0.80±0.1 (ASP). When S2 was performed in aCSF lacking Ca^{++} ions, the S2/S1 ratios were reduced to 0.4±0.1 (GLU, n=4, P= 0.004 unpaired t-test) and 0.3±0.06 (ASP, n=4, P= 0.006). Phenylephrine 30µg/kg/min. (i.v.) during S2 increased evoked GLU release to 446±120% (n=5) of basal, S2/S1 = 1.7 ± 0.2 (P=0.0037). This dose of phenylephrine also increased the basal efflux of GLU to 148±28% (n=5, P<0.05 ANOVA) of control. No effect was observed on basal or evoked release of ASP. Resting mean arterial pressure (98±5 mm Hg, n=10) was increased to 136±7 mm Hg (n=10, P=0.0004) by phenylephrine.

These data demonstrate neuronal release of GLU and ASP in the dorsomedial medulla. Furthermore, systemic phenylephrine can increase neuronal release of GLU in the area of the brain including the medial NTS. This effect may be the result of vagal baroreceptor stimulation, suggesting GLU may be an important neurotransmitter at these afferent terminals.

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62P GLUTAMATE RECEPTOR-INDUCED CALCIUM TRANSIENTS IN CULTURED HIPPOCAMPAL NEURONES BEFORE AND AFTER SYNAPTOGENESIS

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Using a bioassay for studying synaptic vesicles (SVs) exo-endocytosis, we have demonstrated in cultured hippocampal neurons that SVs can undergo exo-endocytotic recycling even before synapse formation (Matteoli et al., 1992). To verify whether this recycling had implications in neurotransmitter release, we have used Fura-2 imaging of hippocampal cells maintained in cultures for different periods of time. By substituting the standard physiological medium with a solution nominally free of Mg^{2+} ions and containing 1 µM glycine we have revealed activation of NMDA receptors by endogenously released glutamate in neurons before and after the formation of synaptic contacts.The activation of the NMDA receptor was inhibited by specific antagonists (50 µM APV) and was almost completely prevented (>97% inhibition) by the Ca^{2+} channel blocker Cd^{2+} (200 µM). These data indicate that release of glutamate and expression of functional NMDA receptors occur in hippocampal neurons in colture even before synapse formation. We next investigated whether differences in the distribution of glutamate receptors were induced by establishment of synaptic contacts. Three days after plating, when synaptic contacts are absent, $[Ca^{2+}]_i$ transients could be induced by pharmacological activation of kainate and NMDA (10-100µM) but not quisqualate (20-50µM) receptors. The changes in $[Ca^{2+}]_i$ occurred homogeneously in the dendritic and somatic compartments. In cells where synaptic contacts could be recognized either morphologically or by immunocytochemical detection of synaptic vesicle accumulation (7-14 days after plating), NMDA receptor-induced $[Ca^{2+}]_i$ preferentially arised from synapse-enriched portions of dendrites. In a same cell, the patterns of $[Ca^{2+}]_i$ responses induced by challange with NMDA or by the glycine-Mg^{2+}free medium were almost superimposable and coincident with the immunocytochemical distribution of synaptic vesicle antigens. Depolarization induced $[Ca^{2+}]_i$ transients were more widely distributed and were more intense in the axonal compartment of well differentiated hippocampal cells. The data suggest that synapse formation triggers cellular mechanisms responsible for NMDA receptor accumulation at dendritic strategic sytes facing the presynaptic boutons.

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63P ETHANOL WITHDRAWAL IS ASSOCIATED WITH A MARKED INCREASE IN EXTRACELLULAR GLUTAMATE: AN NMDA RECEPTOR-MEDIATED MECHANISM

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Chronic exposure to intoxicating doses of ethanol results in physical dependence and in abstinence syndrome after ethanol withdrawal. Recent evidence suggests that NMDA receptors play an important role in the mechanism of ethanol actions. In vitro, acute ethanol inhibits NMDA-mediated responses whereas chronic ethanol exposure results in a potentiation of NMDA excitotoxicity. In vivo, acute ethanol prevents NMDA-induced seizures in rats whereas chronic ethanol results in an increase in NMDA receptors and NMDA antagonist drugs reverse the symptomatology of ethanol withdrawal. These studies suggest that ethanol dependence is associated with increased activity of NMDA-mediated glutamatergic neurotransmission. To test this hypothesis directly, we measured by microdialysis the extracellular concentrations of glutamate (GLU) in the ventral striatum of ethanol-dependent rats. GLU was measured in 20-min perfusate samples by HPLC coupled to fluorescence detection after pre-column derivatization. In dependent animals (5 g/kg p.o. four times a day for 6 days), 12 h following withdrawal, extracellular GLU levels were 38.3 ± 4.0 pmol/min and 5.0 ± 0.5 pmol/min, in chronic ethanol- and sucrose-treated rats, respectively (means ± S.E.M, n=7; $p<0.001$). An ethanol challenge (5 g/kg p.o.) reduced perfusate GLU concentrations to 17.5 ± 2.2 pmol/min within 2 h. Thereafter GLU levels progressively increased and reached 31.0 ± 3.8 pmol/min 10 h following ethanol administration. The changes in GLU output were temporally correlated to corresponding changes in the severity of withdrawal symptomatology, as assessed by subjective evaluation of behavioral signs of withdrawal. MK-801 (1 mg/kg i.p.), administered to 12 h-withdrawn rats reduced GLU output to 27 ± 3.0 pmol/min (n=6, $p<0.05$, as compared to 12 h-withdrawn rats) and, as expected, markedly reduced the withdrawal symptomatology. The administration of diazepam (5 mg/kg i.p.) reduced the signs of ethanol withdrawal, however produced no effect on extracellular GLU output. In sucrose-treated rats, the focal application of NMDA 0.8 mM (corrected for recovery) through the perfusion medium for 20 min stimulated GLU output to 270 ± 38% of baseline (n=7). In 12 h ethanol-withdrawn animals, NMDA-induced response was potentiated by 1.7-fold (459 ± 60% of baseline, n=3; $p<0.05$). The NMDA-induced response was prevented by ethanol and by MK-801. In contrast, diazepam did not affect the NMDA-induced GLU response in controls nor in ethanol-dependent rats. These results suggest that a) ethanol dependence is associated with increased glutamatergic activity; b) the increase in GLU output is mediated by enhanced NMDA receptor function presumably through a feed-forward mechanism; c) benzodiazepines act as physiological antagonists of NMDA-mediated GLU activity. Thus the functional activation of NMDA receptors, rather than decreased sensitivity of $GABA_A$ receptors plays a primary role in ethanol withdrawal. The increased extracellular GLU concentrations during withdrawal may constitute the neurochemical basis of the neurodegenerative effects of long term ethanol consumption in alcoholics.

64P GLUTAMATE AND GLYCINE ANTAGONISTS FACILITATE THE INHIBITION OF SPERMINE-INDUCED CNS EXCITATION AND CONVULSIONS BY IFENPRODIL

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The NMDA receptor macrocomplex contains binding sites for various agonists including glutamate, glycine and polyamines. Binding studies have shown that polyamines, like glutamate and glycine, potentiate dizocilpine binding (Williams *et al.*, 1991). We have previously examined the effect of the putative polyamine antagonist ifenprodil (30 & 60 mg/kg, ip), the glutamate antagonist D-CPP, (10 mg/kg, ip), and the glycine antagonist 7 Cl-kynurenate (30 nmol, icv) in inhibiting the CNS excitation and convulsions induced by spermine (100 µg, icv), and found no effective antagonism by any of these compounds, indeed 7 Cl-kynurenate potentiated the response. Recent evidence has shown that there appear to be reciprocal allosteric interactions between the binding sites for glutamate or glycine and the polyamines, (Williams *et al.*, 1991; Carter *et al.*, 1992) and in the light of this evidence it was of interest to examine the effect of co-administration of ifenprodil (30 & 60 mg/kg, ip) with D-CPP (10 mg/kg, ip) or 7 Cl-kynurenate (30 nmol, icv).

Table1:

Drug:	Median score of CNS excitation at 5hr (†IQR):	% showing tonic convulsions by 6.5 hr:
Spermine control	3.0 (3.0)	67
+ 7Clkyn	4.5 (2.75)	92
+ Ifn 30 + 7Clkyn	1.5 (4.0)	60
+ Ifn 60 + 7Clkyn	1.0 (0.75)**	31§
+ Ifn 30 + D-CPP	2.0 (1.0)**	25§§
+ Ifn 60 + D-CPP	1.0 (0.5)**	23§§

Co-administration of 7Cl-kynurenate with ifenprodil to female mice (laca strain) caused a dose dependent reduction in CNS excitation and an increase in the time to onset of tonic convulsions. Similarly, co-administration of D-CPP with ifenprodil also produced a dose-dependent reduction in CNS excitation and an increase in the median time to first tonic convulsion. It is concluded that moderate doses of glycine and glutamate antagonists appear to reveal the inhibitory potential of ifenprodil. Moreover, whereas low doses of dizocilpine were ineffective, a high dose (2 x 0.3 mg/kg, ip) produced a modest antagonism suggesting that these effects of spermine may be mediated, at least in part, through an action at the NMDA receptor macrocomplex.

**$p<0.01$ Mann Whitney U-test; §$p<0.05$, §§$p<0.01$ Proportionality test; †IQR: Inter quartile range.

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65P MEASURING EXCITATORY AMINO ACID RELEASE DURING STRESS IN BEHAVING RATS

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Glutamic acid (Glu) is the main excitatory amino acid in the brain. Glu can cause excitotoxic lesions and consequently neuronal death. Its excitotoxic nature is thought possibly to underlie several neurological diseases including Hungtington's chorea, status epileticus, Alzheimer's dementia and olivopontocerebellar atrophy. Glu may potentiate ischemic injury by excessive receptor stimulation and intracellular calcium overload according to the concept of excitotoxicity. We have used two assay systems to measure changes in the extracellular concentration of glutamate in response to a 5 min tail pinch, a mild behavioural stimulus which we have shown previously to increase the release of dopamine and ascorbate in the striatum and 5-hydroxytryptamine and noradrenaline in the hippocampus. The first of these assays is an immobilized enzyme-based flow injection assay system which uses the enzymes glutamate oxidase and horseradish peroxidase and a modified ferrocene as a solution mediator. Using microdialysis and an automatic injector, sampling can be carried out at 2.5 min intervals; the assay was very sensitive with a detection limit of 1.8 pmol injected on column (Boutelle, et al., 1992). The second method is the dialysis electrode (Albery, et al., 1992) in which a fine platinum/iridium wire is placed inside a dialysis probe which also contains the enzyme glutamate oxidase. Hydrogen peroxide produced from the oxidation of glutamate is measured at a potential of 650 mV against a Ag/AgCl reference electrode. This system gives a linear calibration plot and can detect step changes of 0.2 µM. Male Sprague-Dawley rats weighing between 200-300 g were used in all *in vivo* experiments. Experiments were carried out 24 h following probe implantation in the striatum. With microdialysis and flow injection analysis we failed to get any changes in glutamate in response to tail pinch. With the dialysis electrode there was a brisk response in the absence of glutamate oxidase in the probe; we were able to show that this was due to an increase in ascorbate, since it was completely abolished by the injection of ascorbate oxidase into the probe. When glutamate oxidase was now added to the probe, tail pinch produced an increase in the signal (n=7; Student t test $P<0.05$); this was abolished by the addition of catalase, which confirms that the signal is due to glutamate.The dialysis electrode provides a method for the continuous measurement of glutamate and has provided further evidence for the close coupling between changes in glutamate and ascorbate. The enzyme bed assay enables us to use the variation of concentration method to measure the true extracellular concentration of glutamate and to determine how much of this is derived from impulse dependent neuronal activity. The extracellular fluid(ECF) is the biophase for the multitude of drugs acting on cell surface bond receptors.
These methods can be applied to pharmacology to study the effects of various drugs in the ECF of the central nervous system (CNS) and to develop and characterize new drugs as well.

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66P ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTORS REDUCES THE RELEASE OF PURINES STIMULATED BY FORSKOLIN IN RAT HIPPOCAMPAL SLICES

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Metabotropic glutamate receptors (mGluRs) form a family of receptors whose members are either coupled to polyphosphoinositide (PPI) hydrolysis ($mGluR_{PI}$) or negatively linked to adenylate cyclase ($mGluR_{\downarrow cAMP}$) (Nakanishi, 1992). While $1_S,3_R$ aminocyclopentane-1,3-dicarboxylic acid (ACPD) acts as a mixed agonist at all mGluR subtypes, $(2_S,1'_R,2'_R,3'_R)$-2-(2,3-dicarboxycyclopropyl) glycine (DCG IV) potently activates $mGluR_{\downarrow cAMP^s}$ without increasing the basal PPI hydrolysis. In rat hippocampal slices, stimulation of cAMP formation by forskolin (F) was substantially reduced either by $mGluR_{\downarrow cAMP}$ agonists or by adenosine deaminase (ADA), which depletes the extracellular levels of adenosine (Ado). Inhibition of cAMP formation by $1_S,3_R$-ACPD and ADA were less than additive, suggesting that their actions converge into a common pathway or mechanism. In addition, a large component of the inhibitory action of $1_S,3_R$-ACPD on F-stimulated cAMP formation was prevented when experiments were performed in presence of N-ethylcarboxamidoadenosine (NECA), which substitutes for endogenous Ado in activating A_2 sites. As a possible explanation for these results, we have hypothesized that F and $mGluR_{\downarrow cAMP^s}$ interact in modulating purine (P) release. Thus, in slices preloaded with [^{3}H] -Ado, labelled and endogenous P release was assayed by radioactivity measurement and HPLC analysis of the superfusate. 20-80 µM F enhanced P release in concentration-dependent fashion (55% over basal outflow for 80 µM F). A_1 and A_2 Ado subtype receptors are likely to be implicated in the modulation of F-induced P release, since antagonists, such as 200 nM 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) and 1 µM benzene sulfonamide, N-[2(dimethylamino) ethyl] -N-meythyl-4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1H-purin-8yl) (PD 115,199), raised and inhibited the evoked P release, respectively. 3 µM MK-801, a non-competitive NMDA receptor antagonist, reduced the evoked P release by 40%, suggesting that, according to Chen Y. et al. (1992), endogenous glutamate release supports the evoked P release via NMDA sites. The simultaneous pretreatment with DPCPX and MK-801 produced a less than additive effect, confirming the existence of tight interactions between P and excitatory amino acid systems. 100 µM $1_s,3_R$-ACPD and, to a greater extent, 0.1-100 µM DCG IV reduced by 25-65% P release elicited by 80 µM F. Interestingly, DCG IV did not affect the stimulation of P release induced by depolarizing concentrations of K^+, while Ado and NMDA receptor antagonists affected the electrically- and K^+-evoked release. We suggest that, in rat hippocampus, multiple interactions between P and glutamate release induced by cAMP-producing agents occur; in this regard, a specialized population of $mGluR_{\downarrow cAMP^s}$ which controls P release and probably neurotransmitter release, is also involved.

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67P PHARMACOLOGICAL CHARACTERIZATION OF METABOTROPIC GLUTAMATE RECEPTORS ABLE TO MODULATE TRANSMITTER RELEASE

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The stimulation of metabotropic glutamate receptors (mGluR) present in cortical synaptosomes causes an increase of the depolarization-induced transmitter release (Herrero et al., 1992). In order to characterize the subtype of receptors involved in this modulation we undertook in brain slices the study of: a) the depolarization (30 mM KCl) induced output of previously taken up D-^{3}H-aspartate; b) inhibition of forskolin (F) induced cyclic AMP accumulation and c) phosphoinositide hydrolysis. In cortical slices the prototype of mGluR agonist 1S,3R-ACPD (30-300 μM) significantly increased the depolarization-induced output of radioactivity thus basically confirming results obtained in synaptosomes, while in striatal slices 1S,3R- ACPD was a potent inhibitor of the depolarization-induced transmitter output. The maximal degree of inhibition was 60%. Other mGluR agonists had similar effects.

Table 1 Effects of mGluR agonists on the inhibition of transmitter output, inhibition of F (30μM)-induced cyclic AMP accumulation and stimulation of phosphoinositide hydrolysis in striatal slices.

	D^{3}H-ASP output	Cyclic AMP	Phosphoinositide metabolism
L-CCG-1	0.5 (0.3-1.2)	0.9 (0.5-2.2)	30 (10-65)
1S,3R,ACPD	100 (60-180)	30 (15-80)	25 (8-58)
QUIS	100 (70-140)	>300	15 (8-30)

Values are μM and are EC_{50} and 95% confidence limits.; L-AP4 was not active at 500 uM.

The table shows that the order of potency for the inhibition of transmitter output was 2S,3S,4S-carboxycyclopropilglycine (LCCG-1) > 1S,3R-ACPD > QUIS > AP4. A similar order of potency was observed for the inhibition of F-induced accumulation of cyclicAMP (in the presence of IBMX 1mM) while, when the stimulation of phosphoinositide metabolism was measured in the same slices the order of potency was: QUIS > 1S,3R-ACPD > L-CCG-1. Thus, the stimulation of mGluR may either decrease or increase transmitter output. L-CCG-1, was a relatively selective agonist of the receptors linked to the inhibition of cyclic AMP formation, and a potent inhibitor of transmitter output.

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68P SECOND MESSENGER GENERATION BY THE PUTATIVE METABOTROPIC AGONIST (2S,3S,4S)-α-(CARBOXYCYCLOPROPYL)-GLYCINE IN GUINEA-PIG CEREBRAL CORTICAL SLICES

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Glutamate receptors may be divided into ionotropic and metabotropic classes. Subtypes of the former group are activated by selective agonists such as *N*-methyl-D-aspartate, kainate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate. As yet there exists few selective agonists for subclasses of the metabotropic receptors. Recent evidence from cells transfected with metabotropic glutamate receptor subtypes indicates that a rigid glutamate analogue (2S,3S,4S)-α-(carboxycyclopropyl)-glycine (L-CCG-I) may be a potent and selective agonist at mGluR2 receptors linked to inhibition of adenylyl cyclase activity (Hayashi *et al.*, 1992) compared to mGluR1 and mGluR5 receptors linked to phosphoinositide turnover. We have investigated the potency of this agonist in guinea-pig cerebral cortical slices for stimulation of phosphoinositide turnover, potentiation of receptor-stimulated cAMP generation (apparently mediated through the products of phosphoinositide turnover (Hill & Kendall, 1989)) and inhibition of forskolin-stimulated cAMP formation.

cAMP generation and phosphoinositide turnover were assessed in [^{3}H]adenine or [^{3}H]inositol pre-labelled guinea-pig cerebral cortical slices (Alexander *et al.*, 1989).

Phosphoinositide turnover stimulated by L-CCG-I failed to reach a maximal response at concentrations up to 1 mM (response at 1 mM 11.2 ± 2.9-fold over basal, n=3). L-CCG-I failed to alter basal accumulation of [^{3}H]cAMP, but enhanced the [^{3}H]cAMP accumulation elicited by the A_{2B} adenosine receptor agonist 5'-*N*-ethylcarboxamidoadenosine and histamine H_2 receptor activation (1 mM histamine in the presence of 3 μM mepyramine and 10 μM rolipram) with EC_{50} values of 15 ± 3 and 19 ± 2 μM, and maximal responses of 297 ± 14 and 438 ± 42 %, respectively. L-CCG-I inhibited forskolin-stimulated [^{3}H]cAMP accumulation in a concentration-dependent manner with an IC_{50} value of 0.24 ± 0.06 μM (n=5). The inhibitory effect of L-CCG-I was unaltered in the presence of 100 μM 6,7-dinitroquinoxaline-2,3-dione, 100 μM L-2-aminophosphonopentanoate or 1 mM DL-2-aminophosphonopropionate.

It is apparent from these data that the metabotropic glutamate receptor agonist L-CCG-I exhibits greater selectivity for the metabotropic subtype linked to inhibition of cAMP formation in guinea-pig cerebral cortex compared to phosphoinositide turnover than 1S,3S-ACPD (Cartmell *et al.*, 1993). These results suggest that L-CCG-I will be a useful tool in the characterization of metabotropic glutamate receptor subtypes.

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69P DIFFERENCES IN C-*FOS* EXPRESSION IN SEIZURE MODELS ARE RELATED TO SEIZURE SEVERITY AND CONVULSANT

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It has been established that there is increased expression of immediate early genes (IEG's) following chemically and electrically-induced seizures (Morgan *et al.*, 1987). The pattern of increased expression is different from that observed following ischaemia or trauma. Recently, it has been demonstrated that there are differences in Fos-like immunoreactivity with the mode of seizure induction (Jensen *et al.*, 1993). The present study extends these findings to examine changes in mRNA for several of the IEG in three models of seizure. Mice (BKTO, 30g) were infused via the tail vein with convulsant and seizure activity monitored by observation of each animal (Singh *et al.*, 1991). Pentylenetetrazole (PTZ) or NMDLA were given to produce mild seizure (20 mg/kg PTZ) which was taken as the first myoclonic jerk and severe seizures (25-30 mg/kg PTZ) as the clonic phase, no animal in the group experienced a full tonic/clonic seizure. Mice were given ethanol (8% v/v) as a liquid diet for 7 days, the ethanol withdrawn and 16 hours later tested for handling seizures using a rating scale (Grant *et al.*, 1990). Animals killed 1 hour following seizure and IEG distribution measured by *in situ* hybridisation using (^{35}S)-oligonucleotides specific for the mRNA of interest. Following seizure mRNA for c-*fos*, c-*jun*, *jun*B, *jun*D and NGFI-A were increased in the cortex and hippocampus. Ethanol withdrawal seizures gave rise to the smallest increases, eg with NGFI-B the cortex increased 149±3, CA1 157±3, and DG 137±3% (n=9 sections from 3 animals: control 100%). The results summarised below show variations in the distribution pattern of c-*fos* mRNA with respect to the hippocampus and the hypothalamus. These changes were related to both the class of convulsant and the severity of the seizure.

Treatment	Expression of c-*fos* in O.D units (x100)				
	CORTEX	CA1	CA3	DG	HYPOTHALAMUS
Saline	49±6	45±5	45±5	50±5	43±4
PTZ-mild	302±20	153±14	144±20	143±8	154±9
PTZ-severe	306±19	147±23	154±14	245±30	148±9
NMDLA-mild	420±12	210±4	230±5	400±8	189±13
NMDLA-severe	500±6	240±20	270±30	420±30	810±50

n=9 sections from 3 animals, all values significantly different from controls Mann Whitney U test

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70P NMDA RECEPTOR SUBUNIT mRNAs ARE DIFFERENTIALLY-REGULATED IN KINDLING-INDUCED EPILEPTOGENESIS

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A consistent abnormality associated with seizure activity is an increased functional sensitivity of NMDA receptors (Mody & Heinemann, 1987). Attempts to attribute this enhanced sensitivity to increases in the densities of the receptor have so far yielded equivocal results. In the absence of clear evidence for alterations in receptor density, enhanced NMDA receptor function may conceivably be due to seizure-induced modulations of the subunit composition of the receptor. The aim of the present study was to determine the effects of rapid hippocampal kindling on the levels of mRNAs encoding the NMDA receptor subunits, NR1, NR2A and NR2B. Rapid kindling is a robust model of epileptogenesis which mirrors human complex partial seizures in which severe limbic seizures develop within 1 to 3 hours of stimulation. This technique therefore offers the advantage that events not superseded by the adaptive processes, known to take place during the extended time of conventional kindling, can be monitored.

Male Sprague-Dawley rats, implanted bilaterally with stimulating/recording electrodes in the ventral hippocampus, were subjected to 40 unilateral electrical stimulations at 5 minute intervals (400uA; 1ms pulses of 10Hz and 10 seconds duration). Animals were sacrificed at 0, 1, 2, 4, 12, 24, 48 and 120 hours following the last kindling seizure. An additional group of animals were sacrificed 2 hours after receiving only 10 stimulations (partially-kindled). *In situ* hybridisation was performed essentially according to the methodology of Persohn *et al* (1991) with oligonucleotide probes complementary to the cDNA sequences of the NR1, NR2A and NR2B subunits. Hybridised sections together with calibrated standards were apposed to β-max Hyperfilm to generate autoradiograms. Cellular resolution of subunit mRNA distribution was achieved by dipping sections in emulsion. Optical densities of hybridisation signals were quantified by computer-digitised image analysis.

In partially-kindled animals (10 stimulations), while the NR1 subunit mRNA remained unaltered, NR2A and NR2B subunit mRNAs were bilaterally reduced in dentate gyrus granule cells by 46 ± 3.7% and 54 ± 16.5% below control values, respectively (n=4; P<0.01; Dunnett's multiple comparisons test).

In fully-kindled animals (40 stimulations), a progressive reduction in NR1 subunit mRNA levels in the dentate gyrus was observed, being maximal after 4 hours (-67 ± 7.0% (n=6; P<0.01). At the same time-point, NR2A and NR2B transcript levels were transiently increased by 102 ± 5.0% and 46 ± 0.7% above control values, respectively (n=4; P<0.01). These data point to a differential regulation of NMDA receptor subunit mRNAs. No significant alterations were detected in pyramidal cells. Long-term maintenance of the kindled state is not associated with prolonged alterations in NMDA receptor subunit mRNAs since each mRNA returned to baseline by 12 hours and persisted as such for at least 5 days.

To summarize, this study demonstrates a seizure-induced, cell-specific, time-dependent modulation of NMDA-receptor subunit mRNAs which is almost entirely restricted to the granule cells of the dentate gyrus. This phenomenon may contribute to kindling-associated plasticity by mediating a structural reorganisation of NMDA-receptors, leading to an altered excitability of granule cells.

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71P NMDA RECEPTORS AND STRAIN DIFFERENCES IN A SPATIAL LEARNING TASK (MORRIS WATER TEST)

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Wistar (Crl:(WY)BR), Wistar Kyoto (WKY/NCrl BR) and spontaneously hypertensive (SHR/NCrl BR) rats, were trained in the Morris water maze (one trial/day; the position of the platform was held in a fixed position and the starting point was changed every day). NMDA receptor binding was performed according to Reynolds I.J. and Miller R.J., 1988. Tissues were obtained from prefrontal cortex, striatum and hippocampus; [^{3}H]-MK 801 was used as ligand. Kd and Bmax values and their statistical comparison were calculated according to Munson P.J. et Rodbard D., 1984. It has been shown in 72 days old rats that the curves representing the escape latency of Wistar and SHR were significantly different after ten days of training from that of WKY. After this period of time learning of the spatial task has been completely acquired by Wistar (escape latency 20± 5 sec) and SHR (14±3 sec) and the training was stopped. At this point of time the WKY latency was still very high (101±11 sec) and the acquisition of the task was not completely reached when the trial was still continued for 7 days (81±15 sec). This result was confirmed by another experiment in younger rats (47 day old). No significant changes in affinity (Kd; nM) or density (Bmax; fmol/mg prot.) of NMDA receptors were observed in the different strains in animals of different ages (see table). The significance of the differences of the binding parameters and of the coefficient of variation ($p<0.05$) were assessed by using the "Ligand Program" of Munson P.J. and Rodbard D., 1984.

	1 month			4 months			6 months		
	WY	WKY	SHR	WY	WKY	SHR	WY	WKY	SHR
Kd	2.15	2.00	2.39	2.56	2.66	2.56	3.06	3.05	3.12
% CV	9	15	9	9	9	10	9	12	19
Bmax	2983	2528	2739	2626	2819	2876	3279	3024	3167
% CV	5	7	5	5	5	6	5	7	11

CV= coefficient of variation.

In addition, Kd and Bmax values were not changed, when determined in WKY and SHR rats that underwent the Morris task (WKY Kd 2.65 nM; Bmax 2309 fmol/mg prot.; SHR Kd 2.91; Bmax 2830).
In conclusion, these results indicate that Wistar and SHR differ from WKY in the acquisition of a learning task. These variations are not associated to a different affinity or density of NMDA receptors.

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72P DEXAMETHASONE INDUCES THE TRANSLOCATION OF LIPOCORTIN-1 TO THE MEMBRANE OF HUMAN MONOCYTIC U-937 CELLS

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Lipocortin-1 (LC1) is a member of a family of proteins (annexins) with calcium and phospholipid binding properties which is a putative mediator of the anti-inflammatory action of glucocorticoids (Flower, 1988). Altough LC1 is an intracellular protein lacking hydrophobic signal sequences, it has also been observed extracellularly (Christmas *et al.*, 1991). We have previously shown that in U-937 cells, differentiated into macrophage-like cells by incubation with phorbol 12-myristate 13-acetate (PMA), dexamethasone (DEX) induces increased expression of LC1 mRNA as well as the release of the protein (Solito *et al.*, 1991). To further investigate the effect of glucocorticoids on LC1 synthesis and/or release, we have studied by Flow Cytometric Analysis (FACS) the cytosolic and membrane positivity to a LC1 polyclonal antibody (no. 842, Pepinsky *et al.*, 1990) of U-937 cells incubated with PMA and DEX.

U-937 cells incubated for 24h with 10 nM PMA acquired macrophage-like features like high membrane positivity for an anti-CD14 antibody and decreased proliferating capability. The differentiated cells also showed an increased intracellular content of LC1 (60.3 ± 0.4 Fluorescence Units, FU, n=6) compared to not-differentiated cells (2.4 ± 0.03 FU, n=6) whereas the membrane expression of LC1 was very similar (36.4 ± 2.4 and 38.0 ± 3.1 FU for differentiated and not-differentiated cells respectively, n=6). DEX (1 µM) caused a decrease of the LC1 cytosolic content in PMA-treated cells (6.9 ± 0.1 FU after 2 h, n=6). Conversely LC1 membrane positivity was time-dependently increased by DEX (1 µM) with a peak at 4 h in both PMA-treated and control cells with an highly significant difference between the two cell populations (290.6 ± 6.5 FU for PMA-treated cells and 184.0 ± 4.2 for control cells, n=6, $P < 0.001$).

These results indicate that DEX induces the translocation of LC1 to the membranes of U-937 cells and that differentiated cells respond to the glucocorticoid with a significantly higher LC1 membrane expression compared to not-differentiated cells. It is likely that the membrane translocation precedes the release of the protein induced by DEX (Solito *et al.*, 1991). Since extracellular LC1 possesses anti-inflammatory effects (Cirino *et al.*, 1989) the regulation of the protein translocation/release process by glucocorticoids may be part of their mechanism of action.

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73P METABOLISM OF ARACHIDONATE CATALYSED BY HAEMOGLOBIN

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In a previous study we have shown that some peroxidases (POD) and haemoglobin (Hb),aerobically incubated with 5,8,11,14-eicosatetraenoic acid (arachidonic acid,AA),give rise to the formation of compounds with prostaglandin-like activities.Among these,PGE_2 and $PGF_{2\alpha}$ have been identified by TLC and Gas Chromatography-Mass Spectrometry (Zilletti et al.,1989).Recently we have characterised the cyclooxygenase activity of haemoglobin from human (HbAo)(Zilletti et al.,1993) and other species (rat,pigeon,snake).In standard conditions (air as gas phase,25°C in shaking water bath,50 mM sodium carbonate buffer pH 9.5,total volume 4 ml,20 min) 0.1 μM Hb incubated with different concentrations (10-300 μM) of AA,promoted the formation of PGE_2- and $PGF_{2\alpha}$-like substances.The kinetic parameters determined by non-linear regression analysis of the initial rate data are :

	Human	Rat	Pigeon	Snake
Km (μM)	36.8	31.2	29.8	31.7
Vmax (ng PGE_2-like $s.min^{-1}$ $nmol\ Hb^{-1}$	25	28.9	28	31.2

The amount of PG-like substances formed was found in the range of 180 to 304 ng/sample for PGE_2-like substances and in the range of 80-120 ng/sample for $PGF_{2\alpha}$-like substances.This cyclooxygenase activity was inhibited by phenylhydrazine (IC_{50}= 0.17 μM) but not by indomethacin.Furthermore a significant inhibitory effect was shown by 1 μM d-penicillamine,deferoxamine and 21-aminosteroid U 74500A.PGE_2 and $PGF_{2\alpha}$ have been identified by direct analysis on Fast Atom Bombardment in the negative ion mode and by Gas Chromatography-Mass Spectrometry after derivatization with trimethylsilylimidazole,following TLC.These data provide a possible explanation for the presence of high levels of $PGF_{2\alpha}$ and other PGs in human cerebrospinal fluid following subarachnoid haemorragies (Macdonald & Weir,1991).Furthermore they suggest a possible role for Hb in pathological conditions in which intra- and extra-vascular erytrocyte lysis occurs.

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74P EFFECTS OF SELECTIVE PHOSPHODIESTERASE INHIBITORS ON HUMAN ALVEOLAR MACROPHAGE CYCLIC NUCLEOTIDE HYDROLYSIS AND RESPIRATORY BURST

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We have previously reported that theophylline inhibits human alveolar macrophage (AM) respiratory burst through the inhibition of cyclic nucleotide phosphodiesterase (PDE) and the consequent elevation of intracellular cyclic AMP levels (Dent *et al.*, 1992). Since PDE activity is expressed by at least five 'families' of isoenzymes, the present study was undertaken in order to determine the isoenzyme profile of the AM through the use of selective inhibitors and to evaluate the effects of these inhibitors upon the AM respiratory burst.

AM were obtained at routine bronchoscopy by bronchoalveolar lavage of the middle lobe or lingula with 5 x 20 ml sterile isotonic saline. No patients were receiving oral or inhaled glucocorticosteroids prior to bronchoscopy. Cells were purified by differential centrifugation over discontinuous Percoll density gradients and fractions of > 90% purity (92.9 ± 0.8% (mean ± s.e.m.); n = 26) and > 85% viability (89.6 ± 1.4%) were used immediately for respiratory burst measurements or stored at -80°C for later PDE analysis. Opsonised zymosan (OZ; 1 mg 10^6 $cells^{-1}$ ml^{-1})-stimulated hydrogen peroxide (H_2O_2) generation and cyclic AMP/cyclic GMP PDE activity (substrate concentration = 1 μM) were measured as described (Dent *et al.*, 1991).

The mixed PDE III/IV inhibitor zardaverine, the selective PDE III inhibitor motapizone and the PDE IV-selective inhibitors denbufylline and rolipram each inhibited cyclic AMP hydrolysis by AM homogenates, while the PDE V/I inhibitor zaprinast inhibited cyclic GMP hydrolysis. Zardaverine gave a monophasic concentration-inhibition curve with an IC_{50} of 1.44 ± 0.29 μM and a maximum inhibition of 89.5 ± 5.3% (n = 3). Motapizone gave a shallow (n_H = 0.56 ± 0.17) inhibition curve with an IC_{50} of 0.024 ± 0.011 μM and maximum inhibition of 54.2 ± 4.9% (n = 3); these data could be fitted to a two-site inhibition with components of IC_{50} = 0.014 ± 0.004 μM (max. 46.4 ± 5.4% inhibition) and IC_{50} ≈ 80 μM. Denbufylline and rolipram gave IC_{50} values of 1.24 ± 0.67 μM (max. 36.5 ± 1.3% inhibition) and 0.22 ± 0.17 μM (max. 35.7 ± 2.8% inhibition), respectively (n = 3); the rolipram curve was shallow (n_H = 0.42 ± 0.09) and could be fitted to a two-site model with IC_{50} values of 0.004 ± 0.003 μM (max. 17.9 ± 3.1% inhibition) and approximately 25 μM. Zaprinast exhibited an IC_{50} of 5.47 ± 3.37 μM and maximal inhibition of 53.7 ± 11.7% (n = 3); this curve was also shallow (n_H = 0.45 ± 0.12) and a two-site fit could be made, giving IC_{50} values of 0.22 ± 0.21 μM (max. 18.9 ± 2.92% inhibition) and approximately 30 μM. Zardaverine inhibited OZ-stimulated H_2O_2 generation significantly ($p < 0.005$) at concentrations of 10 μM and 100 μM (50.8 ± 6.3% inhibition at 100 μM; n = 5) while rolipram also inhibited ($p < 0.05$) at these concentrations (22.6 ± 5.5% inhibition at 100 μM; n = 5). Motapizone and zaprinast were ineffective at concentrations up to 10 μM but inhibited significantly ($p < 0.05$) at 100 μM (47.6 ± 11.9% and 26.3 ± 10.3%, respectively; n = 4).

We conclude that human AM contain the type-III and type-IV isoenzymes of PDE, with an additional activity representing PDE V and/or PDE I. The inhibition of AM respiratory burst appears to depend more upon the inhibition of PDE IV than of PDE III.

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75P EFFECT OF 5-LIPOXYGENASE INHIBITION ON ANTIGEN-INDUCED AIRWAY HYPERRESPONSIVENESS AND CELL INFILTRATION IN NEONATALLY IMMUNIZED RABBITS

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NZW rabbits were neonatally immunized with the allergen *Alternaria tenuis* in $Al(OH)_3$ gel as previously described (Herd *et al.*, 1992). Airway responsiveness to inhaled histamine was assessed in adult rabbits 24 h prior to and 24 h following allergen challenge (2 min aerosol of 0.9% saline followed by 5 consecutive aerosols of 2 min duration of *Alternaria tenuis* (10,000 PNU ml^{-1})). The concentration of histamine required to elicit a 50% increase in airway resistance (R_L PC_{50}) and a 35% fall in dynamic compliance (C_{dyn} PC_{35}) was calculated. PF 5901 (2-[3(1-hydroxyhexyl) phenoxymethyl] quinoline hydrochloride), a 5-lipoxygenase activating protein (FLAP) inhibitor and LTD_4 antagonist (10 mg) (n=10), or vehicle (n=10), was instilled directly into the lungs 1 h prior to the allergen challenge. Prior to, and 1 h after the drug administration, blood was sampled from the ear vein into heparin, stimulated with A23187, and the resultant plasma assayed for LTB_4 production by radioimmunoassay. Bronchoalveolar lavages (BAL) were performed immediately following histamine and antigen challenges (ie. Pre, 1 h and 25 h post antigen) from which cells were enumerated and LTB_4 levels determined.
Antigen-induced airway hyperresponsiveness (AHR) to inhaled histamine 24 h following antigen challenge was significantly inhibited in animals pretreated with PF 5901.

	PC_{50} (mg ml^{-1}) Pre	24 h	PC_{35} (mg ml^{-1}) Pre	24 h		PC_{50} (mg ml^{-1}) Pre	24 h	PC_{35} (mg ml^{-1}) Pre	24 h
Vehicle:	23.7±1.5	9.3±1.3*	21.4±1.3	4.3±1.1*	PF 5901:	20.2±1.5	17.0±1.4	17.7±1.4	12.3±1.3

values represent geometric mean ± SEM. * $P < 0.05$ compared with PRE value

A significant increase in the total number of neutrophils and eosinophils recovered in BAL fluid was observed 1 h and 25 h following allergen exposure in both the vehicle- and PF 5901-treated groups ($P < 0.05$). In animals pretreated with PF 5901, there was a reduction in the numbers of neutrophils and eosinophils 25 h post antigen compared to the vehicle control, however, this was not stastistically significant ($P > 0.05$).
PF 5901 had no effect on *ex vivo* LTB_4 synthesis in whole blood ($P > 0.05$). In contrast, the antigen-induced increase in LTB_4 levels in BAL 25 h following challenge was significantly inhibited ($P < 0.05$).

From these results we suggest that PF 5901 possesses topical activity as an anti-allergic agent and provides further evidence for a role for 5-lipoxygenase metabolites of arachidonic acid in allergen-induced airway hyperresponsiveness.

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76P EFFECTS OF NABUMETONE AND ITS ACTIVE METABOLITE, 6-METHOXY-2-NAPHTHYLACETIC ACID (6-MNA), ON THROMBOXANE BIOSYNTHESIS *IN VIVO* AND *IN VITRO*

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Nabumetone is a nonacidic nonsteroidal antiinflammatory drug with an unusual pharmacological profile (Blower, 1992). After absorption, nabumetone is converted in the liver to the active metabolite, 6-MNA. In cos-1 cells transfected with the plasmid expression constructs for murine prostaglandin endoperoxide (PGH) synthase-1 and PGH synthase-2, 6-MNA was about a 7-fold better inhibitor of the latter than the former enzyme (Meade et al., 1993). The aims of the present study were: 1) to examine the effects of 6-MNA on the cyclooxygenase activity of human platelet PGH synthase-1 in vitro; 2) to evaluate the effects of a short-term treatment with nabumetone on in vivo thromboxane (TX) A2 biosynthesis in healthy subjects.

For this purpose, we studied 6 healthy volunteers (4M,2F; 34±9yr) who gave informed consent to participating in the study. Multiple 1-ml aliquots of peripheral venous whole blood were allowed to clot at 37°C for 1h in the presence of varying concentrations of 6-MNA. Eight-hour urine samples were obtained on 4 successive days prior to and after the 1st, 3rd, 5th and 7th administration of nabumetone (1 g daily). Peripheral venous blood samples were obtained before and 8h after the first and last drug administration. Whole blood TXB2 production was measured in vitro, as a reflection of the cyclooxygenase activity of platelet PGH synthase-1 (Patrono et al., 1980). The urinary excretion of 11-dehydro-TXB2, a major enzymatic metabolite of TXB2, was measured as a reflection of the in vivo biosynthesis of TXA2 (Ciabattoni et al., 1989).

6-MNA reduced dose-dependently whole blood TXB2 production in vitro, with an IC50 of 49±18 μg/ml (Mean±SD). However, even at very high concentrations (500 μg/ml), 6-MNA only partially inhibited (by 80±4%) the cyclooxygenase activity of platelet PGH synthase-1. Before nabumetone administration, serum TXB2 levels averaged 338±93 and 359±69 ng/ml, on two separate occasions. Oral dosing with this drug was associated with 48±11% reduction after the first dose and 54±17% reduction after the 7th dose ($p<0.01$ vs control). The urinary excretion of 11-dehydro-TXB2 averaged 15.6±6.2, 17.6±3.6, 23.4±9.7 and 22.6±11.1 ng/h on 4 separate occasions (intrasubject coefficient of variation: 27±14%). Metabolite excretion averaged 15.2±3.8 ng/h (NS), 10±3.6 ($p<0.02$), 9.9±7.6 ($p<0.05$) and 7.1±3.9 ($p<0.01$) after the 1st, 3rd, 5th and 7th dose, respectively.

In the light of the non-linear relationship between inhibition of platelet biosynthetic capacity and TXA2 synthesis in vivo (Reilly & FitzGerald,1987) and of the time-related dissociation of the effects of nabumetone on serum TXB2 and urinary 11-dehydro-TXB2, we conclude that: a)6-MNA is a weak inhibitor of platelet PGH synthase-1; b)the reduction in TXA2 biosynthesis in vivo associated with nabumetone administration is likely to reflect the effect on extra-platelet sources of TXA2 possibly expressing PGH synthase-2.

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77P ANAPHYLAXIS IN HUMAN PLATELETS

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Recently we have shown that human platelets are capable of synthesising histamine (18.6 ± 5 pmol/h/g protein, n=8) and of taking up the preformed amine with an energy dependent process (Mannaioni et al., 1992a). 50% of endogenous histamine is released by thrombin (60 mU) during aggregation "in vitro" and by the specific antigen from platelets isolated from sensitised guinea-pigs. The pro-aggregatory effect of exogenous histamine is mediated by the increase in both cytosolic calcium and by the uptake of extracellular calcium (Mannaioni et al., 1992b). This background prompted us to evaluate immunological release of histamine in isolated human platelets.
Purified suspensions of platelets, prepared from fresh blood samples from healthy volunteers, were preincubated for 3 hours at 37° C in a metabolic shaker with different concentrations of IgE protein obtained from a myeloma patient (100 μg protein corresponding to $5x10^4$ U per 10^9 platelets). Platelet aggregation was monitored and thrombin (10-60 mU, n=6) or anti-human IgE (0.5-1 $\mu g.ml^{-1}$, n=6) were used as aggregatory agents. To measure the release of histamine, the appropriate stimulus was added to duplicate aliquots of platelet suspensions and the reaction was stopped by chilling the tubes in an ice-water bath. Histamine was measured fluorimetrically in platelets and supernatants.
Unstimulated human platelets contain histamine in picomolar range (57.15 ± 8.85 pmoles/10^9 platelets, n = 9). Their exposure to different concentrations of thrombin produces a progressive aggregation, which runs in parallel with the release of histamine. Incubation with increasing concentrations of anti-human IgE antibody was ineffective on both aggregation and histamine release. The addition of 0.5-1 µg/ml anti-human IgE antibodies to platelets which have been preloaded with 10-50 or 100 μg of IgE myeloma protein produces a significant concentration-dependent increase both in aggregation and histamine release (n = 8, $p<0.001$).
The electron microscopical features of platelet aggregation induced by thrombin (60 mU) was similar to that induced by the immunological stimulus.
Thrombin (60 mU)-induced platelet aggregation and histamine release are enhanced of 34.27 %± 4.3 (n=8, $p<0.001$) in platelets preloaded with IgE. The release of histamine from platelets is an active process blocked by cyanide (10^{-7} M, n=6, $p<0.001$), dinitrophenol (DNP, 10^{-6} M, n=6, $p<0.001$) and hypoxia (n=4, $p<0.001$).
N,N-diethyl-2-[4-(phenylmethyl)phenoxy] ethanamide (DPPE, 10^{-4} M, n=5), a blocker of intracellular histamine receptors and loratadine (10^{-4} M, n=5), a non sedative anti-histaminic which possess anti-aggregatory effects, significantly block ($p<0.001$) the release of histamine.
These results more firmly establish platelets among the inflammatory cells and also envisage a possible link between allergic inflammation and thrombosis.

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78P ALTERED PROSTACYCLIN SYNTHESIS BY AORTAE FROM RATS WITH HEPATIC PORTAL HYPERTENSION: EVIDENCE FOR EFFECTS ON PROTEIN KINASE C AND CALCIUM

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Portal hypertension is characterised by a decrease in systemic vascular reactivity. Since the mechanisms underlying these alterations are unknown, the effect of ligation of the portal vein of the rat (7 days duration; a model of portal hypertension) on adrenoceptor - and signal transduction - linked PGI_2 (a vasodilator) synthesis (measured as 6-oxo-$PGF_{1\alpha}$ by radioimmunoassay) was studied in the aorta (ex vivo). Rats were killed and aortae excised and processed for in vitro assessment of PGI_2 release as previously described (Jeremy et al., 1993). PGI_2 synthesis was stimulated by adrenaline (via heterogenous alpha-adrenoceptors), phorbol ester dibutyrate (PDBU; a protein kinase C activator [PKC]), Ca^{2+} ionophore A23187 (A23187) and thapsigargin (both elevate intracellular Ca^{2+} which elicits the release of arachidonic acid [AA]; substrate for PGI_2 synthesis) and AA. Maximal PGI_2 release by the aortae from rats with portal hypertension compared to sham-operated controls was: 1) unchanged in response to adrenaline although there was a marked left shift in the dose response curve, 2) reduced in response to PDBU, A23187 and thapsigargin but with no change in the EC_{50}s of the dose reponse curves and 3) unchanged in response to AA.

table: A) EC_{50}s and B) maximal outputs of PGI_2 (pg 6-oxo-$PGF_{1\alpha}$ / mg tissue / min ± SEM, n = 10) of different stimulators on aortic PGI_2 synthesis in HPH and sham-operated rats. * $p < 0.001$

		adrenaline	PDBU	thapsigargin	A23187	arachidonate
A)	CONTROL	0.7×10^{-6}	5×10^{-8}	4×10^{-6}	1.5×10^{-6}	1.7×10^{-6}
	HPH	5.8×10^{-7}	6×10^{-8}	4×10^{-6}	1.3×10^{-6}	1.7×10^{-6}
B)	CONTROL	127 ± 12	128 ± 14	85 ± 7	130 ± 15	120 ± 14
	HPH	125 ± 13	82 ± 8*	68 ± 6*	95 ± 10*	120 ± 13

These data indicate that in aortae from rats with experimental portal hypertension: i) there are no changes in the enzymes involved in PGI_2 synthesis (cyclooxygenase, PGI_2 synthase), ii) there is a specific increase in adrenoceptor - linked PGI_2 synthesis in aortae which may contribute to the known tendency toward vaso-dilation in this experimental model and 3) the diminished response of PGI_2 synthesis to A23187, PDBU and thapsigargin indicates that there is a generalised attenuation of PKC activity and of activator Ca^{2+}. Since PKC and Ca^{2+} are key components of excitation-contraction coupling, attenuation of these systems may also explain, at least in part, the known reduced vasoactivity of aortae from rats with portal hypertension.

Jeremy JY, Thompson CS, Mikhailidis DP. Br J Pharmacol. 1993; 108: 1131-1136.

79P FURTHER INVESTIGATION OF PROSTANOID EP RECEPTORS IN RABBIT JUGULAR AND PIG SAPHENOUS VEINS

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Prostanoid EP receptors are currently classified into four different subtypes; EP_1, EP_2, EP_3 and EP_4 (Louttit *et al.*, 1992). The EP_4 receptor is the most recent of these to be described and at present very little is known about this subtype. Our aim is to compare the EP receptors of the rabbit jugular vein (RJV), reported to be atypical of EP_2 receptors (Lawrence & Jones, 1992), with those of the pig saphenous vein (PSV) using agonist potencies for PGE_2, 16,16-dimethyl PGE_2, 11-deoxy PGE_1, AH13205, and butaprost, as well as AH 23848B, a putative EP_4 receptor antagonist. Male New Zealand White rabbits were anaesthetised with Sagatal® (30mg/kg), and the external jugulars excised. Segments (2-4 mm), suspended at a tension of about 1g in oxygenated, overflow organ baths, were washed with Krebs containing indomethacin 1.0 μM. After equilibration for one hour, the preparations were made to contract with a sub-maximal dose of histamine in the presence of the TP receptor antagonist GR 32191B (10 μM). Drugs were added cumulatively and responses measured using the Maclab® data acquisition system. PSV, obtained from freshly culled Chinese Meishan animals, were treated as per the RJV but phenylephrine was used as the contractile agent. Contraction of both tissues is susceptible to fade and this was minimised using indomethacin with the RJV and adenosine deaminase (1U/ml) with the PSV. All results were calculated as percentages of the maximal relaxation achieved with PGE_2, and are given in Table 1.

Table 1 Effect of AH 23848B (30μM, 30 min) and N^G-monomethyl-L-arginine (L-NMMA, 100μM, 15 min) on relaxation induced by EP analogues

RJV	PGE_2	11-deoxy PGE_1	16,16-diMe PGE_2	Butaprost	AH13205
pIC50	8.50±0.10	7.74±0.37	7.32±0.28	6.64±0.24	4.92±0.26
pIC50(AH 23848B)	8.63±0.30	7.79±0.15		6.56±0.28	
pIC50(L-NMMA)	8.58±0.16		7.43±0.38		
PSV					
pIC50	9.01±0.18	8.70±0.23	8.56±0.22	7.38±0.19	5.19±0.12

Since block of nitric oxide production by L-NMMA, or removal of the endothelial tissue (IC_{50}=8.6 & 8.66), did not significantly affect the relaxation mediated by PGE_2, it suggests that this is independent of the endothelium in the RJV. Our results confirm that butaprost is a weak agonist on the RJV, and show that the EP_2 selective agonist AH 13205 is exceptionally poor (EMR = 3330). No antagonism was observed in the RJV with the EP_4 antagonist AH 23848B, and block on the PSV was weak (pIC_{50} of 9.39±0.28 for PGE_2, and 8.56±0.26 with AH 23,848 (pA_2=5.27)). In terms of agonist potency the two preparations are similar; 11-deoxy PGE_1 and 16,16 dimethyl PGE_2 are potent agonists, and AH 13205 a weak agonist. However butaprost is considerably more active on the PSV than RJV. Clearly, more potent and selective compounds are required to help discriminate between EP_2 and EP_4 receptors.

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80P ROLE OF ADHESION GLYCOPROTEINS CD 18 AND ICAM-1 IN INFLAMMATORY REACTIONS OF RABBIT SKIN

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Adherence of leukocytes to vascular endothelium is a fundamental event in the host response to inflammatory stimuli (Springer, 1990). We investigated the role of adhesion glycoproteins CD18 and ICAM-1 in oedema formation and red blood cell (RBC) accumulation caused by different, complement-mediated, inflammatory reactions of rabbit skin; namely, the reversed passive Arthus (RPA) reaction and responses to zymosan particles. Responses to zymosan-activated plasma (ZAP, a source of preformed C5a des Arg), platelet-activating factor (PAF) and bradykinin (BK) were also studied. Oedema formation and RBC accumulation were quantified using ^{125}I-albumin and ^{111}In-labelled RBC respectively. Rabbits were given *i.v.* injections of 2 mg/kg monoclonal antibody (mAb) R15.7 (anti-CD18), mAb RR1/1 (anti-ICAM-1) or a non reactive mouse IgG as a control. Arthus (anti-bovine-gamma-globulin) antiserum (25, 50 & 100%), zymosan (30, 100 & 300 μg/site), ZAP (100%), PAF (10^{-9} mol/site)± PGE_2 ($3x10^{-10}$ mol/site) and BK (10^{-10} mol/site) ± PGE_2 ($3x10^{-10}$ mol/site) were injected *i.d.* Arthus (bovine-gamma-globulin, 5mg kg^{-1}) antigen, was given *i.v.* 5 min after *i.d.* injections. Animals were killed after 4 h. Accumulation ^{125}I-albumin and ^{111}In-RBC was quantified and expressed as μl plasma/skin site and μl blood/skin site respectively. RPA reactions and responses to zymosan and ZAP were associated with marked accumulation of albumin and RBC while PAF and BK co-injected with PGE_2 caused accumulation of albumin but not RBC (Table 1).

Table 1. Oedema formation and RBC accumulation in inflammatory reactions of rabbit skin.

	μl plasma/skin site (mean ± s.e. mean)			μl blood/skin site (mean ± s.e. mean)		
Treatment	Control	+R15.7	+RR1/1	Control	+R15.7	+RR1/1
RPA reaction	82.3±10.6 (5)	16.5±2.0 (5)**	46.6±3.7 (5)**	31.5±1.0 (5)	5.8±1.0 (5)**	16.8±6.4 (5)*
300μg/site zymosan	99.7±12.5 (5)	25.7±4.7 (5)**	57.0±9.1 (5)*	34.3±3.3 (5)	6.6±1.8 (5)**	15.6±4.3 (5)**
ZAP	49.0±12.9 (6)	14.5±2.0 (5)*	34.9±6.7 (5)	23.8±4.0 (6)	6.4±1.7 (5)*	12.3±3.3 (5)*
PAF+PGE_2	71.4±17.8 (4)	56.4±15.2 (4)	63.3±11.3 (4)	13.5±2.4 (5)	5.6±1.1 (3)	6.1±1.4 (3)
BK+PGE_2	101.7±30.3 (4)	97.4±24.4 (4)	89.3±21.1 (4)	12.0±1.9 (5)	4.4±2.2 (3)	5.9±2.8 (3)
Saline	15.2±2.4 (6)	13.0±2.7 (5)	12.9±2.7 (5)	9.0±2.2 (6)	3.8±0.8 (5)	4.5±1.0 (5)

Significant difference from the appropriate control is denoted by *p<0.05, **p<0.01.

The mAb R15.7 effectively abolished oedema formation and RBC accumulation in the RPA reaction and in response to zymosan and ZAP. These responses were also inhibited by the anti-ICAM-1 mAb RR1/1. Oedema formation in response to the neutrophil independent mediators PAF and BK in the presence of PGE_2 were not affected by either antibody. These results show that oedema formation and RBC accumulation in complement-mediated reactions of rabbit skin are completely CD18 dependent, and are, at least in part, ICAM-1 dependent.

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81P ROLE OF CD11/CD18 IN MEDIATING CUTANEOUS INFLAMMATION IN THE GUINEA PIG

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Neutrophils express adhesion molecules, including the integrin CD11/CD18 which is essential for the migration of these cells into inflammatory sites (Williams & Hellewell, 1992). However, much less is known about the role of this integrin in the accumulation of eosinophils *in vivo*. In this study, we have investigated the role of CD11/CD18 in mediating the accumulation of eosinophils and neutrophils, and local oedema formation in cutaneous inflammation in guinea pig skin.

Horse serum-elicited eosinophils (> 95% pure) and casein-elicited neutrophils (> 99% pure) were radiolabelled with ^{111}In (Teixeira *et al*, 1993). The ^{111}In-leukocytes (2.5 x 10^6cells per animal), together with ^{125}I-human serum albumin were given i.v., followed by the i.d. inflammatory stimuli. Two hours later, the animals were killed and cell accumulation (^{111}In-leukocyte per site) and oedema formation (μl of plasma) were assessed. We used both the whole (6.5Ewho) and the F(ab')$_2$ fragment (6.5EFab) of the anti-CD18 monoclonal antibody 6.5E which cross-reacts with guinea pig CD11/CD18.

The i.v. injection of 6.5Ewho (0.12 mg/Kg), but not 6.5EFab (2.4 mg/Kg), resulted in an almost complete loss of circulating ^{111}In-eosinophils at 2 hours (control, 7.4 ± 1.8%; 6.5Ewho, 0.5 ± 0.1%; 6.5EFab, 5.5 ± 1.4%; n= 6 pairs). The percentage of circulating ^{111}In-neutrophils at 2 hours was not altered by administration of 6.5EFab (control, 2.0 ± 0.5%; 6.5EFab, 2.4 mg/Kg, 2.1 ± 0.5%, n= 5 pairs). There was no change in the distribution of radiolabelled leukocytes as assessed by the total counts in the spleen, lung and liver. Both the cutaneous accumulation of ^{111}In-neutrophils and ^{111}In-eosinophils induced by zymosan-activated plasma (ZAP) and in a passive cutaneous anaphylaxis (PCA) reaction were markedly suppressed by 6.5EFab (2.4 mg/Kg, n= 5-6 pairs). For example, ^{111}In-neutrophil accumulation induced by 10%, 30% and 90% ZAP was inhibited by 89%, 82% and 80%, respectively by 6.5EFab. ZAP-induced ^{111}In-eosinophil accumulation was also suppressed by up to 78%. However, only the oedema response induced by the top dose of ZAP (90%) was inhibited by 6.5EFab. Oedema formation induced by bradykinin, histamine and in the PCA reaction were not significantly affected.

The importance of VLA-4 in the accumulation of ^{111}In-eosinophils in the skin of guinea pigs has been recently reported (Weg *et al*, 1993). Here, we have shown that a Fab' fragment of an anti-CD11/CD18 antibody potently inhibited the accumulation of both ^{111}In-neutrophils and ^{111}In-eosinophils in the skin of the same species, suggesting that CD11/CD18 also plays an important role in eosinophil accumulation. Oedema formation in guinea pig skin induced by ZAP was mostly neutrophil-independent in contrast to ZAP-induced oedema responses in rabbit skin which are totally neutrophil-dependent (Wedmore & Williams, 1981).

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82P SUPPRESSION OF EOSINOPHIL ACCUMULATION IN CUTANEOUS INFLAMMATION IN GUINEA PIG BY A TYPE IV PHOSPHODIESTERASE INHIBITOR

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Phosphodiesterases (PDE) are a group of enzymes responsible for the metabolism of cyclic nucleotides. There are five major groups of PDE which are distributed throughout different tissues and cells (Torphy & Undem, 1991). The major PDE activity of guinea pig eosinophils has been identified as belonging to the cAMP-specific (type IV) family (Dent *et al*, 1991). In this study, we have investigated the effects of local and systemic administration of rolipram, a type IV PDE inhibitor, on the accumulation of ^{111}In-eosinophils and oedema formation in guinea pig skin.

The accumulation of ^{111}In-eosinophils and ^{125}I-albumin leakage in response to i.d. inflammatory agents or in a passive cutaneous anaphylaxis (PCA) reaction were measured simultaneously (Texeira *et al*, 1993). Rolipram was co-injected with the mediators or antigen (local treatment) or given systemically (5 mg/Kg i.p and 0.5 mg/Kg i.v., 30 min and 10 min prior to injection of the ^{111}In-eosinophils, respectively). Oedema formation (μl of plasma) and ^{111}In-eosinophil accumulation were assessed two hours after the i.d. stimuli. Local injection of rolipram (4 x 10^{-8} to 4 x 10^{-10}moles per site) had no effect on the ^{111}In-eosinophil accumulation or oedema formation in a PCA reaction (1 μg of BGG per site) but significantly inhibited ^{111}In-eosinophil accumulation induced by PAF (10^{-9} moles per site) at the dose of 4 x 10^{-8}moles per site. In contrast to its weak effects when given locally, systemic rolipram potently inhibited the ^{111}In-eosinophil accumulation induced by PAF (10^{-10} to 10^{-9}moles per site), ZAP (10%, 30% and 100% in saline) and in a PCA reaction (0.01 to 1.0 μg per site) (Table 1). However, oedema responses in the same sites were not altered.

	PCA (μg)			ZAP (% in saline)			PAF (moles)	Saline
	0.01	0.1	1.0	10	30	100	10^{-9}	
Control	683 ± 126	1745 ± 391	2902 ± 442	2356 ± 330	6372 ± 898	16308 ± 3123	2997 ± 919	277 ± 52
Rolipram	244 ± 11*	219 ± 22*	406 ± 113**	410 ± 59**	1453 ± 263**	5971 ± 1055*	890 ± 353*	173 ± 14

Results are mean ± s.e. mean ^{111}In-eosinophils per site for n= 5 pairs. * P< 0.05, ** P< 0.01.

In summary, systemic administration of rolipram effectively suppressed the accumulation of ^{111}In-eosinophil into sites of inflammation in guinea pig skin while it did not have any effect on oedema formation. Rolipram has also been shown to inhibit eosinophil function *in vitro* (Dent *et al*, 1991). These inhibitory actions of rolipram may be relevant to the control of disease states in which accumulation of activated eosinophils are important.

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83P INDUCTION OF NITRIC OXIDE SYNTHASE DURING INFLAMMATION: STUDIES IN THE CHRONIC GRANULOMATOUS TISSUE OF THE MURINE CROTON OIL-INDUCED AIR POUCH

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Nitric oxide (NO) is formed by both constitutive (calcium-dependent) and inducible (calcium-independent) isoform of NO synthase (NOS). The deleterious effects of large amounts of NO produced by the inducible isoform of NOS in septic shock has been the basis for the development of isoform-selective inhibitors of this enzyme. The induction of NOS in pathophysiological states other than septic shock, such as atherosclerosis (Lang et al., 1993) and haemorrhagic shock (Thiemermann et al., 1993) has been the subject of recent studies. Here we have investigated the possibility that NOS is induced during the inflammatory processes associated with the murine croton oil-induced air pouch model (Kimura et al., 1991). Female Tuck original mice (30±2g) were injected with 3 ml of air into the dorsal subcutaneous tissue, followed on day 1 by injection of 0.5 ml of 0.1% v/v croton in Freund's complete adjuvant. For the acute phase of inflammation the skin of the pouch was removed before the development of granulomatous tissue at 0, 6, 12 and 24 hours. For the chronic phase of inflammation air pouch sites were removed at 3,5,7 and 21 days after croton oil injection. The chronic air pouch was dissected into two regions the top site, containing skin and granulomatous tissue and the bottom site consisting of granulomatous tissue. Inflamed sites were assayed for NOS activity by the ability of tissue homogenates to convert ^{3}H-L-arginine to ^{3}H-L-citrulline in the presence of co-factors and NOS activity was expressed as pmol of citrulline/mg protein (Mitchell et al., 1991).

Table 1: NOS activity (pmol Cit/mg protein) in the skin and granulomatous tissue from the croton oil murine air pouch.

time	0	6 hour	12 hour	24 hour	3 day	5 day	7 day	21 day
total NOS	52±13	65±15	78±4	86±14	top; 120±13	top; 281±64	top; 113±72	top; 45±9
Ca-independent	14±4	7.9±1	23.9±8	35.4±3	top; 118±22	top; 248±98	top; 94±26	top; 52±11
total NOS					bot; 409±44	bot; 249±44	bot; 539±75	bot; 324±23
Ca-independent					bot; 406±48	bot; 258±45	bot; 379±65	bot; 312±20

The table shows mean NOS±s.e.mean from 4-8 determinations from 3-4 seperate animals.

Control skin contained mainly calcium-dependent NOS activity, however, 12 and 24 hours after croton oil injection significant levels of calcium-independent NOS was present (table 1). The top and bottom (bot) regions of the inflammed site contained NOS activity which was mainly independent of calcium (table 1) and comparable in magnitude to that found in the spleen and lung of rats treated with endotoxin (5 mg/kg; 6 hours; Mitchell et al., 1993). These results show that the inducible isoform of NOS is expressed in the acute (0-24h), chronic (3-7 days) and healing (21 days) stages of inflammation. We speculate that inhibition of the inducible isoform of NOS with selective inhibitors is a novel approach for the therapy of inflammation.

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84P HISTAMINE LIPOLYTIC ACTIVITY IN WHITE ADIPOSE TISSUE (WAT)

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In addition to the well studied functions of histamine in inflammation (Larsen and Henson, 1983), on cardiovascular and immunological systems (Altura and Halevy, 1978; Beaven, 1978), a putative activity of the autacoid as neuromodulator has also been proposed (Prell and Green, 1986). All histamine activities seem modulated by the stimulation of specific receptors located on the cell membrane. At least three types of histamine receptors have been described so far (Hill, 1990). Although the features of H_1 and H_2 receptors have been studied in several tissues, some authors have recently found discrepancies in their characteristics (Hill, 1990). For this reason, in the case of histamine receptors, it is possible to hypothesize the existence of multiple forms of the proteins which regulate the transduction of the signal elicited by the amine.

Several observations have been produced to demonstrate the activity of histamine in controlling metabolic pathways, via the stimulation of H_2 and H_1 receptors (Garcia-Sainz et al., 1987). We recently reported on histamine activity at adipocyte levels (Raimondi et al, 1992). Histamine activity in WAT seems regulated by two cellular receptors which are distinguishable by the use of more selective agonists and antagonists. Both regulate lipolytic activity of the autacoid. We are now interested in the pharmacological characterization of the receptor which is responsible for the positive stimulation of lipolysis. To this aim the lipolytic activity of H_2 agonists was followed on isolated adipocytes.

Adipocytes were prepared from epididymal rat fat pads (Rodbell, 1964) and production of glycerol was measured (according to Eggestein and Kuhlman, 1974) after 30 min of incubation in standard conditions of temperature (37 °C) in Krebs-bicarbonate buffer pH = 7.4 in a humidified atmosphere. Under these conditions we compared the pD_2 of histamine (7.04 ± 0.31), 4 methyl-histamine (5.39 ± 0.25) and dimaprit (7.90 ± 0.42) on adipocyte lipolysis).

The activity of these agonists was inhibited by cimetidine and ranitidine. The effects of these two antagonists on the lipolysis induced by the H_2 agonists seemed to reveal an atypical picture of the receptor features. These observations could confirm the existence of sites on adipocyte membrane with a pharmacological profile quite distinct from the typical H_2 receptor described in other districts.

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85P HISTAMINASE ACTIVITY OF SOME TISSUE-BOUND SEMICARBAZIDE-SENSITIVE AMINE OXIDASE (SSAO) ACTIVITIES

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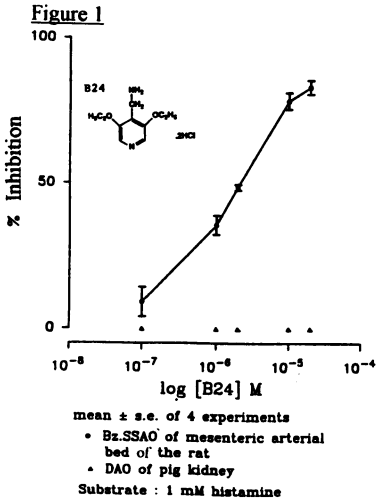
In the late 1964 Buffoni and Blaschko first observed that the plasma amine oxidase (benzylamine oxidase) of the pig also had histaminase activity.

In recent years similar amine oxidase activities with high affinity for benzylamine have been described in many tissues of different species (see Callingham et al., 1990). Benzylamine (B) is not a physiological substrate and up to now it is not yet clear what are the physiological substrates of these amine oxidases. Histamine (H) is quite a good substrate of some tissue-bound SSAO enzymes: rat white adipocytes (Raimondi et al., 1991), rat lung (Ignesti et al., 1992), mouse, rabbit, pig white adipose tissue (Raimondi et al., 1992), guinea pig skin (Buffoni et al., 1993), rat mesenteric arteries (Banchelli et al., 1993).

The SSAO activities of pig aorta and guinea pig skin have been purified by column chromatography and the oxidation of H and B assayed at every step of purification. Both substrates are oxidized by these enzymes although at different rates. Both these enzymes also oxidize acetylputrescine and tele-methylhistamine. In situ experiments on the perfused mesenteric arterial bed of the rat according to the method already described (Banchelli et al., 1990) have shown that the amount of imidazolacetic acid (IAA) that can be detected in the perfusate when H was perfused is strongly reduced if the SSAO activity is fully inhibited by B24, a selective reversible inhibitor of SSAO enzymes (Buffoni et al., 1988, Fig. 1). IAA was identified and assayed in the perfusate by HPLC. During the perfusion with 10^{-4} M H 5.06 ± 0.90 µg of IAA (mean ± s.e. of 8 experiments) were released in the perfusate in 30 min. In the presence of $5x10^{-5}$ M B24, a concentration which fully inhibits the SSAO activity, the release of IAA was reduced of 57.5 ± 8.9 % (mean ± s.e. of 8 experiments, $p < 0.01$).

These results suggest a role of tissue-bound SSAO in the degradation of H in the circulation.

Figure 1



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86P COMPARATIVE RECEPTOR AUTORADIOGRAPHY OF *EX VIVO* AND *IN VITRO* [^{3}H]-DIZOCILPINE BINDING IN MOUSE BRAIN AFTER MIDDLE CEREBRAL ARTERY OCCLUSION

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Excitatory amino acid (EAA) receptor activation has been implicated in the neurodegenerative process which follows periods of ischaemia in mammalian brain and EAA receptor antagonists, in particular at the NMDA receptor, have been demonstrated to be neuroprotective in a variety of animal models of ischaemic damage (Meldrum & Garthwaite, 1990). However, if the NMDA receptor is to be a target for neuroprotective agents administered post-ischaemia it is important to determine not only the time course of the viability of the receptor but also the period post-ischaemia during which drugs can gain access to the receptors in the region of the infarct. ^{3}H-dizocilpine binding provides a useful marker of the integrity of NMDA receptors, thus in the present study, we have compared the *in vitro* and *ex vivo* distributions and densities of ^{3}H-dizocilpine binding sites in mouse brain after focal ischaemia using receptor autoradiography. Studies were performed in male CD1 mice (25-30g) following middle cerebral artery occlusion (MCA-O) as described by Birch *et al.* (1991). Receptor autoradiography was performed *in vitro* as previously described (Bowery *et al.*, 1988) and *ex vivo* 10 min after i.v. injection of 50µCi ^{3}H-dizocilpine (53.1Ci/mmol, 1mCi.ml), (Price *et al.* 1988). Background binding was determined by prior injection of unlabelled dizocilpine (1mg/kg i.v. 15 min before).

The distribution patterns of ^{3}H-dizocilpine binding sites obtained *in vitro* and *ex vivo* in normal mouse brain were the same with the highest densities occuring in the hippocampus and cerebral cortex. MCA-O had little or no effect on the *in vitro* binding density for at least 24 hours post-ischaemia. Binding in the region of the infarct at that time was not significantly different from the contralateral cerebral cortex. However after 2-3 days binding densities in the ipsilateral cortex were significantly reduced by 55.2 ± 15% (n=3 $p<0.05$ ANOVA) and 53.1 ± 13% (n=4 $p<0.05$) respectively. Further reductions occurred after 5 (69.8 ± 8% n=3) and 7 (85.3 ± 6% n=3) days.

By contrast, in the same groups of mice, *ex vivo* ^{3}H-dizocilpine binding was reduced in the infarcted area by 78.6 ± 4% within 2 hours of the ischaemic insult. At all subsequent times binding was reduced by more than 75%. However, at 5 min *ex vivo* binding was not significantly different from control. *Ex vivo* binding after ischaemia was always much less than 30% of *in vitro* binding and this decrease was apparent within 2 hours of the ischaemic insult whereas *in vitro* binding was maintained at control levels for at least 24 hours.

In conclusion, these data suggest that although the population of NMDA receptors is maintained in the infarct region for some days access to them *in vivo* may be sufficiently impaired within 2 or 4 hours of ischaemic insult to reduce the neuroprotective activity of NMDA antagonists after this time.

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87P NITRIC OXIDE MEDIATES NMDA-EVOKED CELL DEATH IN CHP100 HUMAN NEUROBLASTOMA IN CULTURE

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In the mammalian brain, the Ca^{2+}-calmodulin-dependent enzyme nitric oxide (NO)-synthase (NOS) converts L-arginine (L-arg) into citrulline with stoichiometric production of NO (see Moncada et al., 1991). Stimulation of N-methyl-D-aspartate (NMDA) receptors enhances neuronal Ca^{2+} and activates NOS leading to NO production and cGMP accumulation (Garthwaite et al., 1988). L-arg-NO-cGMP pathway has been involved in long-term potentiation (Schuman & Madison, 1991) and electrocortical arousal control (Bagetta et al., 1993). NO has also been implicated in the pathophysiology of cell death produced in vitro by excessive NMDA receptor stimulation (Dawson et al., 1991). We now report that NMDA receptor activation leads to cell death in CHP100 human neuroblastoma cultures via NO production.
Exposure of CHP100 neuroblastoma cells to NMDA (0.25-1.5 mM; n=3-7 experiments per dose) significantly ($P<0.01$) increased nitrite production and cGMP accumulation and these were accompanied by Ca^{2+}- and concentration-dependent cell death. Lethal effects of NMDA (1 mM) were significantly ($P<0.01$) reduced by removing glycine (0.2 mM; n=4) from the exposure solution or by adding competitive (CGP 37849; 0.1 mM; n=4) and non-competitive (MK 801; 200 nM; n=4) NMDA receptor antagonists. Substitution of L-arg (1 mM) with D-arg (1 mM; n=4) or coincubation with L-NAME (0.2 mM; n=5), an inhibitor of NOS, or haemoglobin (0.01 mM; n=3), a NO-trapping agent, prevented NMDA-induced cell death. Superoxide dismutase (SOD; 100 UI/ml; n=6) potentiated the effects of NMDA (0.5 mM) and this was reverted by coincubating SOD with a concentration of catalase (100 UI/ml; n=6) which per se did not affect NMDA-evoked (n=6) response.

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88P ANTISENSE STRATEGY UNRAVELS TAU PROTEIN AS A NOVEL PUTATIVE MOLECULAR RISK FACTOR FOR NEURODEGENERATION

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One of the emerging concept in the field of neurodegeneration is that the selective neuron vulnerability which can be detected both in vivo and in vitro stands on the history of the neurons which may result in an accumulation of molecular risk factors for the disease.Accumulation of destabilizing factors over time, with the loss of adaptive or protective responses, provides one possible explanation for the increased sensitivity of selective neurons to a potential neurotoxic input. We propose tau proteins to be considered as a risk factor for neurodegeneration which contributes, together with others, to define the threshold for disciminating an excitatory from a neurotoxic glutamatergic input.

We found that a brief pulse of either toxic (100 μM) or subtoxic (10 μM) concentrations of glutamate to primary culture of rat cerebellar granule cells induced a short lasting accumulation of mRNA for tau proteins. Time course experiments revealed a bell-shape curve with the maximal increase (two folds over basal) of tau mRNA levels 2 hr after the glutamate pulse. Tau mRNA is further processed to protein as suggested by the significant increase of tau immunoreactivity detectable 2 and 4 hr after the pulse. Immunohistological experiments were done using either TAU-1 (1:100 dilution) or TAU-2 (1:1,000 dilution) antibodies. The glutamate-induced increase in tau immunoreactivity was detectable with both tau antibodies, and resulted in the same magnitude (about + 120% over basal). Values were analysed by the Magiscan Image Analysis System. We then invistigated the possible involvement of the newly synthesized tau protein in the neurotoxic process activated by glutamate using the oligonucleotide antisense strategy. Pretreatment of granule cells with an antisense oligonucleotide complementary to 26 nucleotide sequence comprising the ATG translation initiation codon of tau gene completely prevented the increase of tau immunoreactivity induced by glutamate. Significantly, the concentration response curve of glutamate for inducing neuronal death in cells pretreated with 25 μM tau antisense oligonucleotide showed a shift to the right when compared to those obtained in untreated or tau sense oligonucleotide-treated cells. Particularly, the EC_{50} values of glutamate for inducing cell death were 20 μM, 25 μM and 150 μM for vehicle-, sense-, and antisense treated cell, respectively.
Since inhibition of tau synthesis does not completely prevent but only decrease the neuronal sensitivity to glutamate it is tempting to speculate that accumulation of tau within the neuron represents one of the mulecular risk factors lowering the safety margin of neurons to excitotoxic-induced injury.

89P 7-Cl-THIOKYNURENIC ACID PROTECTS CA1 PYRAMIDAL CELLS AGAINST GLOBAL ISCHEMIC DAMAGE

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We have proposed that excitatory amino acids and oxygen-derived free radicals may cooperate in the genesis of glutamate-mediated neuronal damage (Pellegrini-Giampietro *et al.*, 1990). Glutamate receptor antagonists and free radical scavengers have been demonstrated to protect vulnerable cells against delayed injury following transient but severe forebrain ischemia. In the present study, we examined whether 7-Cl-thio-kynurenic acid, a potent antagonist at the glycine site of the N-methyl-D-aspartate (NMDA) receptor that also inhibits lipid peroxidation (Moroni *et al.*, 1992), protected CA1 pyramidal cells against postischemic damage.

Sham-operated gerbils and gerbils subjected to 5 min of bilateral carotid occlusion were treated i.p. with either vehicle (0.5 ml) or 7-Cl-thio-kynurenic acid (100 mg/kg at 0, 30, 90, 180 and 270 min following reperfusion). Gerbils' temperature was maintained at 37°C throughout the surgery and for 30 min after reperfusion. Five days later, the extent of CA1 cell loss in brain sections was evaluated by examining sections stained with toluidine blue.

Transient bilateral carotid occlusion induced neurodegeneration of almost all (95±0.7%) CA1 pyramidal cells. 7-Cl-thio-kynurenic acid dramatically attenuated ischemia-induced CA1 cell loss to 5±2.6%: the protection was associated with a 4 degree reduction in the animals' temperature that developed between 90 and 180 min post-ischemia and lasted for several hrs. However, when the temperature was maintained at 37°C until 6 hrs following reperfusion, 7-Cl-thio-kynurenic acid still provided partial (54±11.8%) protection, indicating that the latter could not be simply ascribed to hypothermia alone. Gene expression of NMDAR1, a recently cloned subunit of the NMDA receptor, was then examined in damaged and protected CA1 cells by *in situ* hybridization. Qualitative analysis of hybridized sections is currently in progress in our laboratory.

Our results indicate that molecules capable of blocking both excitotoxic and free radical-mediated damage could open new avenues in the search for anti-excitotoxic drugs.

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90P *IN VIVO* NEUROPROTECTION BY 7-CHLORO KYNURENIC ACID WITHOUT AN AMNESIC EFFECT

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Overactivation of NMDA receptors plays a major role in neuronal cell death following cerebral stroke and because of that NMDA antagonists are of great potential as antistroke drugs. However, since NMDA receptors are also involved in other functions, such as memory, NMDA antagonists can cause side-effects such as memory disruption. In the present study we will present evidence that blocking NMDA receptor function in vivo with antagonists at the strychnine-insensitive glycine site associated to the NMDA receptor complex neuroprotection can be achieved in a rodent model of stroke at doses which do not affect memory function. 7-Chloro Kynurenic acid (7-Cl KYNA) was used as a reference glycine antagonist and MK 801 was used as reference NMDA antagonist. The following models were used: NMDA-induced convulsions, as a model of NMDA receptor overactivation in vivo; Middle Cerebral Artery occlusion (MCAo) in mice as a model of cerebral stroke in rodents and dark acquisition avoidance test as a model of memory. The methods used have been described elsewere (Chiamulera *et al*, 1990; Chiamulera *et al*, 1993). Against NMDA induced convulsions (1.2 nmol/ intracerebral) both compounds were effective. At 100 mg/kg ip 7-Cl KYNA inhibited by 54.5 % convulsions (n= 10, higher doses could not be tested) whereas the estimated ED_{50} for MK 801 was 0.51 (0.3-0.8) mg/Kg ip (4 doses, n=10 each dose). In the MCAo model in mice both compounds were found neuroprotective. The compounds were administered before the occlusion (30 min) and the maximal protection with 7-Cl KYNA was seen at 100 mg/Kg ip (average 40% reduction of the infarct size, n=12) whereas with MK 801 the maximal protection was at 1 mg/kg ip (average 50% reduction, n=10). Different results were seen in the memory model. In fact, no activity was detected with 7-Cl KYNA up to the dose of 100 mg/kg ip (n=10) whereas a dose-dependent disruption of the retention performance in the dark avoidance task was seen with MK 801, ED_{50} = 0.13 (0.05-0.3) mg/Kg ip (4 doses, n=10 each dose). The present results demonstrate that 7-Cl KYNA as well as MK 801 can be neuroprotective in a rodent model of stroke at doses which block NMDA receptor overactivation. In contrast, at these doses, 7-Cl KYNA was devoid of a potential side-effect such as memory impairment whereas MK 801 was also amnesic. Since in the literature is reported that also competitive NMDA antagonists causes amnesia (Lawlor and Davis 1992, Chiamulera *et al*, 1990) and since amnesia could be a possible limitation in the use of these compounds in man it can be concluded that glycine antagonists can potentially be a better target for antistroke agents.

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91P CEREBROPROTECTIVE EFFECT OF LAMOTRIGINE AFTER FOCAL CEREBRAL ISCHAEMIA IN THE RAT

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Lamotrigine (3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine) is anticonvulsant and reduces veratrine-induced glutamate release by a possible interaction with Na^+ channels (Leach *et al.*, 1991; Lang & Wang, 1991; Cheung *et al.*, 1992).
We achieved proximal left middle cerebral artery (MCA) occlusion by bipolar coagulation in anaesthetised (2% halothane in 70% N_2O & 30% O_2) male Fischer rats. A 36-38°C core temperature was maintained. Rats received (blind) immediately vehicle (0.25% w/v methylcellulose in H_2O) or lamotrigine as the isethionate salt (3.2, 8, 20, or 50 mg kg^{-1}, i.v. over 10 min), or vehicle or lamotrigine (20 mg kg^{-1}) with a 1h or a 2h delay after occlusion. 24h later a blind neurological test was performed (0 "normal", 1 forelimb flexion, 2 decreased resistance to lateral push, 3 circling) then ten 1mm thick serial brain sections were incubated in 2%w/v triphenyltetrazolium Cl^{-1} in saline (37°C) (Non-infarcted tissue becomes red) then stored in PBF. Volumes were estimated from photographed sections by image analysis. Results are mean(±s.e.mean) infarct volumes (mm^3) or neuroscores in Table 1 and were analysed using Student's unpaired *t* test. * P≤0.05, ** P≤0.01 with respective control (n=8).

TABLE 1.

	0h delay					1h delay		2h delay	
Treatment	Veh	L3.2	L8	L20	L50	Veh	L20	Veh	L20
Total	118±12	96±8	93±14	81±13*	96±11	131±21	91±21	83±15	98±18
Cortical	50±5	49±4	31±4*	24±5**	35±6	56±9	33±6*	39±7	46±9
Noncortical	66±8	47±5	60±11	57±11	59±6	72±12	58±9	44±8	52±11
Neurology	1.8±0.2	1.5±0.3	1.6±0.2	0.8±0.3*	1.5±0.2	1.8±0.2	1.4±0.2	1.8±0.2	1.4±0.3

Lamotrigine (20 mg kg^{-1}, i.v.) only reduces total infarct volume by 32% and improves neurological deficit when administered immediately after MCA occlusion. Cortical infarct volume is reduced after delayed (1h but not 2h) administration of lamotrigine. Lamotrigine (50 mg kg^{-1} but not lower doses) reduces mean arterial blood pressure by 20 mmHg compared with vehicle-treated controls (P≤0.05) which may limit its potential cerebroprotective effects. The "time window" for effective therapy with lamotrigine in permanent focal cerebral ischaemia in rats is short (<2 h).

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92P EFFECTS OF UNILATERAL AMPA LESION OF THE NUCLEUS BASALIS MAGNOCELLULARIS (NBM) AND SUBSEQUENT FETAL BASAL FOREBRAIN TRANSPLANTS ON CORTICAL GABAergic MECHANISMS IN RATS

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Cholinergic neurones in the basal forebrain, including the nbm, project to the frontal cortex and receive an inhibitory GABAergic input (Zaborsky *et al.*, 1986). There is also a GABAergic projection from the nbm which terminates on cortical GABAergic parvalbumin-containing interneurones (Freund and Meskenaite, 1992). The effects of unilateral S-AMPA (2 injections of 0.93 μg 0.5 μl, Abdulla *et al.*, 1992) lesions of nbm and of subsequent intracortical transplants of cholinergic-rich E15 basal forebrain or non-cholinergic E17 hippocampal tissue on the sensitivity of frontal cortex neurones to iontophoretic application, for 20s, of muscimol (10 mM, a $GABA_A$ agonist), baclofen (10 mM, a $GABA_B$ agonist) and glutamate (200 mM) were studied in anaesthetized rats (urethane 1.5 mg/kg).

Eight doses (ejection currents 0-90 nA) of the two GABA agonists were applied to frontal cortex neurones. Responses were considered significant (Wilcoxon, $P < 0.05$) when firing rates differed from control either during or within 20s of drug application. Dose (current)-response curves were plotted (log current v. % inhibition) for control, lesioned and transplanted groups.

Eight-10 weeks after lesion, when acetylcholinesterase (AChE)-positive fibre were almost completely absent from the area, the dose response curve to muscimol was shifted to the left (indicating an increase in $GABA_A$ sensitivity) whereas the dose response to baclofen was shifted to the right (indicating a decrease in $GABA_B$ sensitivity); the spontaneous firing rate of the neurones was significantly reduced. Six-8 weeks after cholinergic-rich transplants to the lesioned frontal cortex, when transplant-derived AChE-positive axons could be seen in the recording area, sensitivity of frontal cortex neurones to both $GABA_A$ and $GABA_B$ was partly restored to the prelesioned level; non-cholinergic transplants were without effect. However, cholinergic transplants did not alter basal firing rate. The sensitivity of frontal cortex neurones to glutamate (30nA) was not affected by any of the procedures.

The extensive neuronal loss within the nbm suggests that the lesion substantially damaged both the cholinergic and GABAergic projections to the neocortex. Destruction of the GABAergic pathway would indirectly increase GABAergic inhibitory tone on frontal cortex pyramidal cells, because it projects primarily onto GABAergic interneurones, and lead to a decreased firing rate. The increased sensitivity to muscimol could be due to an upregulation of $GABA_A$ receptors on GABAergic interneurones while decreased sensitivity to baclofen could be explained by a loss of those presynaptic $GABA_B$ receptors located on cholinergic terminals which degenerated after nbm lesion.

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93P PREVENTION BY THE $GABA_B$ ANTAGONIST CGP35348 OF PROMETHAZINE ANTINOCICEPTION

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Several H1 antihistaminic drugs have been reported to be antinociceptive in laboratory animals and analgesic in the clinic. Nevertheless, their antinociceptive effect might not be due to action on histamine H1 receptors, since histamine itself has been demonstrated to be antinociceptive (Glick & Crane, 1978; Chung et al., 1984). In a previous study on diphenhydramine, promethazine and pyrilamine we reported that their antinociceptive action is not due to an involvement of the opioid, cholinergic, adrenergic or serotoninergic systems (Meini et al., 1988). Lately we demonstrated that the selective GABAB receptor antagonist CGP35348 is able to prevent specifically the antinociception induced by the GABAB agonist baclofen (Malcangio et al., 1991). Taking into account that some of the side effects of the H1 antagonists resemble those of baclofen (e.g. drowsiness, weakness, ataxia), it seemed worthwhile to investigate whether CGP35348 is also able to prevent antinociception induced by promethazine. The study was performed on two animal species by three tests inducing different kinds of pain stimulus (0.6% acetic acid writhing and 52.5°C hot plate tests on mice and paw pressure test on rats with permanent i.c.v. cannulae), and animal motor coordination was assessed by the ability of staying balanced on a rotating rod (24 r.p.m.) for 30 s.

Promethazine HCl was antinociceptive at 3 and 6 $mg \cdot kg^{-1}$ s.c. in the writhing (18.8±2.2 writhes in 10 min and 10.1±3.3 respectively, 15-25 min after treatment; controls: 36.2±1.1) and at 3 $mg \cdot kg^{-1}$ s.c. in the paw pressure (78.0±5.2 g; controls: 57.4±2.8 g 30 min after treatment), but neither of the two doses was effective in the hot plate test. A higher dose (10 $mg \cdot kg^{-1}$ s.c.) elicited antinociception in the hot plate test (23.8±3.4 s of reaction latency; controls: 13.4±0.5 s 30 min after treatment). At the same dose mice were unable ($P<0.05$) to remain on the rotating rod. In all three tests promethazine antinociception was highly significant ($P<0.01$). CGP35348 was hyperalgesic ($P<0.05$) in both the hot plate and paw pressure tests (2.5 µg per mouse or 25 µg per rat i.c.v.; 10.7±0.6 s and 44.6±10.4 g respectively, 30 min after treatment) and slightly antinociceptive in the writhing test (32.3±1.5 writhes). Nevertheless, CGP35348, administered 15 min before promethazine, was able to completely prevent the antinociceptive effect (35.5±3.3 and 35.4±2.7 writhes respectively for promethazine 3 and 6 $mg \cdot kg^{-1}$ s.c. in the writhing test; 51.6±3.8 g in the paw pressure test; 11.0±1.0 s in the hot plate test), but not the motor incoordination.

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94P EVIDENCE FOR $GABA_B$ RECEPTOR-MEDIATED INHIBITION IN RAT SPINAL CORD *IN VITRO*

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The role of $GABA_B$ receptors in synaptic transmission in the dorsal horn of immature rat spinal cord has been investigated in the present study using a whole cell patch clamp technique. A layer of dorsal spinal cord containing the insertion zone of L4 and 5 dorsal roots was trimmed from rat (3-6 day old) hemisected spinal cords. Preparations were perfused with medium containing (in mM); NaCl 118, KCl 3, $CaCl_2$ 1.5, $MgSO_4$ 0.75, glucose 12, $NaHCO_3$ 24, gassed with 95%/5% O_2/CO_2. Whole cell recordings were made from dorsal horn neurones using patch pipettes (4 to 10 MΩ) containing (in mM); Potassium gluconate 130, KCl 10, EGTA 11, HEPES 10, $CaCl_2$ 1, $MgCl_2$ 2, adjusted to a pH of 7.4 with 1M NaOH. Dorsal roots were stimulated once every 40s via glass suction electrodes and this evoked a compound action potential (recorded from a suction electrode on the same dorsal root) and inward synaptic currents (EPSCs) from neurones with a holding potential of -60 mV (Axopatch-1D amplifier). Recordings were made from 17 neurones with a mean input resistance of 452±80 MΩ (s.e.m.) and a mean resting potential (I=0) of -55±4.7 mV. The $GABA_B$ agonist baclofen produced reversible depression of EPSCs and the mean EC_{50} for this depression was 70±13 nM (n=6). No change in membrane conductance was observed in the presence of baclofen at levels up to 1 µM indicating a presynaptic site for the depressant effect. In the presence of the $GABA_B$ antagonist P-(3-aminopropyl)-P-diethoxymethylphosphinic acid (CGP35348, 200 µM) the EC_{50} for baclofen was increased to 3.4±1 µM (n=5). This concentration ratio yields an apparent Kd of 4.2 µM for the CGP35348 receptor complex. Under similar conditions a novel antagonist (Froestl et al, 1992), 3-N[1-(S)-(3,4-dichlorophenyl)ethyl] amino-2-(S)-hydroxypropyl-P-benzyl-phosphinic acid (CGP55845, 100 nM), increased the EC_{50} of baclofen to 10±0.4 µM (n=4) (Kd 0.7 nM). These antagonists as well as reversing the effect of baclofen also directly effected the EPSCs. In 5 out of 7 preparations, 100 nM CGP55845 caused a significant increase in the inward current, integrated over a time course of 500 ms, which ranged from 34 to 183% above control. This potentiation was fully reversed in 30 min (n=2). These results show that the $GABA_B$ antagonists reverse the presynaptic depressant action of baclofen. The antagonist-induced potentiation of the EPSCs indicates the presence of intrinsic inhibition at spinal $GABA_B$ receptors which may operate at pre- or post-junctional sites.

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95P ANTAGONISM BY PIRACETAM OF BACLOFEN ANTINOCICEPTION

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The ability of the first-orally active $GABA_B$ antagonist CGP-36742 to facilitate learning and memory processes was shown by Mondadori et al. (1992) in different tests such as mouse passive avoidance, rat social learning and rhesus monkey colour-spatial task. A potentiation of short-term memory has also been reported for the $GABA_B$ antagonist Phaclofen when this is applied directly to monkey frontal cortex (Sawaguchi et al. 1992).
Since the exact mechanism of the nootropic action of Piracetam, a cyclic derivative of GABA, is still unknown, we considered it interesting to investigate if this action, like that of CGP-36742 and Phaclofen, might depend on the antagonism of $GABA_B$ receptors. To verify this hypothesis we tried to antagonize the antinociceptive effect of the $GABA_B$ agonist Baclofen with Piracetam, since it is known that Baclofen antinociception may be prevented with the $GABA_B$ antagonist CGP-35348 (Malcangio et al.,1991).

Experiments were performed in male Swiss Webster mice (25-30 g) with two different noxious stimuli: thermal (hot-plate test termostatically set at 52.5 ± 0.1°C) and chemical (writhing test - 10 ml kg^{-1} of 0.6% acetic acid solution). The results showed that doses of Piracetam of 10 or 50 mg kg^{-1} i.p. produce, in mouse hot-plate, a significant ($p<0.001$) reduction (49.5±5.3 or 56.8±6.3% respectively) of Baclofen (4 mg/kg s.c.) antinociception 30 min after its administration. At higher doses, Piracetam was able to block Baclofen antinociception completely but at these doses it also prevented Morphine (7 mg kg^{-1} s.c.) and Clomipramine (25 mg kg^{-1} s.c.) analgesias. Piracetam alone, in the dose range of 10-50 mg kg^{-1} i.p., did not modify either pain threshold or mice normal behavior. In the mouse writhing test Piracetam, at the tested dose of 10 mg kg^{-1} i.p., reduced Baclofen (4 mg kg^{-1} s.c.) antinociception without interfering with Morphine (7 mg kg^{-1} s.c.), Clomipramine (25 mg kg^{-1} s.c.) or Ketorolac (10 mg kg^{-1} i.p.) analgesias. The antagonism of Baclofen antinociception by Piracetam in this test was 77.8±5% ($p<0.001$)

Furher experiments are now in progress to elucidate the exact mechanism of action of Piracetam. The present results however indicate an involvement of the GABAergic system in Piracetam effect.

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96P EFFECTS OF L-ARGININE ANALOGUES AND NICORANDIL ON LUMINOL-ENHANCED CHEMILUMINESCENCE GENERATED BY PMA-ACTIVATED PORCINE LEUKOCYTES

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Although chemiluminescence (CL) is widely used for the measurement of free radicals produced by leukocytes, the involvement of NO in this response has not been well characterized. The objectives of this study were to investigate the effects of L-arginine, L-arginine analogues and a NO-donor nicorandil on free radicals generated by porcine leukocytes and to examine the direct free radical scavenging activity of these substances in a cell-free system of free radicals generated by xanthine (X) plus xanthine oxidase (XO).

Leukocytes were isolated from citrated porcine venous blood and luminol-enhanced CL was measured according to the method described previously (Demiryurek et al., 1992). The effects of L-arginine analogues (1 mM) and nicorandil (1mM) were examined in the leukocyte assay either by preincubation for 20 min prior to addition of phorbol myristate acetate (PMA, 0.8 μM) or when added at the peak of the CL. In the X-XO assay, drugs were added before the stimulants. The peak chemiluminescence was measured and the effects of the drugs expressed as % change (mean ± s.e. mean) from the response to the stimulant alone.

Table 1. Drugs effects on PMA and X-XO induced CL (* $p<0.05$).

	PMA-induced CL		X-XO-induced CL
Drugs	Preincubated	Added at peak	Added before X-XO
NICORANDIL	-59 ± 8 (2)	-42 ± 0.2*(3)	-30 ± 6* (6)
L-ARGININE	-24 ± 3* (4)	-12 ± 3* (6)	-3 ± 4 (4)
D-ARGININE	-10 ± 4 (3)	-1 ± 3 (4)	-1 ± 6 (3)
L-CAN	82 ± 26*(4)	46 ± 6* (8)	-12 ± 4 (3)
L-NAME	29 ± 6* (5)	18 ± 3* (6)	-4 ± 6 (3)
L-NNA	26 ± 18 (3)	15 ± 4* (4)	-5 ± 3 (3)

The NO-donor, nicorandil, markedly inhibited CL generated by both PMA-stimulated leukocytes and X-XO (Table 1). L-, but not D-, arginine inhibited PMA-activated free radical release. These results suggest that NO is capable of inhibiting CL. L-canavanine, L-NAME, and N^{ω}-Nitro-L-arginine (L-NNA), inhibitors of the NO pathway, augmented PMA-induced CL. These effects are due to inhibition of endogenous NO which inactivates the superoxide radical. However, these NO-synthetase inhibitors had no effect on X-XO-induced CL, suggesting that these substances have no direct free radical scavenging activity.

It is concluded that contribution of the endogenous NO in leukocyte free radical release can be detected by CL and NO release may play an important role in the measurement of luminol-enhanced CL by interacting with superoxide radical.

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97P THE RELEASE OF VASODILATOR QUANTITIES OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) IS NITRIC OXIDE (NO) DEPENDENT IN RABBIT SKIN

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The increase in microvascular blood flow induced by intradermal (i.d.) capsaicin in rabbit skin is mediated by endogenously released CGRP (Hughes et al, 1992). Preliminary experiments suggest that L-nitro arginine methyl ester (L-NAME) inhibits the increase in blood flow induced by i.d. capsaicin but not i.d. CGRP in rabbit skin (Brain et al, 1993). We have now further investigated the possibility that NO is involved in the release but not the vasodilator action of CGRP.

Local blood flow changes induced by i.d. test agents (0.1 ml site^{-1}) were measured over a 15 min period in the shaved dorsal skin of anaesthetised (pentobarbitone sodium 30 mg kg^{-1}) New Zealand White rabbits (male, 2.5-3 kg), using a multiple site ^{133}Xenon clearance technique (Hughes et al, 1992). L-NAME (30 nmol site^{-1}) had no inhibitory effect on dose related increases in microvascular blood flow induced by i.d. CGRP (0.1-3 pmol site^{-1}), but significantly inhibited the responses induced by i.d capsaicin (10-300 nmol site^{-1}). Table 1. shows that the inactive enantiomer D-NAME (30 nmol site^{-1}) had no inhibitory effect on capsaicin (100 nmol site^{-1}) induced blood flow, while the inhibitory effect of L-NAME (30 nmol site^{-1}) was reversed by L-arginine (300 nmol site^{-1}). In addition, the α-adrenoceptor agonist phenylephrine (10 pmol site^{-1}), which reduced basal blood flow to the same extent as L-NAME, had no significant effect on capsaicin (100 nmol site^{-1}) induced blood flow.

TABLE 1. Selective Inhibition of Capsaicin Induced Blood Flow by L-NAME. (Results are % increase in blood flow at test as compared to control, saline injected sites. P= modified t-test.)

	% increase. (mean ± s.e.mean)	n	P
Capsaicin (Cap)	41±2	5	
Cap + L-NAME	18±6	5	P<0.01
Cap + D-NAME	39±9	5	
Cap + L-NAME + L-arginine	35±9	3	
Cap	34±4	5	
Cap + LNAME	9±6	5	P<0.01
Cap + Phenylephrine	32±3	5	

These results show that L-NAME selectively inhibits the increase in microvascular blood flow induced by i.d. capsaicin but not CGRP in rabbit skin. Since $CGRP_{(8-37)}$ completely inhibits the response induced by intradermal capsaicin, these results suggest that NO has a role in the release but not the mechanism of action of endogenous CGRP in the rabbit cutaneous microvasculature.

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98P PROSTAGLANDIN D_2 IS AN INTERMEDIATE IN AGONIST-STIMULATED NITRIC OXIDE RELEASE IN THE RABBIT SKIN MICROCIRCULATION *IN VIVO*

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We investigated the mechanisms by which acetylcholine, ATP and bradykinin increase microvascular blood flow when injected locally in rabbit skin *in vivo*. Test agents were injected intradermally and changes in skin blood flow measured with a laser Doppler flow probe at 30 min (Warren *et al.*,1993). Skin blood flow increased by 75% at 7.33, 6.77, 11.63, 10.30, 10.55 and <7 (-log moles/site) with acetylcholine, ATP, bradykinin, PGD_2, PGE_2 and nitroprusside respectively.

Co-injection of the cyclooxygenase inhibitor indomethacin $3x10^{-9}$ moles/site, or the nitric oxide synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME) 10^{-7} moles/site, abolished the effects of acetylcholine, ATP and bradykinin. This suggests a link between nitric oxide and prostaglandin release. The possibility that PGD_2 is a stimulus to nitric oxide release (Braun & Schror,1992) was tested with the specific PGD_2 antagonist BW A868C (Giles *et al.*,1989). Arachidonic acid $3x10^{-9}$ moles/site increased blood flow by 156±20% which was attenuated by either L-NAME (85±27%) or BW A868C 10^{-9} moles/site (86±20%), and abolished by indomethacin (-2±11%), $P<0.05$ in each case. PGD_2-mediated vasodilation, but not PGE_2-mediated vasodilation, was abolished by L-NAME or BW A868C. L-NAME, BW A868C, or indomethacin had minimal non-specific constrictor effects when co-injected with pituitary adenylate cyclase activating polypeptide (PACAP), which acts independently of nitric oxide (Warren *et al.*,1991). PACAP 10^{-13} moles/site increased basal blood flow by 128±19% and when co-injected with L-NAME, BW A868C, or indomethacin, increased blood flow by 101±18, 121±31 & 136±22% respectively. The increase for PACAP 10^{-12} moles/site was 182±9% and 153±13, 156±21 & 180±27% with each of the antagonists respectively. Blood flow responses to intradermal acetylcholine and bradykinin were abolished, and the effects of ATP attenuated, by co-injection with BW A868C. In all experiments for each dose of each agent $n \geq 6$ animals, 4 replicates per rabbit, data are mean ±s.e.mean. In studies of rabbit aortic rings *in vitro* pre-contracted with phenylephrine 10^{-6}M, the endothelium-dependent relaxation induced by acetylcholine was abolished by L-NAME 10^{-4}M and unaffected by indomethacin 10^{-5}M. ATP also caused relaxation (-log M EC_{50} 4.25) which was reduced by indomethacin (-log M EC_{50} 3.79) and to a greater extent by L-NAME (EC_{50} <3).

The results suggest that in the microcirculation in rabbit skin *in vivo*, acetylcholine, ATP and bradykinin increase blood flow by production of PGD_2, which in turn stimulates the nitric oxide pathway.

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99P ENDOTHELIUM-DEPENDENT HYPERPOLARIZATION IN THE RABBIT ISOLATED FEMORAL ARTERY

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Endothelium-dependent relaxation of arterial smooth muscle is usually accompanied by hyperpolarization of the smooth muscle cell membrane which appears to reflect the release of a diffusible factor distinct from nitric oxide (NO; Taylor & Weston, 1988). In isolated segments of the rabbit femoral artery, acetylcholine (ACh)-evoked relaxations are depressed by the nitric oxide (NO) synthase inhibitor L-N^G-nitroarginine methyl ester (L-NAME; Plane *et al.*, 1992) whereas relaxations evoked by the calcium ionophore A23187 are resistant to L-NAME (Plane *et al.*, 1992).

In this study, the relative contribution of NO and membrane hyperpolarization to smooth muscle relaxation evoked by ACh and A23187 in the rabbit isolated femoral artery was assessed by simultaneous recording of tension and membrane potential in arterial segments (2 mm in length) mounted in a myograph under a normalized tension. In tissues pre-contracted and depolarized with noradrenaline (0.1-1 μM), ACh (0.01-10 μM) and A23187 (0.01-100 μM) evoked dose-dependent increases in membrane potential which were associated with smooth muscle relaxation. The maximal hyperpolarization and relaxation responses evoked by ACh (10 μM) and A23187 (100 μM) were 9.0 $\pm$ 2.7 mV and 96.8 $\pm$ 2.3 % (n=4), and 23.0 $\pm$ 2.0 mV and 95.0 $\pm$ 4.5 % (n=4), respectively. Pre-incubation with L-NAME (100 μM; 20 mins) abolished the change in membrane potential evoked by ACh and reduced the maximal relaxation to 2.0 $\pm$ 1.4 %; (n=4; $p<0.01$) but had no effect on responses to A23187. In the presence of potassium chloride (20-25 mM) to induce contraction and depolarization, the maximal hyperpolarization and relaxation evoked by A23187 (100 μM) were significantly reduced to 69.0 $\pm$ 10.1 % and 12.0 $\pm$ 1.5 mV, respectively (n=4; $p<0.01$). Subsequent exposure to L-NAME caused a further reduction in the maximal relaxation evoked by A23187 (42 $\pm$ 5.6%; n=4; $p<0.01$) but had no effect on the accompanying hyperpolarization. In the presence of glibenclamide (10 μM; 10 mins), ACh-evoked hyperpolarization was abolished but the accompanying changes in tension and both the hyperpolarization and relaxation to A23187 were unaffected. Indomethacin (10 μM) was without effect on responses to either ACh or A23187.

These data indicate that NO is involved in both the change in membrane potential and relaxation to ACh in the rabbit femoral artery. In contrast, membrane hyperpolarization and relaxation induced by A23187 are resistant to inhibitors of NO synthase.

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100P LOCALISATION OF INDUCIBLE ISOFORMS OF NITRIC OXIDE SYNTHASE AND CYCLOOXYGENASE IN RAT FALLOPIAN TUBES

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Nitric oxide (NO) and prostanoids are released from endothelial cells when intracellular calcium is elevated, for instance, by receptor activation. NO and prostanoids are formed respectively by constitutive isoforms of NO synthase (NOS) and cyclooxygenase (COX-1). In addition, cytokines and/or endotoxin coinduce a different isoform of NOS and cyclooxygenase (COX-2) (Mitchell et al., 1993). It has been suggested that an inducible isoform of COX plays a role in ovarian function (Wong and Richards, 1991). Here we have investigated whether NOS and COX-2 are colocated in normal female genital tract.

Vaginal smears were taken from female rats (220-250g) to assess the stage of the oestrous cycle. Rats were killed by cervical dislocation. Subsequently, ovaries and fallopian tubes (at different stages of the oestrous cycle) were dissected out and frozen in hexane cooled by liquid nitrogen. Cryostat sections (10μm) were prepared and fixed in 4% paraformaldehyde (30 mins) or in acetone (10 mins). Sections were stained either for NADPH diaphorase, an indicator of NOS activity (Dawson et al., 1991), or for NOS using a horseradish peroxidase immunohistochemical technique with antibodies to the induced and constitutive (brain; Schmidt et al., 1992) isoforms. In addition, a polyclonal antibody to COX-2 (Cayman Chemicals, USA) was investigated on serial sections. NOS activity was measured in semi-purified (DEAE anion exchange chromatography) pooled fallopian tube samples (n=12-14) by the conversion of [^{3}H]-L-arginine to [^{3}H]-L-Citrulline. NOS activity represents the mean of 3 determinations and is expressed as pmol of citrulline produced/mg protein in 30 minutes (Mitchell et al., 1991).

Positive diaphorase staining was found in the epithelial cell lining and smooth muscle cells of the fallopian tubes. Staining was also present in the endothelium and smooth muscle of blood vessels and the endocrine cells of the ovary. During di-oestrous, pro-oestrous and met-oestrous no differences were detected in the density of staining. However, in sections from oestrous, tissue staining was reduced, particularly in the fallopian tubes (n=3). Immunohistochemistry demonstrated immunoreactivity to both constitutive and inducible NOS isoforms in both the epithelial lining and smooth muscle of the fallopian tubes at all stages of the oestrous cycle (n=4). NOS activity was present in both constitutive (calcium-dependent; di-oestrous, 10.7, pro-oestrous, 7.2, oestrous, 11.0 and met-oestrous, 0) and inducible (calcium-independent; di-oestrous, 8.8, pro-oestrous, 17.3, oestrous, 8.2 and met-oestrous, 13.9). COX-2 staining was also present in the fallopian tube smooth muscle and epithelial cell lining, but no differences were seen across the oestrous cycle (n=4).

These results show that in addition to constitutive NOS the cytokine inducible isoforms of both NOS and COX are present in all stages of the oestrous cycle. The relative role of NO and COX metabolites produced by constitutive and inducible isoforms of NOS and COX during the reproductive cycle remains the subject of further investigation.

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101P EFFECTS OF DEXAMETHASONE ON ENDOTHELIN-1 RELEASE AND GROWTH OF RAT AORTIC SMOOTH MUSCLE CELLS

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High affinity receptors for glucocorticoids are present in blood vessels (Dural et al., 1977). Glucocorticoids may inhibit the vascular synthesis of nitric oxide (NO) and prostacyclin (PGI_2), two inhibitors of the tone and growth of vascular smooth muscle cells (VSMC), by suppressing the expression of the inducible prostaglandin endoperoxide (PGH) and NO synthases (Bailey *et al.*, 1988 and Knowles *et al.*, 1990). Endothelin-1 (ET-1), secreted by endothelial and VSMC, represents a potent vasoconstrictor and mitogenic peptide (Bobik *et al.*, 1990). The aims of the present investigation were: 1) to compare the release of ET-1 from quiescent and proliferating rat VSMC in culture;2) to study the effects of dexamethasone on ET-1 release and serum-induced proliferation of VSMC; 3) to examine whether endogenous NO and PGI_2 regulate ET-1 release from VSMC.

Rat aortic SMC were subcultured for 48h in the presence of 10% fetal calf serum (FCS). Cells were made quiescent by replacing FCS in the medium with 0.5% albumin for 48h. ET-1 was measured by a radioimmunoassay technique (Patrignani *et al.*, 1991). Cell numbers were counted using a Coulter counter.

Proliferating VSMC released significantly lower amounts of ET-1 than quiescent cells: 0.5 ± 0.4 vs 5.7 ± 2 pg/10^4cells/24h (mean$\pm$SD, n=4, $p<0.01$). At 48h, exogenous ET-1 (10^{-8}-10^{-10} M) significantly ($p<0.05$) stimulated VSMC proliferation in the presence of serum (by 13-23%) but not in quiescent cells. Dexamethasone (2 μM) significantly ($p<0.02$) stimulated ET-1 release both in quiescent and proliferating VSMC by 3- to 12-fold. Despite this effect, dexamethasone significantly ($p<0.05$) decreased (by 37%) VSMC growth in response to 10% FCS. Indomethacin (1.4 μM), an inhibitor of PGH synthase, did not significantly affect ET-1 release or serum-induced proliferation of VSMC. Treatment of VSMC with N^Gmonomethyl-L-arginine (L-NMMA, 200 μM), an inhibitor of NO synthesis, alone or in combination with indomethacin did not significantly alter ET-1 release while causing a significant ($p<0.05$) stimulation (by 50%) of serum-induced VSMC proliferation.

We conclude that in proliferating rat aortic SMC: a) ET-1 release is down-regulated to a rate unlikely to contribute to the effect of serum on cell growth; b) NO appears to modulate the effect of serum; c) dexamethasone stimulates ET-1 release by a mechanism independent of the inhibition of endogenous NO and PGI_2.

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102P DIFFERENTIAL EFFECTS OF OXIDISED LOW-DENSITY LIPOPROTEINS AND LYSOPHOSPHATIDYLCHOLINE ON THE ACTIVITY OF THE CONSTITUTIVE AND INDUCIBLE NO SYNTHASE IN ISOLATED RAT AORTA

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Atherosclerotic lesions are characterised by the accumulation of oxidised low-density lipoproteins and inflammatory cells, such as macrophages, in the blood vessel wall. The production of cytokines by these inflammatory cells may lead to the induction of nitric oxide synthase (NOS). Previous studies in this laboratory have shown that Cu^{2+}-oxidised low-density lipoproteins (OXLDL), prepared separately from the plasma of individual donors, inhibits endothelium-dependent relaxations in isolated rabbit aortic rings (Jacobs *et al.*, 1990) which are mediated by NO produced by the constitutive NOS (see Moncada *et al.*, 1991). This study investigated whether OXLDL or lysophosphatidylcholine (LPC), a major constituent of OXLDL, could inhibit the activity of an isoform of NOS induced in aortic rings from Wistar rats.

Thoracic aortic rings were suspended in organ baths containing Krebs' buffer maintained at 37°C and bubbled with 95% O_2/ 5% CO_2. The effect of OXLDL and LPC on the activity of the inducible NOS was investigated by two protocols. 1) after 4 hours incubation in Krebs' buffer, L-arginine (1-500 μM)-evoked relaxations of phenylephrine (PE)-contracted intact, aortic rings and were assessed before and after addition of OXLDL (2 mg protein/ml) or LPC (10 μM). Cycloheximide (10 μM) and L-nitroarginine methylester (100 μM) inhibited the L-arginine-evoked responses. 2) PE (1nM-10 μM)-evoked contractions were assessed in denuded aortic rings from rats 4 hours after injection with lipopolysaccaride (LPS; S. typhosa; 4 mg/kg i.p.). Cumulative concentrations of PE were added to tissues in the presence and absence of OXLDL (2 mg protein/ml) or LPC (10 μM).

OXLDL (2 mg protein/ml; Donor 1) inhibited acetlycholine (ACh; 0.01-5 μM)-evoked relaxations, significantly reducing the maximum response from 95.7 ± 4.3 % to 67.7 ± 8.9 % (n=4; $p<0.05$). In contrast, OXLDL (2 mg protein/ml; Donor 1) had no effect on relaxations evoked by L-arginine (1-500 μM). The maximum response in control tissues was 90.0 ± 3.8 % and 91.2 ± 4.6 % (n=4; $p>0.05$) in the presence of OXLDL. Similar results were obtained with OXLDL from Donor 2. LPC (10 μM) abolished ACh-evoked relaxations (n=4), whereas L-arginine-evoked responses were potentiated (n=5; $p<0.05$).
In endothelium-denuded tissues from LPS-treated rats, PE-evoked contractions were depressed. The maximum response in aortic rings from LPS-treated rats was 0.44 ± 0.1 g (n=7) and the maximum contractions in control rats was 1.67 ± 0.07 g (n=8 $p<0.05$). OXLDL (2 mg protein/ml; donors 3 and 4) did not significantly influence PE-evoked contractions. The maximal response in the presence of the lipoproteins was 0.65 ± 0.23 g (n=4; donor 3; $p>0.05$) and 0.36 ± 0.13 g (n=4; donor 4; $p>0.05$). Similarily, LPC (10 μM) did not affect contractions to PE in tissues from LPS-treated rats (n=5; $p>0.05$).
In conclusion, this study has shown that in rat aortic rings, OXLDL and LPC inhibit relaxations mediated by the activity of the constitutive NOS. In contrast, these agents do not influence the activity of the form of this enzyme induced *in vitro* and *in vivo*.

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103P NITRIC OXIDE MODULATES SUBSTANCE P-INDUCED MOBILIZATION OF CORONARY CAPILLARY ENDOTHELIAL CELLS

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Nitric oxide (NO) release by endothelium has been identified as the main mediator of vasorelaxation. At microvascular level Substance P (SP) induces endothelium-dependent vasodilation and plasma extravasation which can be modulated by NO synthase inhibitors (Hughes *et al.*, 1990). We have recently reported that SP promotes angiogenesis in vivo and capillary endothelial cell growth and migration in vitro (Ziche *et al.*, 1990; Ziche *et al.*, 1991). In this study we have investigated whether NO could affect endothelial cell mobilization and could modulate SP induced migration. Endothelial cells were isolated from coronary postcapillary venules (CVEC) (Shelling *et al.*, 1988). Cell migration was evaluated by the use of a micro-chemotaxis chamber (Neuroprobe). The method is based on the passage of endothelial cells across porous filters against a concentration gradient of the effector. Mobilization was measured by the number of cells moving across the filter. The nitric oxide generating drug sodium nitroprusside (SNP) induced a significative mobilization of CVEC at 10 μM concentration (50% increment over basal; $p<0.001$). SP induced a dose-dependent migration of the endothelial cells with maximal effect at 10 nM (169.7±7 migrated cells/well vs. 109.4±2.3 of control condition). Pretreatment of the cells with the NO synthase inhibitors L-NAME and L-NMMA (100 μM) completely blocked SP effect. The inhibition was reverted by 100 μM L-Arginine. NO synthase inhibitors did not affect basal or basic Fibroblast Growth Factor induced endothelial cell mobilization. At microvascular level endothelium mobilization plays a major role in angiogenesis and in the evolution of tissue repair processes. Our results indicate that the mobilization produced by SP on capillary endothelial cells is mediated by NO production at endothelial cell level and suggest a novel autocrine effect of NO on capillary endothelium which might be relevant for angiogenesis in vivo.

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104P ACUTE VASODILATOR ACTION OF BACTERIAL LIPOPOLYSACCHARIDE IS MEDIATED VIA RELEASE OF BRADYKININ IN THE PERFUSED RAT HEART

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We have reported previously that perfusion of bacterial lipopolysaccharide (LPS) through the rat isolated heart results in a rapid and sustained fall in coronary perfusion pressure which was blocked by inhibitors of NO synthase (Baydoun et al., 1993). Our findings suggested that LPS may cause acute release of NO from the coronary microvasculature of the rat, mediated presumably by intermediates which activate the constitutive Ca^{2+}/calmodulin-dependent NO pathway. We have extended these studies by investigating whether bradykinin, a potent vasodilator released basally in the rat heart (Baumgarten et al., 1993) and in response to LPS (Katori et al., 1989), mediates the vasodilator action of LPS observed in our previous studies.

Hearts obtained from male Sprague-Dawley rats were perfused by a modified Langendorff technique at a constant flow rate of 10 ml min^{-1} with Krebs-Henseleit buffer maintained at 37°C and gassed with 95% O_2/5% CO_2. Following an equilibration period of 20 min, coronary tone was raised from basal levels (44 ± 6 mmHg) to 120 ± 14 mmHg (n=9) by continuous infusion with 9,11-dideoxy-11α,9α-epoxy methanoprostaglandin $F_{2\alpha}$ (5 nM; U46619). Bradykinin (1 nmol) or sodium nitroprusside (10 nmoles; SNP) were applied as a bolus injection 5 cm proximal to the aortic cannulae. *Escherichia coli* lipopolysaccharide (serotype: 0111-B4; 20 μg ml^{-1}) was added to the buffer reservoir and perfused through the heart either in the presence or absence of the kinin B_2 receptor antagonist, D-Arg-[Hyp^3, Thi^{5,8}, D-Phe^7]bradykinin (200 nM). In some experiments the antagonist was perfused through the hearts after the response to LPS had reached a maximum.

LPS (20 μg ml^{-1}) perfusion caused a rapid endothelium-dependent drop in coronary tone, reducing perfusion pressure by 53 ± 5 mmHg (n=13). As observed previously, the response was maximal within 5 min and reversible upon the removal of LPS from the perfusate, but sustained over a prolonged period (90 min) in the continued presence of LPS. Perfusion of D-Arg-[Hyp^3, Thi^{5,8}, D-Phe^7]bradykinin through hearts 30 min prior to LPS reduced the vasodilator response to LPS from 53 ± 5 to 18 ± 1 mmHg (n=3) and partially blocked (56% inhibition) the vasodilatation induced by exogenous bradykinin. Responses to SNP, a vasodilator acting directly on guanylate cyclase, remained unaltered. Perfusion with the bradykinin antagonist after the LPS response had reached a maximum partially reversed the vasodilatation to LPS, causing a recovery of 61 ± 3% (n=3) which was maintained in the continued presence of the antagonist. In conclusion, LPS appears to cause rapid release of bradykinin in the rat coronary microcirculation which in turn activates constitutive NO synthase resulting in NO release.

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105P EVIDENCE FOR EPISODIC PLATELET ACTIVATION IN ACUTE ISCHEMIC STROKE

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We have previously reported enhanced thromboxane biosynthesis in acute ischemic stroke and provided evidence for its platelet origin (Koudstaal et al, 1993). We have now examined the relationship of thromboxane biosynthesis to time after the onset of symptoms in an attempt to characterize the dynamics of platelet activation in this setting. Thus, we have obtained 4 to 8 consecutive 6-hr urine samples from each of 14 stroke patients (9 males, 5 females; aged 20 to 93 years) during the first 48 hr after the onset of symptoms. The urinary excretion of 11-dehydro-thromboxane (TX) B_2 was measured by a previously validated radioimmunoassay (Ciabattoni et al, 1987 & 1989). Urinary 11-dehydro-TXB_2 averaged 340±319 pmol/mmol creatinine (mean±SD, n=90) with 12 of 14 patients having at least one value >2SD of control mean (119±66, n=20). The prevalence of episodes of enhanced 11-dehydro-TXB_2 excretion was evenly distributed among the eight 6-hr collection periods and averaged 51±11 % (range: 36 to 67 %). No uniform pattern of time-related changes in metabolite excretion was apparent with 3 patients having peak 11-dehydro-TXB_2 values (207 - 2,002 pmol/mmol) at 0 - 12 hr, 3 at 12 - 24 hr, 6 at 24 - 36 hr and 2 at 36 - 48 hr. The level and dynamics of 11-dehydro-TXB_2 excretion were not related to the nature of symptoms nor to the type or site of cerebral infarction on CT-scan. We conclude that during the first 48 hr after the onset of symptoms of an acute ischemic stroke platelet activation occurs episodically. Given its dynamic nature, this ongoing process may be amenable to pharmacologic modulation.

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106P ROLE OF ENDOTHELIUM IN THE MEDIATION OF ANGIOTENSIN II-STIMULATED CONTRACTION AND PROSTACYCLIN RELEASE IN THE HUMAN SAPHENOUS VEIN

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In the human saphenous vein (HSV) Angiotensin II (A II) causes vasoconstriction and the release of prostacyclin, both of which are augmented in the absence of endothelium (Barker et al 1993). Nitric oxide (NO) is also released by A II from other vascular preparations (Gruetter et al 1988) and may be involved in the vascular actions of A II in HSV. We have investigated the effects of piroxicam (PC) and L-NAME, inhibitors of cyclo-oxgenase and NO synthase respectively, on the responses of HSV to A II, in order to elucidate the nature of the endothelium dependent augmented responses previously seen.

HSV (2-4mm rings) were mounted in organ baths in Krebs Henseleit solution. Contractions of the rings (recorded isometrically) were caused by potassium chloride (KCl, 90mM) and A II (10^{-9} M - 10^{-7} M). Endothelial integrity was assesed from the relaxation induced in the precontracted ring (phenylephrine 10^{-5}M) to bradykinin (10^{-5}M). Pre-incubation with PC ($5x10^{-6}$M) and L-NAME ($2x10^{-4}$M) was for 20 minutes. The organ bath fluid was collected and the content of PGI_2 (as 6-oxo-PGF_{1a}) was measured by radioimmunoassay. Contractions are given as percentages of the maximum response to KCl (medians with interquartile ranges). PGI_2 release is expressed as 6-oxo-PGF_{1a} measured after 20 minutes with A II, corrected for basal release.

Contractile responses to A II were unaffected by treatment with PC or L-NAME individually. In the presence of both PC and L-NAME there was an increase in response to A II (10^{-7} M), 51 % (42,67) vs 31 % (21,54) n=8 ($P<0.05$, Wilcoxon test). On stimulation of HSV by A II or treatment with L-NAME seperately, PGI_2 release was small, about 0.3 ng. However, simultaneous treatment with L-NAME and A II (10^{-7} M) caused an increase in PGI_2 release to 2.1 ng ($P<0.05$, n=6). The finding that PC and L-NAME together augmented the contractile response to A II, whereas individually they had no effect suggest that either PGI_2 or NO may attenuate contraction to A II. Further, since inhibition of NO by L-NAME caused a seven fold increase in PGI_2 release, NO would appear to inhibit PGI_2 release in HSV.

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107P EFFECT OF 5-METHYLURAPADIL AND CHLOROETHYLCLONIDINE ON α-ADRENOCEPTOR-INDUCED CONTRACTIONS OF CANINE SAPHENOUS VEIN

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Controversy surrounds the proposed existence of α_1-adrenoceptor subtypes in vascular smooth muscle (Oshita *et al.*, 1993). With the current available pharmacological probes, α_{1A}- and α_{1B}-adrenoceptors have been suggested to be present in a wide range of various tissues (Minneman, 1988), however they have not been exclusively identified in vascular smooth muscle (Oshita *et al.*, 1993). Accordingly, we undertook a study to examine α_1-adrenoceptor responsiveness in canine saphenous vein. Noradrenaline (NA) and phenylephrine (PE) were used as agonists and 5-methylurapidil (5-MU) and chloroethylclonidine (CEC) were used as selective antagonists for the α_{1A}- and α_{1B}-adrenoceptors respectively.

Ring preparations of saphenous vein from at least 5 male mongrel dogs (20-25kg) were used. Following equilibration, concentration-effect curves were generated to either NA or PE in the presence of yohimbine (10^{-7} M) and propranolol (10^{-6} M). Once completed, rings were washed and the tension was allowed to return to baseline. Concentration-effect curves were then repeated to the agonists in the presence of increasing concentrations of 5-MU (10^{-8} - 10^{-6} M) and CEC (10^{-8} - 10^{-6} M). At the same time, in a separate set of rings, control concentration-effect curves were constructed to the agonists alone to assess time-dependent changes in sensitivity and responsiveness.

The results are shown in the table below for maximum response (max; g.mm^{-2}, mean ± s.e.mean) and EC_{50} (uM; geometric mean with 95% confidence intervals). An asterisk (*) signifies $P < 0.05$ versus control.

Table 1. The effect of 5-MU and CEC on NA and PE-induced contractions of canine saphenous vein.

	max - 5-MU -	EC_{50}	max - CEC -	EC_{50}
NA control	33.6±1.3	0.8(0.4-1.5)	27.1±1.3	0.7(0.4-1.2)
10^{-8} [Antag]	33.0±1.3	1.7(1.0-2.8)	25.7±2.4	0.9(0.7-1.8)
10^{-7} [Antag]	31.2±1.4	3.2(1.8-5.7)*	29.2±2.2	2.6(1.4-4.6)*
10^{-6} [Antag]	23.8±1.4*	8.0(4.7-16.7)*	22.3±1.6	4.1(2.6-6.5)*
PE control	35.6±2.2	5.7(3.6-8.9)	35.6±2.3	7.3(4.5-11.8)
10^{-8} [Antag]	32.1±2.7	8.1(3.5-18.7)	29.9±3.0	7.9(4.3-14.7)
10^{-7} [Antag]	37.2±2.4	11.9(9.4-22.1)*	30.0±4.0	6.4(3.5-11.7)
10^{-6} [Antag]	29.2±2.5	45.1(21.1-96.2)*	30.2±2.1	4.2(2.4-7.2)

The results show a) both antagonists (with the exception of 10^{-6} M 5-MU do not significantly alter the maxima to either NA or PE. b) *Only* CEC significantly increased the EC_{50} to NA, pA_2 values for these data were 8.3 with a slope of -1.3, which was not different from unity. c) 5-MU increased the EC_{50} to both NA and PE, pA_2 values were 7.2 (slope -1.2) and 7.0 (slope -0.8) respectively.

Thus it can be concluded that in the canine saphenous vein, NA simulates both α_{1A}- and α_{1B}-adrenoceptors, whereas PE, under these experimental conditions, stimulates only α_{1A}-adrenoceptors.

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108P THE ROLE OF EXTRACELLULAR Ca^{2+} FOR THE NORADRENALINE-INDUCED CONTRACTIONS OF RAT THORACIC AORTA, SPLEEN AND EPIDIDYMAL VAS DEFERENS

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The subtype(s) of α_1-adrenoceptor responsible for the noradrenaline (NA) induced contractions in rat thoracic aorta have been classified into either an α_{1A}- and α_{1B}-involvement or a putative receptor which shares the properties of both the $_{1A}$- and the $_{1B}$-subtypes (Wenham and Marshall, 1992). The nature of the vasoconstrictor response to NA in the aorta is in the form of an initial rapid phasic contraction followed by a slowly developing tonic contraction. It has been suggested that the Ca^{2+} required for the phasic response is derived from an intracellular source but for the tonic response extracellular Ca^{2+} is utilised. (Godfraind *et al.*, 1982). In this study, the effects of Ca^{2+}-free Krebs solution and the dihydropyridine Ca^{2+}-channel blocker, nifedipine, were observed on the individual phasic and tonic contractions of the aorta to NA and compared to functional tissues which have been characterised as either predominantly α_{1A} (epididymal rat vas deferens) or predominantly α_{1B} (rat spleen) (Burt *et al*, 1992).

Thoracic aortae, spleen and epididymal vas deferens were removed from male Sprague Dawley rats (300-400g). The aortae were cut into 4-5mm rings and the endothelium was removed. Aortic rings, epididymal vas deferens and longitudinal splenic strips were mounted in organ baths containing Krebs buffer under a 0.5g resting tension and maintained at 37°C under oxygenation with 95%:5% O_2:CO_2 for the recording of isometric tension. Non-cumulative concentration response curves to NA were performed in aorta and vas deferens and cumulative curves were performed in spleen. In aorta the phasic component was measured at 15 secs after the initiation of the contraction and the response following was considered to be tonic and measured at its maximum (approx.10 mins). After the first curve tissues were equilibrated in either Ca^{2+}-free Krebs + 0.1mM EGTA or 1μM nifedipine for 30 mins followed by a repeat concentration response curve. Data are given as the mean±s.e.mean of between 4 and 8 separate experiments.

All of the tissues contracted dose dependently to NA, with pD_2 values of 8.0, 5.4 and 5.4 for aorta, epididymal vas deferens and spleen respectively. Both nifedipine and Ca^{2+}-free treatments completely abolished the responses in the vas deferens. In the spleen, nifedipine did not affect the response but Ca^{2+}-free treatment caused a 50±3% reduction in maximal response and a 10-fold shift to the right. Phasic contractions in the aorta contributed to 43±6% of the maximal response. Nifedipine reduced this response by 48±3% of the maximum and Ca^{2+}-free treatment caused an 87±2% reduction in the maximum. Tonic contractions were abolished by Ca^{2+}-free treatment but nifedipine caused an 100-fold shift to the right of the control curve.

The results from these experiments show that the α_{1A}-mediated responses of the epididymal vas deferens are totally dependent on extracellular Ca^{2+} entry through nifedipine sensitive channels, but the α_{1B}-linked contractions of spleen were 50% dependent on Ca^{2+} entry through non-nifedipine sensitive channels. The phasic and tonic contractions of aorta were both partially nifedipine sensitive and dependent on extracellular Ca^{2+}.

In conclusion, the sources of Ca^{2+} mobilised for the phasic and tonic contractions to NA in the thoracic aorta cannot be clearly sub-divided. Instead, it seems that both components of the contraction share similar mechanisms for Ca^{2+} mobilisation.

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109P ROLE OF α_2 ADRENOCEPTORS ON INSULIN SECRETION AND BLOOD LACTATE INCREASE RESULTING FROM α_1 AND β ADRENOCEPTOR STIMULATION IN RABBITS

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β and α_1 adrenoceptor stimulation evokes an increase in circulating lactate and immunoreactive insulin (IRI) plasma levels in conscious rabbits (Reverte *et al*, 1991). Interestingly, when animals were exposed to dual ($\alpha_1+\beta$) adrenoceptor stimulation the insulin secretory response was potentiated (García-Barrado *et al*, 1992). The present work has been conducted to reassess the role of α_1 adrenoceptors on insulin secretion and potentiation, to explore the dual adrenoceptor interaction on lactate release and finally to analyse the influence of α_2 inhibitory adrenoceptors on the excitatory responses under investigation.

In conscious 24 h fasted rabbits the i-v infusion of amidephrine (AMID, 3 or 10 μg/Kg/min, for 30 min) induced a dose related increase in arterial IRI levels (peak effect=52.30+6.50%, n=5 $p<0.001$ and 115.80+36.40%, n=7 $p<0.001$, when compared to saline treated animals Δ=-0.96+3.30%, n=6. Preinfusion absolute values of plasma IRI for control and drug treated animals ranged between 12.90+1.62 and 7.15+1.35 μU.l.lml). Indomethacin (0.66 mg/Kg/min, for 15 min) i-v infused 45 min before AMID suppressed the insulin secretory response mediated by the higher dose of the agonist (Δ at 45 min in the presence of indomethacin was:- 13.80+14.25%, n=5, $p<0.001$). The simultaneous infusion of the lower AMID dose and isoprenaline (ISO: 0.3 μg/Kg/min) for 30 min evoked a potentiated insulin secretory rise (Δ at 30 min=421.80+74.20%, n=5, $p<0.001$; Δ after AMID alone 52.30+6.50%, n=5, Δ after ISO alone=91.24+17.62%, n=4), which was maintained for 15 min after the end of the infusion. Changes in plasma glucose did not differ from those found in saline control animals. This potentiated response was suppressed by the concomitant administration of clonidine (CLO: 2 μg/Kg/min, i-v, for 30 min), Δ at 30 min=36.75+32.60%, n=5, $p<0.001$. AMID (10 μg/Kg/min) or salbutamol (SALB: 0.3 μg/Kg/min, i-v, for 30 min) elevated arterial lactate plasma levels (Δ at 45 min after AMID or SALB administration respectively was 1.62+0.26 mmol/l, n=6, $p<0.001$, and 2.20+0.37 mmol/l, n=8, $p<0.001$, vs 0.09+0.20 mmol/l, n=7, in saline treated rabbits; preinfusion absolute values of plasma lactate for control and drug treated rabbits varied between 1.00+0.18 and 0.77+0.13 mmol/l). The combined infusion of both agonists did not potentiate the response. However, CLO reduced the rise in plasma lactate mediated by either agonist (Δ at 45 min after CLO+AMID or CLO+SALB respectively was: 0.19+0.26 mmol/l, n=7, $p<0.05$ and -0.08+0.48 mmol/l, n=5, $p<0.01$). Our results add evidence to support the selectivity of the insulin potentiated secretory response. They also indicate the role of α_2 adrenoceptors in opposing potentiation and reducing the excitatory effects of α_1 and β_2 adrenoceptors on circulating lactate levels.

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110P EFFECTS OF AN ANABOLIC β_2-ADRENOCEPTOR AGONIST, CLENBUTEROL, ON CREATINE TURNOVER

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Clenbuterol, and other β_2-adrenoceptor agonists, increase skeletal muscle mass in animals (Emery *et al*, 1984) via unknown mechanisms. As there is indirect evidence linking creatine with regulation of myofibrillar protein synthesis (*eg* Ingwall, 1976), experiments were undertaken in rats to study the effect of clenbuterol on creatine turnover. Results are shown as means (±sd), n=6.

A single injection of clenbuterol (250μg/kg sc) caused large increases in serum concentrations of creatinine (55 ± 13, control 22 ± 2 μmol/l; $p<0.001$) and creatine (420 ± 25, control 121 ± 25 μmol/l; $p<0.001$) which were maximal after 2 and 12 hours respectively. The clenbuterol-induced rise in serum creatine was preceded, 1 hour after injection, by a significant and reproducible decrease (89 ± 20, control 124 ± 6 μmol/l; $p<0.005$). Serum creatine kinase activity, an indicator of muscle damage, was unaffected by clenbuterol.

After repeated injection for 7 days, clenbuterol (250μg/kg sc) induced a similar peak (2h) increase in serum creatinine concentration (38 ± 6, control 22 ± 2 μmol/l; $p<0.005$). Baseline serum creatine for clenbuterol-treated rats (85 ± 13 μmol/l) was significantly lower than the control (131 ± 18 μmol/l; $p<0.001$). No post-injection rise in serum creatine occurred, as was observed after a single injection, but the initial decrease at 1 hour was reproduced (29% compared to baseline; $p<0.005$).

The effects on serum creatinine and creatine were not explained by alterations in 24h urinary elimination.

In conclusion, clenbuterol causes marked changes in circulating creatine and creatinine levels that, in the absence of obvious muscle damage or altered renal elimination, may indicate effects on creatine turnover via synthesis, transport or interaction with the creatine phosphate shuttle.

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111P EFFECTS OF INDOLEAMINES ON ALDOSTERONE SECRETION FROM RAT ZONA GLOMERULOSA CELLS

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5-hydroxytryptamine, (5-HT) stimulates aldosterone secretion from the adrenal cortex in man (Mantero et al.(1982)) and in rat via a 5HT receptor linked to adenylate cyclase (Bishop and Williams (1990)). Since previous studies with a restricted number of indoleamines have employed whole rat adrenal capsules (Muller, 1988), we have tested a range of indoleamines to determine the structural requirements of agonists necessary to stimulate aldosterone output in acutely isolated rat zona glomerulosa cells.

Cells were prepared from rat adrenal capsules using collagenase digestion followed by 3 washes in Krebs-Ringer bicarbonate buffer containing 2% bovine serum albumin. The final preparation contained at least 95% pure zona glomerulosa cells with minimal contamination by zona fasciculata cells. Following 1 hour static incubation of the cell suspensions at 37°C with a range of indoleamines (10^{-10}-10^{-5}M) , aldosterone secretion into the medium was measured by radioimmunoassay.

Basal secretion of aldosterone was 1.0±0.18 (mean±SEM) nM per 20,000 cells per hour. 5-HT caused a dose-dependent increase in aldosterone secretion with a maximum of 4.76±1.6 nM at 1μM 5-HT. Results from three experiments for each agonist are expressed as the mean EC_{50}±SEM and the mean±SEM maximal aldosterone stimulation , expressed as a percentage of the maximal aldosterone stimulation by 5-HT. Concentration-dependent stimulation of aldosterone occurred with 5-HT (0.145μM±0.027;100%), 5-methoxytryptamine (0.266μM±0.018;82%±6%), 5-methyltryptamine (0.139μM±0.038;74%±12%), N-methyltryptamine (0.127μM±0.023;100%±5%), tryptamine (2.21μM±0.352;140%±28%), 5-hydroxytryptophan (1.93μM±0.273;32%±9%) and 6-methoxytryptamine (3.01μM±0.069;60%±4%). No effect on aldosterone secretion was detected for L-tryptophan and 5-hydroxyindoleacetic acid.

These results show that for agonist activity of indoleamines the structural requirements are (1) a basic terminal NH_2 group, (2) substitution at C5 with an OH, OCH_3 or CH_3 group. Compounds lacking both these features have low aldosterone stimulating activity

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112P PHORBOL ESTER POTENTIATES CALCIUM BUT NOT GUANINE NUCLEOTIDE-EVOKED ACTH SECRETION FROM ELECTRICALLY-PERMEABILISED AtT-20 CELLS

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GTP-binding proteins may mediate calcium-evoked secretion of ACTH from the anterior pituitary tumour cell line AtT-20 and may be a site of action for regulation by cAMP-dependent protein kinase (PKA) (Guild, 1991). Activation of protein kinase C (PKC) stimulated ACTH secretion from AtT-20 cells by mechanisms different from that of cAMP but did share with this nucleotide a post-calcium site of action (Reisine & Guild, 1987). Whether this site of regulation by PKC is at the level of GTP-binding proteins was investigated by studying the effect of the PKC activator phorbol 12-myristate 13-acetate (PMA) upon calcium- and guanine nucleotides-evoked ACTH secretion from AtT-20 cells.

The effect of PMA upon ACTH secretion from intact AtT-20 cells was investigated using the methods of Hook et al, 1982. The effect of PMA upon calcium- and guanine nucleotide-evoked ACTH secretion from electrically-permeabilised AtT-20 cells was studied using the methods previously described for cAMP (Guild, 1991). PMA (10^{-12}-10^{-7}M) stimulated secretion from intact AtT-20 cells in a concentration-dependent manner. Guanosine 5'-O-(3-thiotriphosphate) (GTP-γ-S) stimulated secretion from permeabilised cells at concentrations greater than 10^{-6}M (4-fold increase at $3x10^{-4}$M). Calcium stimulated secretion from permeabilised cells in the concentration range 10^{-7}-10^{-5}M (EC_{50} = 10^{-6}M, 4 fold increase at maximum) and was additive with GTP-γ-S (10^{-5}M) up to a maximum which could be stimulated by calcium alone. Co-incubation of calcium with PMA (10^{-7}M) evoked a 2-fold enhancement of secretion at all concentrations of calcium. GTP-γ-S (10^{-5}M) evoked secretion was additive to that obtained to the combination of PMA (10^{-7}M) and calcium (10^{-9}-10^{-5}M) up to a maximum which could be stimulated by PMA and calcium together.

The potentiation of calcium-evoked ACTH secretion, by PMA, from permeabilised cells indicates a post-calcium site of action for PKC in stimulating ACTH secretion. The data also support a role for GTP-binding proteins in mediating calcium-evoked ACTH secretion and that an action at a the level of, or distal to, such proteins may be involved in stimulus-secretion coupling for PKC activation .

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113P GROWTH FACTORS FROM ASTROGLIAL CELLS INFLUENCE LHRH NEURONAL CELL PROLIFERATION AND SECRETION

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Astrocytes are involved in the differentiation, migration and maturation of neurons, and establishment of appropriate axonal connections. While the trophic role of the glial cell compartment in support of neurons has long been suspected, more recent studies have demonstrated that astroglial cells can respond to the T-cell and macrophage-derived cytokines including, interleukin 1 (IL-1), interleukin 2 (IL-2), interleukin 3 (IL-3) and interleukin 6 (IL-6), and when activated can produce factors resembling or identical to that same cytokines. Exposure of brain cells to these growth factors results in cell survival, growth and differentiation (Fontana et al. 1981). In view of the important hormonal sensitivity of the glia and the marked synaptic plasticity that occurs over the course of the estrous cycle, we have studied the possible interactions between the glia and the luteinizing hormone-releasing hormone (LHRH) neuron. In the present study we have addressed the question of whether growth factors released from astroglial cells at different stages of their differentiation and maturation in vitro may influence LHRH neuronal cell growth and/or secretion.

In this experimental design LHRH neuronal cells were cultured alone, in the presence of astroglial cells, or in medium conditioned (CM) from astroglial cell cultures. Moreover, the ability of CM from astroglial cells to influence leukocyte cell growth was assessed. For this aim, we have used the immortalized hypothalamic neuronal cell line (GT1-1 subclone) recently developed by genetically targeting the expression of the simian virus-40 large T-antigen in the LHRH neurons (Mellon et al. 1990). Primary astroglial cells were obtained from cerebral hemispheres of newborn rats and split in DMEM with 10% FCS. At least 80% of the cells in these cultures are astrocytes, as confirmed by immunocytochemical localization of the astrocytic marker, glial fibrillary acidic protein (GFAP). Conditioned media from astrocyte cultures were collected at different time intervals (7-55 days of culture) and assayed on the cultured LHRH neuronal cells. Media from cultured LHRH neuronal cells were collected 2, 4, 6 and 8 days following coculture with glial cells or after addition of astroglial cell CM. The present data show a significant stimulation of GT1-1 neuronal cell proliferation, paralleled by increased LHRH secretion following coculture with astrocytes, or addition of CM. The effect of CM was characterized by a stimulation at early stages, and an inhibition at later stages of glia maturation. When the effect of CM was investigated on resting splenocytes, a significant stimulation ($p^{*}< 0.01$) of cell proliferation mimicking the one obtained following a subactive dose of a T-dependent mitogen, was observed (Basal 732±0; ConA 0.3 µg/ml 1547±204; 12 days CM 1537±149), suggesting that the astroglial cell compartment produces factors able to modulate both neuronal and immunocyte cell function.

GT1-1 Neuronal Cells	Astroglial cells CM		
	Control	Days of Culture	
		12	40
LHRH Secretion (pg/ml)	16.15± 0.388	50± * 0.565	9.45± 0.106
LHRH Proliferation (dpm)	82053.5± 1904.6	146537± * 21442	78404± 4264.2

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114P MATERNAL HYPERCORTICOSTERONAEMIA IMPROVES COGNITION AND INCREASES CORTICOSTEROID RECEPTORS IN THE HIPPOCAMPUS OF MALE OFFSPRING: A MULTIVARIATE APPROACH

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The effects of the increase of plasma concentrations of glucocorticoid hormones during neonatal life on learning and corticosteroid receptors (CR) in Central Nervous System have been generally studied using procedures involving physical manipulations or separation stress in mother and newborn rats. In this study, the increase of plasma levels of corticosterone (B) from birth to weaning was obtained through maternal milk, by adding 200 µg/ml of B (hemisuccinate) in the drinking water of lactating mothers (dams significantly from 4.3±0.5 to 9.5±1.8 µg/100ml, namely mild psychic stress levels; pups from 0.7±0.1 to 1.2±0.2 µg/100ml at 10 days of lactation). Maternal behavior was not affected by the treatment. Learning capacity in the Water Maze test at 22 days of life, and in the Active Avoidance test at 12 months of age, and Bmax and Kd of CR in the hippocampus at the age of 14-months were measured. Learning was significantly improved in both behavioral tests and the Bmax of type I CR was significantly higher in B-nursed male rats compared with controls. The number of trials to reach a criterion for the learning tests (i.e. 3 consecutive trials with latencies lower than 30 secs in the Water Maze, and four consecutive avoidances in the Active Avoidance test) and the Bmax of type I CR, were analyzed by a multivariate approach, which identifies natural groups of subjects similar for the characteristics considered. To study the structure of the data set variability in the space of the three variables a Cluster analysis was used. All six controls were in the Cluster 1 (low learning performances/low Bmax of type I CR) while five out of 7 B-nursed rats were in the Cluster 2 (high performances/high Bmax of type I CR) and this distribution of the two groups of animals was significantly different (Fisher test $p<0.01$). To study the relation structure of the data set, a Principal Component Analysis (PCA) was executed. This procedure generated a first Component that explained the 54% of the total variability linking together all the three variables; this Component was the consequence of the correlation generated by the difference between the two groups due to the treatment. The Cluster analysis on this Component gave the same results of the previous Clustering procedure. We conclude that an increase in plasma levels of B during neonatal life might affect the maturation of brain systems involved in spatial and conditioned learning, likely through an increase in CR in the hippocampus.

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115P CICLETANINE-INDUCED ABBREVIATION OF CARDIAC ACTION POTENTIAL: A ROLE FOR K_{ATP} CHANNELS?

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The precise mode(s) of cardiovascular action of cicletanine, a novel antihypertensive drug with cardioprotective actions, remains unclear. Its direct actions on vascular smooth muscle are thought to partly involve inhibition of Ca influx and/or mobilisation and opening of K^+ channels, but this remains controversial (Deitmer *et al.*, 1992; Ebeigbe and Cabanie, 1991). We examined whether cicletanine opened K^+ channels in cardiac tissues by comparing its effects on the cardiac action potential with those of levcromakalim (a K_{ATP} channel opener), in the absence and prescence of glibenclamide (a K_{ATP} channel blocker).

Sheep Purkinje fibres were superfused *in vitro* with Tyrode's solution at 36°C and paced at 1.5 Hz. Action potentials were measured before and after 30 min of exposure to vehicle (DMSO) of glibenclamide (10 μM) and at 5, 15 and 30 min of exposure to cicletanine (100 μM) or levcromakalim (20 μM). Cicletanine (100 μM) and levcromakalim (20 μM) both induced significant time-dependent abbreviation of action potential duration measured at both 50% (APD_{50}) and 90% (APD_{90}) repolarisation, respectively. Thus, cicletanine (100 μM) reduced APD_{90} from 275 ± 13 to 242 ± 15 ms (n = 6), and levcromakalim (20 μM), from 269 ± 4 to 170 ± 20 ms (n = 3), within 30 min of expsoure. Cicletanine, but not levcromakalim, also caused slight but significant reductions in action potential amplitude from 121 ± 1 to 116 ± 1 mV and its differential from 605 ± 10 to 570 ± 16 Vs^{-1}. Glibenclamide (10 μM) had no effect on Purkinje action potentials but completely abolished levcromakalim-induced abbreviation of the action potential. Thus, APD_{90}, was 277 ± 8 and 275 ± 12 ms, respectively before and 30 min after levcromakalim (n = 3). By contrast, glibenclamide (10 μM) only partially antagonised cicletanine-induced abbreviation of the action potential, with APD_{90} being reduced from 277 ± 14 to 268 ± 15 ms after 30 min of exposure (n = 5).

These results suggest that cicletanine may open K_{ATP} channels in sheep Purkinje fibres and that this action may contribute, at least in part, to its ability to shorten the cardiac action potential.

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116P MEMBRANE ELECTRICAL PROPERTIES OF SKELETAL MUSCLE FIBRES FROM DYSTROPHIC MICE: A PHARMACOLOGICAL APPROACH

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Lack of dystrophin and associated glycoprotein complex in muscle sarcolemma may cause ion channel malfunction which in turn could contribute to the degenerative condition occurring in dystrophin related myopathies (Ibraghimov-Beskrovnaya *et al.*, 1992). We measured *in vitro* membrane cable properties, resting conductances to chloride (G_{Cl}) and potassium (G_K) ions and excitability characteristics of extensor digitorum longus (EDL) muscles from 6 week to 5-6 month old dystrophic (mdx) and control mice, by means of computerized two microelectrode technique. At 6 weeks of age no significant differences were observed in all the parameters studied. At 8 weeks of age the input resistance (R_{in}) was significantly lower in mdx (0.22±0.02 Ohm; n=20) with respect to age matched controls (0.34±0.04 Ohm; n=20); such decrease was paralleled by a marked decrease of time constant and membrane resistance (R_m). The conversion of R_m values to conductances showed that G_{Cl} was significantly higher in mdx (3003±241 μS/cm^2; n=20) than in the age related controls (2257±164 μS/cm^2; n=24), whereas G_K was unchanged. Accordingly, in mdx muscle the threshold current (I_{th}) was significantly increased, the latency of action potential shortened and the firing capability reduced. Similar changes, although attenuated, were observed at 10 weeks of age. Actually, at 5-6 months of age R_{in} and R_m were higher and G_{Cl} was sligthly lower in mdx than in control mice. We attempted to characterize such temporary increase of G_{Cl} by using drugs highly specific for muscle chloride channels, such as the S-(-) and R-(+) enantiomers of 2-(p-chlorophenoxy) propionic acid (CPP) (De Luca *et al.*, 1992). These compounds have been tested *in vitro* on EDL muscles from both mdx and control mice aged 10 weeks or 5-6 months. S-(-)CPP tested at 5μM and 20μM produced the classical concentration dependent decrease of G_{Cl} in EDl muscles from control mice at both ages. On the contrary 5μM S-(-) CPP was without effect and 20 μM only moderately decreased G_{Cl} on EDL muscle from 10 week old mdx mice. At 5-6 months the effects of S-(-) CPP in control and mdx mice were almost overlapping. The R-(+) CPP at 3μM and 40μM produced in control muscles the expected biphasic effect on G_{Cl} (De Luca *et al.*, 1992), whereas in the 10 week old mdx muscles we observed only an increase of G_{Cl}. In this phenotype the biphasic effect of R-(+) CPP started to be detectable at 5-6 months. The present results suggest that in muscles from mdx mice GCl, and consequently the passive and the active membrane properties related to it, change transiently after regenerative process. Such changes may be caused by compensatory mechanisms related to regeneration of mdx phenotype other than to a direct consequence of the lack of dystrophin. The pharmacological investigation suggests that appearance of chloride channels with different pharmacology, whose expression may be related to factors controlling regeneration, could contribute to the transient increase of muscle G_{Cl} in this phenotype.(Supported by Telethon-Italy, 1991/92).

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117P CHARACTERIZATION OF POTASSIUM CHANNELS ON THE SURFACE MEMBRANE OF SKELETAL MUSCLE FROM AGED RATS

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The population of K^+ channels present on the surface membrane of skeletal muscle fibers from 7, 15 and 25 month old rats (Wistar Kioto), were surveyed by using patch clamp technique. Single fibers were isolated enzimatically at 30° C from Flexor Digitorum Brevis toe muscle of the rats (Bekoff & Betz, 1977). Continuous recording of the channel activity was performed in cell-attached (C-A) and inside-out (I-O) patch configurations at constant voltage (steady state) at 20° C in symmetrical KCl (150 mM) and in high bath KCl concentration (400 mM). The C-A recordings showed a typical activity from Ca^{++} activated K^+ channel of 253±2 pS (n=3) of conductance; indeed when the bath solution was enriched with Egta (5 mM), the excision of the patches from the cells, led to the loss of channel activity. This channel was rarely found in the fibers from 7 month old rats showing a frequency of about 1% of the patches, while there was a 30% increase of its frequency in the patches from 25 month old rats. Typical activity from ATP-sensitive K^+ channels was commonly found in I-O configuration; indeed, these channels were reversibly blocked by internal application of 20-50 μM ATP. However, the frequency of occurrence of these channels increased with aging: indeed, they were releaved in 36%, 50% and 54% of I-O recordings respectively from 7, 15 and 25 month old rats. Single channel conductance was unaffected by aging, being 57±1.3 pS (n=4), 54±2.3 pS (n=4) and 56±2 pS (n=4) in symmetrical KCl respectively in 7, 15 and 25 month old rats. In contrast, the Popen of the channel in aged rats was about 100 times lower than the Popen calculated in 7 month old rats (Figure 1).



Figure 1. K^+_{ATP} channel from 7 (a) and 15 (b) month old rat. I-O configuration, Holding potential: -80 mV; Acquisition rate: 20 KHz; filter: 2 KHz (-3dB).

The application to the cytoplasmic side of the channel of aged rat of cysteine (200 μM), a disulfide reducing agent, led to a transient increase of channel activity. Our preliminary results indicate that the frequency of occurring and the gating properties of the K^+_{ATP} channels are altered by aging. The high success rate, can be partly due to an increase of channel density. The modification of the gating properties can be explained by an altered oxidation state of sulfhydryl (thiol) groups of the channel protein; indeed, these groups are functionally important in regulating the activity of the K_{ATP} channel in various tissues (Islam *et al.*, 1993). Another possibility may involve a mutation of the channel subunits. Finally, it should be underlined that the increase of activity of K^+_{Ca}++ channel in the aged fibers, may confirm an alteration of the metabolic state of the cell. (Supported by Italian CNR P.F. Invecchiamento).

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118P INFLUENCE OF PRENATAL EXPOSURE TO CARBON MONOXIDE ON THE BIOGENESIS OF VOLTAGE-SENSITIVE SODIUM CHANNELS IN RATS

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Voltage-sensitive sodium channels are glycoproteins which undergo several steps of processing and assembly during periods of active membrane biogenesis (*Schmidt & Catterall, 1987*). In particular, the oligosaccharide residues of the sodium channel must be processed to the mature complex type for attainment of a functional conformation, and it is well known that any interference with glycosylation may affect the normal expression of functional channels in the surface membrane (*Barchi, 1988*). In this report, we show that prenatal exposure to relatively mild concentration of carbon monoxide (75 and 150 ppm) induces in rat offspring subtle changes in the functional properties of voltage-sensitive sodium channels as well as in the carbohydrate structures associated with surface-bound glycoconjugates. Electrophysiological and histochemical analyses were carried out on the nodal axon membrane of sciatic nerve fibres which exhibits a high density of voltage-sensitive sodium channels (*Black et al., 1990*). In CO (75 and 150 ppm)-exposed rats, the voltage-clamp analysis showed that inactivation, which controls rate and extent of channel closure, was notably slowed [τ_{h1} (ms): control 0.4 ± 0.01; 75 ppm 1.3 ± 0.11*; 150 ppm 1.6 ± 0.13*; τ_{h2} (ms) : control 1.9 ± 0.1; 75 ppm 4.9 ± 0.2*; 150 ppm 5.1 ± 0.2*; *p<0.01]. In CO-exposed rats a considerable fraction of sodium channels was in a conducting state at the end of a 12 ms depolarization; moreover, the peak sodium current reversed at more negative voltages. Unlike the change in the inactivation kinetic, the negative shift of sodium reversal potential was a persistent effect [V_{revip}(mV) : control 129 ± 2.9; 75 ppm 104 ± 4.3*; 150 ppm 99 ± 4.1*; *p<0.01]. In CO-treated rats, the histochemical staining method revealed two major changes in the glycoprotein profile of nodal axon membrane: (i) sialylation of glycoconjugates as shown by the positive reaction to sialidase-wheat germ agglutinin; (ii) sulfation of glycoconjugates as demonstrated by affinity for high iron diamine-Alcian Bleu sequence. These findings indicate that acidic glycoconjugates are mainly expressed in the nodal membrane of CO-exposed rats. In sum, our results show that prenatal exposure to relatively mild CO concentrations induces in rat offspring significant changes in the carbohydrate structures associated with surface-bound glycoconjugates and this, in turn, could impair the normal expression of functional channels in the surface membrane.

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119P EVIDENCE FOR DISTINCT PRESYNAPTIC NICOTINIC RECEPTORS WHICH FACILITATE ACTION-POTENTIAL EVOKED ACETYLCHOLINE RELEASE FROM MOUSE CORTICAL SLICES

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It is well recognised that cholinergic neurones possess presynaptic muscarinic receptors, activation of which inhibit action-potential evoked acetylcholine release as well as presynaptic nicotinic receptors which when activated induce acetylcholine release (Raiteri *et al.*, 1990; Wilkie *et al.*, 1993). The aim of the present study was to investigate the operation of these systems in mouse cortical slices which were pre-incubated with [^{3}H]-choline to label the transmitter stores with [^{3}H] acetylcholine and then superfused with physiological salt solution. The release of radioactivity by electrical fleld stimulation (3 Hz, 2 ms pulses for 1 min) was used as an index of acetylcholine release. Exogenous acetylcholine had three distinct effects on stimulation-induced acetylcholine release. Firstly, at a low concentration (1 μM) it enhanced the release by 49±8%, n=6 without affecting the spontaneous release. This effect was attenuated by mecamylamine (30 μM) and thus appears to involve nicotinic receptors. At higher concentrations (10 and 30 μM), exogenous acetylcholine inhibited the stimulation-induced release (by 25±12%, n = 7 at 10 μM) and this effect was reversed to a facilitatory effect by atropine (0.3 μM), indicating that the inhibitory effect was due to muscarinic receptor activation and also that the inhibitory effect was able to mask the underlying activation of release-enhancing nicotinic receptors. The third effect of exogenous acetylcholine was that at the highest concentration (30 μM) where the spontaneous outflow of radioactivity was increased, presumably due to activation of release-inducing nicotinic receptors which are well described. When these experiments were repeated with the muscarinic agonist bethanechol (0.01-100 μM), only an inhibitory effect on stimulation-induced acetylcholine release was observed. The cholinesterase inhibitor neostigmine (0.001-100 μM) concentration dependently inhibited stimulation-induced acetylcholine release (IC_{50} = 0.02 μM) without affecting the spontaneous outflow. After atropine this inhibitory effect was reversed to a facilitation, indicating that endogenous acetylcholine could activate release-enhancing nicotinic receptors. The spontaneous outflow of radioactivity was not altered by neostigmine, even after atropine.

In conclusion, the present study suggests that cholinergic neurones possess release-enhancing nicotinic receptors which can be activated by exogenous and endogenous acetylcholine. These are different to release-inducing nicotinic receptors in that they respond to lower concentrations of acetylcholine (1 versus 30 μM). Release-inhibiting muscarinic receptors are operative at higher acetylcholine concentrations and are able to overide the facilitatory nicotinic effect. The physiological significance and precise receptor characterization of the nicotinic receptors remains to be elucidated.

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120P ALTERATIONS OF LTP AND PHOSPHORYLATION PROCESSES IN AN ANIMAL MODEL OF COGNITIVE IMPAIRMENT

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The use of appropriate animal models to study specific pathologies of the brain will be of great importance in better defining the molecular mechanism underlying these brain defects and the subsequent pharmacological strategies to treat them.
Rat embryos exposed on gestational day 15 to methyl-azoxy-methanol acetate (MAM) develop a microencephaly characterized primarely by a hypoplasia of the necortex and CA area of the hippocampus so that in adulthood they show a marked alteration of cognitive processes, as evidenced in several tests for learning and memory, without any other confounding behavioural alteration (Di Luca and Cattabeni, 1991). To investigate whether the observed behavioral impairment was paralleled by alterations in Long Term Potentiation, one of the most suitable electrophysiological model of synaptic plasticity related to information storage processes in the CNS, we have studied the capacity of hippocampal slices from microencephalic rats to express a high frequency stimulation induced LTP. Our results indicate a lack of induction of LTP in the CA1 region but not in the Dentate Gyrus of treated animals (CA1 region: 7 slices from 7 different control rats at t=60 min showed an increase in the field EPSPs; $p<0.05$ in Wilcoxon signed-rank test with respect to the baseline. None of 7 slices from 7 different treated animals exhibited an increase. Dentate Gyrus: One way MANOVA of the slope of field EPSPs showed no significant differences in the two experimental groups; 7 slices from 7 different animals; 3 independent experiments).

At the molecular level, phosphorylation processes are thought to play a fundamental role in neuronal plasticity and particular attention has been given to PKC and its neuronal substrate B-50/GAP-43 involved in the development and plasticity of synaptic connections (Skene, 1989; Gianotti et al., 1992). We have studied the phosphorylation of B-50 in cortex and hippocampus of microencephalic rats by means of in situ phosphorylation and quantitative immunoprecipitation; these experiments revealed a marked alteration on basal phosphorylation of B-50 in treated animals (increase of 51.4±7.4% vs controls; $p<0.05$, 3 independent experiments). Accordingly, the PDBu binding signal as measured by autoradiography is enhanced by about 40% in brain areas of cognitively impaired animals. This observation is confirmed by immunoblot analysis performed on soluble and particulate fraction of synaptosomal preparations that revealed an enhanced translocation of PKC to the membrane (+ 56.6±7.2, $p<0.0001$ vs controls).

These results, correlating brain damage to alteration of phosphorylation processes, LTP and behavioural observations in MAM-treated rats, suggest that this animal model could be of particular interest to study molecular correlates of cognitive functions and new pharmacological strategies to treat disorders of cognition.

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121P WEANING ACTIVATES THE DEVELOPMENT OF A δ-OPIOID RECEPTOR SUBTYPE IN THE RAT

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Pharmacological evidence using selective antagonists shows that the opioid form of stress-induced antinociception is mediated by μ-opioid receptors in preweanling rats (20 days) but is operated by δ–receptors in postweanling animals (25 days) (Kitchen & Pinker,1990). Recently we showed that delay of weaning can prevent the transition from μ- to δ–opioid receptor control of stress-induced antinociception between day 20 and day 25 (Muhammad & Kitchen 1993). We have hypothesised that weaning may be the stimulus for the development of a δ–opioid receptor subtype which develops in the fourth postnatal week, since binding studies show that 25% of δ–receptors do not develop until after weaning (McDowell & Kitchen, 1986). We have studied the antinociceptive activity of putative δ_1 (DELT I) and δ_2 (DSLET) selective agonists in 25 day old Wistar albino weaned and non-weaned rats. Weaning was carried out at day 21 except in non-weaned groups where rats remained with their mothers at all times other than during drug administration and nociceptive testing. Nociceptive responses were recorded 15 min before drug administration by the use of the neonatal tail immersion test at 50°C and over a 15 min period after i.p. administration of agonists. Where studied, the δ-opioid antagonist naltriben was injected 10 min before agonist administration.

Table 1. Peak tail immersion latencies (s) after administration of selective δ–agonists in weaned and non-weaned 25 day old rats

	[D-Ser^2,Leu^5,Thr^6]-enkephalin (DSLET)			[D-Ala^2]-deltorphin I (DELT I)		
Dose	0.5 mg kg^{-1}	0.75 mg kg^{-1}	1 mg kg^{-1}	0.01 mg kg^{-1}	0.025 mg kg^{-1}	0.05 mg kg^{-1}
Weaned	3.9 ± 0.23 *	4.4 ± 0.44 **	4.7 ± 0.49 **	3.4 ± 0.26 *	4.1 ± 0.19 **	4.8 ± 0.60 **
Non-weaned	2.5 ± 0.12	2.6 ± 0.15	2.6 ± 0.12	3.5 ± 0.16 **	3.8 ± 0.14 **	4.2 ± 0.12 **

Values are mean ± s.e.mean, n=6. Saline control latencies for all groups were 2.4 - 2.5 sec. * P<0.05 ** P<0.01 vs saline control.

In weaned rats DSLET produced dose related antinociception which was significantly antagonised by naltriben at a dose (5μg kg^{-1}) which shows selectivity for the δ_2-receptor (Crook et al., 1992) but in non-weaned animals DSLET had no effect. In contrast, dose related responses to DELT I were evident in both weaned and non-weaned rats and these responses were not antagonised by naltriben (5μg kg^{-1}), but are reversed by higher doses of this antagonist (Crook et al., 1992). This data provides the first indirect *in vivo* evidence that δ–receptor subtypes develop independently and suggest that weaning may be the stimulus for the development of the δ_2-receptor subtype. Regional binding studies and *in situ* hybridisation experiments are underway to corroborate these findings at the molecular level since weaning may activate expression of the δ-receptor gene.

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122P ANTINOCICEPTIVE EFFECTS AND MECHANISM OF OPIOID ACTIVITY OF (LYS7) DERMORPHIN, A NATURALLY OCCURRING PEPTIDE WITH HIGH AFFINITY AND SELECTIVITY FOR μ-OPIOID RECEPTORS

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[Lys7]dermorphin (Tyr-D-Ala-Phe-Gly-Tyr-Pro-Lys-NH_2, m.w. = 845), a new Amphibian peptide, showed affinity and selectivity for μ-opioid receptors higher than that of dermorphin and DAGO.. The analysis of the displacement of [H^3]DAGO binding to rat brain membranes by graded concentrations of [Lys^7]dermorphin revealed two μ-binding sites, one of high and the other of low affinity (Negri et al., 1992).

Drugs were administered intracerebroventricoularly (i.c.v.) and subcutaneously (s.c.) in male rats (Wistar, 250 g b.w.). Antinociception was evaluated by tail-flick test, catalepsy by measuring the time the animal maintained an abnormal posture. [Lys7]dermorphin produced a long lasting antinociception both by central and peripheral route, but induced catalepsy only at high i.c.v. doses. The antinociceptive potency of [Lys^7]dermorphin exceeded that of morphine

Table 1. Antinociceptive and cataleptic effects of [Lys7]dermorphin compared to morphine and dermorphin.

TREATMENT	ANALGESIA ED_{50}		CATALEPSY ED_{50}
	i.c.v. nmol/rat	s.c. nmol/kg	i.c.v. nmol/rat
Morphine	10 ± 1.2	7000 ± 541	
Dermorphin	0.032 ± 0.005	2000 ± 185	0.12 ± 0.02
[Lys7]dermorphin	0.030 ± 0.004	300 ± 39	0.6 ± 0.07
• +NLX 0.1 mg/kg i.p.	0.30 ± 0.04	4500 ± 375	
• +NLXZ 10 mg/kg i.v.	0.35 ± 0.04	2600 ± 193	0.62 ± 0.08
• +NLT 10 mg/kg i.p.	0.04 ± 0.003		

300 times, by i.c.v. injection, and about 25 times by s.c. administration. The dose-response curves for antinociception were shifted to the right by pretreatment with naloxone (NLX, 0.1 mg/kg, i.p., -30 min) but naltrindole (NLT, 10 mg/kg, i.p., -20 min), a δ-opioid selective antagonist, did not modify the antinociceptive response. The μ_1-selective, non-equilibrium antagonist naloxonazine (NLXZ, 10 mg/kg i.v., -24 h) shifted to the right the [Lys^7]dermorphin dose-response curve by twelve times, when the peptide was administered i.c.v., and nine times when it was given s.c. Naloxonazine was not able to antagonize catalepsy induced by i.c.v. administration of high doses of [Lys^7]dermorphin. These data indicate that, in rats, the analgesic action of [Lys^7]dermorphin is largely mediated through naloxonazine-sensitive μ_1-opioid receptors, while its cataleptic action results from the activation of μ_2 opioid receptors, insensitive to naloxonazine.

i.c.v. daily injection of 100 and 200 ng/rat of [Lys^7]dermorphin reduced the antinociceptive effect by only 50% after 9 days treatment and shifted the s.c. dose-response curve to the right three times only. Catalepsy disappeared in the first four days.
There was no loss of body weight and naloxone precipitated withdrawal signs were negligible.

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123P MODIFICATION OF INTRACELLULAR MECHANISMS AFTER LONG TERM ANTIDEPRESSANT AND LITHIUM TREATMENT

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cAMP dependent protein kinase (PKA) is a central component of signal transduction for receptors coupled to activation of adenylcyclase. The enzyme is a tetramer consisting of two catalytic (C) subunits and a dimeric regulatory (R) subunit, which represents the receptor for cAMP. Binding of cAMP to R subunits causes the dissociation of the holoenzyme enabling free monomeric C subunits to phosphorylate specific substrate proteins which in turn are involved in the control of cellular functions (Taylor, 1989). Accordingly, the regulation of cAMP dependent phosphorylation system represents a possible target for psycotropic drugs. Our data show that, in rat cerebral cortex repeated administration of desmethylimipramine (DMI) (10 mg Kg^{-1} i.p. twice daily for 10 days), a tricyclic antidepressant, induces an increase in the amount of a protein band with Mr 52 KDa that for its biochemical properties can be the R subunit of PKA (Brunello *et al.*, 1990; Racagni *et al.*, 1992). Further characterization of this result was obtained by covalent binding with 8-N_3-[^{32}P] cAMP. Chronic DMI treatment enhanced by 187% the binding of [^{32}P] cAMP into 52 KDa cAMP receptor when compared to control animals (Perez *et al.*, 1989).
Similarly to what we observed after DMI treatment, prolonged administration of antidepressants that inhibit 5HT or NE uptake, such as fluoxetine and (+) oxaprotiline (10 mg Kg^{-1} i.p. twice daily for 10 days), was also able to increase by 150% the covalent binding to R subunits (Perez *et al.*, 1991). Since phosphoproteins represent the effectors of the biological response in a target cell, we analyzed the pattern of cAMP dependent phosphoproteins in rat cerebral cortex. We have found that chronic, but not acute DMI treatment modified the cAMP endogenous phosphorylation of a microtubule associated protein MAP_2 (Perez *et al.*, 1989). The amount of radioactive phosphate incorporated into MAP_2 after 8 and 15 days of DMI treatment was compared with that observed in control animals. The increase in the ^{32}P incorporation induced by DMI was 100% and 140% respectively ($P<0.001$) under basal conditions. Moreover an increase of 120% ($P<0.001$) in the incorporation of ^{32}P was observed under stimulated conditions (cAMP 5μM) when compared to the controls.
We have also studied the effects of Lithium (Li^+) on protein phosphorylation. Since the biochemical action of Li^+ could be due to competition with Mg^{++} at the level of Mg^{++} dependent reaction, we have investigated the in vitro action of Li^+ on cAMP dependent phosphorylation system, which is Mg^{++} dependent. The results have shown that the presence of Li^+ at therapeutic concentrations (1 mM) decreased of 40% ($P<0.001$) the cAMP dependent endogenous phosphorylation of MAP_2.
Experiments are in progress in our laboratory to investigate the effect of in vivo long term Li^+ treatment on cAMP dependent phosphorylation system.
In conclusion, our results demonstrate that cAMP dependent phosphorylation system can be a target for the action of antidepressant drugs and lithium suggesting its role in the pathogenesis of affective disorders.

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124P REGION-SPECIFIC DIFFERENCES BETWEEN SUBCELLULAR DISTRIBUTION AND ACTIVATION OF BRAIN ADENYLATE CYCLASE-CYCLIC AMP-PROTEIN KINASE A SYSTEM

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We found interesting differences in basal and forskolin-stimulated adenylate cyclase (AC) activity between discrete brain regions, with basal activity significantly higher in hypothalamus, cerebellum and brainstem (hb-AC) (277±12, 90.5±4.1, and 113.1±3.6 pmoles cAMP/mg protein/min, respectively), than in neocortex (frontal, parietal and occipital), striatum and hippocampus (lb-AC) (50.6±2.9, 75.8±1.4, and 53.6±2.6 pmoles cAMP/mg protein/min, respectively); hb-AC regions were characterized by a reduced response to stimulation by forskolin (3nM to 300nM) (+17.5±2.7%, +54±3.8%, and +37±1.5%), while lb-AC regions responded more potently to the diterpene compound, with a higher potency in the striatum (+91.8±11.2%, +287.5±4.7%, and +85.6±5%). These results led us to evaluate whether a region-specific pattern of PKA activation followed this varied AC response. lb-AC regions showed high levels of cytoplasmic holoenzyme, while low levels were found in cerebellum, brainstem and hypothalamus. Conversely, hb-AC regions showed cytosolic and nuclear levels of PKA catalytic subunit much higher than lb-AC regions. Also, the lowest levels of A Kinase Anchoring Protein (Glantz et al., 1992) were found in particulate fractions derived from lb-AC areas. These data could predict the existence of two functionally distinct modes of response to cAMP in the central nervous system. Furthermore, we stimulated slices (175 μm) obtained from neocortex, cerebellum, hippocampus and striatum with forskolin (10 μM) (15, 30, and 60 min) and found that, while cAMP-induced dissociation of the holoenzyme occurred in all areas, the traslocation to nucleus of PKA catalytic subunit only occurred in neocortex and striatum (+125±14.5%, and +35±5.3% at 15 min, respectively), suggesting a regional specificity also for PKA-mediated nuclear events. The semiquantitative PCR amplification of the mRNA encoding PKA regulatory subunits (RIα,β;RIIα,β) (Cadd & McKnight, 1989) demonstrated a higher degree of expression of RIIβ in areas where the stimulation by cAMP induced nuclear traslocation of PKA catalyst (striatum>neocortex>hippocampus>hypothalamus>brainstem>cerebellum). Taken together, our data point out that brain AC-cAMP-PKA system is characterized by region-specific differences, conceivably related to their different functions. The characterization of these region-specific differences of the AC-cAMP-PKA system could allow to evaluate the possible involvement of this system in the pathophysiology of neurological disorders such as neurodegenerative diseases and may represent an useful model to develop new pharmacological strategies.

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125P FUNCTIONAL ALTERATION OF G PROTEINS BY OPIATE EXPOSURE CORRELATES TO REDUCED NEURONAL PLASTICITY AND REGENERATION

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Perinatal treatment with morphine greatly affects reactive neuronal plasticity and regeneration in the rat brain and spinal cord following selective neurotoxic lesions. Following neonatal 5,7-hydroxytryptamine (5,7-HT) lesions (LES) serotonin (5-HT) axons regrow in the cortex and along the spinal cord. Such a process is preceeded by a transient hypergrowth of the short axon collaterals in the pons-medulla oblongata, as indicated by the increased 5-HT levels (C 8.1±0.6, LES 14.6±0.9 ng/mg prot.) a process which has been termed "pruning effect" (Gorio et al., 1993a). Neonatal 6-hydroxydopamine (6-OHDA) lesions (LES) cause noradrenalin (NE) denervation in the frontal cortex (C 7±0.1, LES 2.8±0.3). This loss is compensated by a reactive increase in 5-HT (C 24.6±3.2, LES 35.2±2.6), dopamine (C 6.6±0.2, LES 9.8±0.8) and met-enkephalin (C 5.4±0.6, LES 8.4±0.7) innervation. In morphine exposed rats the reactive compensatory events caused by 5,7-HT and 6-OHDA lesions are markedly suppressed as shown by biochemical and immunohistochemical data (Gorio et al., 1993a;1993b). By *in situ* hybridization we have monitored the expression of several molecules which might be involved in these effects. No changes were observed for trophic factors BDNF, NT3 and NGF gene expression, in any experimental condition evaluated, while morphine suppressed synapsin I and increased G_s and G_o protein expression, suggesting a potential role for G protein in neuronal plasticity. This hypothesis was directly confirmed by the ability of GTP-γ-S, added to the culture medium at the concentration of 10^{-8} M, of restoring NGF-induced neuritogenesis of pheocromocytoma PC12 cells, which was markedly impaired by morphine. PC12 cells were plated and grown as previously reported (Ferrari et al, 1983), morphine was added 6 hours after plating and the number of cells forming neurites after NGF addiction was scored according to established procedures (Ferrari et al, 1983). The inhibitory effect of morphine was dose dependent with a minor effect at concentrations of 10^{-10} M and with an inhibition of 50% at 10^{-6} M; higher doses were toxic. 10^{-8} M GTP-γ-S reversed the inhibitory effects of morphine applied at the dose of 10^{-6} M. Selective opiate agonists and antagonists effects on *in vitro* NGF induced neuritogenesis suggest the involvment of a receptor with mixed μ and δ properties. On the other hand the data also suggest that G proteins are directly responsible for the impaired neuronal plasticity triggered by opiates, without apparent involvement of trophic factors.

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126P PRODYNORPHIN GENE EXPRESSION IN DIFFERENT RAT BRAIN AREAS IN THE KINDLING MODEL OF EPILEPSY

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The term "kindling" refers to the phenomenon by which the repeated application of an initially subconvulsive stimulus evokes a progressive seizure activity which culminates in generalized tonic-clonic-convulsions. Previous investigations have suggested that electrical kindling induces specific alterations in the neuronal content of the opioid peptides (Iadarola *et al.*, 1986; Lee *et al.*, 1989). However, varying results have been obtained depending upon the site of application of the stimulus, the stage of kindling, the time elapsed after the last seizure and the brain areas investigated. Therefore, the aim of the present study was to evaluate the involvement of one of the three opioid precursors, prodynorphin, in amygdaloid kindling.
Male Sprague-Dawley rats (300±30 g) were stereotaxically implanted in the right amygdala with a bipolar electrode and stimulated once daily with a single one second train of pulses (bipolar 0.5 msec, 60 Hz, 100 μA above afterdischarge threshold) until three subsequent tonic-clonic convulsions were evoked. Animals were divided into 4 groups: *controls*, *sham-operated*, *kindled* (killed one week after the last convulsion) and *convulsive* (like the kindled group but receiving one more stimulation and killed one hour later). Brains were rapidly removed, areas were dissected and frozen at -80 °C. Total RNAs were extracted, electrophoresed and transferred on nylon membranes for hybridization to a specific cDNA probe (pBgBa), complementary to the 5'-translated region of the main exon of the prodynorphin gene. Messenger RNA levels were quantitated by densitometric analysis and reported as a percentage of control (equal to 100%).
In all tissues studied no significant differences were observed in sham-operated rats compared to controls. In the hippocampus, the levels of prodynorphin mRNA were significantly increased in the convulsive rats (275% vs controls) but not changed in the kindled group (120% vs control). In the striatum, the levels of prodynorphin mRNA were increased in the convulsive rats (143% vs control), but again unchanged in the kindled group (115% vs control). No significant differences among the groups were detected in the spinal cord. Other brain areas are presently under investigation.
Taken together, these results support the hypothesis that the dynorphinergic system is involved in kindled seizures in some rat brain areas. The possibility exists that the increased prodynorphin mRNA levels observed might be the expression of an increased release of dynorphin peptides requiring activation of the biosynthesis, immediately following kindling convulsions.

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127P MOLECULAR MECHANISMS CONTROLLING JUNCTIONAL AChR DEGRADATION RATE IN MUSCLE

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Mammalian muscles express two types of nicotinic AChRs, one made up by $2\alpha\beta\gamma\delta$ subunits and the other by $2\alpha\beta\varepsilon\delta$ subunits. The different subunit composition confers distinct electrophysiological properties to the receptor. In embryo, AChR molecules contain the γ subunit, are evenly distributed and metabolically unstable (t 1/2= 1-3 days). Following innervation, the receptor is clustered in the postsynaptic membrane, becomes metabolically stable (t 1/2= 8-10 days) and contains the ε subunit; in extrajunctional regions the synthesis of the receptor is suppressed. Following denervation, γ subunit containing and metabolically unstable AChRs appear extrajunctionally while the receptor at the endplate loses its metabolic stability. Two distinct kinetic population of junctional AChR (1) co-exist at the denervated neuromuscular junction : a) a slowly degrading population (R_S) is synthesised in innervated muscles or in denervated and electrically stimulated muscles (2); b) a rapidly degrading population (R_f) present in denervated muscles or in innervated muscles where activity had been suppressed by TTX block of the nerve or intramuscular injection of Botulinum toxin. Half-life of the R_f is about 1-2 days and is not stabilised by reinnervation or electrical stimulation (3). The subunit composition of the junctional receptor synthesised in denervated endplates and its possible role in controlling AChR metabolic properties have not been defined. To address these questions, we have used in situ hybridisation and immunofluorescence techniques to study the expression of the rat ε subunit in muscles where the R_f/R_S ratio had been experimentally manipulated. Antibodies against extracellular or intracellular epitopes of the ε receptor where obtained by immunising rabbits with specific synthetic peptides. Following denervation there is a progressive reduction of the intensity of the immunofluorescent labelling of the extracellular epitope of the ε subunit; similar results were obtained following chronic pharmacological denervation (TTX block of the sciatic nerve). Reinnervation or direct electrical stimulation of denervated muscles restored the intensity of the immunostaining. When antibodies directed against the intracellular epitope were used, no changes in the intensity of the immunoreaction were observed; the expression level of the ε subunit mRNA was also unaffected by the different experimental manipulations. The data suggest 1) that denervation modifies AChR subunit composition by interfering with assembly of the ε subunit in the mature receptor molecule; 2) subunit composition may be an important factor in defining metabolic stability of individual AChR molecules.

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128P MOLECULAR AND FUNCTIONAL PROPERTIES OF NICOTINIC RECEPTOR IN HUMAN SMALL-CELL LUNG CARCINOMA CELL-LINES

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Small-cell lung carcinoma (SCLC) cells express a number of membrane reccptors for hormones and neurotransmitters. These receptors control the generation of intracellular second messengers, some of which are involved in the control of physiological processes such as secretion or cell proliferation (Sorenson *et al*, 1991; Woll, 1991). Cholinergic receptors of the muscarinic type have been found in SCLC (Cunningham *et al.*, 1985). More recently we have shown that SCLC also expresses cholinergic receptors of the nicotinic type (Chini *et al.*, 1992; Tarroni *et al.*, 1992).

SCLC cells bind both [^{125}I]-α-bungarotoxin (B_{max}=130 fmoles/mg protein; K_d=4.9x10^{-8} M for NCI-N-592 cells) and [^{125}I]-κ-bungarotoxin ([^{125}I]-κ-bgtx; B_{max} = 44 fmoles/mg protein; Kd = 1x10^{-8} M), two selective snake polypeptides targeting specific nicotinic receptor subtypes. This binding is tissue-, time- and dose-dependent. The α-bgtx binding site present in SCLC is similar to its neuronal counterpart, not being recognized by a monoclonal antibody (mAb35) recognizing muscle but not neuronal α-bgtx receptors. Also, κ-bgtx defines a nicotinic receptor subtype previously believed to be expressed only in neurons. Specific [^{125}I]-κ-bgtx binding is displaced by only 45% with 1 μM α-bgtx, suggesting the presence of both as κ-selective receptors. Furthermore, SCLC cells bind the non-competitive nicotinic receptor antagonist [^{3}H]-TPMP, and this binding is allosterically stimulated in a dose-dependent manner by carbachol (250% stimulation at a carbachol concentration of 1 μM). SCLC cells do not express the muscle α_1 nicotinic receptor subunit, but express several other nicotinic receptor subunits such as α_3, α_5, α_7, β_2 and β_4: all these subunits are typically expressed in peripheral neurons.

Nicotine stimulates [^{3}H]-5-HT release from three different SCLC cell lines in a time- and dose-dependent and stereoselective way. Maximum release is achieved after 5 min at 37° with 1 nM of (-)nicotine, while the (+)isomer at the same dose is only half potent. Nicotine-stimulated release is completely dependent on external calcium and is blocked by the ganglionic nicotinic receptor antagonist mecamylamine (1 μM). Furthermore, nicotine-stimulated release is blocked by both α- and κ-bgtx, α-bgtx being more potent (IC_{50} = 1 nM and 10 nM for α- and κ-bgtx, respectively) and more effective (maximal inhibition of 80% and 60% for α- and κ-bgtx, respectively) than κ-bgtx, their effects being additive. This result indicates the involvement of both these toxin receptors in the control of the secretion process.

Taken together, these data suggest that different types of nicotinic receptors are expressed in human SCLC cell lines and that nicotine, interacting with specific nicotinic receptor subtypes, might induce the release of endogenous hormones such as 5-HT from SCLC cells and possibly in this way participate in the autocrine control of cell proliferation.

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129P EFFECTS OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASE INHIBITION BY ROLIPRAM AND ORG 9935 ON HISTAMINE-STIMULATED INOSITOL PHOSPHATE ACCUMULATION IN AIRWAY SMOOTH MUSCLE

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Inhibition of cyclic nucleotide phosphodiesterase (PDE) isoenzymes can attenuate the generation of agonist-stimulated total [^{3}H]inositol phosphate ([^{3}H]InsP) accumulation in bovine tracheal smooth muscle (BTSM) (Adams et al., 1992). We have previously shown greater than additive effects when the isoenzyme-selective PDE III and IV inhibitors, ORG 9935 and (ORG) rolipram (ROL), were used in combination to inhibit tonic or phasic contractions evoked by histamine (HIST) in this tissue (Adams et al., 1993). Here we have further investigated the nature of PDE III/IV inhibition on the HIST-stimulated [^{3}H]InsP response.

BTSM slices were maintained in culture medium for 24-48 h (Adams et al., 1992). Where indicated slices were labelled with 0.8 μCi [^{3}H]inositol/ml for 24 h. For incubations, slices were washed into oxygenated Krebs-Henseleit buffer (KHB) and 75 μl packed slices dispensed into 400 μl KHB ($\pm$ 0.8 μCi [^{3}H]inositol/ml and 5 mM LiCl). PDE inhibitors were added 30 min before addition of histamine (100 μM) for 30 min. Incubations were terminated and prepared for cyclic AMP or [^{3}H]InsP assay as described previously (Challiss et al., 1992).

Additions of either PDE inhibitor at concentrations considered to be PDE isoenzyme-selective (1 μM ORG and 100 μM ROL) failed to significantly increase cyclic AMP above basal levels (2.3 + 0.2 pmol/mg protein). However, despite this, ROL concentration-dependently (EC_{50} 120 + 27 nM) inhibited HIST-stimulated [^{3}H]InsP accumulation causing a maximum 76% inhibition at 100 μM (HIST, 23790 + 2922; HIST+ROL, 8099 + 512 d.p.m./mg protein; $P<0.01$). ORG (1 μM) alone caused an insignificant 19% inhibition of the HIST-stimulated [^{3}H]InsP response. Addition of ORG (1 μM) and varying concentrations of ROL caused a concentration-dependent increase in cyclic AMP (EC_{50} 280 + 180 nM: ORG + 100 μM ROL, 14.0 $\pm$ 2.5 pmol/mg protein). In the presence of ORG (1 μM), a maximal 77% inhibition of HIST-stimulated [^{3}H]InsP accumulation was observed at 0.1 μM ROL, with the concentration-response curve for inhibition by ROL being shifted approximately 100-fold to the left (EC_{50} 1.5 $\pm$ 0.1 nM). The ability of ORG (1 μM)-plus-ROL (0.1 μM) to inhibit HIST-stimulated [^{3}H]InsP accumulation was completely reversed by increasing the KHB concentration of KCl from 4.7 to 65 mM, but had no significant effect on BTSM cyclic AMP levels (in the presence of 100 μM HIST/1 μM ORG/0.1 μM ROL: 4.7 mM KCl, 8.0 $\pm$ 1.3; 65 mM KCl, 9.3 $\pm$ 1.8 pmol/mg protein).

These data demonstrate that although rolipram alone can cause marked inhibition of HIST-stimulated [^{3}H]InsP accumulation, the concentration of rolipram required to inhibit this response is dramatically decreased by co-addition of the PDE III inhibitor ORG 9935. Furthermore, the rolipram/ORG 9935 effect on the phosphoinositide response is completely prevented by elevating KCl, suggesting that membrane potential may be important in 'cross-talk' between the cyclic AMP and phosphoinositide second messenger pathways.

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130P ROLE OF INOSITOL (1,4,5)-TRISPHOSPHATE IN 5-HT-INDUCED CONTRACTION OF RABBIT MESENTERIC ARTERY

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5-Hydroxytryptamine (5-HT) stimulates phosphatidylinositol hydrolysis and inositol (1,4,5)-trisphosphate (IP_3) production in various cell types. This event may be an important component of 5-HT-induced contraction of vascular smooth muscle cells, as IP_3 stimulates contraction of blood vessels by releasing intracellular Ca^{2+} (Hashimoto *et al.*, 1986). However, our previous work suggests that 5-HT-induced contraction of permeabilized rabbit mesenteric artery strips did not involve intracellular Ca^{2+} (Seager & Garland, 1992). The present study examined 5-HT-induced contraction and IP_3 production in intact rabbit mesenteric arteries.

In functional studies, segments of endothelium-denuded, third order rabbit mesenteric arteries were mounted in a Mulvany-Halpern myograph in warmed, oxygenated Krebs' solution. 5-HT (0.01-100 μM) did not induce contraction of mesenteric arteries under normal conditions (n=4). However, in Krebs' solution containing 25.2 mM added K^+, 5-HT did induce concentration-dependent contraction (maximum tension = 1.81 $\pm$ 0.37 g, or 134.8 $\pm$ 27.6 % of contraction to 100 mM KCl, n=4). The high K^+ Krebs did not alter the basal tone of the arteries (14.7 mN). In biochemical studies, segments of rabbit mesenteric arteries were incubated in oxygenated, warmed Krebs' solution. Drugs or vehicle solution were added and the reaction terminated at varying times between 5 s and 5 min with 1 M ice-cold trichloroacetic acid. Aliquots were taken and the acid extracted by repeated washing with water-saturated diethylether. The amount of IP_3 in the samples was determined by competition binding to bovine adrenal cortex microsomes in the presence of [^{3}H]IP_3. 5-HT (100 μM) did not significantly increase IP_3 accumulation in rabbit mesenteric arteries at any time up to 5 min following its application (n=8). In contrast, noradrenaline (10 μM) caused a rapid, transient increase in the amount of IP_3 in rabbit mesenteric arteries, which peaked after 15 s at 19.15 $\pm$ 2.16 pmol/mg protein (n=5) representing 314 % of the corresponding basal level. In Krebs' solution containing 25.2 mM K^+, 5-HT (100 μM) did stimulate a transient increase in IP_3 levels, which peaked after 30 s at 14.96 $\pm$ 5.15 pmol/mg protein (n=7; 1500 % of the corresponding basal level). The 5-HT-induced IP_3 increase was abolished by the α_1-adrenoceptor antagonist prazosin (0.1 μM). A lower concentration of 5-HT (10 μM) failed to stimulate IP_3 accumulation in the rabbit mesenteric arteries in high K^+ Krebs.

These results suggest that IP_3 is not involved in the contractile effects of 5-HT in rabbit mesenteric artery. Depolarization of the mesenteric artery smooth muscle enhanced the contractile effect of 5-HT, but this was not due to IP_3 production. At high concentrations, 5-HT did stimulate IP_3 accumulation in high K^+ Krebs' solution, most probably mediated through activation of α_1-adrenoceptors.

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131P HOMOLOGOUS AND HETEROLOGOUS DESENSITISATION OF CO-EXISTING P_{2Y}-PURINOCEPTORS AND NUCLEOTIDE RECEPTORS ON ENDOTHELIAL CELLS

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Vascular endothelial cell function is regulated in part by receptors for ATP and ADP. On cultured bovine aortic endothelial cells (BAECs) these are co-existing P_{2Y}-purinoceptors and nucleotide receptors (Wilkinson *et.al.*, 1993; Motte *et.al.*, 1993) linked to phospholipase C. 2-Methylthio ATP (2MeSATP) is a selective agonist at P_{2Y}-purinoceptors and UTP is selective for nucleotide receptors. Here we report on the desensitisation of these two types of receptor by prior exposure to 2MeSATP or UTP.

Confluent BAECs at passage 2-5 in 24 well multiwells were stimulated, after 5 min preincubations, with either 2MeSATP (30μM) or UTP (300μM) and mass $Ins(1,4,5)P_3$ determined by the assay of Challiss *et.al.*, 1988. Preincubations were with either 2MeSATP or UTP at concentrations indicated, followed by 2 washes over 2 min, followed by stimulation.

Preincubation with 30μM 2MeSATP gave homologous desensitisation: the subsequent response to 2MeSATP was reduced by 45.2 ± 7.1% at 5s and 46.0 ± 4.8% at 10s. For example, at 5s the stimulation by 2MeSATP was reduced from 14.59 ± 2.4 pmols/well to 8.24 ± 0.61 pmols/well of $Ins(1,4,5)P_3$ (mean ± s.e. mean, n=4 separate experiments). Similar homologous desensitisation was seen with UTP (reduced by 64.6 ± 5.6% at 5s and 54.2 ± 6.4% at 10s). Preincubations with 2MeSATP and stimulation with UTP showed no heterologous desensitisation. Preincubation with UTP did cause heterologous desensitisation, with a subsequent response to 2MeSATP reduced by 44.7 ± 4.84% at 5s and 38.7 ± 1.9% at 10s. The EC_{50} of UTP as a desensitising agonist was essentially the same when stimulation was with either UTP or 2MeSATP.

To investigate a possible role of protein kinase C (PKC), the selective inhibitor Ro 31 8220 (10μM) was included in the preincubation, with subsequent stimulation for 5s. The response to stimulation with 2MeSATP was increased by 57 ± 4.5% (n=4) when Ro 31 8220 was included in the stimulation, while the response to UTP was unaffected. Ro 31 8220 had no effect on the homologous desensitisation caused by 2MeSATP preincubation. However, PKC inhibition did reduce both homologous and heterologous desensitisation caused by preincubation with UTP.

The results show that activation of P_{2Y}-receptors produced homologous desensitisation by a mechanism independent of PKC, and no heterologous desensitisation. However, activation of nucleotide receptors gives desensitisation of both nucleotide and P_{2Y}-receptors by a PKC dependent mechanism.

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132P SELECTIVE ADENOSINE A_1 AND A_2 AGONISTS INDUCE DIFFERENT HEMODYNAMIC EFFECTS AFTER SINGLE AND REPEATED ADMINISTRATION IN SPONTANEOUSLY HYPERTENSIVE RATS

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The acute administration of adenosine receptor agonists produces different hemodynamic effects in spontaneously hypertensive rats (SHRs), in accordance with their different selectivity for either A1 or A2 receptors (Webb et al.,1990). We compared the cardiovascular effects of four adenosine analogues, following acute and chronic intraperitoneal administration in conscious SHRs. We tested the A1 agonist CCPA (2-chloro-N6-cyclopentyladenosine; Lohse et al., 1988), the A2 agonists 2-hexynyl-NECA (2-hexynyl-5'-N-ethylcarboxamido adenosine; Cristalli et al., 1992) and CGS 21680 (2-[4-(2-carboxyethyl) phenethylamino]-5'-N-ethylcarboxamido adenosine; Hutchinson et al., 1989) and the non selective agonist NECA (5'-N-ethylcarboxamido adenosine). Systolic blood pressure (SBP) and heart rate (HR) were measured by the tail cuff technique. All agonists examined produced a dose-dependent decrease in SBP. The relative potency of the agonists in lowering blood pressure with respect to NECA (with 90% confidence limits) was found to be as follows: 2-hexynyl-NECA [2.06 (1.36-3.11)] > NECA [1] > CGS 21680 [0.08 (0.05-0.14)] = CCPA [0.08 (0.04-0.15)]. The A1 agonist CCPA induced a dose-dependent bradycardia, whereas the A2 agonists had only minimal influence on HR. The non-selective agonist NECA showed a biphasic effect, being inactive at low doses, which instead were effective on SBP, and inducing bradycardia at higher doses.
In the chronic study, the compounds were given twice daily for 21 days at doses which reduced SBP by 30% in acute experiments: NECA and 2-hexynyl-NECA at 0.01 mg/kg, CCPA and CGS 21680 at 0.1 mg/kg.
Baseline SBP values ranged between 197 ± 3 and 210 ± 2 mmHg. In treated groups there was a similar decrease in SBP after single dosing (114 ± 11, 109 ± 4, 115 ± 13, 132 ± 13 mmHg for CCPA, 2-hexynyl-NECA, CGS 21680 and NECA, respectively). This effect declined over time in the CCPA group, and SBP returned to baseline value at day 21 (201 ± 2 mmHg). Conversely, the other compounds maintained a significant antihypertensive activity throughout the experimental period. Treatments with 2-hexynyl-NECA, CGS 21680 and NECA did not influence HR, whereas CCPA induced marked bradycardia (from 406 ± 7 to 210 ± 24 beats/min), which gradually underwent tolerance, reaching 350 ± 23 beats/min at day 21. These data show that blood pressure lowering activity is not influenced by chronic treatment with compounds interacting selectively with A2 receptors, whereas prolonged exposure to a selective A1 agonist leads to tolerance to the hemodynamic effects.

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133P EFFECT OF ADENOSINE RECEPTOR BLOCKADE DURING ISCHAEMIC PRECONDITIONING ON A "SECOND WINDOW OF PROTECTION" IN RABBIT MYOCARDIUM

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Brief periods of ischaemia with intermittent reperfusion protect the myocardium against subsequent longer periods of ischaemia-reperfusion ("ischaemic preconditioning", PC, reviewed by Walker & Yellon, 1992). There is evidence that PC involves adenosine (A1) receptor activation, and that the protection following PC is an acute response, lasting no more than 1 to 2 h. However, using a standard PC protocol, we have observed a "second window of protection", evident *24 h following PC* (Marber et al, 1993) and have investigated if this delayed adaptive response, like the early short-term protection, is linked to adenosine receptor activation.

Male New Zealand White rabbits (2.0-3.5 kg) underwent midline thoracotomy and were assigned to either PC or sham operated groups. The pericardium was opened and a branch of the left coronary artery identified. In PC groups, the artery was ensnared with a silk ligature; in sham operated animals no ligature was positioned. The PC protocol consisted of four, 5 min occlusions each separated by 10 min reperfusion. During PC or the sham period, animals received i.v. treatment with either saline (controls) or the adenosine receptor antagonist 8-sulphophenyltheophylline (SPT; given as a 7.5 $mg.kg^{-1}$ i.v. bolus followed by infusion of 20 $mg.kg^{-1}$ over 1h). 24 h later animals were re-anaesthetised and the coronary artery branch was occluded for 30 min followed by 120 min reperfusion. Rate pressure product (RPP) was monitored throughout. The ischaemic risk zone (R) was determined *ex vivo* with fluorescent microspheres and the infarcted zone (I) determined with triphenyltetrazolium staining. Computerised planimetry was used to determine the volumes of these zones and the ratio I/R was calculated. Data are presented in Table 1 as mean± s.e.m. * $p<0.01$ compared with PC control group.

TABLE 1	Sham control	PC control	Sham + SPT	PC + SPT
n	9	9	6	6
Baseline RPPx10^3 ($mmHg.min^{-1}$)	18.8 ± 1.3	20.9 ± 1.4	21.1 ± 1.8	20.2 ± 1.8
R (cm3)	1.1 ± 0.1	1.2 ± 0.2	0.9 ± 0.1	1.0 ± 0.1
I/R (%)	53.0 ± 5.0*	30.6 ± 6.0	61.7 ± 5.4	56.8 ± 3.8*

24 h following the PC protocol, baseline RPP was similar in all groups. Although there were no significant differences in R between groups, the ratio I/R was significantly reduced in the PC control group, clearly demonstrating myocardial protection 24 h following the PC protocol. Moreover, the complete abolition of this protection by SPT treatment during PC suggests that this "second window of protection", as with the early phase of protection. may be mediated by an adenosine receptor-coupled pathway.

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134P A_1 ADENOSINE RECEPTOR SUBTYPE ACTIVATION SELECTIVELY INDUCES MEMBRANE HYPERPOLARIZATION IN CORTICAL BRAIN SYNAPTOSOMES

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Adenosine exerts a powerful inhibitory influence on neuronal activity which is mediated by a high affinity A_1 receptor and a lower affinity A_2 receptor . These receptor subclasses were originally characterized by their ability to inhibit (A_1) or stimulate (A_2) cyclic-AMP formation (Lindon, 1991) and by their pharmacological profiles to a variety of adenosine-related agonists and antagonists. Recently it has been suggested that activation of adenosine receptors in brain may increase K^+ conductance leading to membrane hyperpolarization (Trussel & Jackson, 1985). In this study we investigated the effect of high selective A_1 and A_2 agonists on K^+-induced membrane depolarization in rat cortical brain Percoll purified synaptosomes. To this aim, the lipophylic anion bisoxonol, that has been widely used to monitor membrane potential changes because its fluorescence emission increases when it moves from polar solution to non polar environments, has been utilized (Taglialatela *et al.*, 1990). The high selective A_1 agonist, 2-chloro-N_6-cyclopenthyladenosine (CCPA) decreased K^+-induced membrane depolarization in a concentration-dependent manner in cortical brain synaptosomes (Fig. 1). This effect was counteracted by 8-cyclopenthyl-1,3-dipropylxantine (DPCPX), a high selective A_1 receptor antagonist, (Fig. 2) whereas 9-chloro-2-(2-furanyl)-5,6-dihydro-1,2,4-triazolo(1,5-c)quinazoline-5-imine (CGS 15943A) a selective A_2 receptor antagonist was uneffective.

Figure 1

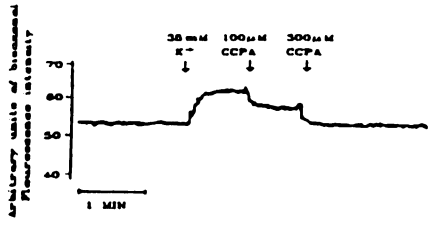
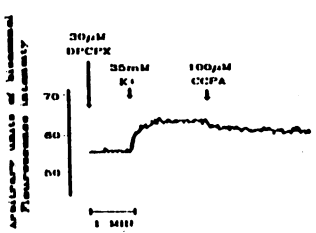


Figure 2



Finally, 2-p-(2-carboxyethyl)phenethylamino-5-N-ethylcarboxamido adenosine (CGS 21680) a selective A_2 receptor agonist did not induced any changes of K^+-induced membrane depolarization. Collectively these results seem to suggest that A_1 rather than A_2 receptor subtype activation induces membrane hyperpolarization.

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135P EFFECTS OF CHRONIC TREATMENT WITH SELECTIVE A_1 AND A_2 ADENOSINE AGONISTS ON ADENOSINE AND DOPAMINE RECEPTOR BINDING IN RAT BRAIN

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There is considerable evidence that adenosine acts as an inhibitory modulator of neuronal activity in the mammalian central nervous system (Williams, 1989). Recent behavioral and biochemical studies have also demonstrated the existence of interaction between adenosine and dopamine systems (Ferre' et al., 1991). Thus, we have designed experiments in order to assess changes of adenosine and dopamine receptor parameters after repeated administration of selective adenosine receptor agonists. Sprague-Dawley rats were treated for 12 days by two daily intraperitoneal injections of the A1 selective agonist 2-chloro-N6-cyclopentyladenosine (CCPA) and the A2 selective agonist 2-hexynyl-5'-N-ethyl--carboxamidoadenosine (2-HE-NECA) at doses of 0.3 and 0.03 mg/Kg, respectively. The doses were two times the ED50, that is the dose producing protection to pentylenetetrazole-induced lethal seizures. Saturation experiments at A1 and A2 adenosine receptors were carried out on cortex and striatal membranes using the agonists [3H]CHA and [3H]CGS 21680. As for striatal D1 and D2 dopamine receptors [3H]SCH 23390 and [3H]raclopride were used as radioligands. Results are summarized in the following table:

Drug Treatment	A1		A2		D1		D2	
	Kd (nM)	Bmax (fm/mg prot)	Kd (nM)	Bmax (fm/mg prot)	Kd (nM)	Bmax (fm/mg prot)	Kd (nM)	Bmax (fm/mg prot)
VEHICLE	1.1	468	26	1281	0.2	1400	1.4	267
CCPA	1.2	467	24	1228	0.2	1385	1.0 *	269
2-HE-NECA	1.3	510	24	1328	0.2	1137	1.2	283

* $p < 0.05$ as compared to control (Dunnett' test)

Neither binding affinity (Kd) nor number of binding sites (Bmax) for A1 and A2 receptors was affected by the repeated treatment with selective A1 or A2 agonists. Likewise, no changes occurred in the affinity or density of [3H]SCH 23390 binding sites. Conversely, treatment with CCPA increased [3H]raclopride binding affinity by 29%, but did not change the number of binding sites. The data indicate that the repeated administration of selective agonists for either A1 or A2 receptors does not lead to change of adenosine receptor binding parameters. Moreover, the results provide support to other studies showing an interaction between adenosine and dopamine systems.

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136P OPPOSITE EFFECTS OF ADENOSINE AND ATP ANALOGUES ON ASTROGLIAL CELLS IN RAT BRAIN PRIMARY CULTURES

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Purines, namely adenosine and ATP, exert potent and specific biological activities through the activation of P1 and P2 receptors, respectively (Burnstock, 1993). Whereas neuromodulation of brain function by adenosine is well documented (Rudolphi, 1992), it was only recently suggested that ATP could also play a key role in CNS neurotransmission (for review, see Burnstock, 1993). Although most studies have focussed on the possible effects of purines on neuronal cell functions, P1 and P2 receptors are also expressed by astrocytes, which might suggest a role of purines in physiological regulation of glial cell functions and in reactive gliosis following trauma, ischemia and hypoxia (Rathbone, 1992). Based on this, we deemed it interesting to study the effects of purine analogs on astroglial cells in rat CNS primary cultures obtained from the c. striatum of 7 day-old rats. Cultures were grown in complete medium in the absence or presence of the purine analogs 2-chloro-adenosine (2-CA) or αβ-methylene-ATP (αβmeATP) for 2-4 days, fixed and processed for immunocytochemistry utilizing primary antibodies raised against glial fibrillary acidic protein (GFAP, an astroglial marker) or protein gene product (PGP 9.5, a neuronal marker). Cultures were then analyzed with a fluorescence microscope. Exposure to αβmeATP resulted in a significant and dose-dependent increase in the number of GFAP-positive cells, whereas 2-CA exerted opposite effects on the same parameter. For example, after 3 days in presence of 10^{-6}, $5x10^{-6}$ and 10^{-5} αβmeATP, the number of astroglial cells (% of control cell number set to 100%) was 109±4 ($P=0.05$), 128±5 ($P<0.02$) and 147±3 ($P<0.007$), respectively. Conversely, after growing cells for 3 days in presence of 10^{-5}, $5x10^{-5}$ and 10^{-4} M 2-CA, the number of GFAP-positive cells (% of control cell number set to 100%) was 85±3 ($P<0.05$), 72±5 ($P<0.002$) and 57±3 ($P<0.0001$), respectively. Effects were selective for glial cells, since neither the P1 nor the P2 agonist significantly affected the number of neuronal cells in culture, as determined by PGP 9.5 immunolabelling. Finally, the αβmeATP effect was antagonized by culturing cells in the presence of suramin, suggesting the involvement of either P2x or P2y receptor subtypes; conversely, effects induced by 2-CA were not reversed by xanthine derivatives, suggesting the involvement of a xanthine-insensitive adenosine receptor subtype (Abbracchio et al., 1993). These results therefore suggest that purines may modulate astrocyte proliferation in opposite ways in vivo through the activation of P1 and P2 receptors. This might be of importance in regulating astroglial cell function in CNS responses to injury and ischemia.

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137P ADENOSINE RELEASE MEASURED BY TRANS-STRIATAL DIALYSIS IN ADULT AND AGED FREELY MOVING RATS

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The extracellular adenosine level in the striatum of adult (3 months) and aged (24 months) Charles River rats was measured by transverse microdialysis (Pazzagli et al., 1993). During surgery rats were anaesthetized with chloral hydrate (400 mg/Kg i.p.). The fibre was perfused at a flow rate of 2 µl/min. Adenosine recovery through the dialysis membrane "in vitro" was 13%. Adenosine concentration was determined using reverse phase HPLC with a fluorimetric detector. The minimal detectable amount of adenosine was 0.1 pmol. The adenosine concentration (values not corrected for recovery) 24 h after surgery in freely moving rats was 0.0151±0.0009 µM in adult rats and 0.0153±0.0015 µM in old rats, calculated as the mean of the first 4 samples. The inhibitor of adenosine deaminase erythro-2-(hydroxy-3-nonyl)adenine (100 µM, EHNA) induced a mean percent increase of 168±11% in adult rats and of 125±11% in aged rats (Fig. 1). Two-way ANOVA showed that EHNA affected adenosine release in old and young rats differently ($P<0.01$). The percent increase of adenosine release evoked by perfusion with 100 mM potassium over the control value (calculated from the areas under the curve) was 316±45% in adult rats and 208±40% in old rats (Fig. 1). The difference was statistically significant (Student's two-tailed t test, $P<0.05$). The findings demonstrate that the basal extracellular striatal adenosine levels of adult and aged rats are similar, and suggest lower activity of the enzyme adenosine deaminase and that less adenosine is available for release by K^+ in aged rats.

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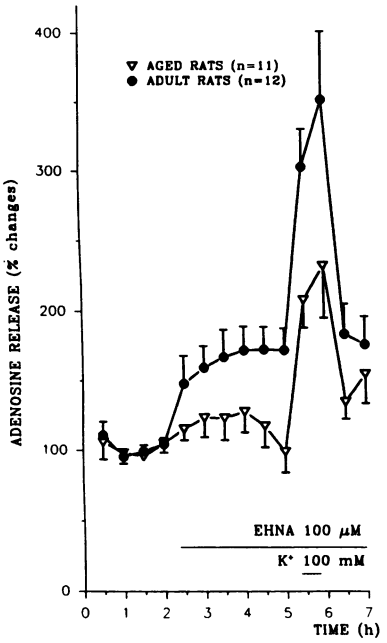


Fig. 1 Effect of EHNA and potassium on adenosine release from adult and aged rats.

138P ADENOSINE SELECTIVITY IN REVERSING DRUG-INDUCED CONTRACTIONS IN RAT DUODENUM

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The relaxation of vascular smooth muscle induced by adenosine via P_1-receptors of the A_1 subtype involves the opening of K^+ channels (Markel et al., 1992). Also in rat duodenum adenosine exerts a relaxing effect that is mediated by P_1-purinergic receptors (Gaion et al., 1988; Nicholls et al., 1992) localized on smooth muscle cells (Serio et al., 1990).

In order to stress the possible role of K^+ channels in adenosine action in this intestinal preparation, experiments were performed on rat duodenum segments mounted in isolated organ baths containing 10 ml of Tyrode solution, as previously described (Gaion et al., 1988). Changes in tension were recorded isometrically. Data are means ± SEM of the results obtained in 8 to 12 preparations.

The three K^+ channel blockers, tetraethylammonium (TEA), 4-aminopyridine (4-AP) and glibenclamide exerted different effects, in as much as TEA, up to 10 mM caused concentration-dependent contractions, the contractile effect of 4-AP was lower as compared to TEA and it was converted into a relaxing one at concentrations higher than 1 mM, while glibenclamide induced only smooth muscle relaxation. Addition of 0.5 mM adenosine during the tonic phase of drug-evoked contraction caused a rapid decrease of muscle tension: the effect of 4-AP (1 mM) was completely reversed (-94.7±8.1 %) by adenosine and contractions induced by 5 mM TEA were reduced by 73.1±4.7 %. Similarly, adenosine markedly relaxed (-70.4±8.1 %) preparations contracted by 50 µM ouabain, an inhibitor of Na^+-K^+ ATPase. By contrast, the response to 30 mM KCl, that originates from a direct depolarization of smooth muscle cell membrane, was insensitive to adenosine (-8.3±3.0 %). Contractions induced by the two receptor agonists, acetylcholine (1 µM) and 5-hydroxytryptamine (1 µM), were only partially reversed by the nucleoside: -18.7 ±2.4 % and -41.6±5.4 %, respectively. Verapamil (0.2 µM), a drug that blocks voltage operated calcium channels, fully reversed the effects of TEA, 4-AP, ouabain, KCl, 5-hydroxytryptamine and reduced by 85.2±5.3 % the tonic response to acetylcholine.

These results show that the tonic component of the contractile action exerted by a variety of agents on rat duodenum preparations strictly depends on calcium entrance through voltage operated channels, a process that is inhibited by verapamil. On the other hand the ability of adenosine to counteract the muscle response to these contracting agents is related to the specific cellular mechanism leading to calcium influx. The responses evoked by 4-AP, by TEA and by ouabain were by far the most sensitive to the action of adenosine, indicating that K^+ flux across the plasma membrane might be selectively affected by the nucleoside.

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139P PRELIMINARY EVIDENCE FOR A NOVEL ENDOTHELIN RECEPTOR SUBTYPE IN HUMAN SMALL BLOOD VESSELS

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ET_A and ET_B receptors mediate contractions of blood vessels from animals (Sumner et al., 1992; Moreland et al., 1992), however little is known about the receptor subtype mediating responses to endothelin in human vessels. In the present study, the receptor mediating responses to endothelin in small arteries and veins from human omentum was characterized using endothelin-1 (ET-1), endothelin-3 (ET-3), the ET_B receptor agonist sarafotoxin 6c (S6c) and the ET_A receptor antagonist BQ123 (Ihara et al., 1992).

Small arteries (413 $\pm$ 22 μm) and parallel running small veins (646 $\pm$ 35 μm) were dissected from human omentum and mounted in a myograph under a normalized tension equivalent to 90% of a transmural pressure of 100 mm Hg and 19 mm Hg, respectively. After equilibration, endothelial integrity was determined by measuring relaxations to acetylcholine (ACh; 1 μM) and bradykinin (BK; 1 μM). After washout, contractions to S6c (0.01-100 nM), ET-1 (0.01-100 nM) and ET-3 (0.1-1000 nM) were measured.

In arteries submaximally precontracted with U46619 (9α,11α-methanoepoxy-prostaglandin $F_{2\alpha}$; 0.1 μM), ACh caused 43 $\pm$ 6% relaxation. No relaxation to ACh was seen in veins, however. Arteries and veins relaxed virtually completely to BK. Neither small arteries nor veins contracted to S6c. In small arteries and veins, ET-1 caused a concentration-dependent increase in wall tension (E_{max} = 3.90 $\pm$ 0.56 mN/mm and 1.90 $\pm$ 0.32 mN/mm resp., $P<0.05$) and was equipotent (arteries: pD_2 = 8.91 $\pm$ 0.11; veins: pD_2 = 8.63 $\pm$ 0.08, NS). BQ123 (0.03 - 3 μM) produced a rightward parallel displacement of the ET-1 concentration response curve, yielding pA_2 values of 7.09 and 7.48 in arteries and veins respectively. In veins, the concentration response curve to ET-3 was biphasic showing a BQ123 resistant, high sensitivity component (pD_2 = 8.80 $\pm$ 0.20) and a low sensitivity component (pD_2 = 7.11 $\pm$ 0.06) which was blocked by 3 μM BQ123. In contrast, small arteries did not contract to ET-3 (up to 1000 nM).

These data suggest the presence of at least two different endothelin receptors mediating contractions in human small vessels. One subtype has the profile of an ET_A receptor, is present in arteries and veins and mediates the most part of the ET-1 induced contractions. The other subtype is present in veins and mediates contractions to ET-3 but not S6c, thus being different from the ET_B receptor subtype previously described.

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140P PRE- OR POST-ADMINISTRATION OF BQ123 AND FR139317 ANTAGONIZES ENDOTHELIN-1 (ET-1)-INDUCED CONTRACTION OF HUMAN BLOOD VESSELS *IN VITRO*

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We have compared the potency of the modified tripeptide FR139317 (Sogabe et al., 1992) to the human ET_A receptor antagonist BQ123 (Molenaar et al., 1992, Davenport et al., 1993) in preparations of isolated human saphenous vein (SV), internal mammary artery (IMA) and coronary artery (CA). The long lasting vasoconstrictor activity of ET-1 may limit the therapeutic usefulness of such antagonists. We therefore tested the ability of BQ123 to relax saphenous vein preconstricted by ET-1.

Sections of human SV, IMA and CA, cut into 2mm rings with endothelium removed, were mounted under 2g resting tension in 25ml organ baths containing oxygenated Krebs at 37°C. ET-1 dose-response curves (DRC's) were constructed in the absence and presence of BQ123 (0.3-3μM) or FR139317 (0.1-3μM) added to the bathing medium 30 minutes previously. ET-1 responses were expressed as a % of the contraction elicited by 50mM KCl.
Rings of SV were preconstricted with ET-1 (10^{-8}M) and at peak response BQ123 (1-3μM) was added to the bathing medium. The time required for the contraction to be reduced by 50% ($t_{1/2}$) was determined and compared to control preparations run in parallel (Student's paired t-test).

BQ123 and FR139317 produced dose-dependent rightward shifts of the ET-1 DRC's with Schild derived pA_2 values in SV, IMA and CA for BQ123 of 6.4, 7.4 and 7.0 and for FR139317 of 6.9, 7.9 and 7.2 respectively ($n \geq 6$). In each case the slope of the regression line was not significantly different from unity.
10^{-8}M ET-1 produced a long lasting contraction of SV with $t_{1/2}$ = 70±20 minutes (n=5). On addition of 1μM BQ123 this was reduced to 12±2 minutes (n=4) and by 3μM BQ123 to 8±2 minutes. In both cases this was significantly different from control ($p \leq 0.05$).

These data show that a linear tripeptide structure is sufficient to retain potent ET-1 antagonist activity and may provide useful structural information for the synthesis of novel non-peptide antagonists. It is also clear that ET-1-mediated vasoconstriction can be attenuated by pre-or post-administration of antagonists in human isolated tissues.

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141P QEEG ANALYSIS OF BENZODIAZEPINE-LIGANDS WITH DIFFERENT CLINICAL-PHARMACOLOGICAL PROFILES

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Evidence, from pharmacology to molecular biology, suggests the presence of a heterogeneous population of $GABA_A$ receptors, each of which has been hypothesized to subserve different physiological functions. A major interest studying benzodiazepines (BZs), a class of molecules acting through GABA-gated chloride channels, is thus to determine if a correlation exists between specific receptor activation and the associated physiological outcome. Understanding these relationships would be of great relevance when developing new molecules with a predetermined receptor subtype selectivity. To characterize the molecular properties of diazepam (the BZ prototype), triazolam, zolpidem (hypnotic BZs), bretazenil, and imidazenil (high potency-low efficacy BZs), we applied a quantified electrocortical activity (QEEG) analysis in freely moving rats (n=5). The spectral profiles were studied over a 30-minute period after administration of increasing doses (0.3, 1.0 and 3.0 mg/Kg i.p.) of the above substances. All rats were treated with all drugs according to a Latin-squared design. Data (mean % changes ± S.D.) were analyzed by means of a 2-way-ANOVA and post-hoc Tukey's test. The lowest dose of diazepam produced a selective increase in the energy of the high β band (46±11), whereas 1 and 3 mg/Kg raised ($P<0.05$) the power of both α (63±32, 71±26) and β (75±28, 148±21) bands, confirming the anxiolytic effect of this drug. Triazolam, with greater efficacy than diazepam, increased ($P<0.05$) the energy of α (19±7, 61±15, 65±9) and β (128±11, 189±13, 245±21) bands and concomitantly reduced ($P<0.05$) the power of δ (-2 ±4, -34±12, -40±8) and τ (-39±3, -47±3, -56±4) bands. On the other hand, zolpidem brought about an increase ($P<0.05$) in low δ waves (7±5, 18±3, 16±4). These data indicate that differences exist in the mechanisms of action of triazolam and zolpidem, which are otherwise marketed with analogous clinical indications. Finally, bretazenil and imidazenil similarly enhanced, at the two higher doses, the energy of α (bretazenil: 21±16, 5±15; imidazenil: 18±15, 20±19) and β (bretazenil 47±9, 142±37; Imidazenil 60±41, 109±58) bands, the effects being significant ($P<0.05$) only in the β band. The above results are viewed in the context of the proposed classification of positive modulators of $GABA_A$ receptors as: i) full allosteric modulators (FAM), ii) selective allosteric modulators (SAM), iii) partial allosteric modulators (PAM) (Ducic *et. al*, 1993). Accordingly, triazolam should be considered as a FAM; diazepam and zolpidem as SAMs; bretazenil and imidazenil as PAMs. In conclusion, our findings confirm that the QEEG technique could be particularly useful in predicting or exploring the pharmacological effects of new BZ ligands.

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142P MODULATION OF $GABA_A$ RECEPTOR FUNCTION BY IMIDAZENIL AND ABECARNIL: TWO NEW ANXIOSELECTIVE AND ANTICONVULSANT DRUGS

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The imidazobenzodiazepines, imidazenil (IMZ) and bretazenil (BTZ) and the ß-carboline derivative abecarnil (AB) have been recently presented as benzodiazepine (BZ) receptor ligands able to induce anxiolytic and anticonvulsant effects with less sedation, no motor impairment and reduced ethanol potentiation. We studied the pharmacological activity of these drugs by evaluating "in vitro" and "in vivo" their effects on $GABA_A$ receptor function. Added "in vitro" to rat and mouse cortical membrane preparations, AB increased [^{3}H]GABA binding (+35% at 3 μM, $p < 0.01$), enhanced 5 μM muscimol-stimulated $^{36}Cl^-$ uptake (+35% at 10 μM, $p < 0.01$) and reduced [^{35}S]t-butylbicyclophosphorothionate (TBPS) binding (-60% at 30 μM, $p < 0.01$), BTZ showed very week efficacy (less than 15% changes at 30 μM) while IMZ was completely uneffective in these biochemical tests. In vivo administration of IMZ to mice failed to affect [^{35}S]TBPS binding measured "ex vivo" in the cerebral cortex. In contrast, AB induced a time-dependent (15-180 min, $p < 0.01$) and a dose-related decrease of this parameter. AB started to decrease [^{35}S]TBPS binding at the dose of 0.05 mg/kg (-38%, $p < 0.01$), its effect was maximal (-70%, $p < 0.01$) at the dose of 1 mg/kg and higher doses (up to 20 mg/kg) failed to further decrease [^{35}S]TBPS binding. IMZ (0.05-1 mg/kg, $p < 0.05$), like AB (0.05-01, $p < 0.01$) reduced the increase (+45%, $p < 0.01$) of [^{35}S]TBPS binding in the brain of mice treated with isoniazid (200 mg/kg). IMZ at very low doses (0.05-0.3 mg/kg) markedly delayed the onset of convulsions and death elicited by isoniazid (20-45 min, $p < 0.01$). Moreover, an higher dose (1 mg/kg) of IMZ and a low dose (0.1 mg/kg) of AB completely antagonized the effect of isoniazid. On the contrary BTZ was uneffective in antagonizing the effects of isoniazid up to 15 mg/kg. Finally, IMZ and AB (0.5-1 mg/kg) significantly antagonized ($p < 0.01$) the increase of [^{35}S]TBPS binding induced by foot shock or carbon dioxide exposure (+30%, $p < 0.01$). Our results demonstrate that the efficacy of the new anxiolytic and anticonvulsant compounds IMZ and AB on $GABA_A$ receptor function is quite different. AB enhances "in vivo" and "in vitro" GABAergic transmission with an efficacy comparable to its pharmacological activity while IMZ shows a great pharmacological activity in spite of its very low intrinsic efficacy in enhancing the function of GABAergic transmission.

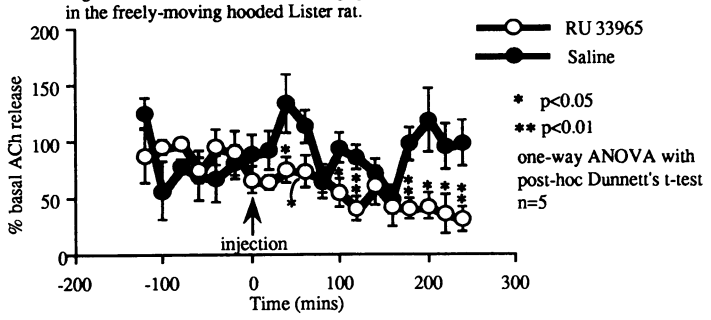
This study was in part supported by grant 92.00022.PF41 from the National Research Council (CNR) [Target project "Prevention and Control Disease Factor"]; Subproject "Stress".

143P EFFECTS OF THE BENZODIAZEPINE PARTIAL INVERSE AGONIST, RU 33965, ON HIPPOCAMPAL ACETYLCHOLINE RELEASE IN THE FREELY-MOVING RAT: A MICRODIALYSIS STUDY

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On the water maze, cognitive deficits induced by atropine can be reversed by benzodiazepine (BZ) inverse agonists in a dose dependant manner (Guy *et al.*, 1990). This putative procognitive action of the BZ inverse agonists can in turn be reversed by BZ antagonists. These studies suggest that there may be a possible interaction at a functional level between systems which utilize cholinergic and BZ receptors. This study investigated the effects of the BZ inverse agonist, RU 33965 (5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1, 5-a] [1,4] benzodiazepin-3-yl-cyclopropyl methanone) on acetylcholine (ACh) release in freely-moving rats in an attempt to correlate the behavioural effects with those seen *in vivo*, using the technique of microdialysis.

Figure 1. Effect of RU33965 (50 mgkg^{-1} i.p.) on hippocampal ACh release in the freely-moving hooded Lister rat.



A microdialysis probe was perfused at a rate of 2µlmin^{-1} with a modified Ringer's solution (containing 2µM neostigmine bromide) prior to surgery. Male hooded Lister Rats (250-350g), were anaesthetised using a halothane (2%), N_2O/O_2 (1/2, v/v) mixture and the probe stereotaxically implanted (while flowing) into the hippocampus. After surgery and overnight the perfusion rate was reduced and the next day returned to 2µlmin^{-1} for at least one hour prior to sample collection. Samples were then taken every 20 min and snap frozen. The ACh content of dialysates was measured by an HPLC-ED system based upon the methodology of Damsma *et al*, 1987 and modified by the addition of a second immobilised enzyme reactor, containing choline oxidase alone, before the analytical column (Aspley *et al.*, 1991).

The BZ partial inverse agonist, RU 33965 (50 mgkg^{-1} i.p., n=5) significantly decreased ACh release in freely-moving hooded Lister rats when compared with saline controls (n=5) over a post injection period of 4h (Figure 1) while at a lower dose (10mgkg^{-1} i.p., n=5) the drug had no significant effect. In addition, the ACh levels increased immediately after injection in all controls and animals treated with the low dose, due to handling; this increase was not apparent in animals treated with the high dose. The ACh levels measured where shown to be of neuronal origin as they were abolished by the administration of TTX (10^{-6}M).
These results suggest that the putative procognitive effects of RU 33965 in behavioural tests may be due to a post-synaptic action (since microdialysis measures pre-synaptic events) and since the present experimental conditions suggest that the drug acts as an BZ agonist rather than as an inverse agonist.

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144P SB 200646A: A SELECTIVE 5-HT$_{2C}$ RECEPTOR ANTAGONIST

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Although the 5-HT$_{1C}$ (now called the 5-HT$_{2C}$) receptor was first identified in 1984 (Pazos et al) no selective antagonists for this receptor have yet been described. Indeed the 5-HT$_{2C}$ receptor is closely related to the 5-HT$_{2A}$ receptor and many so-called selective 5-HT$_{2A}$ (formerly the classical 5-HT$_2$) receptor antagonists display marked affinity for the 5-HT$_{2C}$ receptor. We now report the biochemical profile of the first selective 5-HT$_{2C}$ receptor antagonist, SB 200646A (N-(l-methyl-1H-indol-5-yl)-~-(3-pyridyl) urea.HCl (SB 200646A).

Radioligand binding studies were as outlined in Blackburn, et al., (1993), notably using [^{3}H]-mesulergine (in the presence of 30nM spiperone) in pig choroid plexus and [^{3}H]-ketanserin (both at 0.5 nM) in rat frontal cortex to label 5-HT$_{2C}$ and 5-HT$_{2A}$ receptors respectively. Phosphoinositide (PI) hydrolysis was followed according to Brown, et al., (1991).

SB 200646A had moderate affinity for the 5-HT$_{2C}$ receptor (pK$_I$ 6.87 ± 0.12, mean ± SEM (6)) and low affinity for the 5-HT$_{2A}$ receptor (pK$_I$ 5.18 ± 0.07 (10). Further SB 200646A showed negligible affinity (pK$_I$ <5) for a wide variety of binding sites including 5-HT$_{1A}$, 5-HT$_{1D}$, 5-HT$_3$, 5-HT$_4$, adrenergic-α_1,-α_{2A},-α_{2B},M_1,M_2,D_1,D_2,opioid-µ,k,δ,and GABA$_A$/benzodiazepine. In the pig choroid plexus, SB 200646 attenuated the potency of 5-HT without affecting the maximal response with an apparent pK$_B$ of 7.0 ± 0.18 (3). SB 200646A had no agonist-like activity in this model.

In conclusion, SB 200646A appear to be a selective and competitive antagonist at the 5-HT$_{2C}$ (formerly 5-HT$_{1C}$) receptor. It should prove to be a useful tool in elucidating the pathological and physiological role for this receptor.

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145P EVIDENCE INDICATING THAT 5-HT_{1A} RECEPTOR MECHANISMS ARE NOT INVOLVED IN IDAZOXAN-INDUCED HYPERPHAGIA

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The α_2-adrenoceptor antagonist, idazoxan, increases food intake in freely-feeding rats (Jackson et al., 1991). The mechanism whereby α_2-adrenoceptor antagonists increase feeding is unclear but is correlated with affinity for the non-adrenergic idazoxan binding site (Jackson et al., 1991). 5-HT_1 receptors may also be involved, since the hyperphagia is blocked by (-)-propranolol (a non-selective 5-HT_1 and β-adrenoceptor antagonist) but not by selective β-adrenoceptor antagonists (Jackson & Nutt, 1992). We have investigated idazoxan-induced hyperphagia and compared it with the hyperphagia induced by the 5-HT_{1A} receptor agonist, 8-OH-DPAT, and the α_2-adrenoceptor agonist, clonidine. Thus, we have examined the effect of pretreatment with: i) the selective 5-HT uptake inhibitor, WY-27587 (Fletcher et al., 1988), ii) (-)-propranolol, iii) a dose of 8-OH-DPAT which appears to desensitise 5-HT_{1A} somatodendritic autoreceptors (Kennett et al., 1987) and iv) the selective 5-HT_{1A} antagonist, (±)-WAY-100135 (Fletcher et al., 1993), on the hyperphagia induced by idazoxan, 8-OH-DPAT and clonidine.

Free feeding rats (male 250-400g; n=8-10) were individually housed. All compounds were injected s.c. (at 10.00 h), with WY-27587, (5, 10 or 15mg/kg), (-)-propranolol (10mg/kg), 8-OH-DPAT (1mg/kg), (±)-WAY-100135 (10mg/kg), or the appropriate vehicles administered 30 minutes (or 1 day later for 8-OH-DPAT) before the hyperphagic agents or vehicle. The animals were then allowed access to food contained in weighed food hoppers which were reweighed 1 and 3 h after dosing to allow calculation of food intake.

Idazoxan (10 & 30 mg/kg), 8-OH-DPAT (0.1 & 0.3 mg/kg) and clonidine (0.01 & 0.03 mg/kg) induced dose related increases in food intake (1.4 - 2.7g greater than the control intake of 0.3 - 0.4g) 1-3 h after dosing. Pretreatment with WY-27587, or (-)propranolol, significantly ($p<0.05$) attenuated the hyperphagic response to all three agents. 24h after a dose of 1 mg/kg of 8-OH-DPAT there was a significant ($p<0.001$) reduction in the amount of food consumed in response to a subsequent 8-OH-DPAT challenge (0.3 mg/kg), (3 h intake = 2.1g) compared with control animals (3h intake = 4.2g). However, the response to clonidine or idazoxan was unaffected by 8-OH-DPAT (1mg/kg) pretreatment. The 5-HT_{1A} antagonist, (±)-WAY-100135, (3 & 10mg/kg s.c.) blocked ($p<0.05$) the hyperphagic effect of 8-OH-DPAT (0.1mg/kg), but had no effect on the response to clonidine or idazoxan.

Inhibition of 5-HT uptake blocked the hyperphagic effect of idazoxan, 8-OH-DPAT and clonidine implicating serotonergic mechanisms in the mediation of increased food intake induced by these compounds. Furthermore, the non-selective 5-HT antagonist, propranolol, attenuated the hyperphagic response to all three drugs. However, as pretreatment with 8-OH-DPAT, or blockade of 5-HT_{1A} somatodendritic autoreceptors did not attenuate the responses to idazoxan or clonidine it appears that their hyperphagic effects are not mediated via activation of the 5-HT_{1A} receptor. These data indicate that another 5-HT receptor subtype (possibly 5-HT_{1B} or 5-HT_{1C}) maybe involved, either directly or indirectly, in the hyperphagic effects of idazoxan and clonidine.

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146P DESENSITISATION OF POSTSYNAPTIC 5-HT_{1A} RECEPTORS FOLLOWING SINGLE ADMINISTRATION OF 5-HT_{1A} LIGANDS

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Somatodendritic 5-HT_{1A} receptors appear to undergo rapid desensitisation *in vivo* within 24h following a single dose of selective 5-HT_{1A} receptor agonists (e.g. Kennett et al., 1987), although more recent studies have yielded conflicting results. Adaptational responses involving postsynaptic 5-HT_{1A} receptors are even less clearly defined. The present study uses the behavioural syndrome response to the 5-HT_{1A} receptor agonist, 8-OH-DPAT (Tricklebank et al., 1984), to investigate adaptational changes of postsynaptic 5-HT_{1A} receptors following a single treatment with 5-HT_{1A} ligands.

Male Sprague-Dawley rats (250-350g; n = 12) were dosed s.c. with 5-HT_{1A} agonists (8-OH-DPAT, WY-48723 (Andree et al., 1988)), partial agonists (buspirone, ipsapirone), antagonists (SDZ 216-525 (Schoeffter et al., 1993), (+)-WAY-100135 (Fletcher et al., 1993)) or vehicle 24h before assessment of the 8-OH-DPAT syndrome. 8-OH-DPAT was administered via a lateral tail vein and the syndrome (flat posture, forepaw treading) assessed as present or absent during the next 5 min. Animals were tested sequentially using an up/down procedure, and ED_{50} values (95% confidence limits) calculated from quantal data using modified probit analysis (Kimball et al., 1957).

Pretreatment with 8-OH-DPAT, WY-48723 or ipsapirone significantly increased the ED_{50} for induction of the syndrome by 8-OH-DPAT compared with their vehicle-control groups. Pretreatment with buspirone, SDZ 216-525 or (+)-WAY100135 did not significantly modify the response to 8-OH-DPAT.

	8-OH-DPAT ED_{50} (95% confidence limits) mg/kg i.v.	
Pretreatment (mg/kg s.c.)	Vehicle	5-HT_{1A} ligand
8-OH-DPAT (1)	0.048 (0.038-0.060)	0.10 (0.084-0.12) *
WY-48723 (1)	0.062 (0.054-0.071)	0.12 (0.11-0.14) *
Ipsapirone (30)	0.053 (0.049-0.059)	0.080 (0.070-0.092) *
Buspirone (10)	0.053 (0.049-0.059)	0.068 (0.056-0.082)
SDZ 216-525 (3)	0.062 (0.053-0.073)	0.067 (0.049-0.090)
(+)-WAY-100135 (3)	0.048 (0.038-0.060)	0.071 (0.052-0.098)

* significant difference from respective vehicle control group (on the basis that 95% confidence limits do not overlap)

These data indicate that postsynaptic 5-HT_{1A} receptors undergo rapid desensitisation *in vivo* following a single dose of a selective 5-HT_{1A} receptor agonist, as determined by increases in the ED_{50} for induction of the syndrome by 8-OH-DPAT. A less marked desensitisation was observed following administration of the 5-HT_{1A} receptor partial agonist, ipsapirone. This study did not provide any evidence for adaptational responses to acute treatments with selective 5-HT_{1A} receptor antagonists.

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147P BIOCHEMICAL CHARACTERISATION OF THE CLONED HUMAN 5-HT_{2C} RECEPTOR EXPRESSED IN THE HUMAN NEUROBLASTOMA CELL LINE SH-SY5Y

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The 5-HT_{2C} receptor is a member of the 5-HT_2 receptor family which utilises the phosphoinositide hydrolysis second messenger system. The cDNA for the human form of this receptor has recently been cloned and sequenced and shows 90% amino acid homology with the rat 5-HT_{2C} receptor cDNA (Saltzman et al., 1991). We report here the biochemical characterisation of the human 5-HT_{2C} receptor cDNA stably expressed in the human neuroblastoma cell line SH-SY5Y.

A mammalian expression vector containing the 5-HT_{2C} receptor cDNA and encoding resistance to geneticin was transfected into the cells using the calcium phosphate precipitation technique and stable clones selected for resistance to 450μg/ml geneticin. Clones were screened for the presence of a functional receptor by investigation of intracellular calcium ion levels in fura-2 loaded cells. Functional clones demonstrated increased intracellular calcium ion levels in response to application of 5-HT(10μM) with fluorescence intensity monitored using an image analysis system. Levels of 5-HT_{2C} receptors in clones demonstrating a positive calcium response to 5-HT were quantified by ligand binding using [^{3}H]mesulergine. Untransfected cells did not respond to 5-HT nor did they possess specific [^{3}H]mesulergine binding.

A stable clone expressing [^{3}H]mesulergine binding at 110±10 fmol/mg protein and with a pKd of 9.02±0.09, n=3 (mean ± s.e.m.) was chosen for further characterisation. This affinity agrees well with data in human choroid plexus (Elliott et al., 1993). Inhibition of [^{3}H]mesulergine binding revealed the expected 5-HT_{2C} receptor binding profile with antagonist potency (pKi ± s.e.m. in parentheses, n=3) decreasing in the order: mianserin (8.20±0.05) > ketanserin (7.00±0.27) > spiperone (6.46±0.19). 5-HT showed high affinity binding (pKi=7.86±0.14, n=3) which agrees with previous data obtained for the 5-HT_{2C} receptor in the rat (Hoyer et al., 1985).

Phosphoinositide (PI) hydrolysis was investigated by growing cells on 24 well plates and then incubating for 48 hours with medium supplemented with 1μCi/ml [^{3}H]inositol. The cells were then washed with DMEM and incubated in the presence of LiCl (10mM) for 4 minutes prior to the 30 min application of agonist. PI hydrolysis was terminated by addition of ice-cold methanol and tritiated inositol phosphates ([^{3}H]IP_X) were separated on Dowex AG1-X8 columns. 5-HT stimulated [^{3}H]IP_X production in a concentration-dependent manner with pEC_{50}=7.64±0.06 and maximal stimulation of 400±230% basal level (n=3). 1-(3-chlorophenyl) piperazine (m-CPP) also stimulated [^{3}H]IP_X production with a maximal stimulation 1.23±0.18 (n=3) times that of 5-HT, thus demonstrating full agonist behaviour.

This cell line should therefore provide a useful model system for further studies on the human 5-HT_{2C} receptor.

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148P THE EFFECT OF PARACHLOROAMPHETAMINE ON THE EXPRESSION OF THE IMMEDIATE-EARLY GENE C-FOS IN RAT BRAIN

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Recent studies have shown that serotonergic neurones from the dorsal and median raphe nuclei give rise to projections that differ in axon morphology and vulnerability to certain psychotropic drugs. In particular, a class of substituted amphetamines which includes parachloroamphetamine (PCA) causes an acute release of serotonin in the forebrain and appears to act preferentially on the terminals of fine axons arising from the dorsal raphe nucleus (Molliver, 1987). It seems possible therefore, that the 5-HT projection from the dorsal raphe plays an important role in the control of affect. Although little is known of the postsynaptic targets of these projections, Blue *et al.*, (1988) noted that the cortical distribution of 5-HT_2 receptor binding sites closely matched that of fine serotonin- immunoreactive axons with small varicosities. Previously we have shown that c-Fos immunochemistry is a useful tool for studying 5-HT_2 receptor activation resulting from administration of the selective agonist DOI (2,5-dimethoxy-4-iodophenylisopropyl-amine) (Leslie *et al.*, 1993). To investigate the possible association of the 5-HT_2 receptor subtype with these specific fine axons further, we have compared the distribution of DOI-induced Fos expression with that induced by PCA.

Adult male Spague-Dawley rats were injected with 10mg/kg PCA, 8mg/kg DOI or saline 160 minutes before fixation for Fos immunohistochemistry. Fos protein was localised in 100μM-thick Vibratome sections using an antiserum (Cambridge Research Biochemicals) to the N-terminal region of the Fos molecule. PCA administration resulted in a highly localised pattern of Fos-like immunoreactivity (FLI) with particularly high levels in olfactory tubercle, islands of Calleja, amygdala and mammillary nuclei. The FLI distribution following PCA was similar to the Fos expression resulting from DOI in some, but not all, brain areas; for example, PCA administration results in dense FLI throughout the caudate putamen whereas DOI induces Fos expression only in a small ventromedial region of this nucleus.

PCA administration results in a distribution of FLI similar to that of 5-HT_2 receptor-mediated Fos expression in some, but not all, brain areas. The reason for this discrepancy is unclear; however, it may reflect the fact that the 5-HT release caused by PCA administration would affect the functioning of all the 5-HT receptor subtypes in a single brain area. Conversely, DOI is only an agonist at 5-HT_{1C} and 5-HT_2 receptors and would not be expected to be active at other subtypes such as 5-HT_{1A}, 5-HT_{1B}, 5-HT_3 or 5-HT_4. It could also be that the differences in Fos distribution may occur due to activation of non-serotonergic receptors by PCA. Further studies will be required to address this question.

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149P BRAIN 5-HT RECEPTOR SUBTYPES IN HYPERTHYROID RATS

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It has been suggested that thyroid hormones influence receptor sensitivity as well as synthesis and/or turnover rate of many neurotransmitters in the brain (for review see: Vaccari, 1988), and it has long been known that hypo- and hyperthyroidism are often associated with behavioural disorders (Prange *et al.*, 1987). We recently demonstrated that experimentally-induced hypo- and hyperthyroidism in rats are associated with variations in the characteristics of adrenergic, GABAergic and imipramine receptors in discrete brain areas (Sandrini *et al.*, 1991). The aim of the present study was to evaluate the influence of experimentally-induced hyperthyroidism on the characteristics of brain serotonin receptors in adult rats. Hyperthyroidism was induced by daily administration of L-triiodothyronine (100 μg/kg s.c. for 7 days). To assess the thyroid status serum levels of T_4 and T_3 were determined by radioimmunoassay at the end of the experiments (Sandrini *et al.*, 1992). Data were analyzed by means of Student's *t* test.

In a first set of experiments we observed that a condition of hyperthyroidism causes a decrease in the number of 5-HT binding sites in cortex and hippocampus, using [^{3}H]5-HT as ligand. However the data showed a non-linear bifasic distribution, suggesting that at least two receptor subtypes were affected. So, in a second set of experiments, we focused our attention on 5-HT_{1A} and 5-HT_2 binding sites, which are involved in several of the behavioral changes. We observed a significant decrease in the number of 5-HT_2 receptors, using [^{3}H] ketanserin as ligand, in the cerebral cortex (B_{max} being 223.84±18.4 in controls and 148.6±16.65 in hyperthyroid rats; P=0.015) but not in the hippocampus (B_{max} being 54.8±1.44 in controls and 55.04±3.06 in hyperthyroid rats; P>0.05). On the other hand the characteristics of 5-HT_{1A} receptors, using [^{3}H]-8OH-DPAT as ligand, are not affected both in the cerebral cortex (B_{max} being 269.3±27.4 in controls and 219.9±9.8 in hyperthyroid rats; P>0.05) and in the hippocampus (B_{max} being 437.7±8.0 in controls and 450.7±16.7 in hyperthyroid rats; P>0.05). K_D values were never modified.

These results may indicate that thyroid hormones are able to enhance 5-HT concentration in certain brain areas, so provoking a down regulation of 5-HT_2 receptors and suggest that the serotoninergic system takes part in the complex brain neurotransmitter imbalance underlying hyperthyroidism-linked behavioral changes.

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150P EFFECT OF 5,7-DIHYDROXYTRYPTAMINE ON 5HT_{2C} RECEPTOR PROTEIN-LIKE IMMUNOREACTIVITY IN THE RAT BRAIN AND SPINAL CORD

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5-Hydroxytryptamine$_{2C}$ receptors (formerly 5-HT_{1C}) were originally identified in the choroid plexus but *in situ* hybridisation and autoradiography have shown the 5-HT_{2C} receptor to be widely distributed in the rat brain. The present study examined the effects of intracerebroventricular 5,7-dihydroxytryptamine (5,7-DHT) treatment on 5-HT_{2C} receptor protein-like immunoreactivity (5-HT_{2C}-LI) in rat brain and spinal cord by radioimmunoassay (Sharma *et al.*,1992).

Guide cannulae were implanted above the lateral ventricle in adult male Wistar rats (320-400g) under sodium methohexitone (60 mg kg^{-1} i.p) anaesthesia. Following 10 days recovery, rats received 5,7-DHT (150μg, 5μl in 0.154M sodium chloride containing 0.57mM ascorbic acid, n=8) or vehicle, (n=10) once daily for 2 days, both 30 mins after pretreatment with desipramine (15 mg kg^{-1} i.p) to prevent uptake into noradrenergic neurones. Ten days after 5,7-DHT, selected CNS regions were divided into bilateral halves to measure 5-hydroxytryptamine (5-HT) by HPLC with electrochemical detection and 5-HT_{2C}-LI in membranes by RIA (Sharma *et al.*,1992).

Table 1. Effect of 5,7-DHT on 5-HT (pmol mg protein^{-1}) and 5-HT_{2C}-LI (fmol mg protein^{-1}; mean ±s.e.mean) in the rat CNS

		Choroid plexus	Frontal cortex	Brainstem	Midbrain	Dorsal Thoracic	Ventral Cervical
5-HT	VEHICLE	130.9±38.7	66.3±14.9	31.0±4.1	29.9±10.9	100.5±33.9	84.9±9.4
	5,7-DHT	45.7±.14.9	22.2±7.9	19.6±7.9	17.7±4.2	9.6±5.5	13.4±6.7
	% depletion	65% (p<0.05)	66% (n.s)	37% (p<0.05)	41% (n.s)	90% (p<0.05)	84% (p<0.001)
5-HT_{2C}-LI	VEHICLE	2724.1±438	894.9±147.2	107.1±8.7	92.4±6.6	84.2±12.8	44.3±9.4
	5,7-DHT	4418.5±649.8 (p<0.05)	965.3±157.8 (n.s)	51.6±18.2 (p<0.05)	55.7±10.9 (p<0.01)	26.7±7.5 (p<0.01)	134.2±29.8 (p<0.001)

5,7-DHT produced significant depletions of 5-HT in the choroid plexus (65%) and spinal cord (>80%) but less reductions marked in the midbrain and brainstem (Table1.). Following 5,7-DHT, 5-HT_{2C}-LI was significantly increased in the choroid plexus and ventral cervical cord yet significantly reduced in the midbrain, brainstem, and dorsal thoracic cord and unaltered in any other brain region.

The postsynaptic location of 5-HT_{2C} receptors in the choroid plexus suggested from the current findings, agrees with the reported increase in 5-HT_{2C}-mediated phosphoinositide hydrolysis in the choroid plexus following 5,7-DHT (Conn *et al.*,1987). However, the current data also suggests that 5-HT_{2C} receptors may be located on 5-HT nerve terminals in the midbrain, brainstem and dorsal spinal cord.

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151P CORRELATION OF *IN VIVO* FUNCTIONAL AND ANXIOLYTIC EFFECTS OF 5-HT_{1A} RECEPTOR LIGANDS IN THE MOUSE

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Many reports suggest that an attenuation of central 5-HT neurotransmission is anxiolytic in animal models (e.g. Iversen, 1984). Consequently, the anxiolytic effects of 5-HT_{1A} receptor ligands may result from an agonist action at somatodendritic 5-HT_{1A} autoreceptors or antagonism of postsynaptic 5-HT_{1A} receptors, or a combination of both actions. In an attempt to investigate this issue, we have compared the activities of several 5-HT_{1A} ligands, ranging from the agonists/partial agonists, 8-OH-DPAT, ipsapirone, buspirone, BMY-7378 and MDL-73005, to the putative antagonists, NAN-190, (+)WAY-100135 and SDZ 216-525 (see Cliffe et al., 1993), in models of 5-HT_{1A} receptor function (8-OH-DPAT-induced hypothermia) and anxiety (light/dark box) in the mouse.

Female Tuck (T/O) mice (24-35g; n=8 per treatment) were used. In the hypothermia experiments, the 5-HT_{1A} ligands or saline were administered s.c. 20 min before the s.c. injection of a challenge dose of 8-OH-DPAT (0.5 mg/kg). Rectal temperatures were recorded immediately before each injection, and at 15 and 30 min after 8-OH-DPAT. Hypothermic responses (mean ± s.e.m.) to 8-OH-DPAT in saline-pretreated animals ranged from -2.1 ± 0.4 to -3.4 ± 0.5°C. At doses (0.1-3.0 mg/kg s.c.) that had no intrinsic effect on body temperature, SDZ 216-525, BMY-7378, buspirone, MDL-73005 and (+)WAY-100135 dose-dependently antagonised 8-OH-DPAT-induced hypothermia, with ID_{50} values (doses to inhibit hypothermia by 50%) of 0.25 (0.1-0.6), 0.6 (0.2-1.7), 0.9 (0.5-1.9), 1.5 (0.8-2.6) and 1.6 (1.0-2.6) mg/kg s.c. respectively. Ipsapirone (1.0 mg/kg) partially attenuated (by 47%; $P<0.05$) the hypothermic response to 8-OH-DPAT. Higher doses of ipsapirone (3 & 10 mg/kg) also attenuated (by 39-82%) 8-OH-DPAT-induced hypothermia, but caused a significant ($P<0.05$) decrease in body temperature (1.0-1.8°C) when given alone. NAN-190 (1.0-3.0 mg/kg) elicited a dose-related hypothermia (0.9-2.0°C) but, in contrast to the other ligands, had no effect on the hypothermic response to 8-OH-DPAT. Furthermore, pretreatment with (+)WAY-100135 (3 & 10 mg/kg s.c.) failed to modify the hypothermia induced by NAN-190 (3 mg/kg).

The light/dark box apparatus has been previously described by Bill et al. (1992). Animals were dosed s.c. with vehicle or the 5-HT_{1A} ligands (0.01-10 mg/kg) 30 min before being placed in the centre of the light compartment. Locomotor activity (LMA), rearing and time spent in each compartment of the box were recorded over the following 5 min test period. Vehicle control mice were markedly less active in the light compartment relative to the dark, spending between 30-44% of the total time in the light. The ranges of control values (mean ± s.e.m. per 5 min) in these experiments were:- light (rears 6.5 ± 1.9 - 14.0 ± 1.8; LMA 47.4 ± 7.7 - 70.6 ± 11.6), dark (rears 28.0 ± 3.2 - 44.6 ± 3.2; LMA 95.8 ± 15.0 - 125.8 ± 4.5). With the exception of NAN-190, the 5-HT_{1A} ligands were active in the mouse light/dark box model, inducing significant ($P<0.05$; ANOVA/t-test) increases in LMA and rearing in the light compartment. The minimum effective doses in mg/kg (with percentage increases relative to the vehicle controls, in light compartment rears/LMA) were:- 8-OH-DPAT 0.05 (134/97), SDZ 216-525 0.1 (152/84), BMY-7378 1.0 (121/55), buspirone 1.0 (86/54), (+)WAY-100135 1.0 (88/52), ipsapirone 1.5 (123/83) and MDL-73005 2.0 (58/36). In conclusion, the potencies of SDZ-216,525, BMY-7378, buspirone, MDL-73005 and (+)WAY-100135 as antagonists of 8-OH-DPAT-induced hypothermia in the mouse were correlated closely with their anxiolytic potencies in the mouse light/dark box model of anxiety. The inactivity of NAN-190 in the light/dark box may be due to its apparent lack of activity at 5-HT_{1A} receptors in the mouse, a finding consistent with that previously reported by Moser (1991).

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152P EFFECT OF THE ACUTE ADMINISTRATION OF THE 5-HT_{1A} RECEPTOR LIGAND LESOPITRON ON CENTRAL 5-HT AND DOPAMINE TURNOVER

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Lesopitron (E-4424) (2-[4-[4-(4-chloro-1-pyrazolyl)butyl]-1-piperazinyl]pyrimidine) is a 5-HT_{1A} receptor ligand and is more potent than buspirone to reduce behavioural responding to aversive situations in animal models of anxiety (Costall et al., 1992). 5-hydroxytryptamine (5-HT) systems have a known involvement to inhibit responding in many behavioural paradigms and the present study compares the effect of lesopitron and buspirone to modify neurotransmitter release using the microdialysis technique.

Measurements of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) as well as dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the extracellular fluid were made in the frontal cortex of freely moving rats 24h after microdialysis probe implantation using the technique of Adell et al. (1991).

Table 1. The effect of lesopitron and buspirone to modify levels of 5-HT, 5-HIAA, DOPAC and HVA in the rat frontal cortex.

Treatment mg kg^{-1} i.p.	5-HT(1) %	5-HIAA(2) %	DOPAC(3) %	HVA(4) %
Vehicle Control	88 ± 11	81 ± 3	86 ± 5	93 ± 10
Lesopitron 0.03	59 ± 7*	100 ± 7	97 ± 7	96 ± 8
Buspirone 0.03	77 ± 10	107 ± 12	105 ± 17	123 ± 18
Lesopitron 5.0	42 ± 7*	79 ± 3	120 ± 18	127 ± 11
Buspirone 5.0	24 ± 6*	91 ± 6	324 ± 46*	388 ± 106*

The results are expressed as percentages, mean ± s.e. mean, n = 6, of the average value of four samples collected before injection of the drug. The average basal values for each group are: (1) 8.40 ± 1.76 fmols; (2) 4.71 ± 0.57 pmols; (3) 236.45 ± 63.68 fmols; (4) 554.32 ± 88.35 fmols. Values are taken 90 min after injection at the time of maximum effect. *$P<0.05$ compared to vehicle controls (ANOVA two factors repeated-measurements design using GLM procedures of SAS SYSTEM).

The peripheral administration of lesopitron and buspirone at doses reducing anxiety-like behaviour (30 μg Kg^{-1} i.p. for lesopitron and 5.0 mg kg^{-1} i.p. for bispirone) reduced the levels of 5-HT with no change in 5-HIAA levels. However, unlike lesopitron, the use of buspirone caused approximate 300-400% increases in DOPAC or HVA (see also Hamon et al., 1988). Indeed, even at doses 100 fold greater than minimally effective behavioural doses, lesopitron (5.0 mg kg^{-1} i.p.) failed to significantly increase DOPAC and HVA levels. In summary, acute injection of lesopitron, causes a marked reduction in cortical 5-HT levels at doses causing an anxiolytic-like action in the rodent (Costall et al., 1992). This is achieved in the absence of an effect on the dopamine system, the selectivity of action for the 5-HT system indicating the greater importance of a 5-HT involvement to the drug induced behavioural effects.

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153P DOPAMINE RELEASE IN THE N. ACCUMBENS AFTER SYSTEMIC AND INTRAHIPPOCAMPAL INJECTIONS OF 5-HT_1 AGONISTS WHICH DIFFERENTIALLY AFFECT ATTENTION IN THE RAT

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Many of the positive symptoms observed in acute schizophrenia can be explained by a state of "overattention", which can be modelled in the rat by disruption of latent inhibition (LI). Gray et al. (1991) have proposed that the structural basis of schizophrenia is to be found in an abnormality in the connections between the limbic forebrain (especially, the hippocampal formation, via the subicular area) and the basal ganglia (especially, the n. accumbens), leading to an increase in dopaminergic transmission in the n. accumbens. Beside the leading hyperdopaminergic hypothesis, an underactivity of the serotonergic system has also been proposed in schizophrenia. Following the findings reported by Cassaday et al. (1993) of a disruption of LI after systemic injections of a mixed 5-HT1B and 5-HT1A agonist (RU 24 969, Roussel Uclaf), but no clear disruption with a specific 5-HT1A agonist (8-OH-DPAT), we tested the neurochemical effects of these drugs in the hippocampo-accumbens system using microdialysis in freely moving rats. Systemic injections of RU 24 969 (0.1, 0.5, 2 mg/kg, n=5-7/dose) induced a 10 to 40% decrease in the serotonergic metabolite 5-hydroxy-indoleacetic acid (5-HIAA; $P<0.0001$), a 30-40% increase in dopamine (DA) release ($P<0.01$ at 0.5 mg/kg), and a 5-30% increase in dopamine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC; $P<0.05$) and homovanillic acid (HVA; NS) in the shell of the n. accumbens. Similar changes in 5-HIAA and DA levels were measured in the core of the n. accumbens. 8-OH-DPAT injections (0.25 and 0.5 mg/kg, n=5-8/dose) induced a 20-30% decrease in 5-HIAA ($P<0.0001$), failed to alter DA and HVA, but induced a small increase in DOPAC (5%, $P<0.05$) in the shell of the n. accumbens. Initial results obtained with local injections (n=3) of RU 24 969 (10 μg/μl, 1 μl in 1 min) into the ventral subiculum show a long lasting and slightly delayed increase in DA (about 70%) and of its metabolites (40-50%), but no significant change in 5-HIAA levels in the shell of the n. accumbens. These results are highly consistent with the hypothesis of an interaction between the serotonergic and dopaminergic systems, occuring at least in part in the hippocampus, which could account for some effects of serotonergic agents on LI. The effect of the RU 24 969 on DA release is not secondary to the decrease in 5-HT release, since 8-OH-DPAT reduced 5-HIAA levels without inducing any increase in DA release in the n. accumbens. Of the two drugs tested, only RU 24 969 disrupted LI, and increased DA release in the n. accumbens. This effect is probably mediated by postsynaptic 5-HT1B receptors, which could be located on the intrahippocampal cell bodies and/or dendrites of the glutamatergic neurons projecting to the n. accumbens. The delayed effect of the local injection may however indicate a more indirect mode of action.

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154P SIMULTANEOUS, SELECTIVE DETECTION OF DOPAMINE AND 5-HT USING CYCLIC VOLTAMMETRY WITH PRETREATED MICRO-SENSOR

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To date the simultaneous and selective voltammetric analyses of catechols and indoles has been carried out in vivo and in vitro only by means of slow scanning methods such as differential pulse (DPV) or differential normal pulse (DNPV) voltammetry associated with electrically and/or chemically pretreated carbon fibre micro-electrodes (mCFE) (Crespi, 1990). Faster scanning techniques, such as chronoamperometry or cyclic voltammetry while allowing more rapid and frequent measurements of these chemicals, show poor sensitivity and selectivity in the presence of different electroactive compounds with a similar oxidation potential (for a review see Marsden et al., 1988). In particular, the so called fast cyclic voltammetry (FCV, scan speed 300V/s) technique which is usually applied in vitro with untreated mCFE, measures a µM dopamine (DA) oxidation signal at +586mV and a µM serotonin (5HT) oxidation signal at +579mV (Bull et al., 1990). Here we have joined the methods of cyclic voltammetry (CV, scan speed 500mV/s) or DPV (scan speed 10mV/sec) with either untreated or electrically pretreated mCFE and analysed the in vitro oxidation potential values of DA and 5HT µM. It appeared that as for FCV, CV or DPV associated with untreated mCFE are both unable to separate and therefore selectively detect these two chemicals; the voltammograms recorded with both methods resulted in a broad oxidation signal peaking at about +560mV or +360mV respectively. In contrast, when CV or DPV were performed with electrically pretreated mCFE (n=5 for both methods) µM DA and µM 5HT oxidation signals were monitored simultaneously and respectively at +130 ±19mV or +320 ±27mV with CV and +65 ±11mV or +240 ±12mV with DPV (means ±S.D.). This is the first evidence for the feasibility of concomitant in vitro analyses of DA and 5HT using a fast scanning method such as CV. In addition, the potential values observed with CV-mCFE for DA and 5HT are comparable to those monitored with DPV-mCFE supporting the view that the electrical pretreatment of the sensor is a key point in the attempt to increase selectivity and possibly the sensitivity of the different voltammetric techniques.

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155P THE 5-HT_4 RECEPTOR AGONISTS RENZAPRIDE AND (S)-ZACOPRIDE STIMULATE DOPAMINE RELEASE FROM RAT STRIATAL SLICES

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It is becoming increasingly clear that the 5-HT_4-R (5-hydroxytryptamine$_4$ receptor) plays a modulatory role in the CNS. This receptor, which is positively coupled to adenylate cyclase, is prone to desensitisation and has been largely characterised by its agonist pharmacology although more selective antagonists are becoming available (for review see Bockaert et al., 1992). A recent study has reported relatively high densities of 5-HT_4 receptor recognition sites in the nigro-striatal pathway of rat and guinea pig (Grossman et al., 1993). In the present study, we directly assess the ability of the 5-HT_4 receptor agonists, renzapride and (S)-zacopride (Bockaert et al., 1992), to modulate the spontaneous release of dopamine from rat striatal slices.

Female Wistar rats (190-310 g, Birmingham bred) were killed by cervical dislocation and the striatum removed. Slices of striatum were cut with a McIlwain tissue chopper (400 x 400 μm x thickness of the striatum). Washed striatal slices were loaded into a perfusion chamber (37°C) and perfused (0.5 ml min^{-1}) with gassed (95/5 O_2/CO_2) Krebs buffer (mM; NaCl 120, KCl 4.75, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11) plus tyrosine (50 μM), GBR12909 (1.0 μM) and (-)-sulpiride (1.0 μM) for 120 min before the continuous collection of 2 min perfusate fractions for 60 min. Drugs were added via the perfusing Krebs buffer after 32 min. Dopamine in the perfusates was quantified by high performance liquid chromatography with electrochemical detection. Protein content was estimated by the Coomassie Blue method (Bradford, 1976) using bovine serum albumin as standard.

Administration of renzapride (10 μM) and (S)-zacopride (10 μM) via the perfusing Krebs buffer maximally enhanced the release of dopamine from rat striatal slices by 233 ± 42 % (mean ± SEM, n = 5, $P<0.05$ Student's t test) and 256 ± 66 % (mean ± SEM, n = 8, $P<0.05$ Student's t test) of basal values (basal release = 2.6 ± 0.9 pmol min^{-1} mg^{-1} protein, mean ± SEM, n = 13), respectively. The initial renzapride/(S)-zacopride-induced increase in dopamine release appeared to desensitize rapidly with values returning to near basal levels after 4-6 min in the continued presence of either renzapride or (S)-zacopride.

The present study has shown that the 5-HT_4 receptor agonists, renzapride and (S)-zacopride, stimulate the release of dopamine from rat striatal slices. Whilst this response requires more extensive pharmacological characterisation, the present results may indicate that the 5-HT_4 receptor enhances dopamine release from rat striatal slices.

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156P INCREASED [^{3}H]-5HT RELEASE IN AMYGDALOID SLICES FROM RATS WITHDRAWN FROM CHRONIC DIAZEPAM TREATMENT

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Previous studies have found increases in [^{3}H]-5-HT release from hippocampal slices taken from rats withdrawn from chronic diazepam treatment (Andrews *et al.*, 1992). It has been suggested that such an increase in 5-HT release from terminal regions in the hippocampus may mediate the anxiogenic behavioural response during benzodiazepine withdrawal (Andrews & File, in press). The present study investigated whether changes in [^{3}H]-5-HT could be detected in the amygdala, following withdrawal from chronic diazepam.

Two groups of male hooded Lister rats were tested in the elevated plus-maze test of anxiety for a 5 min period 18-24h following the last of 21 daily i.p. injections of either vehicle (water/Tween-20) or diazepam (2 mg/kg/day). They then received at least one more day of treatment and were sacrificed 18h after their last injection. The rats were killed by cervical dislocation and the hippocampus and amygdala dissected. Slices (0.2 mm) were taken from both these areas and changes in release and uptake of [^{3}H]-5-HT were measured as previously described (Andrews *et al.*, 1992). Results were expressed as mean ± s.e.m. and analysed with a one-way ANOVA.

The rats withdrawn from diazepam spent significantly less % time on the open arms of the elevated plus-maze compared with the control group (19.4 ± 5.5 % and 39.5 ± 5.2 % respectively, [n=8-12], $p < 0.05$) and had significantly increased K^+-evoked release of [^{3}H]-5-HT from the amygdala, but not from the hippocampus, see Table 1. These results suggest that an increased 5-HT release from the amygdala may be sufficient to mediate these anxiogenic responses seen in the plus-maze on withdrawal from diazepam.

TABLE 1 Mean (±sem) basal (FRC's) and K^+-evoked release (FRC's above baseline) and uptake (dpm x 10^{-3}) of [^{3}H]-5-HT from hippocampal and amygdaloid slices taken from rats treated with vehicle (Con) or withdrawn from diazepam (DW, 2 mg/kg/day). * p = 0.05 compared with control group

	HIPPOCAMPUS			AMYGDALA		
	Basal	**Evoked**	**Uptake**	**Basal**	**Evoked**	**Uptake**
Con (n=8)	0.8±0.1	4.6±0.9	166.5±25.2	0.7±0.1	6.9±1.0	173.6±16.6
DW (n=12)	0.8±0.1	3.7±0.4	170.7±11.5	0.7±0.1	10.1±1.1*	185.4±15.0

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157P EFFECT OF THE 5-HT NEUROTOXIN P-CHLOROAMPHETAMINE ON RAT HIPPOCAMPAL 5-HT RELEASE EVOKED BY ELECTRICAL STIMULATION OF THE DORSAL AND MEDIAN RAPHÉ NUCLEI

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There is neuroanatomical evidence (Molliver et al., 1990) that certain substituted amphetamines, including p-chloroamphetamine (PCA) cause degeneration of terminals of 5-HT nerve cells originating in the dorsal raphe nucleus (DRN), whilst sparing those from the median raphe nucleus (MRN). There is as yet, however, little evidence of a loss in function of 5-HT terminals following administration of these amphetamines or that this loss is selective for the DRN versus MRN. Here, using brain microdialysis, we have investigated the effect of PCA on the release of 5-HT evoked in vivo by electrical stimulation of the DRN and MRN.

Male Sprague-Dawley rats (180-200g) were injected with either PCA (2X10mg/kg i.p., 24 h apart; Molliver et al., 1990) or saline vehicle. Two weeks later rats were anaesthetized with chloral hydrate and a microdialysis probe (single cannula) and stimulating electrode were stereotaxically implanted into the ventral hippocampus (co-ordinates (mm): A/P -4, L -4.6, V -9.5) and DRN (A/P -7.5, L 0, V -6.5), respectively. Perfusates (perfusion medium; artificial CSF containing 1.0 µM citalopram, 2µl/min) were collected every 20 min and analysed for 5-HT and 5-HIAA by HPLC-EC (Sharp et al., 1990). Following a control period of perfusion (2-3 h), the DRN was electrically stimulated (1 msec, 300 µA, 5 Hz for 20 min). The electrode was subsequently lowered (2 mm) into the MRN and after 2 h the stimulation was repeated. At the end of the experiment the brain was removed and the final position of the electrode was confirmed to be in the MRN.

Compared to controls (n=8), rats treated with PCA (n=5) had lower basal levels of both 5-HT (controls, 55±9 fmol/20 min; PCA, 26±3 fmol/20 min; mean±s.e.mean, $p<0.01$ Student's t-test) and 5-HIAA (controls, 4845±1058 fmol/20 min; PCA, 839±123 fmol/20 min, $p<0.01$). In controls, electrical stimulation of the DRN and MRN evoked an immediate and short-lasting (20-40 min) release of 5-HT(peak minus baseline: DRN, 71±15 fmol; MRN, 80±22 fmol). In comparison, in rats treated with PCA the amount of 5-HT released by stimulation of the DRN and MRN was reduced by 70-85% (DRN, 21±5 fmol/20 min; MRN, 12±5 fmol/20 min).

In summary, our data indicate that PCA treatment decreases the amount of hippocampal 5-HT released by electrical stimulation of either the DRN or MRN, indicating the presence of a functional 5-HT lesion. However, this observation does not match neuroanatomical evidence that the 5-HT lesion induced by PCA is restricted to 5-HT pathways of the DRN. It should be added that we cannot yet exclude the possibility that our electrical stimulation paradigm does not discriminate between overlapping DRN and MRN 5-HT pathways. Further studies are in progress to address this issue.

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R McQuade is a MRC Student.

158P EFFECT OF THE PUTATIVE 5-HT$_{1A}$ ANTAGONIST SDZ 216-525 ON 5-HT NEURONAL FIRING IN THE GUINEA PIG DORSAL RAPHÉ NUCLEUS

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Putative 5-HT1A antagonists (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspirol[4,5]-decane-7,9-dione di-hydrochloride (BMY 7378) and 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN 190) since their introduction have proved to be antagonists at the postsynaptic 5-HT1A sites and agonists at the presynaptic 5-HT1A sites (Hjorth et al. 1990;Sharp et al. 1990). Recently it was proposed that methyl 4-{4-[4-(1,1,3-trioxo-2H-1,2-benzoisothiazol-2-yl)butyl]-1-piperazinyl}1H-indole-2-carboxylate (SDZ 216-525) was also a selective 5-HT1A antagonist (Schoeffter et al. 1993), however a partial agonist activity at the presynaptic somatodendritic autoreceptor in the dorsal raphe nucleus (DRN) was demonstrated using two *in vivo* models of somatodendritic autoreceptor function suggesting that SDZ 216-525 may be a 5HT1A receptor partial agonist (Gurling et al. 1993). SDZ 216-525 caused an increase in food intake in satiated rats and a decrease in extracellular 5-HT in the hippocampus measured using microdialysis. The present study has used *in vivo* electrophysiology in the guinea-pig DRN to compare the effects of SDZ 216-525 on 5-HT neuronal firing with those of the selective 5-HT1A antagonist N-tert-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2phenylpropanamide dihydrochloride (WAY 100135) (Fletcher et al. 1991).

Male Dunkin-Hartley guinea-pigs (320-450g) were anaestetised with urethane (1.3g kg^{-1} i.p.) and the jugular vein cannulated for i.v. administration of drugs. Single barrelled glass electrodes, filled with 2M NaCl containing 2% pontamine sky blue dye, were implanted into the DRN using stereotaxic coordinates taken from lambda (A +0.7-1.0; L 0.0; V -6.5-7.5mm) (Mundey et al. 1992). 5-HT neurones were identified by their slow, regular, firing pattern (0.5-4.0 spikes sec^{-1}.) and their reversible inhibition (81±12%, n=16) by the specific 5-HT1A agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) (10µg kg^{-1} i.v.). SDZ 216-525 (0.25 mg kg^{-1} i.v.) inhibited unit activity of 8-OHDPAT sensitive cells (75±5%, n=5) whilst WAY 100135 (0.5mg kg^{-1} i.v., n=5) had no effect itself on the neuronal firing rate but fully antagonised 8-OHDPAT and SDZ 216-525 induced inhibition of DR neuronal activity. These results demonstrate that WAY 100135 is a silent and selective antagonist and suggests that SDZ 216-525 has partial intrinsic agonist activity at the somatodendritic 5-HT1A autoreceptor in the guinea-pig DRN.

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159P THE EFFECTS OF FORSKOLIN AND IBMX ON A 5-HT_3 AND A PUTATIVE 5-HT_4 RECEPTOR-MEDIATED DEPOLARIZATION OF THE RAT ISOLATED VAGUS NERVE

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We have previously described a component of 5-hydroxytryptamine (5-HT)-evoked depolarization of the rat isolated vagus nerve that is resistant to blockade by the 5-HT3 receptor antagonist ondansetron and that has characteristics of a 5-HT4 receptor mechanism (Rhodes *et al.*, 1992; Coleman & Rhodes, 1993). In an attempt to differentiate the 5-HT3 and the ondansetron-resistant components of depolarization we have investigated the effects of forskolin and isobutylmethylxanthine (IBMX), compounds known to elevate intracellular concentrations of cyclic AMP. It is known that 5-HT4 receptors may be positively coupled to adenylate cyclase (Dumuis *et al.*, 1988).

Extracellular recording of depolarization of the cervical vagus nerve was carried out using a "grease-gap" technique (Rhodes et al., 1992). Male, Sprague Dawley rats (180-220 g) were killed with a blow to the head and cervical dislocation. The vagus nerve was placed in a two chamber tissue bath, one chamber being perfused (3 ml min^{-1}) with Krebs' solution (2.5 mM $CaCl_2$, at 27°C) to which drugs were added. Contact time for 5-HT was 3 min with a 10-15 min washout between doses. Two non cumulative concentration-response curves (CRC's) were obtained in each tissue. After the first CRC tissues were equilibrated (60 min) with either ondansetron (30 nM) alone or together with IBMX (100 µM). Two separate groups of tissues were used to compare the effects of ondansetron (100 nM) in the absence and presence of forskolin (10 µM).

The mean maximum depolarization to 5-HT in the first CRC in the forskolin study was 513.5 ± 41.2 µV (n=10) and in the IBMX study 490 ± 23.6 µV (n=18). Both forskolin and IBMX evoked prolonged depolarizations of 509 ± 49.7 µV (n=5) and 261 ± 35.3 µV (n= 9) respectively. In the presence of ondansetron the CRC to 5-HT was biphasic, the responses to 5-HT (10 nM-10 µM) in the first phase being enhanced by forskolin. In the presence of forskolin the depolarization evoked by 0.3 µM 5-HT was 14.2 ± 0.91 %, (n=5) of the initial 5-HT maximum compared to 10.4±0.90 %, (n=5, $p<0.05$, Mann-Whitney test) in the presence of ondansetron alone. Similar results were obtained with IBMX. The depolarization evoked by 0.3 µM 5-HT was 15.6 ± 1.82 % of the initial 5-HT maximum (n= 9) in the presence and 11.0 ± 0.80 % (n=9, $p<0.05$) in the absence of IBMX. The responses to 5-HT in the second, 5-HT3-mediated, phase of the CRC were reduced by IBMX and forskolin. The mean responses to 0.1 mM 5-HT in the presence and absence of 10 µM forskolin were 28.4±3.2 and 47.9±7.3 % (n= 5, $p<0.05$) of the initial 5-HT maximum response respectively. In the presence and absence of IBMX responses to 0.1 mM 5-HT were 47.2±3.8 and 62.7 ± 5.0 % (n=9, $p<0.05$) of the initial 5-HT maximum respectively.

The potentiation by forskolin and IBMX of the first, but not the second phase of the 5-HT CRC in the presence of ondansetron suggests different mechanisms of depolarization in each phase and is consistent with an adenylate cyclase-linked 5-HT4 receptor mechanism for the first phase. The effects of forskolin and IBMX alone suggest an adenylate cyclase-linked depolarizing mechanism in this tissue.

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160P G-PROTEIN MEDIATED INHIBITION OF CALCIUM CURRENTS BY 5-HT IN CULTURED RAT MELANOTROPHS

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The effect of serotonin (5-HT) on whole-cell Ca^{2+} currents was studied in primary cultures of rat pituitary intermediate lobe (IL) cells, using the patch-clamp technique. 5-HT (10 µM) reversibly inhibited voltage-dependent Ca^{2+} currents in 75% (n=68) of the cells tested and two different patterns of inhibition were observed. In 18% of the responding cells (9 out of 51), 5-HT produced a parallel reduction of the high-threshold L-type Ca^{2+} current I_{CaL} (mean inhibition 25,7± 11%, n=9). In contrast, in 82% of the cells (42 out of 51), serotonin reduced the peak inward Ca^{2+} current by 33,2± 9% (n=42), whereas it reduced the non-inactivating component of I_{Ca} (recorded just before termination of the voltage step) to a lesser extent (13,9± 12%, n=42). Most importantly, in 15% of the cells (8 out of 42), 5-HT only reduced the peak current, leaving the steady-state component unaffected. Nevertheless, the low-threshold transient component I_{CaT} (evoked by voltage steps to -30 mV, from a holding potential of -100 mV) was not affected by 5-HT, suggesting that the suppression of peak I_{Ca} was due to either a slowing in kinetics of L-type Ca^{2+} channels and/or to inhibition of a high-threshold transient component of the Ca^{2+} current.
The effect of 5-HT was blocked to various extent by mianserin (1 µM) and reproduced by 8-OH DPAT (0,1 µM), suggesting the presence of both 5-HT_{1C} and 5-HT_{1A} receptors in rat IL cells, as previously described in porcine IL cells (Ciranna et al., 1993).
The effect of 5-HT on calcium currents was voltage-dependent: a short (30 ms) depolarizing voltage step to +80 mV preceeding the test pulse was able to relieve 5-HT inhibition completely. When GTP-γ-S (30 µM) was added to the intracellular solution, the effect of serotonin was made irreversible; under these conditions, a second application of 5-HT was completely uneffective in further reducing I_{Ca}.
Our results show that in rat IL cells 5-HT inhibits Ca^{2+} currents by a G-protein dependent pathway. Although the receptors involved in the effect are the same as those described on porcine IL cells, the mechanism of action seems to be different.

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161P THE INFLUENCE OF NON STEROIDAL ANTI-INFLAMMATORY DRUGS ON PAIN THRESHOLD AND 5-HT RECEPTORS IN THE RAT BRAIN

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The prostaglandin hypothesis of the mode of action of non steroidal anti-inflammatory drugs explains only some of the effects of these drugs. Shyu (1985) reported that the central monoaminergic system is involved in antinociceptive action, while Woolf (1985) pointed out that acetyl salicylic acid (ASA) seems to be able to reverse pain-sustained behaviour induced by intrathecally administered substance P. Moreover evidence suggests there exists a dissociation between analgesic and anti-inflammatory activity of NSAIDs (McCormack, 1991).
Pain-threshold was determined using the hot-plate test and 5-HT binding capacity was studied in the cortical and pontine areas of rat brain two hours after administration of two strong cyclooxygenase inhibitors: acetyl salicylate lysine salt (ASL) 900 mg/kg ip and ketorolac (KET) 100 mg/kg ip, one mild inhibitor, phenazone (PHEN) 60 mg/kg po and one weak inhibitor, azapropazone (AZA) 500 mg/kg ip.
PHEN and ASL per se induced analgesia in the hot-plate test and decreased the number of cortical and pontine 5-HT binding sites, whereas AZA and KET modified neither pain-threshold nor binding capacity of serotonin brain receptors. Depletion of serotonin levels with a four-day pre-treatment with parachlorophenilalanine (PCPA) 100 mg/kg ip, prevented both the analgesic effect on the hot-plate test (% MPE: PHEN 16.2±2.7*; PCPA 0.6±3.3; PCPA+PHEN 6.2±2.7; VEHICLE 1.1±2.4, mean±SEM, P=0.03, ANOVA followed by Student-Newman-Keuls' test, * p<0.05 vs VEHICLE) (%MPE: ASL 24.7±7.1*; PCPA 0.5±5.0; PCPA+ASL 7.0±3.2; VEHICLE 2.2±4.5, P=0.02) and the reduction of binding sites caused by PHEN in the cortical (Bmax, as fmol/mg protein: PHEN 62.4±4.2*, PCPA 159.2±14.4*; PCPA+PHEN 142.2±5.4; VEHICLE 114.4±6.0, P=0.002) and pontine areas (Bmax: PHEN 77.4±4.0*; PCPA 149.7±9.1*; PCPA+PHEN 133.4±9.4; VEHICLE 113.3±5.5, P=0.002) and by ASL in cortex (Bmax: ASL 91.3±7.4*; PCPA 200.1±10.0*; PCPA+ASL 188.2±15.2; VEHICLE 173.1±32.5, P=0.002) but not in pons (Bmax: ASL 180.5±8.7; PCPA 219.4±10.1*; PCPA+ASL 200.1±7.6; VEHICLE 172.2±16.1, P=0.03).
These results support the hypothesis that the analgesic action of PHEN and ASL depends at least in part, on the serotonergic system, whereas AZA and KET do not show central analgesic activity and fail to modify the central serotonergic system.

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162P SB 200646: A SELECTIVE 5-HT_{2C} RECEPTOR ANTAGONIST *IN VIVO* WITH ANXIOLYTIC EFFICACY

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SB 200646A (N-(1-Methyl-5-indolyl)-N-(3-pyridyl urea.HCL) is the first selective 5-HT_{2C} receptor antagonist (Forbes et al 1993). We now report on its effects in rats on two putative tests of central 5-HT_{2C} receptor function; m-chlorophenylpiperazine (mCPP)-induced hypolocomotion and hypophagia (Curzon and Kennett 1990), a model of 5-HT_2 receptor activation, (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI)-induced head shakes, and a model of anxiety, the social interaction (SI) test.

Male SD rats were given SB 200646 1h pretest and mCPP (7 mg/kg i.p.) 20 min, before placing in automated locomotion boxes under red light for 10 min. Other rats were 23h food deprived and given SB 200646 and mCPP (7 mg/kg i.p. 30 min pretest), before restoring food for 2 h. Alternatively rats were placed in individual cages for 30 min before giving 3 mg/kg i.p. DOI and counting head shakes over 30 min. The SI test was carried out as in Kennett (1992). Means ± SEM (n>6) are cited. SB 200646 was always given p.o. 1h pretest. ID_{50}'s ± 95% confidence limits were determined by the curve fitting program "Allfit".

MCPP reduced transits from 18.0 ± 1.2 to 5.8 ± 1.3 (P<0.01). SB 200646 (2-40 mg/kg) alone, had no effect, but reserved (ID_{50} 19.2 ± 0.4 mg/kg p.o.) the effect of mCPP also reduced 2h food intake from 4.3 ± 0.4 to 1.2 ± 0.4g (P<0.01). SB 200646 (10-60 mg/kg) had no effect when given alone, but reversed the effect of mCPP with an ID_{50} value of 18.3 ± 10.3 mg/kg. DOI-induced head shakes (23.4 ± 2.8 in 30 min) were unaffected by 200 mg/kg SB 200646. SB 200646 enhanced time (secs) spent in SI (vehicle 70.8 ± 4.7, SB 200646 2 mg/kg, 110.8 ± 8.2 P<0.05, 10 mg/kg 153.9 ± 13.7 P<0.01, 40 mg/kg 134.0 ± 11.0 r<0.01) but not locomotion. The maximal response to SB 200646 equals that of diazepam (2 mg/kg 140.2 ± 11.1 P<0.01).

Thus SB 200646 is a selective 5-HT_{2C} receptor antagonist in vivo. Its anxiolytic properties in the rat SI test are consistent with previous data (Kennett 1992) and with 5-HT_{2C} receptor mediation of mCPP-induced anxiety (Curzon and Kennett 1990).

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163P REVERSAL LEARNING IMPAIRMENTS INDUCED BY AMPHETAMINE IN THE MARMOSET ARE ATTENUATED BY 5-HT RECEPTOR ANTAGONISTS

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Previous work using the marmoset has shown that amphetamine induces cognitive perseveration in a reversal learning task and that this effect can be ameliorated by the neuroleptic agent haloperidol (Ridley *et al.*, 1981). More recently, it has been demonstrated that the D_2 selective atypical neuroleptic raclopride and the mixed D_2/5-HT_2 receptor antagonist risperidone also have the ability to reverse an amphetamine-induced learning impairment in this species (Mason *et al.*, 1992). The present studies were designed to further investigate the ability of agents acting at specific subtypes of the 5-HT receptor to modify amphetamine induced learning impairments in the marmoset. These included the novel 5-HT_2 receptor antagonist RP62203 (2-[3-(4-(4-fluorophenyl)-piperazinyl)propyl]naphto[1,8-cd]isothiazole-1,1-dioxide) (Doble *et al.*, 1992) and the 5-HT_3 receptor antagonist ondansetron; haloperidol was used as a reference control.

The studies used 5 adult marmosets, 4 males and 1 female (320-420g). Prior to inclusion in the study these animals were trained to 90% criterion in a simple visual object discrimination learning task using a Wisconsin General Test Apparatus, where it was found that there was no difference in performance between the sexes. Subsequently these animals were given extensive training in the serial object discrimination reversal learning task. Drug pretreatment and amphetamine treatment were given once weekly 30 mins prior to cognitive assessment. Amphetamine administration (0.5mg/kg s.c.) induced impairments in performance shown as high reversal learning trials to criterion (15.2 ± 0.4 amphetamine, compared to 2.6 ± 0.8 saline). These effects were significantly ($P<0.05$) attenuated by pretreatment with haloperidol (5-10μg/kg s.c.) from 14.6 ± 3.0 to 2.2 ± 1.0 (haloperidol 5μg/kg s.c.), by RP62203 (25-500μg/kg s.c.) from 15.4 ± 2.1 to 2.6 ± 1.2 (RP62203 500μg/kg s.c.) and by ondansetron (1ng-100μg/kg s.c.) from 15.0 ± 1.1 to 2.0 ± 1.1 (ondansetron 10ng/kg s.c.). The more specific errors to criterion were also significantly reduced by haloperidol (5-10μg s.c.) from 8.0 ± 1.9 to 1.4 ± 0.6 (haloperidol 5μg/kg s.c.), by RP62203 (25-500μg/kg s.c.) from 11.0 ± 1.8 to 1.8 ± 0.7 (RP62203 500μg/kg s.c.) and by ondansetron (1ng-100μg/kg s.c.) from 10.5 ± 1.4 to 1.3 ± 0.5 (ondansetron) 10μg/kg s.c.).

This work suggests that while this paradigm is of value in assessing the activities of antidopaminergic agents to modify amphetamine-induced cognitive impairments, it is also apparent that compounds having 5-HT_2 and 5-HT_3 receptor antagonist properties confer ability to attenuate learning impairments arising from overstimulation of the mesolimbic neurotransmitter systems.

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164P THE LACK OF EFFECT OF ONDANSETRON IN A MOUSE MODEL OF CONDITIONED PLACE PREFERENCE (CPP)

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The 5-HT_3 receptor antagonists have previously been shown to block morphine- and nicotine-induced place preferences in the rat (Carboni *et al.*, 1988). A more recent study confirmed the ability of 5-HT_3 receptor antagonists to attenuate morphine CPP but also indicated that varying doses of ondansetron alone caused effects, which appeared to be environmentally specific to the black and white sides of the CPP apparatus (Higgins *et al.*, 1992). Previous studies in this laboratory have shown that the reinforcing properties of morphine and cocaine, and the opposing effects of naloxone can be seen in a mouse model of CPP which has not relied upon the use of black versus white as the discriminative visual cues (Marshall *et al.*, 1990, 1992). The present study investigates the effects of ondansetron *per se* in this model.

Four automated 3-chambered boxes and male adult BKW mice were used in the experiment. The 2 conditioning chambers consisted of diagonally striped wood walls with a textured glass floor and plain metal walls with a plain wood floor. The protocol consisted of 3 x 15 min pre-conditioning exposures; 8 x 45 min drug/saline pairings and a 1 x 15 min post-conditioning test. Ondansetron (0.01, 0.1 and 1 mg/kg i.p.) was administered using a counter balanced design and place preference was determined as time spent (s) in a chamber.The results were analysed by two factorial analysis of variance.

There were no significant changes ($F(2, 34) = 1.280$, $p = 0.291$, $F(2, 36) = 1.584$, $p = 0.22192$, $F(2, 36) = 1.120$, $p = 0.334$, $F(2, 36) = 0.753$, $p = 0.478$) in time spent on the control- or drug-paired side between pre-conditioning times of 296.8±9.5s, 300.2±10.4s, 291.3±7.2s and 292.5±8.9s, to post-conditioning times of 279.1±15.9s, 323.8±15.6s, 313.4±15.6s and 307.6±14.1s for control or ondansetron (0.01, 0.1 or 1.0 mg/kg respectively) treated animals nor were there any signs of specific environmental effects to particular cues or sides of relative preference.

These results suggest that ondansetron is ineffective to induce CPP in a mouse model. The previous finding of environmental specific effects could be a facet of the use of black v white as the visual cue; the present results therefore indicate a possible advantage of using more salient stimulus modalities.

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165P DAU 6215: PRECLINICAL EVIDENCE FOR EFFICACY IN COGNITIVE DISORDERS

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DAU 6215 is a potent and selective 5-HT_3 receptor antagonist belonging to the benzimidazolone chemical class (1), and is characterized by distinctive pharmacokinetic profile in human volunteers, such as relatively long lasting plasma half-life, high absolute bioavailability, low metabolism and considerable elimination through the kidneys (2), as compared to other 5-HT_3 antagonists.

Since antagonism at 5-HT_3 receptors has been reported to enhance cognitive performance in rodents and primates (3), the effects of DAU 6215 in cognitive behavioural paradigms, as well as on those biochemical and electrophysiological parameters believed to play a role in learning/memory processes, were investigated. It was found that:

- Acute treatment with DAU 6215 reverted the scopolamine-induced (0.75 mg/kg i.p.) impairment in the passive avoidance procedure in young rats at i.p. doses of 10 and 30 μg/kg, higher or lower doses appearing, however, to be ineffective. Similarly, DAU 6215 improved spatial memory performance in the Morris water maze test in young rats, when scopolamine (0.2 mg/kg s.c.) was used as amnestic agent: significant antagonism was observed at the i.p. single doses of 10 and 30 μg/kg, but not at 1 and 100 μg/kg.
- After repeated treatment, DAU 6215 (10 μg/kg i.p., b.i.d. for three weeks) significantly improved the basal cognitive performance of senescent rats both in the Morris water maze test and in the multiple choice avoidance model, a paradigm capable of evaluating "discrete memory".
- DAU 6215, 30 μg/kg i.p., counteracted 2-methyl-5-HT-induced inhibition of ACh release in the frontal cortex of the rat, as measured by the microdialysis technique, being ineffective in modulating ACh release when given alone. The ability of DAU 6215 to prevent the ACh release inhibition induced by specific stimulation of 5-HT_3 receptors, would suggest that the cognitive-enhancing mechanisms of 5-HT_3 antagonists are in some way related to facilitation of cholinergic transmission, a function recognized to be fundamental for cognition.

Moreover, it has been recently reported (4) that DAU 6215, 60 nM, antagonizes the 5-HT-induced inhibition of "long term potentiation", an "in vitro" electrophysiologic event, recorded from rat pyramidal neurons of the CA1 hippocampal layers, which is suggested to be at the basis of learning processes.

The profile of DAU 6215 emerging from the present results is consistent with that of a potential cognitive enhancer, confirming that blockade of 5-HT_3 receptors may improve learning/memory processes.

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166P Ca^{2+} ENTRY MECHANISMS ACTIVATED BY N-METHYL-D-ASPARTATE OR K+-DEPOLARIZATION IN CULTURED CEREBELLAR GRANULE CELLS

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We have previously shown that N-methyl-D-aspartate (NMDA) and depolarization (evoked by increasing extracellular K^+ concentration: $[K^+]_o$) cause biphasic elevations of intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in cultured cerebellar granule cells which are due, at least in part, to release of Ca^{2+} from intracellular stores (Simpson et al., 1993). For both NMDA- and K^+-evoked responses, organellar Ca^{2+} release is triggered by Ca^{2+} entry, therefore, we have examined the possibility that there may be common Ca^{2+} entry mechanisms activated by these agents.

Cerebellar granule cells were prepared from neonatal rat cerebella as described previously (Whitham et al., 1991), and cultured on glass cover-slips pre-coated with poly-D-lysine. Cells (used at <10 days in vitro) were incubated with 2 μM Fura 2-AM for 20 min in Krebs-Henseleit buffer (KHB), and $[Ca^{2+}]_i$ measurements performed on small groups of 5-8 granule cells (Simpson et al., 1993). The ability of the Ca^{2+}-surrogate Mn^{2+} (0.1 mM) to 'quench' fura 2 fluorescence excited at 360 nm was also investigated, in Ca^{2+}-free KHB.

The rate of decrease in fura 2 fluorescence (at 360 nm) upon addition of Mn^{2+} either simultaneously with, or 100 s after, increasing from 4.7 to 29.7 mM $[K^+]_o$ was used to assess Ca^{2+} entry during the peak and plateau phases of the $[Ca^{2+}]_i$ response respectively. The rate of quench was markedly increased by elevated $[K^+]_o$, and no significant difference was seen between the two phases of the response (50% decrease in Fura 2 fluorescence: peak 29 ± 9; plateau 50 ± 14 s). However, 340/380 nm ratiometric fura 2 measurements indicated that the types of Ca^{2+}-channel contributing to the Ca^{2+} entry during the two phases of the response differed. Addition of the L-type Ca^{2+} channel antagonist (-)PN 202 791 (1 μM) had no effect on the peak Ca^{2+} response to elevated $[K^+]_o$, but significantly inhibited the plateau phase (by 51 ± 4%). The (+)-enantiomer of PN 202 791 (1 μM), which is a L-type Ca^{2+} channel agonist, potentiated the K^+-evoked response (by 65 ± 13 and 46 ± 9% for the peak and plateau phases respectively). MK 801 (1 μM), which abolished the response evoked by NMDA (200 μM), had no effect on the response to elevated $[K^+]_o$. (-)PN 202 791 caused no significant change in the peak $[Ca^{2+}]i$ response to NMDA (200 μM), and only a small inhibition of the plateau (7 ± 3%), whereas the (+)-enantiomer increased both phases, by 27 ± 12 and 25 ± 2% respectively.

These results suggest that Ca^{2+} entry via L-type Ca^{2+} channels makes a significant contribution to the plateau phase of the $[Ca^{2+}]_i$ response to increased $[K^+]_o$, but little contribution to Ca^{2+} entry evoked by NMDA. As both NMDA and K^+-depolarization can trigger release of Ca^{2+} from organellar stores, it seems likely that Ca^{2+} entry through different classes of Ca^{2+} channel can contribute to this phenomenon.

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167P THE EFFECTS OF SIBUTRAMINE AND ITS METABOLITES ON [^{3}H]DIZOCILPINE (MK-801) BINDING: EVIDENCE FOR INTERACTION WITH NMDA RECEPTORS

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Tricyclic antidepressants exhibit antagonist activity at NMDA receptors. Imipramine and desipramine displace [3H]MK-801 binding in rat brain membranes (Reynolds & Miller, 1989) and desipramine blocks NMDA receptor channels by open channel block in a manner similar to MK-801 (Sernagor *et al.*, 1989). Recently we have reported that BTS 54 505, a metabolite of the non-tricyclic monoamine reuptake inhibitor sibutramine, inhibits NMDA-evoked activity in rat hippocampus and thalamus (Scott *et al.*, 1991). We have therefore used [3H]MK-801 binding to further assess the activity of sibutramine and its secondary and primary amine metabolites (BTS 54 354 and BTS 54 505) on the NMDA receptor; desipramine and other antidepressants have been included for comparison.

Membrane homogenates from rat hippocampus (male Lister hooded, 200-250 g) were incubated with 3 nM [3H]MK-801 with and without test compounds for 1 h at 25°C in 5 mM Tris buffer (pH 7.5) containing 50 µM glutamate and 30 µM glycine; non-specific binding was defined with 100 µM MK-801 (Scott *et al.*, 1992).

Table 1 Effects of compounds on [3H]MK-801 binding to rat hippocampal membranes

Compound	Ki	nH	Compound	Ki	nH	Compound	Ki	nH
MK-801	2.61 ± 0.10 nM	0.95	Phencyclidine	0.88 ± 0.08 nM	0.96	Ketamine	920 ± 30 nM	0.96
Sibutramine	12.2 ± 0.23 µM	1.45	BTS 54 354	15.7 ± 2.14 µM	1.31	BTS 54 505	8.7 ± 1.07 µM	1.49
Imipramine	4.2 ± 0.93 µM	1.24	Desipramine	4.4 ± 0.43 µM	1.25	Amitriptyline	6.7 ± 0.64 µM	1.53
Dothiepin	9.6 ± 1.88 µM	1.78	Fluoxetine	19.2 ± 5.41 µM	1.11	Mianserin	16.2 ± 2.43 µM	1.52

Ki values are mean ± SEM (n = 3) in µM except for MK-801, phencyclidine and ketamine in nM. nH = mean Hill coefficient (n = 3).

The data in Table 1 show that the tricyclic antidepressant desipramine displaced [3H]MK-801 binding with a Ki similar to that reported by Reynolds & Miller (1989). The other standard antidepressants displaced [3H]MK-801 binding with similar or lower potency to desipramine. The relatively high Hill coefficients obtained with these antidepressants are probably related to using high drug concentrations to displace [3H]MK-801 rather than positive co-operativity. The non-tricyclic molecule sibutramine and its secondary amine (BTS 54 354) displaced [3H]MK-801 binding with approximately 3-fold less affinity than desipramine whereas BTS 54 505 exhibited similar potency to desipramine. These results, in conjunction with previous electrophysiological data, support the view that BTS 54 505 acts at the NMDA receptor in a manner similar to the known NMDA receptor antagonist desipramine.

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168P GLUTAMATE REGULATION ON DA RELEASE FROM GUINEA-PIG STRIATAL SLICES

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It has now been established that multiple EAA receptor types are involved in the modulatory actions of the EAAs on the striatal DA release (Ruzicka & Jhamandas, 1993). In the present study the effect of L-glutamic acid (L-glu) on the spontaneous and electrically-induced [^{3}H]-DA efflux from guinea-pig striatal slices was re-investigated, by using some selective antagonists.

The slices were incubated in a medium containing [^{3}H]-DA (0,2 μM) for 45 min, then rinsed and transferred to superfusion chambers. In a first set of trials the slices were kept at rest, drugs were added at the 60th min of perfusion and their effect was evaluated by calculating the resting release as % of the pretreatment value (at 50th min of perfusion). In a second set of experiments the slices were stimulated (S) with retangular pulses of alternate polarity (duration 2 ms, intensity 100 mA) at 0,2 Hz for 2 min at the 45th and 85th min of perfusion. Drugs were added in the interval between the two stimulation periods and their effect was evaluated as ratio between the efflux evoked by S_2 and S_1 (S_2/S_1).

In resting slices, superfused in the presence of Mg^{2+}, spontaneous [^{3}H]-DA outflow was slightly enhanced by L-glu (1-3 mM). As expected, in the absence of Mg^{2+}, L-glu produced a substantial concentration-dependent increase in [^{3}H]-DA outflow. This response was almost abolished by TTX (0,5 μM) and by MK 801 (10 μM), suggesting the prevailing role of NMDA receptors. In electrically stimulated slices, superfused in presence of Mg^{2+}, L-glu (30-1000 μM) increased DA release in a concentration-dependent manner up to + 73% of controls. Kainic acid (up to 1 mM) shared this effect as well. The L-glu-induced increase in [^{3}H]-DA efflux, was not affected by AP5 (100 μM) or MK 801 (10 μM), but was counteracted in a competitive manner both by the non-NMDA receptor antagonist CNQX (0,05 μM to 0,5 μM) and by pyroglutamic acid (L-PCA, 1-3 mM), a putative antagonist of non-NMDA receptors (Beani et al., 1990). The pA_2 values of CNQX and L-PCA were 7,5 and 2,96 respectively. In Mg^{2+}-free medium L-glu was less effective in incresing [^{3}H]-DA.

In conclusion, the present study revealed that, in guinea-pig striatal slices, the spontaneous [^{3}H]-DA release in the presence of Mg^{2+} was slightly stimulated by L-glu via non-NMDA receptors; conversely the [^{3}H]-DA release evoked at low stimulation frequency was mainly facilitated by non-NMDA receptors.

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169P FLURBIPROFEN ENHANCES POTASSIUM-EVOKED RELEASE OF GLUTAMATE FROM RAT CEREBELLUM *IN VITRO*

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N-methyl-D-aspartate (NMDA) has been shown to induce release of arachidonic acid (ArA) in cerebellar primary cultures (Lazarewicz et al 1988). It has been suggested that ArA can raise extracellular levels of glutamate by inhibiting neuronal/glial glutamate uptake (Barbour et al 1989) and/or enhancing release by potentiating the action of glutamate on presynaptic metabotropic receptors (Herrero et al 1992). We report here that inhibition of arachidonic acid metabolism via the cyclo-oxygenase pathway enhances K^+-evoked release of glutamate, presumably by raising free levels of ArA.

Parasaggital slices (250μm) were prepared from the cerebellar hemispheres of female Wistar rats (200-250g) and superfused (1ml/min) with artificial cerebrospinal fluid (ACSF) gassed with 95% O_2 /5% CO_2 at 37°C. Two-minute aliquots of perfusate were collected for analysis of glutamate by high performance liquid chromatography with fluorometric detection. 35mM K^+ ACSF was applied as three, two-minute "pulses" in a 12-minute cycle, with drugs being perfused for a 10-minute period prior to the second pulse (P2). Results are expressed as a percentage of transmitter released by the first pulse (P1) in a minimum of 5 experiments. In control experiments, P2 was 97±9%; flurbiprofen (25-400μM) enhanced K^+-evoked release in P2 in a dose-dependent manner (P2=138±7% with 100μM flurbiprofen; $p<0.001$). Application of 100μM 3[(R)-2-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP), an NMDA receptor antagonist abolished the enhancement of K^+-evoked release induced by 100μM flurbiprofen (P2=83±7%). The metabotropic glutamate receptor antagonist L-2-amino-3-phosphopropionic acid (L-AP3), (50μM), similarly inhibited the potentiation of K^+-evoked release in the presence of 100μM flurbiprofen (P2=83±6%). The effects of flurbiprofen were short term; the third K^+ pulse being enhanced only at the highest concentration of drug used (400μM).

The results show that the cyclo-oxygenase inhibitior flurbiprofen enhances the evoked release of endogenous glutamate, this effect being dependent on both NMDA and metabotropic glutamate receptor activation. It is possible that raised levels of free ArA, induced by NMDA receptor activation by endogenous glutamate, potentiates the activation of presynaptic metabotropic receptors and enhances glutamate release.

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170P EFFECTS OF 7-CHLOROKYNURENIC ACID IN RAT CULTURED CEREBELLAR GRANULE CELLS AND HIPPOCAMPAL SLICES

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7-Chlorokynurenic (7-ClKyn) acid is a potent antagonist of the current elicited by N-methyl-D-aspartate (NMDA). It has been proved to act selectively at the glycine modulatory site of the NMDA receptor complex in cultures of rat (Kemp *et al.*, 1988) and mouse (Henderson *et al.*, 1990) cortical neurones and mouse hippocampal neurones (Vyklicky *et al.*, 1990). In the present study the effect of 7-ClKyn was tested in 8-day-old rat cultured cerebellar granule cells and hippocampal slices.

NMDA-activated whole-cell currents in granules were measured with the patch-clamp technique at a holding potential of -50 mV, in a Mg^{++}-free external solution, in the presence of 10 uM D-Serine and 0.1 uM Tetrodotoxin. D-Serine mimics the effects of glycine acting selectively on the glycine modulatory sites associated with NMDA receptors (Snell et al., 1988). The current evoked by 100 uM NMDA was antagonized by 7-ClKyn acid (0.1-100 uM) in a dose-dependent manner with an IC_{50} of 3.098 uM (inferior confidence limit = 2.367; sup. conf. lim. = 4.078) as derived from the regression line. The action of 7-ClKyn acid (1 uM) on 100 uM NMDA could be reversed by increasing the D-serine concentration (30 uM) while no effect was obtained perfusing a higher dose of NMDA (300 uM). Moreover, 10 uM 7-ClKyn acid had no effect on the currents evoked by 100 uM Kainic acid, 30 uM AMPA and 30 uM Quisqualic acid. These data prove the existence, in this cellular type, of a NMDA receptor complex modulated allosterically by glycine as demonstrated by the selective action of 7-ClKyn acid.

Furthemore, the effects of 7-ClKyn acid were investigated in rat hippocampal slices. Electrical stimulation of Schaffer collaterals and recordings of extracellular synaptic field potentials were carried out in the apical dendritic layer (stratum radiatum) of the CA1 pyramidal cells. To elicit the NMDA receptor mediated component of synaptic trasmission 10 uM of the non-NMDA receptor antagonist CNQX (Honore' et al., 1988) was added to the artificial cerebrospinal fluid and the Mg^{++} was omitted. The NMDA-EPSPs were almost completely blocked by addition of 7-ClKyn acid (30-100 uM) to the chamber bath, and these effects were reversed by D-Serine (30-1000 uM). When D-Serine was applied to the Mg^{++}-free ACSF no significant effects on the NMDA receptor mediated responses could be noted. Moreover, the depression of the NMDA-EPSP observed with CPP 30 uM was insensible to the addition of D-Serine, in agreement with its different site of action. Together, these results suggest that the hippocampal slices and the extracellular recording technique are a useful model to study the glycine modulatory site associated with the NMDA receptor.

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171P GLUTAMATE RECEPTOR ANTAGONISTS REDUCE THE NEURONAL DAMAGE IN A PHOTOTHROMBOTIC MODEL OF RETINAL ISCHEMIA

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We previously described a quantitative "in vivo" model to study ischemic neuronal damage in the rat retina. Neuronal damage was quantified by measuring the activity of the marker enzymes choline acetyl-transferase (ChAT) and glutamic acid decarboxylase (GAD) (Moroni et al 1993). In this model, retinal ischemia was induced by injecting halothane anaesthetized rats i.v. with the photosensitive dye rose bengal (80 mg/kg) and by exposing one of their eyes to cold light filtered at 560 nm. This resulted in profound damage of the retinal tissue; ChAT activity decreased by 75±10% and GAD activity by 78±10%. The inner nuclear and the inner plexiform layers had a diffused swelling and numerous neurons had the chromatin densely packed forming clumps distributed all along nuclear contours. Blood vessels were enlarged and had thrombotic material. With the aim of investigating whether excitatory amino acid antagonists were able to reduce this neuronal damage we intravitreally administered the rats, immediately after the photochemical reaction, with the following compounds: 1) aminophosphono-valeric acid (AP-5); MK801 (competitive and non competitive NMDA antagonists); 2) NBQX and GYKI 52466 (competitive and non competitive AMPA antagonists); 3) 7Cl-thiokynurenic acid (glycine antagonist and free radical scavenger) and 4) thiokynurenic acid (nonselective glutamate antagonist and radical scavenger). The results of these experiments indicated that NBQX (50 nmoles) reduced the decrease of ChAT activity to 50±5%; GYKI 52466 (50 nmoles) to 47±5%, AP-5 (50 mnoles) to 37±6%, MK-801 (50 nmoles) to 19±5%. When NBQX plus AP-5 or GYKI 52466 plus MK-801 were simultaneously injected at 50 nmoles each, the protection was almost complete. Thiokynurenic acid (200 nmoles) reduced the decrease of ChAT activity to 35% while 7-Cl-thiokynurenic acid (200 nmoles) was inactive. In conclusion, the neuronal degeneration caused by ischemic insults to the retina may be reduced by antagonising glutamate receptors. The simultaneous use of both AMPA and NMDA antagonists resulted in a better degree of protection than that of selective antagonists.

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172P THE EFFECT OF D-SERINE ON EXCITATORY NEUROTRANSMISSION IN THE RED NUCLEUS *IN VIVO*

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The magnocellular neurones of the red nucleus (mRN) receive two distinct monosynaptic excitatory inputs, from the interpositus nucleus (IPN) and the sensorimotor cortex (SMC). Both inputs are believed to utilise excitatory amino acids as their transmitters (Nieoullon *et al.*,1984; Davies *et al.*, 1986). Recently we have shown that the IPN input is mediated by non-NMDA receptors whereas the SMC input involves both NMDA and non-NMDA receptors (Harris et al. 1991). The NMDA receptor requires the presence of the co-agonist glycine for activation (Kleckner and Dingledine 1988). In this study we have investigated the effects of glycine, D-serine and 7-Chlorokynurenate (7-ClKyn) on the responses to α-amino-3-hydroxy-5-methylisoxazole-4-proprionate (AMPA), NMDA and to synaptic activation of the mRN neurones.
Cats were anaesthetised with α-chloralose (50 mg/kg i.v.) and extracellular recordings were obtained from the mRN cells using multibarreled micro-electrode; the outer barrels containing the drug solutions for iontophoretic application. Excitatory synaptic responses were obtained by electrical stimulation of the contralateral IPN and the ipsilateral SMC with pulses of 100μs duration, <200μA at 1Hz.
Results were obtained from a total of 29 monosynaptically activated mRN neurones and are summarised in Table 1. (values are mean±SD)

DRUG	Mean current (nA)	% Effect on NMDA	Number Dep / Increased		% Effect on AMPA	Number Dep / Increased		Mean current (nA)	% Effect on SMC	% Effect on IPN
D-serine	18±6	+69±59	1/15	12/15	-14±24	4/8	1/8	43±35	0	0
7-ClKyn	16±18	-72±42	11/12	1/12	-24±49	2/9	0/9	25±22	-49±49	-45±49
D-serine +	9±7	-16±51	0/9	8/9	+4±36	0/7	2/7	9±5	-12±26	---
7-ClKyn	22±12							13±10		

Initially the effects of Glycine, D-serine and 7-ClKyn were tested on the responses to NMDA and AMPA. Glycine was inhibitory on spontaneous activity, NMDA and AMPA responses which may have obscured any modulatory activity it might have on the NMDA receptor. D-serine had little effect on the AMPA response but potentiated the NMDA response at relatively higher currents. D-serine had no direct effects on the synaptic responses. 7-ClKyn was inhibitory to NMDA responses and also to AMPA response in 2 of the 9 cells tested, but was mostly without effect. On both SMC and IPN responses, 7-Cl Kyn was inhibitory (on 5 of 7 cells and 5 of 9 cells respectively). The effects of a combination of 7-ClKyn and D-serine were tested which reduced the inhibitory effect of 7-ClKyn on NMDA and SMC synaptic responses.
These results show that D-serine can enhance NMDA receptor mediated synaptic transmission in the red nucleus.
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173P EXCITATORY AMINO ACIDS MODULATE THE CARDIOVASCULAR EFFECTS OF ENDOTHELIN-1 IN THE PERIAQUEDUCTAL GRAY AREA

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Endothelin-1 (ET-1) is a potent and long-lasting vasoconstrictor peptide which may act as a neurotransmitter within the central nervous system (Matsumura *et al.*, 1991). Our previous studies showed that in the periaqueductal gray (PAG) area glutamate participates in the modulation of cardiovascular function mainly through the NMDA subtype receptor (Maione *et al.*, 1992). The present study was designed to examine the possible relationship, in the left dorsal PAG area, between ET-1 and the glutamergic system in the regulation of the cardiovascular function in freely moving rats.

Male Sprague-Dawley rats (250 - 300 g) were used in this study. Two days before the experiment a stainless steel guide cannula for the intracerebral microinjections (1µl) was implanted in the left dorsal PAG area of rats anaesthetized with ketamine. On the day of the experiment a catheter was inserted into the femoral artery under 2% halothane anaesthesia for the measurement of arterial blood pressure (ABP) by a pressure transducer connected to a Hellige polygraph.

Microinjections of ET-1 (0.1-1-10 pmol/rat) in the left dorsal side of the PAG area significantly ($p < 0.05$) and not immediately increased ABP (respectively 14±2, 48±5 and 55±4 mmHg; n=5). The pretreatment with 2-APV (1µg/rat), a selective NMDA receptor antagonist, but not with CNQX (0.01 µg/rat), a selective non-NMDA receptor antagonist, reduced (-90±3.3 %; n=5; $p < 0.01$) the ET-1 induced cardiovascular changes. Like 2-APV, the non competitive antagonist of NMDA receptor MK-801 (1 mg/kg *ip*), reduced (-100±0.6 %; n=4; $p < 0.01$) the hypertension by ET-1. The pressor effect of ET-1 was always correlated with head twitches, ataxia, and barrel-rolling lasting 35 min. These effects were also prevented by 2-APV and MK-801 but not by CNQX. Finally, the cardiovascular changes induced by ET-1 were also antagonized by systemic administration of prazosin (1 µg/kg *ip*) and propranolol (0.1 mg/kg *ip*) (respectively -90±1.4 and 100±1.9%; n=4; $p<0.01$).

Our study provides the first evidence that stimulation of PAG area by ET-1 activates the glutamergic system, via selective involvement of NMDA receptors. Moreover, it seems that the sympathetic tone is involved in the ET-1 pressor effects. Probably, ET-1 directly activates NMDA neurons in PAG area which in turn modulate sympathetic pathways in the subretrofacial nucleus. The possible relationship between ET-1 and NMDA receptors remains to be clarified.

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174P THE ANTICONVULSANT DIPHENYLHYDANTOIN POTENTIATES SOME *IN VIVO* AND *IN VITRO* ELECTROPHYSIOLOGICAL EFFECTS OF NMDA ANTAGONISTS IN RATS

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N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors are markedly involved in the electrical generalization of epileptiform activity within the brain (1) and in neurodegenerative processes (2).
In the present study, the influence of the prototypic anticonvulsant diphenylhydantoin (DPH) has been tested on some in vivo and in vitro electrophysiological effects induced by NMDA antagonists (PCP, MK-801 and CGS 19755) in rats.
In vivo studies: under Equithesin anaesthesia, rats were chronically implanted with cortical electrodes. The EEG studies were started 5 days later. For each group of treatment, 4-6 animals were used. Even though DPH did not markedly affect basal cortical EEG activity, the drug (10-100 mg/kg i.p.) significantly ($P<0.01$) potentiated all the typical EEG effects induced by the NMDA antagonists consisting of: 1) increase of the cortical desynchronization periods; 2) increase of the amplitude of the fast (20-30 Hz) frequency background cortical activity; 3) appearance of cortical slow (2-3 Hz) wave - sharp wave or spike complexes.
In vitro studies: extracellular recording from rat hippocampal slices (CA1 field) was performed under hypoxia (changing slice perfusion from O_2 to N_2). Both DPH (50 µM, N=6) and MK-801 (50 µM, N=6) significantly ($P<0.01$) preserved the restoring of the CA1 electrical synaptic response (3) after a 45 min hypoxic period of the slices.
Ineffective concentrations of DPH (12 µM) significantly ($P<0.05$) potentiated the protective effects of a low concentration (12 µM) of MK 801 (N=6).
These data support the view of an involvement of the NMDA neurotransmission in the pharmacological effects of DPH.

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175P PALMITOYL CARNITINE SHOWS PROCONVULSANT ACTION WITH N-METHYL-DL-ASPARTATE (NMDLA), BUT NOT WITH BICUCULLINE

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Palmitoyl carnitine is a putative endogenous ligand for certain voltage-operated calcium channels, at which dihydropyridine compounds act (Spedding and Mir, 1987). Some dihydropyridines, such as nitrendipine, are channel antagonists and are anticonvulsant in certain animal models of seizures (Meyer et al., 1987) and protect against the ethanol withdrawal syndrome (Littleton et al., 1990), while Bay K 8644 is a calcium channel activator, which causes seizures in vivo. In the present study we examined the effects of palmitoyl carnitine in seizures produced by two different convulsant drugs, the $GABA_A$ antagonist, bicuculline, and N-methyl-DL-aspartate (NMDLA).

Groups of 7-9 male TO mice (30-35g) were used in all studies. Mice were injected, i.p., with palmitoyl D,L carnitine 12.5 to 50 mg/kg, or saline vehicle. At 30 minutes after this injection, the mice received an i.p. injection of either bicuculline 4 mg/kg, or NMDLA, 320 mg/kg, and the time to reach first convulsion was recorded. There were no significant differences between the convulsion incidences for any of the groups (incidences = 6/8 to 8/8). In separate groups of animals, pretreated 60 min before the test, the doses of intravenously infused bicuculline or NMDLA required to cause the first myoclonic twitch, and a full clonic convulsion, were measured. The behavioural ratings were made by an observer who was unaware of the prior drug treatment.

Table 1. Values are mean ± s.e.m. P.Carn. = palmitoyl D,L carnitine.

Drug pretreatment	Latency (s) to bicuculline convulsions	Latency (s) to NMDA convulsions	Bicuculline thresholds (mg/kg)		NMDA convulsion thresholds (mg/kg)	
			1st twitch	Clonic conv.	1st twitch	Clonic conv.
Saline	169 ± 27	435 ± 84	0.55 ± 0.04	0.70 ± 0.09	622 ± 56	745 ± 41
P. Carn. 12.5mg/kg	108 ± 11 (P = 0.07)	314 ± 44 *	0.60 ± 0.04	0.65 ± 0.05	476 ± 29*	603 ± 38*
P. Carn. 25 mg/kg	144 ± 37	198 ± 18 *	0.48 ± 0.05	0.66 ± 0.11	483 ± 63	720 ± 76
P. Carn. 50 mg/kg	111 ± 17	421 ± 64	0.59 ± 0.04	0.66 ± 0.05	468 ± 21*	644 ± 40

Statistics by Student's nonpaired t-test, * $P < 0.05$, compared with saline values

In both test methods, palmitoyl D,L carnitine was proconvulsant when tested with NMDLA, but not when tested with bicuculline. The lowest dose of palmitoyl D,L carnitine used appeared to have the most proconvulsant effect. The difference between the effects seen with NMDA and bicuculline may be related to palmitoyl carnitine's calcium channel activating properties (Spedding and Mir, 1987), increasing intracellular calcium levels and making neuronal cells more excitable. NMDLA increases calcium conductance, through receptor operated channels, while bicuculline antagonises the action of GABA on chloride channels.

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176P AUTORADIOGRAPHIC ANALYSIS OF CHANGES IN THE $GABA_A$ RECEPTOR COMPLEX AFTER CHRONIC LOW DOSE DIAZEPAM TREATMENT IN RATS

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The evidence for regional changes in the characteristics of the $GABA_A$ receptor complex as a mechanism for benzodiazepine (BZ) tolerance and dependence remains controversial. We have previously reported that chronic intermittent and continuous low-dose treatment with diazepam (DZP) fails to downregulate BZ receptors (Brett and Pratt, 1992). We now report changes in other components of the $GABA_A$ receptor complex induced by such treatment.

3 groups of male Long-Evans rats received daily i.p. injections, either 28 days vehicle (control), 25 days vehicle and 3 days DZP 5 $mgkg^{-1}$ (subacute), or 28 days DZP 5 $mgkg^{-1}$ (chronic). 3 further groups of rats were implanted with silastic capsules, either empty (control and subacute) or filled with DZP (chronic). On days 26-28, the 3 implanted groups received i.p. vehicle (control and chronic) or DZP 5 $mgkg^{-1}$ (subacute). All capsules were removed on day 28. Animals were tested on the elevated plus-maze on day 29 after challenge with vehicle (control groups) or DZP 2.5 $mgkg^{-1}$ (all other groups), and sacrificed 24 h later. Slide-mounted 20 μm cryostat-cut brain sections were incubated with 1 nM [^{3}H]flunitrazepam (FNZP) (in the absence or presence of 100 μM GABA), or with 6 nM [^{3}H]SR 95531 or 50 nM [^{3}H]GABA, rinsed in buffer and distilled water, dried and apposed to ^{3}H-sensitive film together with ^{3}H standards to facilitate quantitative image analysis of the 47 structures measured.

Changes in the % enhancement of [^{3}H]FNZP binding by GABA were found in a small number of structures interconnected with the lateral habenula, accompanied in some cases by a change in [^{3}H]SR 95531 binding (Table 1), but not by changes in [^{3}H]GABA binding (e.g. dorsal tegmental nucleus: control 31±5; subacute 40±5; chronic 37±4; similarly there were no signficant changes in the nucleus accumbens, dorsal raphe, central grey and lateral habenula). In these structures there was no significant effect of treatment route.

Table 1. Effect of DZP treatment on $GABA_A$ receptor complex binding

	GABA enhancement of [^{3}H]FNZP binding (%increase)			[^{3}H]SR 95531 binding (specific binding, $pmolg^{-1}$ tissue)		
	control	subacute	chronic	control	subacute	chronic
dorsal tegmental nucleus	26±7	21±7	48±7*	95±5	78±3*	78±5*
nucleus accumbens	57±8	28±6*	37±5*	117±7	101±5	112±9
dorsal raphe	41±3	22±6°	42±10	119±9	88±7*	102±6
central grey	33±9	17±4*	38±6	90±4	82±5	93±4
lateral habenula	46±9	24±6°	26±5°	43±4	39±4	38±4

Data are expressed as mean ± s.e. mean of 10-12 rats per group (pooled treatment groups from both treatment routes). * $p<0.05$, ° $p<0.1$ compared with control.

The complexity of the pattern of changes would not support the hypothesis that chronic treatment with DZP induces a widespread change in BZ/GABA receptor coupling. In the hippocampus, there were differences in [^{3}H]SR 95531 binding between the two treatment routes, suggesting that handling differences may change low-affinity GABA binding; these changes were not paralleled by changes in [^{3}H]GABA binding.

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177P PLASTICITY OF $GABA_B$ RECEPTOR BINDING SITES IN RAT SPINAL CORD

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$GABA_B$ receptor binding sites in rat spinal cord appear to be located primarily on small diameter afferent fibre terminals and this may, in part, provide the locus for the antinociceptive action of the $GABA_B$ agonist, (-)baclofen, in rodents (Wilson & Yaksh, 1978). Treatment of neonatal rats with capsaicin or rhizotomy in adult rats reduces $GABA_B$ binding in the dorsal laminae (particularly II & III) by approximately 50% (Price *et al.*, 1987) and we have recently demonstrated that $GABA_B$ agonists prevent the release of substance P evoked by dorsal root stimulation in rat spinal cord (Malcangio & Bowery, 1993). Since prolonged administration of (-)baclofen induces tolerance to its antinociceptive effect in rodents (Malcangio *et al.*, 1992) we are currently examining the possible mechanisms underlying this tolerance. In the present study we compare the effects of chronic administration of (-)baclofen or a $GABA_B$ antagonist, CGP 36742 (3-aminopropyl-n-butyl phosphinic acid), with saline on the density of $GABA_B$ receptor binding sites in rat spinal cord using *in vitro* receptor autoradiography.

Wistar male rats (150g) were injected i.p. once daily for 21 days with (-)baclofen (10mg/kg/day, n=3), CGP 36742 (100mg/kg/day, n=4) or saline (n=5). After a further 24 hours the cervical and lumbar enlargements of the spinal cord were prepared for receptor autoradiography (Price *et al.*, 1987). Autoradiograms were prepared using Kodak K5 emulsion-coated coverslips exposed to labelled sections for 12 days. Silver grains overlying laminae II of each section were counted using a Quantimet 970 image analyzer. Results are summarized in the Table.

Treatment	n (rats)	^{3}H-GABA [nM] 10	50	100	150	200
Saline	5	13.2 ± 6.5	25.7 ± 11.9	31.8 ± 12.3	43.0 ± 14.2	47.7 ± 13.8
(-)Baclofen	3	9.1 ± 0.7	5.8 ± 0.7*	11.6 ± 2.7*	-----	13.8 ± 0.01*
CGP 36742	4	11.3 ± 0.5	33.8 ± 5.7	67.2 ± 3.3*	93.6 ± 17.4*	102.7 ± 9.2*

Values are mean number of grains/1000μm^2 ± s.e. mean; *p<0.05 compared with saline (Mann Whitney U Test)

Treatment with (-)baclofen significantly reduced the number of grains whilst CGP 36742 significantly increased the grain count by a factor of 2 (100-200nM, ^{3}H-GABA). These data indicate that $GABA_B$ receptors in the spinal cord can be influenced by prolonged administration of receptor ligands. Whilst this may contribute to the reported tolerance which arises following (-)baclofen treatment, the functional consequences of prolonged administration of $GABA_B$ antagonists remain to be seen.

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178P ON THE MECHANISM BY WHICH THE ADENOSINE A_{2a} RECEPTOR INHIBITS STRIATAL [^{3}H]-GABA RELEASE

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We have previously demonstrated an A_2 receptor mediated inhibition of GABA release from rat striatal nerve terminals, consistent with the localisation of the A_2 receptor in striatal GABA/met-enkephalin containing neurones (Schiffman *et al.*, 1991). In the present study we have characterised the second messenger system involved in this inhibitory action.

Striatal synaptosomes were pre-loaded with [3H]-GABA, drawn onto Whatman GF/B filters and perfused with buffer at a rate of 0.5 ml min-1, maintained at 37°C and aerated with 95% O_2 + 5% CO_2. After an initial equilibration period of 36 min eluent was collected at 2 min intervals for scintillation counting. Release was evoked by a 90 s depolarising stimulus of 15 mM KCl, with modulation of release being assessed by changes in the ratio of the radiolabel released between two stimuli S1 and S2 spaced 12 min apart. Controls were run in parallel where both S1 and S2 contained 15 mM KCl alone; in test experiments putative modulators were also present in the S2 stimuli.

Using this system we found that the A_2 selective agonist 2[p-(2-carboxyethyl)-phenethylamino]-5'-N-ethyl carboxaminoadenosine (CGS 21680) at a concentration of 10^{-9} M inhibited GABA release by 26.6 ± 6.2% (n = 5) in the presence of 1.3 mM calcium, but was ineffective in the absence of calcium 8.6 ± 7.8% (n = 5). Furthermore by increasing the concentration of calcium in the perfusion buffer to 4 mM the inhibitory action of CGS 21680 was abolished. Perfusion 6 min prior to depolarisation with the calcium channel blockers flunarazine (T-type, 1, 10 μM) and nifedipine (L-type, 10, 100, 200 μM) failed to prevent inhibition of release by CGS 21680 although the N-type blocker ω-conotoxin was effective in mimicking the actions of CGS 21680, inhibiting GABA release by 23.0 ± 4.1 (n = 12) and 45.5 ± 4.2 (n = 12) at 0.2 μM and 0.5 μM respectively. CGS 21680 had no effect in the presence of ω-conotoxin.

Inhibition of GABA release by CGS 21680 was prevented by perfusion with the cAMP analogues 8-bromo cAMP (1 mM) and di-butyryl cAMP (1 mM) as well as by forskolin (5, 20, 50 μM) but not its inactive analogue 1,9 dideoxy forskolin (50 μM). None of these compounds affected release on their own. This therefore suggests that stimulation of cyclase activity is not responsible for the inhibitory action of CGS 21680, although it does prevent its action. In vivo administration of pertussis toxin (6 μg) directly into the rat striatum 48 hours prior to experimentation was ineffective in blocking the inhibitory action of CGS 21680, indicating G_i/G_o proteins are not involved in the A_2 mediated inhibition of GABA release. Finally the non-selective K^+ channel blocker 4-aminopyridine (2, 5, 10 mM) was unable to prevent the A_2 mediated inhibition of GABA release. This is consistent with the concept that when evoking release by KCl, the inhibitory action of a potassium channel is unlikely to be observed due to voltage clamping of the membrane potential.

We therefore conclude that the predominant mechanism by which CGS 21680 inhibits KCl stimulated [^{3}H]-GABA release is by interfering with calcium influx possibly via an N-type calcium channel. This finding is consistent with work on the A_{2a} receptor present on neutrophils which also inhibits function by interfering with calcium influx (Tsuruta *et al.*, 1992). An adenosine evoked inhibition of calcium uptake in cortical synaptosomes mediated by an A_2 receptor has also been described (Wu *et al.*, 1982).

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179P EFFECT OF LOCALLY APPLIED KAINIC ACID ON RELEASE OF ENDOGENOUS GABA IN STRIATUM AND SUBSTANTIA NIGRA OF THE AWAKE RAT

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The striatonigral GABAergic system is the major efferent pathway of striatum, providing a direct inhibitory control over output neurones of the substantia nigra. There is evidence that excitatory amino acid (EAA) transmitters influence striatonigral GABA neurones at the level of the striatum. For example, NMDA and non-NMDA receptor agonists stimulate the release of exogenous GABA from rat striatum in vitro (Galli et al., 1992). Striatonigral GABA neurones may also be influenced by EAAs at the level of the substantia nigra given the presence in this region of EAAergic inputs and both NMDA and non-NMDA binding sites (cf. Albin et al., 1992). The present study investigated the effect of direct application of the non-NMDA receptor agonist kainic acid on GABA released locally in striatum and substantia nigra in vivo. Endogenous extracellular GABA was measured in striatum and substantia nigra of conscious rats using microdialysis.

In chloral hydrate anaesthetized rats, microdialysis probes (single cannula) were stereotaxically implanted into the right striatum (coordinates (mm): A/P +0.7, L -3.2, V 5.5), the ipsilateral substantia nigra (A/P -5.4, L -2.2, V 8.9) or both. The next day the probes were perfused continuously (1.4 µl/min) with artificial CSF. GABA in the perfusate (20 µl) was measured by HPLC-EC (Smith and Sharp, 1992). Basal output of GABA in striatal (0.34±0.02 pmol/ 20 µl, n=14) and nigral (0.49±0.04 pmol/ 20 µl, n=15) perfusates was stable for several hours. Perfusion of the striatum with kainic acid (100 µM) for 20 min induced an immediate and short-lasting (20-40 min) increase of GABA in striatal perfusates (+495±121 %, n=9) and also perfusates of the ipsilateral substantia nigra (+534±144 %, n=5). Intra-nigral application of kainic acid (100 µM) induced a similar increase of GABA in nigral perfusates (+232±43 %, n=5). The effects of kainic acid, whether applied locally into striatum or substantia nigra were reduced significantly when the drug was co-perfused with the non-NMDA antagonist DNQX (10 µM; n=4-5 in each case).

In summary, the present experiments show that i) intra-striatal application of kainic acid induces a DNQX-sensitive release of endogenous GABA in both striatum and the ipsilateral substantia nigra and ii) intra-nigral application of kainic acid induces a DNQX-sensitive release of endogenous GABA in substantia nigra. These findings are consistent with the view that both striatonigral and nigral GABAergic neurones are regulated by EAA (non-NMDA) receptors.

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180P DIFFERENTIAL EFFECTS OF 17 β-OESTRADIOL ON [^{3}H]GABA AND [^{3}H]DA UPTAKE AND RELEASE FROM HYPOTHALAMIC NEURONES MAINTAINED IN PRIMARY CULTURE

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It is accepted that gonadal steroids influence the release of hypothalamic neuropeptides which regulate anterior pituitary hormone secretion. For example, release of somatostatin (SRIH) and gonadotrophin releasing hormone (GnRH) are influenced by the prevailing levels of gonadal steroids, which differ between the sexes and during the oestrous cycle. However, oestrogen receptors are not found within these peptidergic neurones (Flugge *et al* 1986), but it has been postulated that the neurotransmitter input to these cells is directly oestrogen responsive (Herbison *et al* 1989).

Therefore we have investigated the uptake and release of [^{3}H] GABA and [^{3}H] DA from primary cultures of dispersed foetal rat hypothalamic cells (taken on day 18 of gestation), maintained in a defined medium, supplemented with a range of concentrations of 17 ß - oestradiol (E_2) from the time of plating (Clarke & Gillies 1987).

The characteristics of the uptake systems differed in many ways. The avidity of the uptake system for GABA was considerably greater than for DA the former requiring 0.1 µCi/well of cells, (specific activity - 91 Ci/mmol), for 10 min and the latter 2.0 µCi/well of cells, (specific activity - 60 Ci/mmol), for 60 min to achieve submaximal loading. With time in culture, no significant differences in [^{3}H] GABA uptake were noted irrespective of the prevailing E_2 concentration, (range 153551 ± 3095 - 1467692 ± 3934 dpm/well of cells, mean ± s.e.mean. n=4, on day *in vitro* 17 (DIV 17). However, the 56mM K^+ stimulated [^{3}H] GABA release was significantly greater ($p < 0.05$, Students t test), from cells grown in 10^{-10}M E_2 (16414 ± 469) compared with 10^{-12}, 10^{-8} and 10^{-6} M (14385 ± 157, 14654 ± 42.27 and 13705 ± 212 respectively), on DIV 18 but not on earlier days studied (DIV 9 and 15), thus indicating a maturation of responsiveness occurring with time in culture.

The time and steroid dependent uptake and release of [^{3}H] DA however, followed distinctively different patterns. Uptake increased with time in culture (Murray & Gillies 1992), was optimal at E_2 10^{-10} M (31279 ± 131) and significantly different from 10^{-12}, 10^{-8} and 10^{-6} M E_2 (21352 ± 152, 28039 ± 123 and 18430 ± 137 respectively), on DIV 8. Release however, was optimal at 10^{-12}M E_2 (5129 ± 209) when compared with 10^{-10}, 10^{-8} and 10^{-6} M E_2 (2418 ± 80, 1982 ± 158 and 2058 ± 67, $p< 0.05$) on DIV 10.

Using this model therefore we have demonstrated a differential steroid responsiveness of GABA -ergic and dopaminergic hypothalamic systems during the postnatal period. We propose that specific differences in the pre-programmed developmental patterns of intrahypothalamic neurotransmitter systems may contribute significantly to the development and emergence of gender specific and cyclical patterns of release of the hypothalamic regulatory factors involved in the control of growth and reproduction and that this model may be used further to investigate factors regulating the ontogeny of the neuroendocrine system.

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181P BIPHENYL ACETIC ACID POTENTIATES THE ANTAGONIST EFFECTS OF FLUOROQUINOLONES AT THE $GABA_A$ RECEPTOR OF THE RAT ISOLATED VAGUS NERVE

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Coadministration of certain non-steroidal antiinflammatory drugs, like fenbufen, may increase the risk of convulsions associated with the use of 4-quinolones (Christ 1990). Recent studies have indicated that such effects may result from a synergistic antagonism at the $GABA_A$ receptor by these drugs (Halliwell *et al.*, 1993). We have examined the actions of 4-quinolones alone and in combination with biphenyl acetic acid (BPAA, the active metabolite of fenbufen) against GABA-evoked responses recorded from the rat excised vagus nerve.

Agonist-evoked changes in membrane polarization were recorded, extracellularly, from the excised rat vagus nerve using conventional grease gap techniques. Drugs were applied to the nerve *via* the perfusion system.

GABA (3-3000μM) dose-dependently depolarized the vagus nerve with an EC_{50} of 42±4μM (mean±s.e.,n=24). GABA-evoked depolarizations were antagonised in a concentration-dependent fashion by bicuculline and picrotoxin (both 0.1-10μM) with IC_{50} values of 1.1±0.4μM (n=4) and 0.9±0.1μM (n=4) respectively. Sodium pentobarbitone (30μM) and diazepam (1μM) potentiated the GABA response to 192±20% (n=4) and 126±3% (n=4) of control respectively. The 4-quinolones alone had little or no effect on the $GABA_A$-evoked response. The IC_{50} values for the 4-quinolones tested were: ciprofloxacin 298±46μM, enoxacin 670±120μM, and ofloxacin, cinoxacin and nalidixic acid >1000μM. BPAA (1-100μM) had little effect on the $GABA_A$ response but significantly ($p \leq 0.02$ for all interactions) enhanced the antagonist potencies of the fluoroquinolones. In the presence of BPAA (30μM), the IC_{50} values for enoxacin, ciprofloxacin and ofloxacin were 0.1±0.03μM, 1.1±0.54μM and 172±63μM respectively. The potentiating effects of BPAA upon ciprofloxacin was concentration-dependent: in the presence of 1μM, 30μM and 100μM BPAA, the IC_{50} values for ciprofloxacin were 14.6±2.8μM, 1.1±0.5μM and 0.07±0.02μM respectively. The antagonist potencies of bicuculline and picrotoxin were not enhanced by BPAA (10μM) or ciprofloxacin (10μM).

The inhibitory effects of the combination of BPAA and certain 4-quinolones on the $GABA_A$ receptor may contribute to the adverse effects associated with these compounds.

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182P MODULATION OF MAMMALIAN $GABA_A$ RECEPTORS BY THE NEUROSTEROID 5α-PREGNAN-3α-20α-DIOL

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Some synthetic and endogenous steroids are potent positive allosteric modulators of the $GABA_A$ receptor. In comparison with 5α-pregnan-3α-ol-20-one (3α,5α,P) the neurosteroid 5α-pregnan-3α-20α-diol (3α,5α,20α-diol) has a limited efficacy in inhibiting the specific binding of [^{35}S]-TBPS to rat brain membranes and in augmenting $GABA_A$ receptor mediated [$^{36}Cl^-$] flux in rat brain synaptosomes (Belelli and Gee 1989; McNeil *et al.*, 1992). In behavioural experiments 3α,5α,20α-diol exhibits properties consistent with an anxiolytic action, but in contrast to 3α,5α,P requires much higher doses to produce sedation (McNeil *et al.*, 1992). Here, the actions of these steroids to enhance GABA-evoked currents recorded from bovine adrenomedullary chromaffin cells in culture and from *Xenopus laevis* oocytes (co-injected intranuclearly 2-12 days previously with cDNAs for human α_1, β_1 γ 2L $GABA_A$ subunits) were determined using whole-cell and two electrode voltage-clamp techniques respectively. Data are reported as the mean ± s.e.mean where appropriate.

In bovine chromaffin cells voltage-clamped at -60mV, inward currents evoked by locally applied GABA(10μM) were potentiated in a reversible manner by bath applied 3α,5α,P (10^{-7} - 10^{-6}M) and by 3α,5α,20α-diol (10^{-7} - 10^{-5}M). However, 3α,5α,20α-diol appeared to exhibit a limited efficacy relative to 3α,5α,P. Hence 3α,5α,P (1μM) enhanced the GABA-evoked current to 635 ± 67% of control (n = 3) whereas 3α,5α,20-diol (3 and 10μM) only potentiated such currents to 340 ± 60% (n = 5) and 312 ± 35% of control (n = 4) respectively. If single bovine chromaffin cells express a heterogenous $GABA_A$ receptor population then such observations might result by 3α,5α,20α-diol exhibiting $GABA_A$ receptor subtype specificity. Hence the influence of these steroids on GABA-evoked currents recorded from *Xenopus* oocytes preinjected with human α_1 β_1 γ_{2L} subunits was determined. In such cells (voltage-clamped at -60mV) GABA (10-30μM) produced a current approximately 10% of the maximum elicited by 3mM GABA. Both 3α,5α,P (10^{-9} - 10^{-6}M) and 3α,5α,20α-diol (3 x 10^{-8} - 3 x 10^{-6}M) produced a dose-dependent enhancement of the amplitude of such GABA (10 - 30μM) - evoked currents. However, the maximal enhancement produced by 3μM 3α,5α,20α-diol (312 ± 35% of control n = 4) was again less than that produced by 1μM 3α,5α,P (641 ± 78% of control n = 5). In conclusion, 3α,5α,20α-diol exhibits a reduced efficacy in comparison with 3α,5α,P on native and recombinant mammalian $GABA_A$ receptors. Such effects may contribute to the distinct behavioural profile of 3α,5α,20α-diol.

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183P MODULATION OF THE $GABA_A$ RECEPTOR BY ORG 20599: A WATER-SOLUBLE PREGNANE STEROID

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ORG 20599 [(2β,3α,5α)-21-chloro-3-hydroxy-2-(4-morpholinyl) pregnan-20-one methanesulphonate salt] is a water soluble steroid which rapidly induces hypnosis when administered i.v. in several animal species (Gemmell *et al.*, 1993). Here, the actions of ORG 20599 and comparative hypnotics upon the $GABA_A$ receptors of rat hippocampal neurones and bovine adrenomedullary chromaffin cells in culture have been investigated under voltage-clamp conditions. Additionally, the ability of these compounds to displace specifically-bound [^{35}S]TBPS from whole rat brain membrane homogenates was studied (Gee *et al.*, 1988). Data are reported as mean ± s.e.mean where appropriate.

Acting upon bovine chromaffin cells voltage-clamped at -60mV, ORG 20599 (30nM-10μM) enhanced the amplitude of submaximal inward current responses elicited by locally applied GABA (100μM) in a concentration-dependent and reversible manner (Figure 1). The comparative hypnotic compounds tested produced a qualitatively similar potentiation, with the order of potency: 5α-pregnan-3α-ol-20-one > ORG 20599 > alphaxalone > propofol > thiopentone (Figure 1). Currents mediated by $GABA_A$ receptors of rat hippocampal neurones in culture were also potentiated in amplitude by ORG 20599 to 179 ± 11.7 (n=8), 297.4 ± 20.4 (n=9), and 330 ± 37.5 (n=4) % of control in the presence of 0.1, 0.3 and 1.0μM of the compound respectively. Over the same concentration range, bath applied ORG 20599 elicited dose-dependent, inward current responses in the absence of exogenous GABA. Such currents were potentiated by pentobarbitone (30μM) or diazepam (1μM), blocked by bicuculline methiodide (10μM) and reversed in polarity at the chloride equilibrium potential. Direct stimulation of the $GABA_A$ receptor, or potentiation of endogenous GABA, seems likely to underlie this effect.

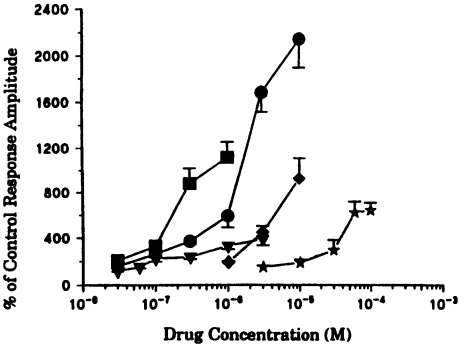


Figure 1. Potentiation of GABA-induced Currents by ■ 5α -Pregnan-3α-ol-20-one ● ORG 20599 ▼ Alphaxalone ♦ Propofol ▲ Thiopentone. Data points are the mean of 4-24 observations.

In the [^{35}S]TBPS binding assay, in the presence of 0.6μM added GABA, ORG 20599 and the other compounds evaluated displaced equilibrium binding with IC_{50} values of: 5α-pregnan-3α-ol-20-one 73.5 ± 3.3nM; ORG 20599 152.0 ± 11.8nM; alphaxalone 332.8 ± 25.9nM; propofol 8.3 ± 0.5μM and thiopentone 29.5 ± 1.6μM, an order of potency identical to that found electrophysiologically. In conclusion, ORG 20599 is a potent positive allosteric modulator of $GABA_A$ receptor function, and may additionally possess a direct GABAmimetic action. Both effects could contribute to the behavioural properties of this compound.

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184P ACTIVATION OF GABA RECEPTORS DIFFERENTIALLY AFFECTS LUTEINIZING HORMONE-RELEASING HORMONE SECRETION FROM IMMORTALIZED HYPOTHALAMIC NEURONS

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Gamma-aminobutyric acid (GABA) is involved in the control of gonadotropin secretion *in vivo* (Donoso et al., 1984). Moreover, recent evidence suggest that GABA can modulate LHRH secretion in vitro (Masotto et al., 1989). In the present study, we have used an immortalized hypothalamic neuronal cell line (GT1-7) (Mellon et al., 1990) to evaluate the effects of the GABAergic agents on LHRH secretion. Cultures from GT1-7 cells were incubated with GABA or the selective $GABA_A$ (muscimol) or $GABA_B$ (baclofen) receptor agonists and LHRH secretion was evaluated by RIA. LHRH secretion was stimulated with increasing concentrations of GABA. This effect was mimicked by muscimol (10-100 μM) leading to a dose-response increase in LHRH release. The stimulatory action of muscimol was antagonized by the specific $GABA_A$ receptor antagonist, bicuculline (100 μM). By contrast, the $GABA_B$ receptor agonist, baclofen (20-200 μM), had no effect on basal LHRH release. Since GABA has been reported to have an inhibitory effect on neuronal function, GT1-7 cells were depolarized with 28 mM [K^+] and the effect of muscimol or baclofen were evaluated. The addition of 50 μM muscimol in the presence of 28 mM [K^+] enhanced the [K^+]-stimulated LHRH secretion in a synergistic manner. When either 50 or 100 μM baclofen was administered in the presence of [K^+], LHRH secretion was significantly depressed relative to cells which had been stimulated with [K^+] alone. These results were further corroborated by binding analysis which revealed that GT1-7 cells contained both $GABA_A$ and $GABA_B$ binding sites. In addition, when Northern blots were hybridized with the cDNAs to the $GABA_A$ receptor α_1 β_3 $_{2L}$ subunits, all 3 subunits were found to be expressed in rat hypothalamus. Transcript for only the β_3 subunit, however, were evident in the GT1-7 cells. These data provide the first demonstration that LHRH neurons are directly responsive to GABAergic agents. Moreover, these results reveal a pharmacologic basis for understanding the dual role that GABA plays in regulating mammalian reproduction. (Supported by the NIEHS Intramural Research Program)

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185P EFFECT OF DIAZEPAM PRETREATMENT ON THE SUPPRESSION OF FOOD INTAKE INDUCED BY CHOLECYSTOKININ (CCK) IN NON-DEPRIVED RATS

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Kubota et al. (1986) have previously reported that the attentuation of feeding caused by intracisternal administration of CCK in mice is antagonised by prior administration of benzodiazepines, such as flurazepam and diazepam. In the present study we investigated whether pretreatment with a benzodiazepine could also reverse the inhibitory effect of systemically administered CCK on food intake in rats.

Non-deprived Male Wistar rats (n=10, b.wt. 360 - 450g) were given seven 60 min habituation sessions during which they were placed individually in experimental cages where they had access to a wet mash. Prior to each of the experimental sessions that followed, the rats were injected with either vehicle solution (veh) followed by saline (sal), veh followed by CCK (2μg kg^{-1}), diazepam (1mg kg^{-1}) (D1) followed by sal, D1 followed by CCK (2μg kg^{-1}), diazepam (2 mg kg^{-1}) (D2) followed by sal, D2 followed by CCK (2μg kg^{-1}). The 1st injection was given s.c., and the 2nd i.p. A period of 60 min separated the 2 injections. Immediately after the 2nd injection, the rats were placed in the experimental cages where they had free access to food and water, and the amount of food consumed by the animals was measured after 30 min. A repeated measures design was used with each animals receiving all 6 treatments. 2 - 3 days separated successive trials. The data were analysed by ANOVA with repeated measures, and post-hoc tests were carried out using the Duncan's multiple range test.

Treatment	Veh + Sal	Veh + CCK	D (1) + Sal	D(1) + CCK	D(2) + Sal	(D2) + CCK
Mean Food Intake (g) ± s.e.mean	11.6 ± 1.2	2.5 ± 0.5**	15.0 ± 1.8	7.2 ± 2.1	23.3 ± 2.2**	8.6 ± 2.6 •

Comparison with Veh + Sal ** P<0.01. Comparison with Veh + CCK •P<0.05

Analysis of the data (see Table) showed significant effects of treatment ($F_{(5,40)}$ = 14.6559, P<0.001). Post-hoc tests revealed that the 2 mg kg^{-1} dose of diazepam significantly attenuated (P<0.05) the reduction in food intake induced by CCK. However, this dose of diazepam also significantly increased food intake (P<0.01) when given alone. The lower dose of diazepam (i.e. 1 mg kg^{-1}) did not produce similar effects. Thus, the results of this study suggest that the hyperphagia caused by diazepam tends to counteract the inhibitory effects of systemically administered CCK on food intake. However, this interpretation of the data does not exclude a complex interaction between CCK and the benzodiazepines.

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186P ALTERATION OF MOBILITY IN FORCED SWIM TEST: EFFECTS OF DESIPRAMINE, ALPRAZOLAM AND MUSCIMOL IN PENTYLENETETRAZOL KINDLED RATS

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The forced swimming test (FST) is an animal model sensitive to antidepressant treatments. Recent studies indicate that stressful situations may play an important role in understanding how responses to stress are modulated by pharmacological treatments in FST (West, 1990). Our previous experiments showed that desipramine (DMI) and alprazolam (ALP) decrease the immobility time in FST, apparently influencing interactions between stress-induced changes and neuronal effects due to drugs (Flugy et al., 1992). Since different neurotransmitter systems, besides GABA one, may be involved in anti-immobility effect, the present series of experiments was undertaken to test influences of DMI, ALP and muscimol (MUS) in FST in rats after termination of chronic treatment with pentylenetetrazol (PTZ), a GABA-A receptor negative modulator. Groups of 10 male Wistar rats (250-280 g) were sensitized by subconvulsant dose of PTZ (20 mg/kg, i.p.) administered three times a week for 5 weeks. Animals received i.p. DMI (2.5-10 mg/kg), ALP (5-20 mg/kg) and MUS (0,5-2 mg/kg) after 15 min from first stressful swimming session and 24-5-1 h before the second session. Data were analyzed with an analysis of variance followed by Tukey's test. Results showed that PTZ sensitization was ineffective in alterating immobility time in FST. Subchronic DMI (10 mg/kg) exerted a slight but significant (P<0.05) reduction in the immobility time in chronic vehicle-treated rats, while a marked potentiation (P<0.001) of DMI effect was noted in PTZ-treated rats. Subchronic ALP, in control rats, reduced significantly (P<0.05) immobility time at the dose of 20 mg/kg. On the contrary, in PTZ-sensitized rats, ALP failed to modify immobility time. Subchronic MUS (2 mg/kg) decreased markedly (P<0.001) immobility time on both vehicle- and PTZ-treated animals. These experimental findings may be regarded as demonstrating that a lasting PTZ-induced reduction in the GABA-A receptor function is not able to alter, like acute reduction, (Cannizzaro et al., 1992), immobility time in FST. The potentiation of DMI effect cannot be attribuited only to an imbalance between a reduced GABA-A receptor activity and a concomitant augmentation of monoaminergic neurotransmission since chronic selective GABA-A negative modulator picrotoxin is not effective (Cannizzaro et al., 1993). The persistent down-regulation of the GABA-A receptor may be responsible for the lack of effect of ALP, while the dopamine-mediated action of MUS does not seem to be influenced by reduction of GABA-A function.

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187P PRENATAL DIAZEPAM ENHANCES THE EFFECT OF DESIPRAMINE AND INHIBITS THAT OF ALPRAZOLAM ON FORCED SWIMMING TEST OF ADULT RAT OFFSPRING

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Several alterations have been showed in animals and humans exposed prenatally to benzodiazepines. Besides, prenatal diazepam (DZ) increased the brain susceptibility to convulsants and induced a reduction in biochemical parameters of the GABA-A receptor in adult rat offspring (Livezey et al.,1986; Kellogg et al., 1991). The GABA-A receptor seems to be involved in the effect either of tricyclic antidepressants, i.e.desipramine (DMI), or of alprazolam (ALP), a GABA/BZ receptor agonist. DMI and ALP decrease the locomotor activity and increase the mobility of rats in forced swimming test (FST) (Flugy et al., 1992) suggested valid animal model of depression. Therefore, the effects of DMI and ALP were studied in FST in adult rats prenatally exposed to DZ. Moreover, bioelectrical activity of the hyppocampus was analyzed following ALP microinjections intra locus coeruleus (L.C.). Female Wistar rats (250 ± 20 g) received a single daily s.c. injection of DZ at 1.25 mg/kg or vehicle from 14th to 20th gestational day. After 15 min from first swimming session and 24-5-1 h before the second session, groups of 10 adult male rat offspring (280 ± 20 g) received DMI (5-7.5-10 mg/kg,i.p.) and ALP (5-10-20 mg/kg,i.p.). Data were analized with an analysis of variance followed by Tukey's test. Prenatal DZ did not affect the immobility time in FST nor locomotor activity. Prenatal DZ significantly ($P<0.01$)increased the anti-immobility effect of DMI (+52% and +28% for DZ and vehicle groups, respectively) and antagonized that of ALP. Locomotor activity was similarly reduced in both prenatally vehicle and DZ treated rats. DMI acts as functional GABA-A receptor antagonist. A reduction in the functionality of GABA-A receptor seems to be related to anti-immobility effect of DMI in FST. Thus,the increased response to DMI of prenatally DZ exposed rats in FST could reflect a lower GABA-mediated neuronal inhibition than in control animals. ALP seems to increase the mobility of rats in FST by reducing the L.C. noradrenaline hyperactivity induced by stressful swimming; prenatal DZ alters the circuitry involved in the mechanisms of stress in adult rat offspring (Simmons et al., 1984). Accordingly in DZ exposed progeny, intra L.C. ALP microinjection was no more effective in modifying bioelectrical activity in the hippocampus, a target structure of L.C. noradrenergic efferent pathway. Thus, an altered response of L.C. neurons to abnormal stimuli induced by stress of forced swimming seems to be the reason for the lack of activity of ALP in prenatal DZ exposed offspring.

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188P AZOLE COMPOUNDS ANTAGONISE THE BICUCULLINE INSENSITIVE, BACLOFEN INSENSITIVE GABA RECEPTOR ON THE MUSCLE CELLS OF THE PARASITIC NEMATODE *ASCARIS SUUM*

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The GABA receptor on *Ascaris* muscle cells gates a chloride channel that is bicuculline insensitive, only weakly antagonised by picrotoxin and not modulated by benzodiazepines nor barbiturates (Holden-Dye *et al.*, 1989). It thus closely resembles a GABA receptor, designated $GABA_C$, recently described in vertebrate visual system (Feigenspan *et al.*, 1993; Qian & Dowling, 1993). Here we describe some novel antagonists of the *Ascaris* GABA receptor that are azole derivatives. Recordings of *Ascaris* muscle cell membrane potential and input conductance were made using conventional electrophysiological techniques. The effects of the compounds on the response to bath applied GABA (30 μM) were determined. Dimethylsulfoxide, as a vehicle (1 %), had no direct effect on the muscle cells. Five of the compounds blocked the response to GABA with similar IC_{50}s of less than 10 μM (Table 1). The block was partially reversible after a wash period.

Table 1. Azole compounds antagonised the increase in input conductance of *Ascaris* muscle cells elicited by bath applied GABA (30 μM). Each value is the mean ± s.e.mean of at least 3 independent determinations.

COMPOUND	CHEMICAL NAME	IC_{50} μM
SN610272	2-(2-nitro-4-trifluoromethylphenyl)-4-(4,5-dicyano-1H-imidazol-2-yl)-5-methyl-2H-1,2,3-triazole	1.3 ± 0.4
SN609997	5-chloro-1-(2,6-dichloro-4-trifluormethylphenyl)-4-(4,5-dicyano-1H-imidazol-2-yl)-3-ethyl-1H-pyrazole	3.7 ± 0.6
SN609899	2-(2,6-dichloro-4-trifluoromethylphenyl)-4-(4,5-dicyano-1H-imidazol-2-yl)-5-ethyl-2H-1,2,3-triazole	2.6 ± 0.5
SN606078	2-(2,6-dichloro-4-trifluoromethylphenyl)-4-(4,5-dicyano-1H-imidazol-2-yl)-2H-1,2,3-triazole	5.5 ± 1.2
SN608154	2-(2,6-dichloro-4-trifluoromethylphenyl)-4-(4,5-dicyano-1H-imidazol-2-yl)-5-methyl-2H-1,2,3-triazole	7.4 ± 3.3
SN609760	2,6-dichloro-4-trifluoromethylphenyl-4-(5-bromo-4-trifluoromethyl-1H-imidazol-2-yl)-2H-1,2,3-triazole	>10
SN608449	2-(2,6-dichloro-4-trifluoromethylphenyl)-4-(4,5-dicyano-1H-imidazol-2-ylthio)-5-methyl-2H-1,2,3-triazole	>10
SN611297	2,6-dichloro-4-trifluoromethylphenyl-4-(5-bromo-4-trifluoromethyl-1H-imidazol-2-yl)-5-ethyl-2H-1,2,3-triazole	>10
SN606011	1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trimethyl-silyl-1H-1,2,3-triazole	>10

These are the first antagonists, apart from the avermectins (Holden-Dye *et al.*, 1990), reported for the *Ascaris* GABA receptor. Because of the striking resemblance between the *Ascaris* GABA receptor and the $GABA_C$ receptor, these compounds may also prove to be useful antagonists of the $GABA_C$ receptor subtype.

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189P Org 20599, A NEW WATER SOLUBLE AMINOSTEROID INTRAVENOUS ANAESTHETIC

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Org 20599 ([2β,3α,5α]-21-chloro-3-hydroxy-2-(4-morpholinyl)pregnan-20-one methanesulphonate salt) selectively modulates the $GABA_A$ receptor and displaces specifically bound [^{35}S]TBPS from rat brain membrane homogenates (Hill-Venning *et al.*, 1993). Here, the ability of the water soluble steroid Org 20599 and other anaesthetics to induce hypnosis following iv administration to a variety of animal species has been investigated.

For these studies the steroids alphaxalone and 5α-pregnan-3α-ol-20-one were dissolved in hydroxypropyl β cyclodextrin (HPCD). Using probit analysis, the hypnotic dose 50 (HD_{50}) in mice and rats for each drug was determined. In mice the LD_{50} was also determined to derive a therapeutic index (TI).

Org 20599, administered iv over 10 seconds, induced a rapid onset loss of righting reflex in mice, rats and dogs. In mice, the three steroids tested were approximately equipotent, and all considerably more potent than propofol and thiopentone (Table 1). Org 20599 possessed a greater TI than either the non-steroidal anaesthetics propofol and thiopentone but a smaller TI than alphaxalone. Org 20599 in repeat dosing studies (data not shown), also in mice, like propofol and alphaxalone, was not cumulative. Org 20599 in rats proved less potent than alphaxalone, but again more potent than propofol and thiopentone. In dogs, the minimal effective dose (MED) for each compound was determined and again the order of potency was alphaxalone > Org 20599 > propofol > thiopentone. In all species onset was rapid, sleeping times were short, and recovery rapid. In dogs the sleep duration for each compound at the MED was <4 minutes.

Table 1 Potency of Org 20599 and Other Intravenous Anaesthetics in Mice, Rats and Dogs

Compound	Mouse (n=8) HD_{50}(μmolkg^{-1}) (95% Fiducial Limits)	Mouse (n=8) Therapeutic Index	Rat (n=8) HD_{50}(μmolkg^{-1}) (95% Fiducial Limits)	Dog (n=3) MED (μmolkg^{-1})
5α-Pregnan-3α-ol-20-one	10 (8.2-12.2)	/	/	/
Alphaxalone	6.3 (5.7-7.2)	23.7	2.4 (2.0-2.8)	3
Org 20599	8.5 (7.6-9.5)	6.1	7.0 (5.6-8.6)	7.4
Propofol	68.2 (64.1-71.8)	3.6	26.3 (23.8-29)	16.8
Thiopentone	83.2 (74.5-91.7)	3.7	41.7 (34.6-49)	30.3

Org 20599, as well as being a fast onset short duration intravenous anaesthetic in animals, is also water soluble; a property considered essential in an "ideal" intravenous anaesthetic (Dundee, 1985). It would seem probable that the anaesthetic activity of Org 20599, like that of alphaxalone, may be explained by its actions at the $GABA_A$ receptor (Hill-Venning *et al.*, 1993).

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190P STUDY ON THE GROWTH HORMONE RELEASING PROPERTIES OF A FAMILY OF GROWTH HORMONE-RELEASING PEPTIDES AND THEIR MECHANISM OF ACTION

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In recent years a number of hexa- and hepta-peptide analogues of GHRP-6 (His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2) have been developed (Momany et al.,1984). GHRPs have been shown to specifically stimulate GH secretion in all the species so far investigated (Bowers et al., 1984). We have studied the GH-releasing activity of GHRPs in which one or more Trp residues were substituted with the more hydrophobic and chemically more stable 2-Methyl-Trp (2-Me-Trp) derivative. In the 10-day old conscious rat, an animal model highly responsive to GH-releasing stimuli, substitution with L-2-Me-Trp resulted in a smaller GH releasing activity, suggesting the existence for some analogues of an antagonistic effect, whereas the D-2-Me-Trp substitution resulted in greater potency and reduced toxicity. In particular, the hexapeptide His-D-2-Me-Trp-Ala-Trp-D-Phe-Lys-NH_2 (Hexarelin) (300 μg/kg, sc) was more potent than GHRP-6 (300 μg/kg, sc) (36.9 ± 22.3,163.5 ± 21.5 and 127.0 ± 18.6 ng GH/ml for controls, Hexarelin and GHRP-6, respectively). GHRP-6 and Hexarelin (32-640 μg/kg, sc) dose-dependently stimulated GH secretion with similar ED_{50} (42 ± 29 and 40 ± 19 μg/kg for GHRP-6 and Hexarelin, respectively) and C_{max} (204 ± 34 and 219 ± 26 for GHRP-6 and Hexarelin, respectively). In time-course studies, GHRP-6 and Hexarelin elicited their maximal effect 15 min after sc administration (Hexarelin being more active than GHRP-6 at 5 and 10 min) and induced plasma GH levels still significantly higher than basal values 60 min post-treatment. In spite of having been characterized as potent GH releasers, the intimate mechanism(s) of action of GHRPs remains still largely unknown. Stimulation of GH secretion by GHRP-6 was independent from stimulation and/or inhibition of endogenous GHRH and SRIF release, respectively. In fact, acute immunization with specific antisera against GHRH (GHRH-ab) and/or SRIF failed to alter GH secretory burst induced by GHRP-6 (300 μg/kg, sc). Moreover, GHRP-6 was capable to stimulate GH synthesis. In fact, in 10-day old rats passively immunized against GHRH from the day of birth, a 3 day-treatment with GHRP-6 (80 μg/kg, sc, daily) potentiated the GH response to the acute challenge with GHRP-6 (10 ± 0.8, 22 ± 1.7 and 41 ± 5.3 ng GH/ml for GHRH-ab, GHRH-ab+acute GHRP-6 and GHRH-ab + chronic + acute GHRP-6, respectively). The results of these studies demonstrate that in the infant rat GHRPs are potent GH releasers whose action is not mediated by endogenous GHRH and/or SRIF. GHRPs, by acting directly on the pituitary or through the mediation of a still unidentified hypothalamic factor, are capable to stimulate both GH release and synthesis. Hexarelin and GHRP-6 should be considered as diagnostic and therapeutic tools in GH secretory disturbances.

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191P THE EFFECTS OF OXYTOCIN ON SOCIAL MEMORY FOLLOW A BIMODAL, SINUSOID DOSE-RESPONSE CURVE

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In the last few years evidence has been presented by different groups that oxytocin modulates several behaviors in animals. This neuropeptide stimulates sexual behavior both in male and female rats, elicits parental care, stimulates foraging, inhibits feeding and drinking, has antidepressant-like activity in animal models of depression, and affects memory. The effects of oxytocin on memory processes are however controversial. Depending on the dose, route of administration, and animal model, oxytocin has been reported either to disrupt or to improve memory. The sole undisputed datum is that oxytocin exerts its action on memory processes at the CNS level.

The aim of the present research was to evaluate the effect of a wide dose-range of oxytocin in the same experimental model (social memory, or recognition of conspecific identity)(Thor & Holloway, 1982; Dantzer *et al.*, 1987), under the same experimental conditions, and using only the intracerebroventricular (i.c.v.) route of administration. The social investigation time (SIT) of adult male rats towards juvenile male ones was measured immediately before i.c.v. administration of oxytocin and 120 min later, and the difference between the SITs of the first and second encounter (Δ SIT) was calculated. Oxytocin was injected at the doses of 0.1, 1, 10, 100, 500 and 1000 ng/rat.

At the lowest doses, oxytocin improved social memory, the maximum effect (Δ SIT=−51.7±6.1 s; n=16; $P<0.01$, Student's t test) being observed after the injection of 1 ng/rat. At the highest doses, oxytocin impaired social memory, the maximum effect (Δ SIT=+36.3±4.8 s; n=16; $P<0.05$, Student's t test) being observed after the injection of 500 ng/rat. After the maximum dose (1000 ng/rat) Δ SIT was similar to that observed in saline-treated rats. So, the effect of i.c.v. oxytocin in the social memory test follows a bimodal, sinusoid-shaped dose-response curve.

Both the memory-improving (1 ng/rat) and the memory disrupting (500 ng/rat) effects of oxytocin were abolished by the concomitant administration of an equal dose of the receptor antagonist $d(CH_2)_5$-Tyr(Me)-Orn^8-vasotocin by the same route.

These data confirm that oxytocin affects social memory by an action at the CNS level involving specific receptors. The opposite effect of low and high doses may be due either to the involvement of different circuitries with opposite activities on memory processes, or to the activation of different subpopulations of oxytocin receptors mediating opposite effects on memory.

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192P AGING ABOLISHES THE BEHAVIOURAL RESPONSE TO ADRENOCORTICOTROPIN

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In experimental animals, adrenocorticotropin (ACTH) and many of its fragments, including α-MSH (melanocortins, melanopeptides) induce a unique and complex behavioral syndrome, which is typically characterized by compulsive cleaning of the body surface (excessive grooming) and by repeated episodes of stretching, yawning and penile erection with ejaculation. Moreover, melanopeptides increase sexual receptivity in females, reduce food intake, increase motivation and attention, facilitate short-term memory processes. Most of these behavioral effects require the functional integrity of brain cholinergic neurons.

Ageing is associated with a progressive impairment of brain cholinergic function, and it is noteworthy that the most typical behaviors stimulated by melanocortins become less frequent with ageing. So, stretching, yawning and spontaneous penile erection occur much more frequently in young than in old animals; in humans, old people rarely yawn and even more rarely stretch or display full penile erection; moreover, self-care and body cleaning (=grooming) are often neglected in the old, and deterioration of short-term memory is typical of ageing.

These considerations prompted us to study the influence of ageing on the behavioral response to melanocortins. Fifteen 28-month-old male Sprague-Dawley rats were intracerebroventricularly (i.c.v.) treated with 4 μg of ACTH-(1-24) (dose and sequence had proved to be the maximum behaviorally active in previous experiments), and grooming, stretchings, yawnings and penile erections were scored for 60 min. Thirty 4-month-old male rats of the same strain were randomly divided into two equal groups and i.c.v. treated either with 4 μg of ACTH-(1-24) or with the vehicle (4 μl of saline). While all the 4 month-old rats displayed the whole, typical behavioral syndrome (penile erections: 3.12±0.78; yawnings: 5.25±0.95; stretchings: 19.50±3.67; grooming score: 148.86±8.26), ACTH failed to elicit any behavioral effect in old rats, and the scores (penile erections: 0.13±0.09; yawnings: 0.25±0.13; stretchings: 1.53±0.45; grooming score: 44.00±6.86; $P<0.05$, at least, in comparison with 4 month-old rats, ANOVA followed by Bonferroni's test) were quite similar to those obtained in saline-treated rats.

These data show that old animals are unresponsive to the behavioral effects of melanocortins, suggesting either an ageing-linked impairment of the involved nervous circuitries or a reduction of the number (or affinity, or both) of brain melanocortin receptors in the elderly. As far as penile erections are concerned, a reduced testosterone production may also be implicated, because this melanocortin-induced behavioral symptom requires the presence of androgens.

193P EVIDENCE THAT BRADYKININ CONTRIBUTES TO THE PRONOUNCED ANTIARRHYTHMIC EFFECTS OF ISCHAEMIC PRECONDITIONING

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Two short (5 min) periods of coronary artery occlusion markedly reduce the severity of arrhythmias that occur during a subsequent longer (25 min) occlusion of the same artery, a phenomenon known as ischaemic preconditioning (Vegh et al. 1990; 1992a). There is evidence that this protection results, in part, from the generation of endothelium-derived substances such as prostanoids (Vegh et al. 1990) and nitric oxide (Vegh et al. 1992b). Because bradykinin is released early in ischaemia, and because it triggers the generation and release of both these substances from endothelial cells, we have examined a possible role for bradykinin in preconditioning by blocking bradykinin $(B)_2$ receptors using HOE 140.

Dogs (mean weight 27 ± 2 kg) were anaesthetised with chloralose and urethane and the anterior descending branch of the left coronary artery (LAD) prepared for occlusion as previously described (Vegh et al., 1992a). HOE 140 (300 μg kg^{-1} iv) was given 10 min before the first of the two 5 min preconditioning LAD occlusions. It was difficult to precondition dogs in the presence of HOE 140 because of the high incidence (50%) of ventricular fibrillation (VF) during the preconditioning procedure and there were more premature beats (VPBs) during the initial 5 min preconditioning occlusion in the presence of the bradykinin antagonist (43 ± 11 v 14 ± 13 in the absence of HOE 140; $P < 0.05$). In those dogs that survived the preconditioning procedure the marked reduction in VPBs following preconditioning (497±109 in control non-preconditioned dogs to 72±21; $P < 0.01$) and the incidence of VT (80% to 21%; episodes of VT 5.9±1.7 to 0.7±0.4) and VF (47% to 0%) were attenuated by HOE 140 (VPBs 390±146; VT 67%; VT episodes 8.0±6.2 per dog; VF 16%). Survival following reperfusion was 0% in the controls, 40% in the preconditioned dogs and 8% in dogs preconditioned in the presence of HOE 140.

These results suggest that bradykinin released early in ischaemia, is cardioprotective and may act as an initial trigger for the antiarrhythmic effect of ischaemic preconditioning by releasing prostanoids and NO.

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194P PHARMACOLOGICAL DISSECTION OF MULTIPLE CLASSES OF CALCIUM CHANNELS IN THE MOUSE AtT20 CELL LINE

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We have recently demonstrated that rat central neurones contain an unique, Ca^{2+} current characterized by its insensitivity to both the dihydropyridine, nimodipine (an L type channel blocker), and the peptide toxins, ω-CTx-GVIA and ω-Aga-IVA (N- and P-type channel blockers respectively). This "Q-type" current can be inhibited by the toxin ω-CTx-MVIIC (Randall et al. 1993). We utilized this toxin to demonstrate that both N-type and Q-type currents have central roles in Ca^{2+}-dependent neurotransmitter release in the hippocampus (Wheeler et al. 1993).

Anterior pituitary cells, although of non-neuronal lineage, share many features of Ca^{2+}-dependent release with neurones. We have, herein, analysed the calcium current complement of the mouse AtT 20 anterior pituitary cell line, to examine if they contain either or both of the two channel types stongly implicated in central neurotransmitter release.

Calcium currents were studied in whole cell recordings from 64 cells, Ba^{2+} ions being used as charge carrier. The extracellular medium consisted, mM; TEACl, 160; $BaCl_2$, 5; HEPES-TEAOH 10, TTX, 0.001; pH 7.3, 313 mOsm. The recording electrodes (2-7 MΩ) contained, mM $CsMeSO_4$, 108; HEPES-CsOH, 24; EGTA, 9; $MgCl_2$, 4.5; ATP, 4; GTP, 0.3, pH 7.3, 297 mOsm. Drug actions were studied on Ba^{2+} currents elicited by a 105 ms voltage step to 0 mV from a holding potential of -80 mV applied every 10 s. All data are presented as mean ± S.E.M.

The total Ba^{2+} current in these cells was 12.7 ± 0.4 pA/pF (capacitance = 12.1 ± 0.42 pF). This current was entirely high-voltage activated (HVA), since no current was observed until depolarizations exceeded -40 mV. The current was also sensitive to Cd^{2+} ions (1 μM and 10 μM depressing 73 ± 2% and 88 ± 4% respectively, n=4). Addition of the N-type current blocker ω-CTx-GVIA (1 μM) irreversibly blocked 3.4 ± 0.2 pA/pF or 25.6 ± 1.7% of the current (n=38). The L-channel agonist FPL 64 176 enhanced control current 395 ± 34% (n = 3), whereas the antagonist nimodipine depressed 4.7 ± 0.2 pA/pF equivalent to 34.7 ± 1.2% (n = 29) of the total current.

Much current in these cells (5.3 ± 0.2 pA/pF or 42.2 ± 1.9%, n = 29) is, therefore, carried by neither L- or N-type channels. This resistant current was entirely high-voltage activated and decayed considerably (eg 54 ± 5%, n = 11 during a 105 ms test pulse from -90 mV to 0 mV). Steady state inactivation analysis of ω-CTx-GVIA/nimodipine insensitive Ba^{2+} current of 12 cells suggested at least two discrete currents remained unblocked. Addition of the P-type channel blocker ω-Aga-IVA (30 nM) had no significant effect on either basal Ba^{2+} current (n = 6) or that recorded in the presence of ω-CTx-GVIA and nimodipine (n = 3). The Q-type channel blocker ω-CTx-MVIIC (5 μM) did, however, block 65.0 ± 8.7% of the current insensitive to L, N and P-type blockers. As observed for its actions on neuronal Q-type current, the block afforded by this compound was slow (τ~70 s). The current left unblocked by all four antagonists exhibited a prominent decay during a 105 ms voltage pulse and probably represents another current we have recently described (Ellinor et al. 1993). These data suggest, that in addition to L-type currents, these non-neuronal excitable cells contain both N- and Q-type currents. Since both of these currents seem to be involved neuronal excitation-secretion coupling, it will be of interest to examine if they play a similar role in Ca^{2+}-dependent pituitary hormone secretion.

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A.D.R is a Lucille P. Markey visiting fellow.

195P COMPARISON OF INTRASEPTAL AND INTRAVENTRICULAR INJECTIONS OF COLCHICINE AND DENDROTOXIN$_I$ ON HIPPOCAMPAL LONG TERM POTENTIATION INDUCED BY MEDIAL SEPTAL STIMULATION IN THE RAT

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The septal complex gives rise to a major cholinergic input to the hippocampus, degeneration of which leads to short-term memory impairment in rats (Flicker et al.,1983) and to the severe cognitive deficits which are a characteristic of Alzheimer's disease (Bartus *et al.* 1982). This study compared the effects of medial septal and intracerebroventricular (icv) injections of colchicine (7 µg) and dendrotoxin$_I$ (22.6 pg) on long-term-potentiation (LTP), evoked by medial septal stimulation, to intraperitoneal injections of physostigmine and oxotremorine. Colchicine, which binds to tubulin, is reported to selectively lesion choline-acetyltransferase positive cells in the medial septum (Ginn & Peterson, 1992) whereas α-dendrotoxin, a selective K^+ channel blocker is known to cause hippocampal damage (Bagetta *et al.*, 1992). LTP is thought to play a role in the encoding of memory and is modulated by the cholinergic system (Krnjevic & Ropert, 1982). Three-4 weeks after either lesion the rats were anaesthetized with urethane (1.75 mg/kg i.p.) and electrodes implanted in the medial septum and dorsal CA1 hippocampal subfield for electrical stimulation and recording, respectively. EPSPs (average of 10 responses at 20 s intervals) were recorded every 10 min with a stimulus current 30% above threshold. LTP was induced after a 30 min control period by applying theta burst electrical stimulation at twice the amplitude used to evoke the EPSP; for statistical purposes, its amplitude was compared to that of the immediately preceding control values .

Dendrotoxin$_I$-treatment significantly increased the current required to evoke an EPSP and prevented the induction of LTP ($P = 0.02$ after icv dendrotoxin$_I$, $n = 8$ and $P = 0.04$ after intraseptal dendrotoxin$_I$, $n = 4$). Post-mortem examination showed there to be considerable cortical thinning and ventricular enlargement. Colchicine-treated animals also required an increased stimulation current to evoke an EPSP but, and in contrast to dendrotoxin$_I$-treated animals, LTP was induced and the potentiation (a 3.2 ± 0.6 fold increase in EPSP amplitude after icv colchicine, $n = 6$ and 3.2 ± 0.3, $n = 6$ after intraseptal colchicine) was similar to that seen in control animals (3.5 ± 0.4, $n = 13$, n.s.); however, maintenance of LTP was reduced over the 2 h observation period. Physostigmine (0.3 mg/kg i.p.) and oxotremorine (0.5 mg/kg i.p.) did not affect the evoked EPSP or the response to theta burst stimulation in animals treated with either colchicine or dendrotoxin$_I$. Thus colchicine, in a dose reported to lesion septo-hippocampal cholinergic fibres, did not affect generation of LTP whereas dendrotoxin$_I$ caused extensive brain damage and prevented induction of hippocampal LTP. Experiments are in progress to elucidate dendrotoxin$_I$'s actions.

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196P INVESTIGATION OF THE MECHANISM OF ATP-STIMULATED CHANGES IN INTRACELLULAR CALCIUM AND pH IN ISOLATED RAT PAROTID ACINI

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ATP stimulates amylase secretion in the rat parotid gland (Arkle *et al.*, 1991). This response is associated with increased cytosolic calcium activity ($[Ca^{2+}]_i$) via a mechanism which is independent of phosphatidylinositol turnover (Soltoff *et al.*, 1990). Stimulation with other Ca^{2+}-mobilising secretagogues is associated with cellular acidification as measured by a decrease in cytosolic pH (pH_i) (Nauntofte & Dissing, 1988) and cellular acidification has previously been reported to increase $[Ca^{2+}]_i$ in parotid acini (Arkle *et al.*, 1988). Here, we have investigated the effects of ATP on pH_i and the relationship of pH_i and $[Ca^{2+}]_i$ in ATP-stimulated parotid acini.

$[Ca^{2+}]_i$ and pH_i were simultaneously determined by spectrofluorimetry in rat parotid acinar suspensions loaded with the pH-sensitive dye BCECF and the Ca^{2+}-sensitive dye Fura-2. The acini were incubated at 37°C in bicarbonate-buffered Krebs Ringer, top-gassed with 95% O_2, 5% CO_2. Addition of ATP to the acini caused a concentration-dependent acidification and increased $[Ca^{2+}]_i$ (table). The acidifying effect of ATP was not mimicked by elevation of $[Ca^{2+}]_i$ with ionomycin. Nigericin did not affect $[Ca^{2+}]_i$ or abolish the Ca^{2+}-mobilising action of ATP. 1mM acetazolamide decreased basal pH_i to 7.13±0.05 and reduced the response to 30µM-ATP (pH_i: 7.09±0.07; $[Ca^{2+}]_i$: 396±15nM) but did not affect basal $[Ca^{2+}]_i$ (183±28nM) (n=5). 20µM amiloride hexamethylene had no effect on basal pH_i (7.22±0.04) but enhanced the 30µM-ATP stimulated acidification (pH_i: 7.10±0.03) (n=5).

addition	none	10µM-ATP	30µM-ATP	100µM-ATP	5µM-ionomycin	5µM-nigericin	5µM-nigericin + 30µM-ATP
$[Ca^{2+}]_i$ (nM)	231±42	420±66	672±95	456±79	>2000	197±48	375±65
pH_i	7.26±0.04	7.20±0.03	7.17±0.03	7.22±0.07	7.23±0.05	7.21±0.03	7.20±0.03

Values are mean ± s.e. mean based on 4-10 observations

We conclude that the Ca^{2+}-mobilising effect of ATP is not absolutely dependent upon cellular acidification and the acidification response to ATP is not dependent upon Ca^{2+}-mobilisation. The potentiating action of the amiloride derivative on the pH_i response is taken to indicate that Na^+/H^+ exchange opposes ATP's acidifying effect. In view of acetazolamide's acidifying action in unstimulated acini, we tentatively conclude that its inhibitory effect on the change in pH_i elicited by ATP indicates that carbonic anhydrase is involved in this response.

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197P INFLUENCE OF MAST CELL HISTAMINE ON THE ANALGESIC EFFECT OF CLOMIPRAMINE IN RATS

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Tricyclic antidepressants(TCAS),developed for the treatment of depression, have also been used to relieve chronic pain conditions in clinical practice. Experimental results in animals indicate that antidepressant and analgesic actions of TCAS are independent of one another. Clomipramine has a strong and specific action on serotonin uptake; in addition both noradrenergic, serotonergic and endorphin central systems seem to be involved in the antinociceptive effect of clomipramine. TCAS and H_2-antagonists, block the facilitatory effect of histamine on a behavioural model of depression, suggesting a link between the central histaminergic system and depression. Evidence also indicate a role for brain mast cells in migraines and in the pathophysiology of specific neuroinflammatory conditions. Previously, we demonstrated a role for mast cell histamine on pain sensitivity (Arrigo-Reina, 1988) and correlations between mast cell histamine and pituitary B-End(ir) content on the reactivity to stressful stimuli (Arrigo-Reina, 1989).
The present study was aimed to evaluate the role played by mast cells in the antinociceptive action of clomipramine in rats, measured by the Tail-Flick and Hot-Plate tests.
Experiments were carried out on male Sprague-Dawley rats (n=6) of 150 g.bw (C.River), kept at 22°C,with a dark-light cycle of 12/12 h and free access to food and water. Rats were pretreated with saline (0.5 ml/animal) or with Compound 48/80 (1 mg/kg,ip) (a non-lethal dose, that produces a characteristic, systemic, anaphylactoid reaction in rats), 15 min before clomipramine (10 - 20 - 40 mg/kg,ip). Analgesia was expressed as % MPE (% Maximum Possible Effect). All data were analyzed by Student's t test,followed by ANOVA. Results showed that clomipramine (10-20-40 mg/kg,ip) did no modified significantly the tail reflex on the Tail-Flick test, or the paw licking reaction on the Hot-Plate test, in saline-pretreated control rats, thus confirming data of literature. In rats pretreated with Compound 48/80, clomipramine (10-20 mg/kg) showed a dose-related highly significant inhibition of reaction: 32±5 vs 7±.8($p<.05$), 54±8 vs 7±.5($p<.01$) in TFT; 42±9 vs 12±2($p<.05$), 79±6 vs 12±1($p<.01$) in HPT. In rats pretreated with Compound 48/80, clomipramine (40 mg/kg) demonstrated symptoms of toxicity , followed by the death of rats. Although the mechanism of the analgesic action of clomipramine is unclear, results of this study are suggestive of a correlation between the tricyclic antidepressant and mast cell histamine.

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198P FURTHER CHARACTERIZATION OF CHOLINERGIC MUSCARINIC RECEPTORS COUPLED TO PHOSPHOINOSITIDE METABOLISM IN IMMATURE RAT BRAIN

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Activation of cholinergic muscarinic receptors in brain is associated with an increased metabolism of membrane phosphoinositides (PtdIns). In particular, the M_1 receptor subtype, which shows a high sensitivity to the inhibitory effect of pirenzepine, has been found to be coupled to this biochemical response (Gonzales & Crews, 1984). In the rat, muscarinic-dependent PtdIns hydrolysis is maximal 7-10 days after birth, in correspondence with the peak of the brain growth spurt, suggesting a primary role for this transducing system in nerve cell maturation and synaptogenesis (Balduini *et al.*, 1987; 1990). In this study, we examined the effects of muscarinic agonists and antagonists on PtdIns metabolism in the cerebral cortex and brainstem of 7-day-old rats, in an attempt to evaluate the role of muscarinic receptor subtypes in this process. [^{3}H]Inositol phosphates ([^{3}H]InsPs) accumulation in [^{3}H]inositol pre-labelled cerebral slices was taken as an index of PtdIns hydrolysis. In cerebral cortex, maximal stimulation induced by 1 mM acetylcholine, carbachol, or metacholine ranged from 750 to 1100% of basal [^{3}H]InsPs accumulation. The effect of the partial agonists betanechol, oxotremorine and pilocarpine varied from 250 to 500%. Muscarinic receptor antagonists inhibited the stimulatory responses in a concentration-dependent manner, with the following order of potency: 4-DAMP > pirenzepine > AF-DX 116 > p-F-HHSiD. Pirenzepine inhibition curves of the responses to acetylcholine, carbachol or metacholine were shallow, with Hill coefficients (n_H) consistently smaller than unity (range: 0.600-0.750). When partial agonists were used, the resulting pirenzepine curves were monophasic ($n_H \approx 1$). In the brainstem, the effect of muscarinic receptor stimulation caused by carbachol was smaller (316 ± 18% of basal), and pirenzepine was approximately 17 times less effective as antagonist. The order of potency was 4-DAMP > p-F-HHSiD > AF-DX 116 ≥ pirenzepine. As in the cerebral cortex, however, inhibition by pirenzepine of the production of [^{3}H]InsPs induced by carbachol and metacholine showed biphasic curves ($n_H < 1$). In both areas, inhibition of the agonist responses by 4-DAMP, AF-DX 116, or p-F-HHSiD resulted in monophasic curves, indicating that these antagonists show no selectivity towards any of the muscarinic receptor subtypes potentially expressed in the immature rat brain. Based on pirenzepine antagonism, it is likely that more than one muscarinic receptor subtype is involved in the activation of PtdIns metabolism in the immature rat brain. The high potency of this antagonist suggests that, when full agonists are employed, M_1 receptors mediate a major component of the PtdIns response. The remaining component is probably activated by other receptor subtypes, which, in the adult rat cerebral cortex, have been postulated as M_3 receptors (Forray & El-Fakahany, 1990). Partial agonists, on the other hand, appear to stimulate PtdIns metabolism by activating M_1 receptors only.

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199P THE EFFECTS OF DEXAMETHASONE ON MORPHINE ANTINOCICEPTION IN DBA/2J AND C57BL/6 MICE

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Studies in our laboratories have recently centered on the steroid-opiate interaction by using different experimental procedures. We have demonstrated that dexamethasone (DEX) was able to modify constipation, hyperactivity, electroencephalographic background effects and epileptiform activity induced by morphine (M). Furthermore, we found that DEX reduced M antinociception in outbred Swiss mice.
In the pharmacogenetic literature, several investigators have found a consistently greater degree of M-induced analgesia in DBA/2J compared to C57BL/6 mice.
The present study was carried out to further investigate the effect of DEX on M-antinociception in two different strains of mice (DBA/2J and C57BL/6 mice) by using the hot plate and tail flick tests (Pieretti et al., 1991; Capasso et al., 1992).
The experiments performed with the hot plate test (HP) test indicate that both intracerebroventricular (i.c.v.) and intraperitoneal (i.p.) injection of DEX did not modify M antinociception in DBA2/J mice (HP latency (s) M 1.0 mg/i.p. = 47.5 ± 5.9; HP latency DEX 10 ng/i.c.v. + M = 50.7 ± 6.3; HP latency DEX 0.1 mg/i.p. = 51.2 ± 7.4) whereas a significant reduction of M effect was observed in C57BL/6 mice after i.p. (but not i.c.v.) injection of DEX (HP latency M 10 mg/i.p. = 45.4 ± 5.3; HP latency DEX 10 ng/i.c.v. + M = 50.3 ± 6.7; HP latency DEX 0.1 mg/i.p. + M = 30.1 ± 2.8, $P<0.05$ vs M).
The tail flick (TF) study, partly confirm the above results as i.c.v. injection of DEX did not modify M antinociception in both strains whereas the DEX i.p. treatment significantly reduces morphine effect in both strains (DBA/2J: TF latency (s) M 1 mg/i.p. = 8.3 ± 3.8; TF latency DEX 10 ng/i.c.v. + M = 9.3 ± 5.8; TF latency DEX 0.1 mg/i.p. + M = 6.1 ± 2.6, $P<0.05$ vs M; C57BL/6: TF latency M 10 mg/i.p. = 7.8 ± 3.5; TF latency DEX 10 ng/i.c.v. + M = 8.0 ± 3.2; TF latency DEX 0.1 mg/i.p. + M = 4.5 ± 1.7, $P<0.01$ vs M).
Our data further show an interference between DEX and M antinociception in inbred strain, thus confirming our previous studies.
The present study suggest that this genetic study may provide an useful approach for studying the steroid-opiate interaction and that the different results from the two analgesic tests may be related to possible mechanisms underlying M antinociception in these strains of mice.

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200P CHANGES IN THE PERMEABILITY OF THE BLOOD-BRAIN BARRIER FOLLOWING SODIUM DODECYL SULPHATE ADMINISTRATION IN THE RAT

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The blood-brain barrier (BBB) arises from epithelial-like tight junctions that virtually cement adjoining capillary endothelium together in the brain microvascolature. Furthermore, numerous specialized enzyme-like transport systems are localized on the luminal side of the BBB. These unique properties limit the free exchange of water-soluble non-electrolytes, electrolytes and proteins from blood to brain (Pardridge, 1988). Besides the osmotic BBB opening, alternative approaches to increasing BBB permeability would be to alter endothelial cell membrane integrity or to activate specific biochemical pathways that regulate BBB permeability. Because of its amphiphilic nature, sodium dodecyl sulphate (an anionic surfactant widely used as solubilizer or stabilizer in several pharmaceutical preparations; SDS) may enter into interactions with the major membrane components, that are lipids and proteins, inducing changes in membrane permeability, loss of membrane lamellar structure, removal of phospholipids from proteins and protein conformational changes (Bartnik, 1992). In addition, the activity of protein kinase C (an enzyme which plays an important role in mediating trans-membrane signalling and can be detected in brain capillaries) may be modified by systemic injection of SDS in the rat.

The aim of the present study was to determine the effect of an intracarotid infusion of SDS on BBB permeability. Adult male Sprague Dawley rats were used; SDS (50 and 100 µg/Kg) was infused into the right carotid artery at a rate of 3 ml/min for 30 sec; the control rats were infused with the same volume of saline. A 4 ml/Kg dose of Evans blue solution (EB; 2% w/v) was injected intravenously, 5 min before SDS infusion; the rats were sacrificed 15 min after, the brain removed, sliced and examined visually for EB extravasation (Rapoport et al., 1984). Furthermore, the local permeability of cerebral blood vessels to [14C]α-aminoisobutyric acid ([14C]AIB), given 2 min after the completation of the SDS infusion, was examined as described previously (Saija et al., 1992), by calculating a unidirectional transfer constant (Ki) for the tracer from blood to brain; the Ki values were corrected for residual cerebral blood radioactivity by using [14C]sucrose. A dose-dependent EB extravasation following SDS infusion was observed in the right brain hemisphere. These results were confirmed by the significant increase in Ki values for [14C]AIB measured in several right sided brain regions after SDS infusion. However, no true increase in regional brain plasma volume seemed to be elicited by SDS infusion. Since the BBB is created by the plasma membrane and tight junctions of the endothelial cells, the change in BBB permeability caused by SDS might be explained as a non-specific surfactant-membrane interaction. Furthermore, the possibility exists that SDS may affect the functional characteristics of brain vascular endothelial cells by an interaction with specific BBB proteins and/or biochemical pathways. In conclusion, one can suggest that intracarotid infusion of SDS may provide a useful clinical approach for the intentional introduction of different substances into the brain. On the other hand these findings should call attention to possible dangerous consequences of using SDS as solubilizer in drug excipients.

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201P ACETYL-L-CARNITINE INCREASES PKC PARTICULATE ACTIVITY IN CORTICAL MEMBRANES *IN VITRO* AND *IN VIVO*

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Drugs belonging to different chemical classes having the ability to improve behavioral performance in learning and memory tests may share the common ability to stimulate protein kinase C activity in rat brain cortex (Govoni et al. 1992). The effect of acetyl-L-carnitine (ALC) on PKC was studied both *in vitro* and *in vivo* following oral repeated administration. Male Wistar rats (Charles River, Calco, Italy) weighing 175-200 g. were used. For *in vivo* studies brain areas were quickly dissected, frozen on dry ice and kept at -80°C until assayed. For *in vitro* studies the brain areas were dissected, kept into oxygenated Krebs Ringer medium and then cross chopped (300 µm) with a Mc Ilwain tissue chopper. Minces suspensions corresponding to roughly 150 mg original w.w. tissue were exposed to the drug as detailed. Particulate (P) and soluble (S) PKC activity was measured as described by Govoni et al. with minor modifications. For behavioral testing we used the Morris maze; rats were submitted to one trial/day and scopolamine (0.3 mg/Kg s.c.) was given daily 30 min. before the trial.

In vitro ALC (100 nM, 10 min) promoted in rat brain cortex slices a significant increase of particulate activity associated with lower soluble PKC activity, suggesting enzyme translocation (Activity as n.moles/min/mg prot.: Control P: 1.91±0.18, S: 2.35±0.22; 100 nM ALC, P: 2.49±0.23, S: 1.86±0.27). Using the same concentration and time of incubation, the effect was not observed in slices prepared from the hippocampus. A longer incubation time (20 min.) decreased phosphorylating activity in both P and S fractions (-42 and -32% in comparison with the respective control activity), possibly suggesting a very early activation.

In vivo ALC was administered orally once a day for 5 days at 10, 60 and 150 mg/Kg. Only the dose of 60 mg/Kg significantly improved the performance of rats in the Morris maze (last day escape latency: 68±15 sec. for scopolamine control; 37±11 sec. for ALC 60; 57±15 for ALC 10 and 56±13 for ALC 150). Immediately after behavioral assessment, rats were sacrificed for the assay of PKC. A significant increase (+37±2%) of particulate PKC activity was observed in the group of rats treated with ALC 60 mg/Kg (P activity in cerebral cortex homogenates prepared from saline treated rats was 3.61±0.51 n.moles/min/mg prot.), but not with the doses that were behaviorally inactive.

The results suggest that ALC as other cognition enhancers increases particulate PKC activity in the cortex both *in vitro* and *in vivo*. This effect in the *in vivo* experiments seems to be observed only using doses that are effective in improving performance of rats in a spatial learning task.

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202P COMPLETE TOLERANCE TO THE ANXIOLYTIC EFFECTS OF DRUGS IN THE ELEVATED PLUS-MAZE PRODUCED IN RATS BY 28-DAY INFUSION OF DRUGS BY OSMOTIC MINIPUMP

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The anxiolytic effect of benzodiazepines in the elevated plus-maze is decreased following repeated treatment (Brett & Pratt, 1990; File *et al.*, 1987), a tolerance which may be at least partly due to the repeated animal handling involved in daily drug dosing. We have, therefore, used osmotic minipumps to investigate the anxiolytic effects in the elevated plus-maze of up to 28 days continuous infusion of chlordiazepoxide and two non-benzodiazepine anxiolytics (buspirone and dothiepin) (Luscombe *et al.*, 1990).

Male CD rats (Charles River), weighing 125-145g at the time of subcutaneous implantation of osmotic minipumps (Alzet), were infused with water or an average daily dose of 3 mg/kg chlordiazepoxide, 1 mg/kg buspirone or 1 mg/kg dothiepin (see Table 1 for evidence of their anxiolytic effects after acute oral administration) for either 14 or 28 days. After these periods, and with the pumps still delivering drug, rats were placed on an elevated plus-maze and arm entries recorded for 10 min; each rat was exposed to the maze only once.

Table 1 Effects of one oral dose or minipump infusion (14 and 28 days) of vehicle or drug on rat behaviour in the elevated plus-maze

	1h pretreatment (oral)		14 days treatment (minipumps)		28 days treatment (minipumps)	
Treatment	Total[a]	Open:Total[a]	Total	Open:Total	Total	Open:Total
Deionised water	16.3 ± 0.8	0.03 ± 0.01	15.8 ± 0.4	0.02 ± 0.01	14.0 ± 0.7	0.01 ± 0.01
Chlordiazepoxide	24.8 ± 1.2**	0.33 ± 0.02**	12.7 ± 2.2	0.27 ± 0.04**	13.4 ± 1.8	0.05 ± 0.02
Buspirone	20.7 ± 1.9*	0.29 ± 0.02**	15.7 ± 1.5	0.21 ± 0.03**	10.5 ± 0.9	0.04 ± 0.03
Dothiepin	22.5 ± 0.9**	0.27 ± 0.01**	16.7 ± 0.7	0.17 ± 0.04**	16.2 ± 1.0	0.01 ± 0.01

a: Total=total arm entries; Open:Total=ratio of open:total arm entries. All values are mean ± s.e.mean. **P<0.01, *P<0.05 (Dunnett's test, following 1-way ANOVA) versus deionised water at that time. n=6 rats/treatment. At 1h and 14 days deionised water data have been combined for clarity (n=18), but statistical changes refer to comparisons against the matched n=6 control.

Chlordiazepoxide, buspirone and dothiepin each produced a marked anxiolytic effect 1h after oral administration, as shown by the increased open:total arm entries ratio. The anxiolytic response was slightly reduced following 14 days drug infusion and abolished after 28 days treatment. Whilst total arm entries were increased by a single dose of drug relative to the vehicle control, they were not increased following 14 and 28 day drug infusion. This is in marked contrast to a separate experiment where 28 days once-daily oral administration of either vehicle or drugs almost abolished total entries. The minipumps therefore eliminated the compromising effects of handling on the potential anxiolytic response to repeated drug administration, but the data nevertheless indicate that complete tolerance does develop to the anxiolytic effects of each of these drugs after 28 days infusion by osmotic minipump.

In conclusion, continuous infusion of chlordiazepoxide, buspirone or dothiepin for 28 days abolished their anxiolytic effect in the elevated plus-maze which, in the absence of a handling influence, suggests that the development of tolerance to their anxiolytic effects may be directly attributable to the actions of the drugs.

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203P A HIGHLY SENSITIVE METHOD FOR THE DETECTION OF BRAIN AMINO ACIDS USING HPLC WITH ELECTROCHEMICAL DETECTION

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Several amino acids including glutamate and gamma-aminobutyric acid (GABA), are considered to act as neurotransmitters in the brain. Traditional methods of detection of amino acids involve ion-exchange chromatography with post-column derivatization using ninhydrin and colorimetric detection. The advent of high-performance liquid chromatography (HPLC) and pre-column derivatization with *o*-phthalaldehyde (OPA) / an alkyl thiol, coupled with the use of fluorescence detection, brought about an increase in detection sensitivity (Lindroth and Mopper, 1979). More recently Joseph and Davies (1983) showed the derivatives to be electrochemically active enabling them to be measured by electrochemical detection (ED). The poor stability of the derivatives due to the thiols employed remained a problem but recently the use of sulphite ions to replace the thiols has been reported (Jacobs, 1987). This method yields derivatives which are both stable and electrochemically active. Here we report a method for the detection of brain transmitter amino acids using HPLC-ED with OPA-sulphite derivatization.

Reversed-phase chromatography was employed using a Rainin Dynamax C_{18}, 25cm, 5µm column. The derivatized amino acids were eluted isocratically with a mobile phase delivered at 0.7ml/min consisting of; 0.1M $NaH_2PO_4.H_2O$, 0.5mM EDTA with 25% (v/v) methanol adjusted to pH 4.5 with 1M phosphoric acid. A glassy carbon working electrode with Ag/AgCl reference electrode was operated at +0.85V using either a Bioanalytical Systems (BAS) or Antec electrochemical system. The derivatizing agent was made following the method of Smith and Sharp (1992) and was stable at room temperature for one week. 20µl OPA reagent was added to 1ml standard and the derivatization proceeded at room temperature.

The derivatizing agent itself is electroactive, resulting in additional peaks to those produced by the amino acids. Initial studies were performed using OPA obtained from Sigma but due to the coelution of GABA with a contaminant peak an alternative source was employed (Aldrich). Therefore, using the above conditions, clear separation of the following amino acids from the peaks due to OPA, could be obtained; serine, taurine, glycine, glutamate, alanine, arginine and GABA, in order of increasing retention time. Total run time was 25 min. The maximal response for taurine, glycine and GABA was obtained with a reaction time of 7 min but the derivatives remained stable for up to 30 min post-reaction. Reproducibility was determined and found to yield coefficients of variation of 4.3% (taurine), 5.4% (glycine), 4.3% (glutamate), 9.3% (alanine) and 7.5% (GABA). Linear standard curves were obtained for the same five amino acids using 2-40 pmol standards (r = 0.9995-0.9999). The limit of detection was 0.01 pmol for GABA which was achieved using an Antec detector but not a BAS.

In summary, we report a simple, highly sensitive and reproducible isocratic HPLC-ED method which may be employed for measuring several amino acids thought to be involved in brain transmission. We are currently using the method in conjunction with microdialysis to study the release of GABA and glutamate from the hippocampus.

H. L. Rowley is a SERC CASE student in conjunction with Boots Pharmaceuticals.

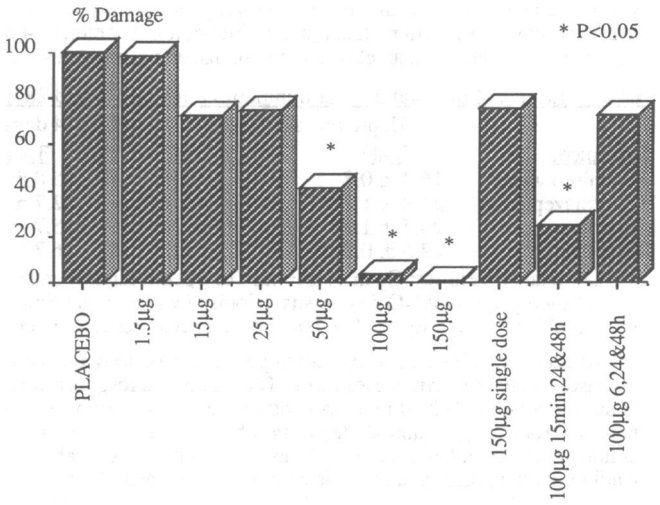
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204P NEUROPROTECTIVE PROPERTIES OF bFGF IN A MOUSE MODEL OF FOCAL ISCHAEMIA

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Neurotrophic factors exhibit a trophic effect on neuronal cells of the brain, characterised as enhancing neuronal survival and maintenance of neuronal cell functions. Therefore they may be useful for treating disorders associated with neuronal cell death eg. cerebral ischaemia. Indeed intracisternal administration of basic Fibroblast Growth Factor (bFGF) to rats following middle cerebral artery occlusion prevents thalamic degeneration (Yamada et al 1991) and acidic Fibroblast Growth Factor (aFGF) prevents hippocampal CA_1 cell death in a gerbil 5 min ischaemia model (Oomora; 1990). In the present series of experiments we have assessed the neuroprotective properties of bFGF in a mouse model of focal cerebral ischaemia

Adult male mice (Swiss CD1, 30-50g) were anaesthetised with 5% Halothane in a 70% nitrous oxide: 30% Oxygen gas mixture, and the left middle cerebral artery was exposed by a burr hole craniectomy and coagulated by bipolar diathermy. Ischaemic damage was assessed in membranes derived from ipsi and contralateral parietal cortices using the ligand [^{3}H]-PK11195 as previously described (Benavides et al 1990). This ligand is a marker of w_3 sites present on non neuronal cells such as astrocytes and macrophages, cell types which are markedly elevated in brain tissue following cerebral ischaemia. A significant increase in [^{3}H]-PK11195 binding is seen 7 days post ischaemia. This increase was significantly reduced by bFGF (50, 100 & 150 µg; iv) administered 15 min post ischaemia once daily on days 2-6 also 100 µg iv administered 15 min post ischaemia and 24 and 48 h later (figure)



The efficiency of bFGF in this model indicates that neurotrophic factors prove to be useful neuroprotective agents, and may be administered systemically due to the compromised state of the blood brain barrier following ischaemia

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205P EFFECTS OF LAMOTRIGINE (LAMICTAL™) IN AN ANIMAL MODEL OF ANXIETY

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Lamotrigine (Lamictal™) [LTG; 3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] is a new antiepileptic drug chemically unrelated to drugs in current use. The anticonvulsant action of LTG may be due to use-dependent blockade of voltage sensitive sodium channels (Leach *et al*, 1991; Lees & Leach ,1993). Manipulations of glutamate-ergic transmission via blockade of NMDA receptors have demonstrated anxiolytic effects in several animal models of anxiety (e.g. Liebman & Bennett, 1988; Stephens & Andrews,1988). LTG and the structural analogue, 524C79 have now been evaluated in the Vögel test of anxiety.

Male hooded Lister rats were water-deprived for 24 hrs prior to test. After drug treatment rats were tested for 5 minutes in a Skinner box containing a drinking spout and a grid-floor and placed inside a ventilated sound-attenuating chamber. Every 20th lick at the spout resulted in a mild shock (0.1mA, 0.5s) delivered between drinking spout and grid-floor. Timers, shock delivery and recording of numbers of licks were controlled by microcomputer. Lamotrigine (20 & 40 mg kg^{-1} p.o.) produced a dose-dependent increase in punished drinking when compared with a vehicle control. 524C79 [3,5-Diamino-6-(2,3,5-trichlorophenyl)-1,2,4-triazine], an analogue of lamotrigine (20 mg kg^{-1} p.o.) also produced a significant increase in punished drinking. These effects were compared to those obtained with diazepam and with the competitive NMDA receptor antagonist CPP (±[3-(carboxypiperazin-1-yl)- propyl] phosphonic acid).

Treatment	Mean Punished Licks/5min (±s.e.m)	% of control	N	p
Vehicle	127.5 (17.5)		11	
Lamotrigine 10	105.6 (14.5)	83%	9	NS
Lamotrigine 20	224.2 (49.3)	176%	9	NS
Lamotrigine 40	357.2 (83.6)	280%	9	**0.021**
Vehicle	125.1 (20.2)		9	
524C79 5	169.2 (20.5)	135%	9	NS
524C79 20	266.2 (55.1)	212%	9	**0.01**

Treatment	Mean Punished Licks/5min (±s.e.m)	% of control	N	p
Vehicle	120.0 (39.6)		8	
CPP 2.5	278.4 (75.6)	232%	8	**0.007**
CPP 10	426.5 (82.7)	355%	9	**0.003**
Vehicle	141.6 (27.8)		8	
Diazepam 0.5	229.6 (23.8)	162%	8	**0.028**
Diazepam 1.0	420.1 (99.2)	350%	10	**0.019**

Doses are in mg kg^{-1} p.o. for LTG and 524C79 and mg kg^{-1} s.c. for CPP and diazepam. p values were derived from a non-parametric comparison with appropriate concurrently run vehicle controls (Mann Whitney U-Test).

Previous studies have demonstrated that lamotrigine does not exhibit the PCP-like effects associated with NMDA receptor blockade (Leach *et al* 1991). From binding data and further behavioural studies, LTG does not appear to be benzodiazepine-like (M.A.E. Critchley, unpublished observations). Lamotrigine-like drugs may therefore provide a new class of anxiolytic which may be devoid of the adverse effects of the benzodiazepines.

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206P EFFECT OF DIHYDROPYRIDINE CALCIUM ANTAGONISTS ON DRUGS OF ABUSE SELF-ADMINISTRATION

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Considerable evidence indicates that dopaminergic neurons, particularly in the mesolimbic system, are critically involved in the rewarding action of drugs of abuse (Johanson and Fischman, 1989; Koob, 1992). Accordingly, recent studies have shown that cocaine and morphine increase the output of dopamine in the ventral striatum and nucleus accumbens, as measured by microdialysis (Di Chiara and Imperato, 1988; Carboni et al., 1989). Both cocaine- and morphine- induced DA output are calcium dependent, as indicated by the fact that removal of Ca++ from perfusion medium has been shown to abolish the effect both of cocaine and morphine on DA output (Carboni et al., 1989). Furthermore, it was shown that two dihydropyridine calcium antagonists, isradipine and nimodipine, are able to inhibit cocaine-induced DA release and cocaine-induced motor stimulation (Pani et al., 1990a; Pani et al., 1990b). We have investigated the effect of isradipine on drugs of abuse such as cocaine, morphine and amphetamine on intravenous self-administration by rats. Isradipine pretreatment (2.5 mg/kg s.c.) significantly increased the number of cocaine injections from 36 ± 1.2 (basal levels) to 53 ± 2.3. We obtained similar results with morphine (basal levels 30 ± 0.5; isradipine pretreatment 45 ± 1.2). On the contrary, isradipine did not change the number of amphetamine injections (39 ± 0.9 vs. 35 ± 1.5). The increased number of cocaine or morphine injections, following isradipine pretreatment, can be explained as the consequence of an attenuation of the cocaine and morphine reinforcing effects. These data suggest that isradipine may act by blunting reward-related brain mechanisms. This activity might be due to the ability of dihydropyridine calcium antagonists to inhibit drugs of abuse-induced dopamine release by interferring with L-type calcium channels. The lack of effect on amphetamine self-administration might support this hypothesis, amphetamine-induced dopamine release being a calcium-independent mechanism (Mc Millan, 1983; Carboni et al., 1989). These results might suggest a possible clinical use of isradipine or related compounds in the treatment of drugs of abuse related disorders.

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207P NITRIC OXIDE AND DRINKING BEHAVIOUR

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Low doses (640 μg/kg/iv) of Escherichia coli endotoxin (LPS) produce inhibition of drinking induced by different stimuli in the rat (Calapai et al., 1990). This effect is mediated by the release of cytokines such as tumor necrosis factor (TNF-α) in the central nervous system (Calapai et al., 1991). TNF-α itself (20-40 ng) is antidipsogenic when given intracerebroventricularly (icv) or into the preoptic area (POA; 1,25-5 ng) of 24 h water deprived rats. Controls: 12.5 ± 2.0 ml rat 1h; icv TNFα 40 ng: 3.6 ± 1.0 ml rat 1h; POA TNFα 5 ng: 2.4 ± 1.0 ml rat 1h. Since both LPS and TNF-α cause nitric oxide (NO) formation by different cellular types, including astrocytes, we studied the involvement of NO in LPS and TNF-α inhibition of water intake. N^{ω}-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase, given icv or into POA (icv L-NAME 10 μg: 8.9 ± 1.5 ml rat 1h; POA L-NAME 11.0 ± 2.0 ml rat 1h), and methylene blue (M.B.), an inhibitor of guanylate cyclase, given into POA, antagonized the antidipsogenic effect of both LPS (M.B. 100 ng: 6.1 ± 1.0 ml rat 1h) and TNF-α (M.B. 100 ng: 8.2 ± 1.0 ml rat 1h) in 24 h water deprived rats. L-arginine (L-arg; 25-100 ng), precursor of NO, but not D-arginine (100 ng; POA) inhibited drinking induced by water deprivation (L-arg 100 ng: 3.4 ± 0.5 ml rat 1h)), when injected into POA of water deprived rats. Moreover, LPS and TNF-α, given at doses (160 μg/kg, iv and 10 ng, icv, respectively) unable to influence drinking in water deprived rats, exhibited a strong antidipsogenic effect in rats treated with a dose of L-arginine (12,5 ng into POA) unable by itself to modify drinking (LPS + L-arg: 6.0 ± 1.0 ml rat 1h; TNFα + L-arg: 5.3 ± 0.8 ml rat 1h). These results indicate that NO into POA acts as inhibitory mechanism on drinking behaviour and plays a role in the antidipsogenic effect of LPS and TNF-α.

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208P PRENATAL EXPOSURE TO LOW DOSES OF ETHANOL PROMOTES SEROTONINERGIC REGENERATION IN THE BRAIN AND SPINAL CORD

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Clinical reports have described a pattern of developmental abnormalities in children of alcohol addicted mothers, the "fetal alcohol syndrome" (Barnes and Walker, 1981). Behavioral deficits observed in humans and animal models of maternal alcoholism have been correlated with persistent alterations of spine distribution in the cerebral cortex of rats prenatally exposed to ethanol (Stoltenburg-Didinger, 1983). It has also been suggested that ethanol administration might affect lesion-induced sprouting in the rat dentate gyrus (West et al, 1982). We have tested the latter hypothesis by monitoring degeneration and regeneration of the serotoninergic system following neonatal neurotoxic lesion in ethanol-exposed rats. Ethanol treatment (Et) was carried out either perinatally, prenatally, or beginning 3 days after birth up to sacrifice by means of a 3% (v/v) acqueous solution. Controls were maintained to a sucrose diet at equicaloric content for the same period of time. Lesioning (LES) of the serotoninergic system was performed with neonatal injection of 5,7-hydroxytryptamine. Serotonin (5-HT) content (ng/mg prot.) was assayed in several brain areas as well as in each spinal cord segment at 1, 4 and 8 weeks (w) after birth. Seven days after birth in the distal portion of lesioned spinal cord there is a marked loss of 5-HT (C 5±0.3, LES 2.8±0.1). A similar depletion occurs in the cerebral cortex (C 4.1±0.2, LES 2.9±0.3) and hippocampus (C 3.4±0.2, LES 2.2±0.1). In perinatal and prenatal ethanol-exposed rats the extent of 5-HT depletion is greater (Et 5.2±0.3, Et+LES 1.2±0.08). However between 4 and 8 weeks, ethanol treated rats show a quick restoration of serotoninergic innervation of the brain and spinal cord, while sucrose-treated lesioned rats still show a profound denervation particularly relevant in the thoracic and lumbar segments (C 4.6±0.2, LES 1.2±0.08, Et 5.1±0.3, Et+LES 4.9±0.2). Ethanol supplied peri- or pre-natally promotes a more extensive 5-HT depletion but restoration occurs faster than in control animals, the latter showing a progressive worsening of 5-HT depletion in the lumbar spinal cord. The compensatory outgrowth of the short 5-HT axons in pons-medulla induced by the lesion is abolished by ethanol exposure (LES 8.1±0.6-1w, 12.2±1.6-4w, 14.6±0.9-8w; Et 8.2±0.9-1w, 8.8±0.6-4w, 8.5±0.9-8w). In the group receiving ethanol postnatally, no effects on 5-HT restoration were observed. (Unless specified, data refer to the thoracic cord). Ethanol exposure seems therefore to enhance CNS susceptibility to damage and to promote serotoninergic restoration in the brain and spinal cord following neurotoxic lesion. The ethanol promoting effect requires prenatal exposure, being the postlesioning treatment ineffective. We suggest that the increased reinnervation induced by ethanol might be originated by the combined effects of inhibition of the compensatory outgrowth of the short 5-HT containing axons and of a more extensive early denervation with no further retrograde degeneration. (Supported by the Italian C.N.R., grant N. 93.00590.PF41)

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209P EVIDENCE THAT IMPROVEMENT IN MEMORY BY NOOTROPIC DRUGS IS NOT SECONDARY TO A REDUCTION IN ANXIETY IN MICE

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Available evidence indicates that improvement in learning and memory may result from a reduction in anxiety. In particular, the possible anxiolytic effects of nootropic drugs have received little attention (File & Hyde, 1979).

In light of foregoing information, the present study was designed to assess the effects of piracetam (PIR) and oxiracetam (OXI) by the use of an animal model predictive of anxiolytic activity: the elevated plus-maze. Anxiolytic drugs would be expected to increase the % of entries into and time spent on open arms. In this test, the acute effects of PIR and OXI were compared with those of an established reference drug, lorazepam (LOR). The putative anxiolytic effects of propanolol (PRO) were also evaluated.

Female Charles River CD1 mice (aged 60 days at the time of experimentation, weighing 24 to 26 g) were used. The following doses were used: PIR (250-500-1000 mg/kg); OXI (50-100-200 mg/kg); LOR (0.025-0.05-0.10 mg/kg); PRO (as hydrochloride) (2.5-5.0-10 mg/kg). All drugs or vehicle were administered intraperitoneally (i.p.) in a volume of 0.01 ml/g body weight, 30 min prior to the test. The 5-min elevated plus maze-test was performed according to Lister (1987).

The results are summarized as follows. Undrugged naive mice had a consistent preference for the closed arms, reflecting animal anxiety. PIR and OXI had no significant effects on the index of anxiety. A pronounced anxiolytic effect for LOR was evident. In fact, LOR increased significantly ($p<0.05$, post hoc Newman Keuls' test) the % of arm entries made into the open arms (LOR 0.05: 75.6 ± 6.3; LOR 0.10: 65.0 ± 5.1 vs controls: 24.1 ± 2.4) and the % of time spent on the open arms (LOR 0.05: 41.8 ± 3.8; LOR 0.10: 53.7 ± 6.1 vs controls 15.7 ± 1.6). Importantly, all doses of LOR were without significant effect on general activity, as measured by the total number of arm entries. In agreement with our previous data (de Angelis , 1991a), PRO displayed also a significant anxiolytic effect.

In summary, these experiments, extending earlier studies (de Angelis, 1991b), show that PIR and OXI are devoid of anxiolytic properties in the plus-maze. Thus, these findings do not support the view that reduction in anxiety is involved in the improvement of learning and memory of nootropic drugs (de Angelis, 1992).

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210P SYNAPTIC NON-MITOCHONDRIAL ATPase ACTIVITIES AS A PHARMACOLOGICAL MODEL FOR THE EVALUATION OF DRUG'S ACTION ON CEREBRAL ENERGY METABOLISM DURING AGEING

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Non-mitochondrial ATPases play a pivotal role in the regulation of presynaptic nerve endings homeostasis and among the other functions: (a) Na^+, K^+-ATPase is involved in the presynaptic transmitter release, in postsynaptic activities and in transmitter uptake; (b) Ca^{++}-ATPase and Ca^{++}, Mg^{++}-ATPase activities located in synaptic plasma membranes pump Ca^{++} ions into synaptic structures or out of the nerve endings to maintain synaptoplasmic Ca^{++} homeostasis, while Ca^{++}-ATPase activity located in synaptic vesicles modulates the intracellular concentration of Ca^{++}, that initiates the interaction between ATP and ATPase for the extrusion of their neurotransmitter content; (c) synaptosomes from mammalian brain tissue have an ATP-hydrolyzing Mg^{++}-ATPase activity which is an ecto-enzyme: the Mg^{++}-ATPase located in synaptic plasma membranes is involved in the hydrolysis of ATP to adenosine, while the enzyme of synaptic vesicles modulates the turnover of neurotransmitters, according to the different nerve terminals involved.

In this research, the catalytic activities of these ATPases were studied during ageing, utilizing the sinaptic plasma membranes of the frontal cerebral cortex of rats aged 2, 6, 12, 18 and 24 months. On synaptic plasma membranes, isolated according to Villa et al. (1989a; 1989b) and Benzi et al. (1993a; 1993b), the following enzyme activities were assayed: (a) Na^+, K^+-ATPase, (b) Ca^{++}, Mg^{++}-ATPase, (c) Mg^{++}-ATPase and (d) Acetylcholinesterase, as a membrane marker (Villa et al., 1989b).

Respect to the value achieved at 2 months of age of Ca^{++}, Mg^{++}-ATPase (10.4 $\pm$ 0.84 μmoles Pi released/hr/mg protein), Mg^{++}-ATPase (8.63 $\pm$ 0.54) and Acetylcholinesterase (0.184 + 0.015 μmoles substrate trasformed/min/mg protein), the catalytic activities were strongly decreased by ageing at 6, 12, 18 and 24 months, respectively of about 56%, 45% and 56%. On the contrary, the activity of Na^+, K^+-ATPase showed a biphasic trend (Villa & Gorini, 1991), being the activity decreased of about 51% only at 6, 18 and 24 months of age respect to the value at 2 months (24.4 $\pm$ 1.29 μmoles Pi released/hr/mg protein).

These data indicate that the catalytic potentialities of ATPases are reduced during ageing and, consequently, the ionic homeostasis of synapses is compromised in frontal cerebral cortex; these results may be of pathophysiological significance for brain diseases related to age and to ageing.

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211P α-BUNGAROTOXIN RECEPTOR SUBTYPES IN CHICK OPTIC LOBE: PURIFICATION AND CHARACTERIZATION

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We have recently demonstrated that αBungarotoxin (αBgtx) receptors, purified from chick optic lobe (COL) and cerebellum, when reconstituted in a planar lipid bilayer form functional nicotinic acetylcholine receptors. After nicotinic agonists bind to these receptors, the formation of cation channels occurs, the opening of which is blocked by antagonists (Gotti *et al.*, 1991; Gotti *et al.*, 1992).

So far, two cDNA related to neuronal αBgtx receptor subunits have been cloned: α7 and α8 (Couturier *et al.*, 1990; Schoepfer *et al.*, 1990). In order to characterize the functional COL αBgtx receptors further, we raised polyclonal antibodies against synthetic peptides of the chick α7 and α8 αBgtx receptor subunits. Using these antibodies we have immunopurified two αBgtx receptor subtypes from chick optic lobe. The predominant subtype (α7) contains the α7 subunit and represents 60+3% receptors present in chick optic lobe; the other subtype (α7-α8) contains both the α7 and α8 subunits and represents only 20±2% of the receptors. In addition to the α8 and/or α7 subunits of Mr 57000+1, both subtypes also have an additional subunit or subunits of Mr 52000+1. These two subtypes have the same affinity for antagonists, but the α7-α8 subtype has higher affinity for agonists, mainly for acetylcholine, than the subtype α7.

When reconstituted in planar lipid bilayer, both subtypes form cationic channels; the α7-α8 subtype gave non-homogeneous traces of channel fluctuations with channel-conductances of between 10-50 pS, the α7 subtype gave more regular traces of channel fluctuation with a conductance of 45 pS. These two subtypes are activated in a concentration-dependent manner by the agonists, the α7-α8 subtype being activated by lower agonist concentrations than the α7 subtype.

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212P PROPIONYL-L-CARNITINE INHIBITS PAF-ACETHER-INDUCED RAT PAW OEDEMA

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It has recently been shown that L-carnitine (LC) and its short-chain acyl esters, acetyl-L-carnitine (ALC) and propionyl-L-carnitine (PLC), may enhance arachidonate metabolism (Cristopherson & Norseth, 1981), leading to a prevalent increase in PGI_2 relative to PGE_2, LTB_4 and TXA_2 (Elliott et al., 1990; 1991). These findings, together with the observation that carnitines inhibit cell response to chemotactic stimuli, would suggest a possible modulatory role of these substances in inflammatory processes (Elliott et al., 1990; 1991). Our preliminary studies (Amico-Roxas et al., 1993) showed that LC, ALC and PLC (50 to 300 mg/kg i.p.) were ineffective either in carrageenin-induced rat paw oedema and peritonitis models or in cotton pellet granuloma test, while only PLC inhibited plasma extravasation in some types of inflammation (i.e. granuloma pouch in rats and ear oedema induced by croton oil, TPA or arachidonic acid in mice).

We report here that PLC (50, 100 and 200 mg/kg i.p.), but not LC or ALC, exerted a dose-dependent inhibition against rat paw oedema induced by PAF-acether (2 μg/paw). These data reinforce our idea that, among the three different carnitines, only PLC could play a protective role in some types of inflammation. Interestingly, PLC (unlike LC or ALC) has been reported to decrease the cytoplasmatic calcium levels in vascular cells (Van Hinsberg & Scheffer, 1991) and to protect endothelial membranes from peroxidative damage (Bertelli et al., 1991), probably due to a specific plasma-membrane stabilization (Hülsmann, 1991). Because of these findings and on the basis of the well known role of PAF-acether on increased vascular permeability, it is likely that the anti-inflammatory effect of PLC may be related to a direct protective action on endothelial cells, rather than to the previously reported modulation of arachidonate metabolism.

Table 1 - Effect of carnitines, indomethacin and betamethasone on PAF-acether rat paw oedema.
Drugs were given i.p. 1 h before testing. Paw swelling was measured 2 h after PAF.
* $p < 0.01$ (Dunnett's t test; n=6-8).

Treatment	Dose mg/kg	Oedema ml±s.e.	Inhibition %
Vehicle	-	0.52±0.03	-
PLC	50	0.44±0.02	15
	100	0.38±0.01*	27
	200	0.31±0.01*	40
LC	200	0.48±0.02	8
ALC	200	0.46±0.02	11
Indomethacin	5	0.48±0.01	8
Betamethasone	0.05	0.30±0.01*	42

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213P POTENTIATION BY NEUROPEPTIDE Y OF VASOCONSTRICTOR RESPONSES TO SYMPATHETIC STIMULATION AND TO NORADRENALINE IN HUMAN SAPHENOUS VEIN

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Neuropeptide Y (NPY), which coexists together with noradrenaline (NA) in sympathetic perivascular nerves, has been found to potentiate the contractile responses of blood vessels to NA and to sympathetic field stimulation in experimental animals. However, the mechanisms underlying this potentiating effect still remain indeterminate, and discrepancies exist on the role of an intact endothelium in this phenomenon (Budai *et al.*, 1989; Hieble *et al.*, 1989). The present study was undertaken to verify the effects of NPY on the contractile responses to NA and to sympathetic stimulation in the human saphenous veins. The role of nitro and nitroso compounds, which activate soluble guanylate cyclase, were also examined.

Ring segments (5 mm) of the veins obtained from patients undergoing coronary by-pass grafting were mounted on L shaped stainless steel rods which permitted the isometric recording of smooth muscle force. The veins were superfused with Krebs solution (5 ml/min) equilibrated with 95% O_2 + 5% CO_2 at 37°C. One min transmural nerve stimulation (TNS) was delivered via platinum wire electrodes placed on both sides of the vessel (14 V, 0.3 ms, 1-16 Hz). Infusions of NA (0.1-3 μM for 1 min) were also performed. After a control series of TNS- and NA-induced responses was elicited, NPY was added to the perfusing medium and allowed to bathe the blood vessels for 30 mins before and during the same repeated sequences of TNS and NA.

Contractile-response curves for TNS and for NA were obtained. The contractions induced by TNS were inhibited by 1 μM tetrodotoxin and 10 μM guanethidine, indicating their sympathetic nature. Adding NPY (10, 50, 100 nM) to the superfusing medium had no direct vasoconstrictor action, but caused a leftward shift of the frequency- and concentration-response curves to TNS and to NA. The potentiating effect of NPY (50 nM) was of the same extent on both responses to TNS (73 ± 25 % at 2 Hz frequency; n=5) and NA (76±16 % at 0.3 μM concentration; n= 5). When the guanylyl cyclase inhibitor methylene blue (10 μM) was added to the perfusing solution together with NPY, both the contractile responses to TNS and to NA were potentiated (74±36% and, respectively, 81±20%; n=6) in the same manner as seen in the control experiments.

The present data on human saphenous veins demonstrate the potentiating effect of low concentration of NPY on the vasoconstrictor responses to both sympathetic nerve stimulation and exogenous noradrenaline, as previously seen in animal studies. Furthermore, it can be excluded that nitro compounds, which have been reported to modulate the constrictor responses to TNS and to NA in blood vessels (Vo *et al.*, 1991), play a role in this potentiating effect.

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214P SIMVASTATIN INHIBITS VASOPRESSIN-INDUCED Ca^{2+} ENTRY IN RAT VASCULAR SMOOTH MUSCLE CELLS

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Alterations in plasma and membrane cholesterol have been shown to change ligand mediated calcium influx mechanisms in vascular smooth muscle cells. Since the introduction of HMG CoA reductase inhibitors to treat hypercholesterolaemia, the actions of this class of drug on intracellular calcium ($[Ca^{2+}]_i$) responses to agonists are unknown. We therefore studied the effects of simvastatin (5 mg/l for 24 h) on the $[Ca^{2+}]_i$ responses to vasopressin in A10 cells, a rat aortic vascular smooth muscle cell line, using fura-2 and microfluorometry to measure $[Ca^{2+}]_i$. Results are presented as 340/380 fluorescence ratios (mean ± s.e.mean).

Simvastatin had no effect on the resting $[Ca^{2+}]_i$ (340/380 ratio 0.79 ± 0.04 vs control, 0.72 ± 0.04). Addition of 1 nM vasopressin caused a rapid increase of $[Ca^{2+}]_i$ to a peak ratio of 2.99 ± 0.19 in controls and 2.67 ± 0.22 in simvastatin treated cells. This initial phase was independent of extracellular Ca^{2+} entry and was presumably due to IP_3 mediated release of cellular Ca^{2+} stores. Subsequently, $[Ca^{2+}]_i$ fell to a plateau that was dependent on extracellular Ca^{2+} entry, since this was completely abolished in media containing 1 mM EGTA. This Ca^{2+} entry was not inhibited by the dihydropyridine isradapine (1 μM) nor pretreatment with pertussis toxin (100 ng/ml for 16 h). However, the 340/380 ratio at the plateau in simvastatin treated cells was 0.77 ± 0.04 compared to controls (1.14 ± 0.06, $P<0.001$) suggesting that vasopressin induced Ca^{2+} entry was much reduced in simvastatin treated cells.

To confirm that divalent cation entry was reduced in simvastatin treated cells, 1 mM Mn^{2+} was used to quench the fluorescence of intracellular fura-2 during vasopressin stimulation. The maximal quench rate at the isosbestic wavelength (360 nm) after vasopressin stimulation in control cells was 5.33 ± 0.33 fluorescence units/s, significantly greater than that of simvastatin treated cells (2.55 ± 0.19 unit/s, $P<0.001$), consistent with reduced vasopressin-mediated Ca^{2+} entry through the divalent cation selective channel. This effect of simvastatin was totally reversed within 2 h by incubating cells with 1 mM mevalonate, suggesting that cells were not irreversibly damaged in a non-specific manner. Simvastatin had no effect on the peak 340 nm fluorescence measurements, indicating that IP_3-mediated release of intracellular Ca^{2+} from stores was unaffected. In conclusion, the effect of simvastatin on vascular smooth muscle cells is predominantly on ligand stimulated Ca^{2+} entry through a receptor operated channel, perhaps via a pertussis toxin insensitive G-protein, since the γ subunits of some G-proteins are isoprenylated.

215P A NEW FUROXAN ANALOGUE OF PRAZOSIN DISTINGUISHES α_1-ADRENOCEPTOR SUBTYPES AND CAUSES RELAXATION OF ARTERIAL SMOOTH MUSCLE BY RAISING cGMP LEVELS

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A novel phenylfuroxan analogue of prazosin(fig. 1)has been synthetized and tested for α_1-adrenoceptor antagonism in the isolated rat thoracic aorta. The antagonism of noradrenaline-induced effects caused by PFX3 did not fulfill the criteria for competitive inhibition at a single receptor system: the Schild plot was biphasic and its slope quite different from unity(0.48 ± 0.24). From this curve we calculated a single pA_2 value(11.5 ± 0.2)relative to the high affinity component(concentrations of PFX3 ranging from 3 pM to 0.1 nM). In order to collect further information, experiments have been performed on rat aorta by using the irreversible blocking agent chloroethylclonidine(CEC). The selective inactivation of α_{1B}-adrenoceptors so obtained yielded a monophasic Schild plot with slope close to unity and a pA_2 of 7.6 ± 0.3. Since our results indicate that the low affinity sites for PFX3 have not been affected by CEC pretreatment, it may be argued that they correspond to the α_{1A}-, CEC-insensitive receptors. On the other hand, the high affinity sites for PFX3 (inactivated by CEC)may be identified as the α_{1B}-adrenoceptors. These results are supported by other competition tests performed in rat tissues where are predominantly expressed either the α_{1B}-subtype(rat spleen) or the α_{1A}-subtype(rat vas deferens). The pA_2 values calculated for PFX3 in these tissues(11.1 ± 0.2 and 7.5 ± 0.2)correspond to those previously obtained in rat aorta in the presence and absence of CEC. Our findings indicate that the α_{1A}- and α_{1B}-adrenoceptors show a large difference in sensitivity to PFX3: in the light of this consideration, this derivative might be regarded as a true subtype-specific compound. An interesting aspect of the pharmacological profile of PFX3 is represented by the dose-dependent relaxation of vascular smooth muscle occurring at concentrations higher than 1.0 μM. Such effect is reversed by addition to the bathing medium of methylene blue(10 μM)and haemoglobin(10 μM), indicating that PFX3 might be responsible as previously reported(Ghigo et al.,1992), for a nitri oxide-dependent accumulation of cGMP in vascular smooth muscle cells. In conclusion our data show that PFX3 may be considered a useful pharmacological probe for the subclassification of α_1-adrenergic receptors and possibly the startin point for the synthesis of derivatives which conjugate α_1-adrenergic blocking activity with cGMP-mediated relaxing effect on the vascular smooth muscle tone.

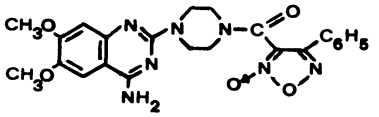


FIGURE 1. 1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(4-phenyl-3-furoxanyl carbonyl)piperazine. (PFX3)

Ghigo,D. et al. J. Med. Chem. 35, 3296-3301

216P EFFECT OF CLONIDINE, MORPHINE AND 4-AMINOPYRIDINE ON EVOKED RELEASE OF [^{3}H]-NA AND MECHANICAL RESPONSES IN MOUSE VAS DEFERENS

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Mouse vas deferens responds to sympathetic stimulation by trains of pulses with a contractile response which has both purinergic (twitch) and noradrenergic (contracture) components (Forsyth & Pollock, 1988). It is unclear whether the transmitters causing these responses are released from a single neurone/vesicle which releases both transmitters or whether some neurones/vesicles release only one transmitter.

Vasa deferentia were taken from Tuck No.1 mice (28-45g) and were incubated with ^{3}H-noradrenaline (0.66 μM) for 45 min as fully described previously by El-Mas & Hughes (1990). The tissues were then placed in 1.5 ml bath where the physiological saline (mM; NaCl 118, KCl 4.75, $CaCl_2$ 2.54, KH_2PO_4 0.93, $NaHCO_3$ 25, glucose 11, EDTA 0.027, ascorbic acid 0.057, 17-β-oestradiol, 0.004; gassed with 5% carbon dioxide in oxygen) was replaced every 2 min. Each tissue was washed for 50 min and then stimulated every 20 min (2 Hz, 2 ms, 200 mA for 90 s). Contractile responses were recorded isometrically (load 0.15g) and consisted of a fast contraction which reached a peak within 3-5 stimuli and which then subsided to a lower sustained plateau. Drugs were applied immediately after a period of stimulation and were in contact with the tissue for at least 15 minutes before further stimulation was applied; drug effects were measured as the ratio (S_2/S_1) of the parameter measured in the presence of the drug to the corresponding value in the preceding drug-free period.

After the wash period the tissues contained 457885±12659 dpm ^{3}H; resting overflow of ^{3}H was 2745±234 dpm; electrical stimulation evoked an overflow of 5815±298 dpm. In fractional terms these represent 0.65±0.02% and 1.22±0.05% respectively (m±sem; n=20). In control tissues resting overflow fell slightly between the two periods of stimulation (S_2/S_1 ratio 0.91±0.03; n=5; $P<0.05$) while evoked overflow (0.99±0.04), peak (1.02±0.05) and plateau (0.99±0.04) contractions were not significantly different from unity (S_2/S_1 ratios; m±sem; n=5; $P>0.1$; t-test). In comparison with control tissues clonidine 1.125×10^{-7}M did not affect resting overflow (0.89±0.04; $P>0.5$) but reduced evoked overflow (0.43±0.08), peak (0.15±0.09) and plateau (0.39±0.07) contractions (S_2/S_1; m±sem; n=5; $P<0.05$; Wilcoxon). Morphine 3.7×10^{-7}M did not affect resting overflow (0.92±0.03; $P>0.5$) but reduced evoked overflow (0.87±0.03), peak (0.21±0.07) and plateau (0.84±0.03) contractions (S_2/S_1; m±sem; n=5; $P<0.05$; Wilcoxon). 4-Aminopyridine (4-AP) 5×10^{-4}M did not affect resting (0.93±0.04; $P>0.5$) but increased evoked overflow (2.84±0.18), peak (2.53±0.12) and plateau (2.37±0.17) contractions (S_2/S_1; m±sem; n=5; $P<0.05$; Wilcoxon).

Although evoked tritium overflow and the peak and plateau contractions were similarly increased by 4-AP and reduced by clonidine the ability of morphine to reduce peak contractile response while having a much smaller effect on evoked tritium overflow and on the plateau contractile response may suggest that at least some portion of the transmitters released can be regulated independently.

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217P δ-OPIOID RECEPTOR SUBTYPES IN THE MOUSE VAS DEFERENS

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There is evidence from *in vivo* studies for subtypes of the δ–opioid receptor. This has so far not been corroborated by isolated tissue studies *in vitro* which has led us to study the effects of three selective δ-agonists [D-Pen^2, D-Pen^5]enkephalin (DPDPE), [D-Ser^2, Leu^5, Thr^6]enkephalin (DSLET) and [D-Ala^2]deltorphin I (DELT I) and their antagonism by the δ-antagonist naltrindole (NTI) in the vas deferens from two strains of mice. Vasa deferentia from Schneider or TO mice were dissected out and mounted in 3.5ml organ baths in Mg^{2+}-free Krebs (containing the peptidase inhibitors; 30μM Bestatin, 10μM Thiorphan, 10μM Captopril and 2mM Leucyl-Leucine) aerated with 95%O_2/5%CO_2. Tissues were maintained at 36°C under a 200mg tension and field stimulation was provided, via linear platinum electrodes, by twin pulses (75ms apart) of 70V, 1ms pulse width at a frequency of 0.1Hz. Inhibition of isometric contractions was recorded and cumulative concentration response curves were carried out on separate tissues for DPDPE, DSLET and DELT I. IC_{50} values were calculated by linear regression analysis. Measures of antagonism by NTI were made by repeating cumulative response curves to the δ-agonists after a 30 min incubation with NTI. On each tissue three concentrations of NTI were studied and pA_2 values were determined by Schild analysis.

Mouse Strain	δ-agonist	IC_{50} (nM)	Naltrindole pA_2	Slope
Schneider	DPDPE	3.32 ± 0.50	9.39 ± 0.44	1.15 ± 0.21
	DSLET	1.98 ± 0.47	9.91 ± 0.37	0.86 ± 0.09
	DELT I	0.75 ± 0.02	8.75 ± 0.07	1.28 ± 0.06
TO	DPDPE	4.20 ± 0.43	9.60 ± 0.22	0.85 ± 0.07
	DSLET	1.12 ± 0.10	9.14 *	1.38*
	DELT I	0.84 ± 0.24	8.78 ± 0.12	1.20 ± 0.12

Values are means ± s.e.mean of 4-7 observations. * Data taken from non-paired experiments due to tachyphylaxis.

In both strains of mice the rank order of potency was DELT I>DSLET>DPDPE. There were significant differences in pA_2 values (ANOVA) for NTI versus the three δ-agonists in both strains of mice with NTI being least effective in antagonising DELT I, although when the slopes were constrained to unity pK_B values were not significantly different. Differences between NTI antagonism of DPDPE and DELT II have previously been shown in antinociceptive studies in the mouse (Jiang et al., 1991), while differences between DSLET and DELT I have been described in the rat (Crook et al., 1992) and these isolated tissue studies raise the possibility of δ-receptor heterogeneity in the mouse vas deferens. Others (Wild et al., 1993) have failed to find evidence for subtypes in the mouse vas deferens using the ICR strain and it could be that strain differences exist, since in our studies the discrimination of pA_2 values was more evident in the Schneider mice.

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218P CHARACTERIZATION OF MUSCARINIC RECEPTORS IN HUMAN PULMONARY ARTERIAL PREPARATIONS

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Acetylcholine (ACh) is known to induce both relaxation and contraction in a number of vascular tissues from a variety of species (Jaiswal et al., 1991). Ach relaxations are associated with stimulation of receptors on endothelial cells while contractions are known to occur by direct stimulation of muscarinic receptors on the vascular smooth muscle. The aim of this study was to characterize and localize the muscarinic receptors on human isolated pulmonary arteries (HPA). HPA were cut as rings and set up in organ baths containing Tyrode's solution gassed with 5% CO_2 in O_2. After equilibration (90 min), the endothelium-intact rings were incubated for 30 min with either pirenzepine (0.1 or 1 μM; M_1 antagonist), methoctramine (10 μM; M_2 antagonist), pFHHSiD (0.2 or 2 μM; M_3 antagonist) or Tyrode's solution. Subsequently, ACh or McN-A-343 (M_1 agonist) concentration-effect curves were produced in precontracted noradrenaline preparations (10 μM; 1.63±0.19 g; mean±SEM of 16 lung samples (n)). This protocol was also performed in rubbed HPA (endothelium removed) on resting tone. The ACh maximal relaxation (R_{max}) was 1.76±0.33 g and the pD_2 value was 7.37±0.08 (n=8). R_{max} for McN-A-343 was 0.94±0.22 g and the pD_2 value was 5.65±0.05 (n=8). In rubbed HPA the maximal contraction induced with ACh was 1.02±0.15 g (n=6) and the pD_2 value was 5.99±0.12 (n=7). McN-A-343 (n=6) did not contract rubbed tissues. The pK_B values obtained with methoctramine against ACh relaxant and contractant responses were 5.38±0.65 and 5.53±0.12, respectively. The pK_B values for pirenzepine and pFHHSiD are presented in the table.

	ACETYLCHOLINE		McN-A-343	
	Pirenzepine	**pFHHSiD**	**Pirenzepine**	**pFHHSiD**
RELAXATION	6.73±0.20 (5)	7.42±0.12 (4)	7.48±0.17 (4)	6.87±0.23 (6)
CONTRACTION	6.95±0.13 (5)	7.55±0.19 (5)	NC	NC

The values are means±SEM and the number of lung samples is shown in parenthesis. NC indicates no contraction.
The relaxations produced either with ACh or McN-A-343 were partially inhibited by indomethacin (1.7 μM) or L-NOARG (0.1 mM). The relaxations with these agonists were markedly reduced in the presence of indometacin and L-NOARG or in rubbed HPA.
A comparison of the pK_B values obtained in HPA with pK_B values obtained in tissues or cells expressing only one type of muscarinic receptor (Dörje et al., 1991) suggests that in HPA the contraction induced by ACh is mediated via stimulation of M_3 receptors located on vascular smooth muscle while the relaxation provoked by cholinergic stimuli in endothelium-intact preparations is mediated *via* release of nitric oxide and a prostanoid, following stimulation of M_1 and M_3 receptors.

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219P INFLUENCE OF AGING ON β-ADRENERGIC RELAXATION OF ISOLATED HUMAN CORONARY ARTERIES (HCA)

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We previously reported that the vasorelaxant effect of isoproterenol in isolated HCAs is reduced with aging (Godfraind & Miller, 1983). The aim of the present study was to further characterize the age-related decrease of beta-adrenergic relaxation in isolated HCAs in relation with the density and function of beta-adrenoceptors.
Rings of HCAs from young and old human subjects (respectively: mean age, 17.1±4.3 years and 50.9±2.5 years; age range, 2.5-29 years and 42-60 years) were suspended under a resting tension of 2 g in 50-ml organ baths filled with a physiological Krebs solution at 37 °C, bubbled with 95 % O_2 - 5 % CO_2. Contractions were evoked by either 100mM K^+-depolarizing solution or by 5-HT 10 μM. Once the contraction had reached a plateau, noradrenaline (NA, 10nM-30μM) or forskolin (FK, 3nM-10μM) were added cumulatively to obtain the corresponding concentration-response curves of relaxation. The affinity and density of beta-receptors were estimated in membrane preparations from HCAs by using the beta-adrenergic antagonist ^{3}H(-)CGP 12177 and the maximum binding (B_{max}) was expressed as fmol per g of tissue wet weight.

The maximum relaxation evoked by NA was significantly higher in young-HCAs than in old-HCAs (for K^+-contraction: 35.8±2.8 % (young, n=6) and 20.1±3.4 % (old, n=7), $P < 0.01$; for 5-HT-contraction: 75.9±3.1 % (young, n=6) and 40.6±9.5 % (old, n=6), $P < 0.01$). The pD_2 values of NA for K^+-contraction were not significantly different (5.79±0.08 and 5.65±0.08 young and old respectively), whereas pD_2 of NA for 5-HT-contraction were significantly higher in young-HCAs (6.04±0.12 and 5.57±0.08 for young and old respectively, $P < 0.01$). Relaxation evoked by NA was not changed by L-NNA (10^{-4} M), an inhibitor of NO-synthase. FK relaxed completely young and old-HCAs stimulated either by K^+-solution or by 5-HT and the pD_2 values of FK were not significantly different in young-HCAs compared to old-HCAs, an indication that the catalytic activity of adenylate cyclase was not affected by aging.

B_{max} of ^{3}H(-)CGP 12177 was equal to 206.8±53.7 fmol/g tissue (young,n=3) and to 196.0±53.6 fmol/g tissue (old, n=3), showing that the density of beta-receptors was not significantly different in young-HCAs compared to old-HCAs.

These results indicate that the age-related decrease of beta-adrenergic relaxation in HCAs could be related to an age-related impairment of the receptor-effector coupling.

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220P NO-INDEPENDENT MUSCULAR RELAXATION BY ATP IN RABBIT THORACIC AORTA

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Vascular endothelial cells synthesize nitric oxide (NO) from L-arginine (L-arg) (Palmer et al., 1987). Two distinct types of NO synthase have been purified: a constitutive Ca^{2+}-calmodulin-dependent enzyme and an inducible Ca^{2+}-independent enzyme. These pathways can potentially be inhibited by various N-substituted analogues of L-arg (McCall and Vallance, 1992) and this inhibition is reversed by the presence of L-arg. The effect of one of these inhibitors, N-nitro-L-arginine methyl ester (L-NAME), was tested *in vitro* on isolated rabbit aorta in order to verify the inhibitory effect of L-NAME on vascular relaxation induced by acetylcholine (ACh, acting at endothelial level) and ATP (acting both at endothelial and smooth-muscle levels). L-arg was used to reverse the effect of L-NAME. Aortic preparations were obtained from 3-month-old New Zealand rabbits. The descending thoracic (chains of 2 rings) were suspended in tissue baths (30 ml) and equilibrated at a resting tension of 35 mN, gassed with a mixture of 95% O_2 - 5% CO_2, pH 7.4, at 37°C. For relaxation studies, ACh and ATP were cumulatively added to aortic rings precontracted with EC_{50} of contracting agents. L-NAME (0.1 mM) and L-arg (0.1 mM) were added to aortic preparations 20 and 60 minutes respectively before relaxation studies. In some aortic preparations endothelium was deliberately removed by gently rubbing the intimal surface with a pipe cleaner. Functional data showed that ACh-induced relaxation (-52.4% ± 9.5% at 10 μM, m ± sem; n=5) was completely abolished by L-NAME (+2.8% ± 4.8% at 10 μM, m ± sem; n=5; $p<0.001$) and the subsequent addition of L-arg was not able to reverse the inhibition by L-NAME (-3.4% ± 7.3% at 10 μM, m ± sem; n=5; $p<0.001$). ATP induced vascular relaxation by acting on P_{2y}-purinoceptors located at endothelial level and on non-P_1-non-P_2-purinoceptors located at smooth-muscle level. L-NAME inhibited ATP-induced relaxation only in the aortic preparations with intact endothelium (-54.2% ± 7.0% *vs* -27.9% ± 7.3% at 1 mM, m ± sem; n=5; $p<0.05$) but not in those deprived of endothelium (-17.7% ± 1.7% *vs* -22.2% ± 2.3% at 1 mM, m ± sem; n=5; p N.S.). L-NAME-induced inhibition on ATP relaxation was not reversed by L-arg (-38.0% ± 5.1% at 1 mM ATP, m ± sem; n=5). In conclusion, the present data indicate that L-NAME inhibited ACh and ATP endothelium-mediated relaxation but not the effect mediated by non-P_1-non-P_2-purinoceptors, suggesting that the muscular relaxation by ATP is not related to the NO pathway. Moreover, the inhibitory effect of L-NAME at endothelial level is not reversed by the presence of L-arg indicating an irreversible inhibition of endothelial NO synthesis.

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221P EFFECT OF DOPEXAMINE HYDROCHLORIDE ON CONTRACTIONS OF RABBIT ISOLATED AORTA EVOKED BY VARIOUS AGONISTS

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Dopexamine hydrochloride is a novel sympathomimetic amine which may prove to be a useful alternative to dopamine in the treatment of acute heart failure and post-operative management of low cardiac output states (Fitton & Benfield 1990). Dopexamine is an agonist at peripheral β-adrenoceptors and dopamine D_1 and D_2 receptors (Brown *et al.* 1985). It is a potent inhibitor of the noradrenaline transport mechanism (uptake-1) (Nedergaard 1989). The purpose of the present study was to examine the effect of dopexamine on contractions of rabbit isolated aorta evoked by various agonists and potassium. The method described by Nedergaard (1986) was used.

Dopexamine (10^{-7}-10^{-6}M) antagonized competitively the contractions of rabbit aorta evoked by noradrenaline (pA_2: 6.60±0.18; mean ± S.E.M.; n=8). Neither cocaine plus corticosterone nor cocaine, corticosterone plus propranolol altered the inhibition (pA_2: 6.77±0.19 and 6.63±0.14, respectively; n=5). The antagonism of dopexamine against noradrenaline-evoked contractions was the same after 1 and 4 h of pretreatment with dopexamine (n=4-5). In the presence of cocaine plus corticosterone, dopexamine antagonized the contractions evoked by phenylephrine (pA_2: 6.94±0.08; n=6). Removal of endothelium did not influence this antagonism (pA_2: 7.06±0.46; n=5). Dopexamine (10^{-7}-10^{-5}M) did not antagonize the contractions of aorta evoked by histamine ($3x10^{-7}$-$6x10^{-5}$M) and by 5-hydroxytryptamine ($3x10^{-7}$-$3x10^{-4}$M) (n=5-7). Dopexamine (10^{-6} and 10^{-7}M) did not alter the contractions of endothelium-free aorta evoked by carbachol (n=5-6). Dopexamine (10^{-7}-10^{-5}M) slightly enhanced the contractions of aorta evoked by potassium (10^{-2}-$5.5x10^{-2}$M) (n=5-8).

These results suggest that dopexamine is an α_1-adrenoceptor antagonist. Furthermore, dopexamine has no affinity to cholinoceptors, histamine and 5-hydroxytryptamine receptors and is apparently not a calcium antagonist.

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222P CHARACTERISATION OF THE 5-HT RECEPTOR WHICH MEDIATES CONTRACTION OF RABBIT PARTIALLY-DEPOLARISED RENAL ARTERIES

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We have reported previously that isolated rabbit renal arteries that are normally unresponsive to 5-hydroxytryptamine (5-HT) and 5-carboxamidotryptamine (5-CT) will show contractile responses to these agents if pre-contracted with threshold concentrations of histamine or the thromboxane mimetic U46619 (Choppin and O'Connor, 1993). Others (Schoeffter and Sahin-Erdemli, 1992) have observed a similar phenomenon in $PGF_{2\alpha}$-contracted guinea pig iliac arteries and proposed that activation of a 5-HT_1-like receptor was responsible. We now describe a preliminary characterisation of the receptor type involved in 5-CT induced contraction of rabbit renal arteries partially depolarised by exposure to a high K^+ (22 mM) physiological solution.

Renal arteries were removed from male New Zealand white rabbits and set up as ring segments for recording isometric tension in normal Krebs solution bubbled with 95% O_2 - 5% CO_2 at 37°C. The buffer solution contained ketanserin, prazosin and idazoxan (all 10^{-6}M) to eliminate the influence of contractile $5HT_2$-, α_1- and α_2-receptors. After a period of equilibration, the physiological solution was changed to one containing 22 mM K^+ which resulted in a moderate contraction (20-30% of the tissue maximum as defined by the response to 80 mM KCl). Once tension had stabilised a single cumulative agonist concentration response curve was constructed in each preparation, either in the absence or in the presence of a receptor antagonist.

Although none of the agonists tested showed significant spasmogenic activity in quiescent renal arteries, all caused contractions in depolarised tissues. 5-HT (10^{-8} - 10^{-5}M), for example, produced concentration-dependent increases in tension achieving 55 ± 6% of the tissue maximum with a pD_2 of 6.91 ± 0.03. The relative order of agonist potencies (pD_2 values) was 5-CT (7.28 ± 0.07) > 5-HT (6.91 ± 0.03) > RU 24969 (6.07 ± 0.20) = sumatriptan (5.94 ± 0.09) > 8-OHDPAT (<5.0). Responses to each agonist were inhibited in the presence of methiothepin $3x10^{-7}$M. The effects of 5-CT were analysed in more depth. 5-CT responses were antagonised by methiothepin (10^{-8} - $3x10^{-7}$M) giving a pA_2 of 7.67 but were unaffected by ondansetron 10^{-6}M, ICS 205930 $3x10^{-6}$M, spiperone $3x10^{-7}$M, pindolol $3x10^{-6}$M, ritanserin $3x10^{-7}$M, SDZ 205-557 $3x10^{-6}$M or l-propranolol 10^{-5}M.

Therefore, 5-HT and related agonists show constrictor activity in partially depolarised rabbit renal arteries. Both the agonist potency order and the antagonist characterisation of 5-CT are consistent with activation of a 5-HT_1-like receptor which, in this tissue, only becomes functional in the presence of another spasmogen.

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223P THE ACTIONS OF THE SELECTIVE 5-HT_{2C} RECEPTOR ANTAGONIST SB 200646 AT 5-HT_2 RECEPTOR SUBTYPES IN RAT CAUDAL ARTERY AND STOMACH FUNDUS

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Structural, transductional and operational similarities have led to a recommendation that 5-HT_2, 5-HT receptors mediating contraction of rat fundus and 5-HT_{1C} receptors should form a 5-HT_2 receptor sub-family and be re-classified as 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors respectively (Humphrey *et al.*, 1993). To further evaluate the operational similarities between these receptors we have examined the actions of a newly described selective 5-HT_{2C} receptor antagonist SB 200646 (Forbes *et al.*, 1993) at functional 5-HT_{2B} receptors in rat stomach fundus and 5-HT_{2A} receptors in rat caudal artery (Van Neuten *et al.*, 1981).

All tissues were isolated from male Sprague Dawley rats (250-350g). Strips (1.5 mm x 20mm) of mucosa denuded longitudinal muscle and endothelium denuded arterial rings (5mm length) were taken from the stomach fundus and the proximal caudal artery respectively. Preparations were mounted under 0.5g tension in oxygenated Tyrode's (fundus) or Kreb's (arteries) solutions at 37°C. Both Krebs and Tyrodes solutions obtained indomethacin (3.0μM) and experiments in caudal artery were conducted in the presence of cocaine (3.0 μM). Antagonist potency was determined after at least one hour equilibration with SB 200646 between successive concentration-effect curves.

5-HT evoked a concentration dependent contraction of both rat stomach fundus and rat caudal artery yielding monophasic concentration effect curves ($-\log_{10} EC_{50}$ (95% C.L.) = 8.9 (8.4-9.2) & 6.5 (6.2-6.7), n= 28 & 6 respectively). In rat stomach fundus, SB 200646 caused a concentration dependent rightward displacement of concentration-effect curves for 5-HT yielding a pA_2 estimate of 7.5 (slope = 0.79). In rat caudal artery SB 200646 (10μM) caused only slight rightward displacements of concentration-effect curves to 5-HT (mean concentration ratio [s.e.m.] = 3.2 [0.7], n=6). SB 200646 did not possess agonist activity in either preparation.

SB 200646 possesses an affinity of approximately 7.03 at functional 5-HT_{2C} receptors (Forbes et al 1993) which is similar to the pA_2 value determined for 5-HT_{2B} receptors in the present study. In contrast, SB 200646 acted as a weak antagonist at 5-HT_{2A} receptors in rat caudal artery. These data support the close pharmacological identity of both 5-HT_{2C} and 5-HT_{2B} receptors.

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224P PLATELET ACTIVATING FACTOR AND TUMOUR NECROSIS FACTOR INDUCE LEUKOCYTE ADHESION IN SPLANCHNIC ARTERY OCCLUSION SHOCK

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The adhesion of circulating neutrophils to vascular endothelia cells is an important event in bowel ischaemia reperfusion injury (Grisham et al ., 1990). It has been shown that depletion of circulating leukocytes ameliorates the severe pathological sequelae associated with splanchnic artery occlusion (SAO) shock (Canale et al ., 1993). The mechanism of the adhesion process has not yet been clarified. Platelet Activating Factor (PAF) represents a potential mediator for neutrophil adhesion (McMannus et al., 1980). Besides PAF, a large body of experimental evidences has pointed out that Tumour Necrosis Factor (TNF α) plays a key role in the developement of leukocyte adhesion to the vascular endothelium (Mantovani & Dejana , 1989). Previous findings have indicated that PAF and TNF-α are involved in the pathogenesis of SAO shock (Squadrito et al ., 1991; Squadrito et al ., 1992). We therefore investigated the role of PAF and TNF-α in the phenomenon of leukocytes adhesion in SAO shock. SAO shock was induced in anaesthetized rats by clamping the celiac trunk and superior mesenteric artery for 45 min. Sham-operated animals were used as controls. The arteries were then released and survival was evaluated. The mean survival time of SAO shocked rats was 93 ± 7 min , while all sham-shocked rats survived more the 6 h. The leukocyte infiltrate was quantitated in the ileum and in the lung using the myeloperoxidase (MPO) assay. MPO activity of the ileum and lung averaged 0.05 ± 0.03 and 0.4 ± 0.2 U/ protein in animals killed before occlusion; MPO did not change in those killed immediately before reperfusion and significantly increased (0.11 ± 0.02 and 1.7 ± 0.6 U/ protein in the ileum and lung, respectivily) in those killed 80 min after the beginning of the reperfusion. The histological examination confirmed the accumulation of leukocytes in the ileum and lung mucosa over the 80 min. Furthermore SAO shocked rats exibited leukopenia and increased circulating levels of both PAF and TNF-α. The administration of TCV-309 (5 μg/kg , i.v. 5 min after reperfusion), a specific PAF-receptor antagonist (Terashita et al . 1992) increased survival time (200 ± 10 min), lowered serum levels of TNF-α , reduced MPO activity in both ileum and lung and ameliorated leukopenia induced by SAO shock . In addition the drug reduced ileum necrosis and lung morphological alterations. Our results suggest that PAF and TNF-α play a important role in leukocyte adhesion in SAO shock.

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225P RADIOIMMUNOASSAY EVIDENCE THAT THE PRESSOR EFFECT OF BIG ENDOTHELIN-1 IN ANAESTHETISED RATS IS DUE TO LOCAL CONVERSION TO ENDOTHELIN-1

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Systemic administration of big ET-1 induces a pressor response comparable in magnitude to that of ET-1. Like ET-1, this effect is markedly attenuated by BQ-123 indicating that it is mediated predominantly by ET_A receptors (McMurdo *et al.*, 1993; Haleen *et al.*, 1993). However, unlike ET-1, the increase in blood pressure produced by big ET-1 can also be blocked by pretreatment with a high dose of the metalloprotease inhibitor phosphoramidon (PHOS) (McMahon *et al.*, 1991), which acts by inhibiting the enzymatic conversion of big ET-1 to ET-1 by the putative endothelin-converting enzyme (ECE). The nature of the enzyme involved is still unclear, and its precise location has yet to be determined. In this study we have used highly specific radioimmunoassays to measure the changes in plasma concentrations of big ET-1 and ET-1 following i.v. administration of big ET-1 in anaesthetised rats.

Male Wistar rats (250-330 g) were anaesthetised with sodium thiopentone (120 mg/kg, i.p.). Intravascular catheters were inserted into the right femoral vein for peptide administration, and into the right femoral artery for measurement of mean arterial blood pressure (MAP) or withdrawal of blood samples. Each rat was used to study only one bolus injection of big ET-1 or ET-1. Heparinised blood was collected into chilled plastic tubes, centrifuged, and plasma was removed and frozen at -30°C until assay. Plasma concentrations of big ET-1 were measured after administration of 1 nmol/kg, or 3 nmol/kg human big ET-1 alone, or 1 nmol/kg big ET-1 in rats pretreated with PHOS (10 mg/kg, -1min). ET-1 levels were measured in these groups, and also for comparison in rats administered ET-1 (1nmol/kg). RIA of big ET-1 was performed, after dilution of the plasma, using an antiserum raised against the C-terminal sequence of human big $ET\text{-}1_{22\text{-}38}$ (crossreactivity with ET-1 <0.001%). For ET-1 measurement, plasma samples were first extracted using disposable C18 columns, and then subjected to RIA using an antiserum raised against $ET\text{-}1_{16\text{-}21}$ (crossreactivity with big ET-1 <0.015%).

Following bolus administration of big ET-1, peak plasma concentrations occurred within 40 sec. These were 12.9 ± 1.3 nM and 52.1 ± 3.0 nM for the 1 and 3 nmol/kg doses of big ET-1 (n=4 for each). At 1 and 3 min these levels had declined to 6.8 ± 0.7 nM and 3.2 ± 0.2 nM after the 1 nmol/kg dose, and 44.7 ± 3.4 nM and 18.3 ± 2.4 nM after the 3 nmol/kg dose. Administration of PHOS did not affect the disappearance rate of big ET-1 (1 nmol/kg), but completely blocked the increase in MAP (+10 min Δ: 43 ± 2 mmHg for big ET-1 alone, n= 6; compared to 3 ± 1 mmHg n=4 with PHOS; $p < 0.001$). Following ET-1 injection, peak plasma levels occurred within 20 sec of administration (10.1 ± 2.5 nM), these decreased rapidly to 2.4 ± 0.2 and 1.2 ± 0.1 nM at 1 and 3 min respectively. After big ET-1 administration, ET-1 was barely detectable in plasma (0.04 to 0.07 nM) and at no time point exceeded 2 % of the big ET-1 concentration. In agreement with the effect of locally applied big ET-1 (Lawrence & Brain, 1993), these results suggest that big ET-1 is converted locally in the vasculature to ET-1, and that the ET-1 generated does not re-enter the circulation to any extent. Furthermore, the fact that PHOS did not alter the rate big ET-1 disappeared from the circulation suggests the conversion step takes place outside the vessel lumen.

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226P THE ET_B RECEPTOR AGONIST, IRL 1620, CAUSES VASODILATATION AND INHIBITS *EX VIVO* PLATELET AGGREGATION BY RELEASING PROSTANOIDS IN THE ANAESTHETISED RABBIT

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Activation of ET_B receptors by ET-1 induces the release of prostacyclin into the circulation, which in turn inhibits platelet aggregation and attenuates the pressor effects of ET-1 (Thiemermann *et al.*, 1988). This conclusion is supported by our findings demonstrating that the non-selective ET_A/ET_B receptor antagonist, PD 145065, but not the selective ET_A receptor antagonist FR 139317, abolishes the ET-1 induced inhibition of *ex vivo* platelet aggregation in the anaesthetised rabbit (McMurdo *et al.*, 1993). Here, we investigate whether the selective agonist for ET_B receptors, N-Succinyl-(Glu^9, $Ala^{11,15}$)-Endothelin (8-21), IRL 1620, (Takai *et al.*, 1992) inhibits *ex vivo* platelet aggregation in the anaesthetised rabbit. In addition, changes in blood pressure and haematocrit induced by IRL 1620 were studied.

NZW rabbits (2-3 kg) were premedicated with Hypnorm (0.1 ml kg^{-1} i.m.; containing 0.315 mg ml^{-1} fentanyl citrate and 10 mg ml^{-1} fluanisone, i.m.). General anaesthesia was then induced and maintained with sodium pentobarbitone (30 mg kg^{-1}, i.v.). The left femoral artery was cannulated for the measurement of mean arterial pressure (MAP) and for blood sampling. The right marginal ear vein was cannulated for injection of indomethacin. Another catheter was placed in the left ventricle via the right common carotid artery for administration of IRL 1620. *Ex vivo* platelet aggregation was measured as previously described (Thiemermann *et al.*, 1988).

Resting MAP was 63 ± 2 mm Hg (n=9). The ET_B agonist (3 nmol kg^{-1}, i.a., bolus; n=5) induced a fall in blood pressure (nadir: -23 ± 2 mm Hg) which was maintained for up to 5 min after peptide injection (5 min: -1 ± 3 mm Hg). This was followed by an overshoot in MAP (19 ± 4 mm Hg at 10 min after injection), with MAP returning to control levels at 30 min after IRL 1620 administration. Pretreatment with indomethacin (5 mg kg^{-1}, i.v., bolus; n=4) at 20 min prior to IRL 1620 injection had no effect on the initial transient depressor response induced by the peptide (-15 ± 5 mm Hg; $p > 0.05$) but, revealed a rapid and sustained increase in MAP (peak: 27 ± 8 mm Hg) at 5 min after ET_B agonist administration. IRL 1620 caused a 64 ± 11 % (1 min) and 92 ± 2 % (5 min) inhibition of ADP-induced platelet aggregation ($p < 0.05$; n=5). The inhibition of platelet aggregation caused by IRL 1620 was prevented by pretreatment with indomethacin (1 min: 11 ± 8 %; 5 min: 9 ± 10 %; $p > 0.05$; n=4). Baseline haematocrit was 38 ± 1 % blood volume (n=9). Administration of IRL 1620 induced a significant rise in haematocrit at 5-30 min after peptide injection (peak at 15 min: 42 ± 1 %; $p < 0.05$; n=5). The increase in haematocrit was unaffected by pretreatment with indomethacin (15 min: 40 ± 0.1 %; $p > 0.05$; n=4).

Thus, in the anaesthetised rabbit, the ET_B receptor agonist has a pronounced depressor action which is converted to a sustained pressor effect in the presence of indomethacin. In addition, IRL 1620 inhibits *ex vivo* platelet aggregation an effect prevented by indomethacin. Thus, ET_B receptor activation inhibits *ex vivo* platelet aggregation in the rabbit, presumably by releasing prostacyclin into the circulation. *We thank Parke-Davis Pharmaceutical Research for funding this work.*

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227P ENDOTHELIN VASOCONSTRICTION IN RAT EXTRALOBAL PULMONARY ARTERIES IS MAINLY Et_A-RECEPTOR-MEDIATED; IN INTRALOBAL RESISTANCE PULMONARY ARTERIES IT IS MAINLY Et_B-RECEPTOR-MEDIATED

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There have been conflicting reports as to the receptor type involved in endothelin-1 (Et-1)-induced vasoconstriction in pulmonary arteries. This may be due to species differences or differences in the location of the pulmonary artery under study. Here we have compared the endothelin receptor subtype/s involved in the vasoconstrictor effect of endothelin-1 in the extra-lobal pulmonary capacitance arteries with those in the intra-lobal pulmonary resistance arteries of the rat. We have used endothelin-1 (Et_A and Et_B receptor agonist), sarafotoxin S6c (SxS6c, selective Et_B agonist) and FR 139317 (Et_A antagonist) to determine the Et receptors involved.

First branches of the main extra-lobal pulmonary artery (2-3mm.i.d.) and second order intra-lobal resistance artery (150-200μm) were isolated from recently killed rats. The large arteries were mounted in 5ml organ baths in Krebs under 2g tension bubbled with $8\%O_2/6\%CO_2$ balance N_2 (bath PO_2=45-50mmHg). The resistance arteries were mounted on a resistance vessel wire myograph under 250mg tension and bubbled with $16\%O_2/6\%CO_2$ balance N_2 (bath PO_2 approx. 100 mmHg). Cumulative concentration-response curves (CCRCs) were constructed to Et-1 and Sx6c (3pM-1μM). A maximal concentration of Et-1 was administered after the last concentration of Sx6c. CCRCs to Et-1 were constructed in the absence or presence of FR 139317 (100nM or 1μM). The results are shown in table 1.

Table 1. ***EC_{50} values (-log M) for Et-1 (± FR) and Sx6c as well as the maximum response to Sx6c as a % of the maximum response to Et-1 in the same preparation. n/n = number of ring preparations/ number of individuals***

Preparation	Agonist ± FR 139317	n/n	EC_{50} values -log M)	Maximum response to Sx6c (% of maximum to Et-1)
Extra-lobal artery	Et-1 control	13/9	8.5 ± 0.1	
Extra-lobal artery	Et-1/FR 100nM	7/7	8.6 ± 0.1	
Extra-lobal artery	Et-1/FR 1μM	10/8	7.8 ± 0.2**	
Extra-lobal artery	Sx6c	8/4	0	9 ± 3
Intra-lobal arteriole	Et-1 control	5/5	9.0 ± 0.2	
Intra-lobal arteriole	Et-1/FR 100nM	5/5	9.0 ± 0.3	
Intra-lobal arteriole	Et-1/FR 1μM	5/5	8.4 ± 0.2*	
Intra-lobal arteriole	Sx6c	9/9	9.7 ± 0.2*	92 ±4

*Statistical difference from relevant control, Student's unpaired t-test *P<0.05; **P<0.01*

The Et-1-induced vasoconstriction in the larger capacitance arteries can be seen to be mediated largely through the Et_A receptor in that they were virtually insensitive to Sx6c and inhibited by FR 139317. The Et-1-induced vasoconstriction in the smaller resistance arteries would appear to have a large Et_B receptor-mediated component: they were approximately five times more sensitive to Sx6c than Et-1. In addition, following a maximum concentration of Sx6c, Et-1 could only induce a residual response some 9% greater than the maximum response to the Et_B agonist. These results indicate that the Et-receptor mediating Et-1 vasoconstriction in the pulmonary circulation varies with the location an/or size of the vessel under study.

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228P MODULATORY EFFECTS OF ENDOTHELINS AT THE OSTEOBLAST

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Endothelin, a newly discovered 21-amino acid peptide, was first identified in vascular endothelial cells, and is considered to be one of the most potent endogenous vasoconstrictor substance yet known. In humans three genes have been identified encoding for peptides of the ET family, ET-1, ET-2, ET-3, which differ each other by few amino acids. Moreover, both ET-1 and ET-3 were found in rats and in other species. Endothelins (ETs) have now been shown to have other biological actions in addition to their effects at cardiovascular system, including the respiratory system, the central nervous system, the hypothalamus, the pituitary and the bone. Recently, in fact, ETs have been implicated in the regulation of bone remodeling. At cellular level ETs stimulate the inositol phospholipid hydrolysis and induce an increase in cytosolic free Ca^{2+}, which is produced both by intracellular mobilization and through influx from extracellular sources. To understand better the mechanism of action of ETs in bone remodelling, we have investigated the effects of ET-3 on osteoblast function in comparison with the already known effects produced by ET-1 in the same cell type. For this purpose we have used the osteoblast-like UMR-106 cell line that represent a good model for the study of osteoblast function *in vitro*. As a first parameter of osteoblast function we have measured alkaline phosphatase (ALP) activity. In these cells, a 48 hour-exposure to ET-3 produced a concentration-dependent increase of ALP activity. A first significant effect was observed at a concentration of 0.1 nM ET-3 (+12%) and maximal stimulation was present when cells were treated with 10 nM ET-3 (+54%). Interestingly, in the presence of parathyroid hormone (PTH), which *per se* reduced ALP activity, ET-3 was able to counteract, in a concentration dependent fashion, the inhibitory effect induced by PTH (178 ± 26.7, 82 ± 6.6, 131 ± 3.3 ALP units/10^5 cells for control, PTH 100 nM and PTH + ET-3 10 nM, respectively). The large stimulation of cAMP accumulation induced by PTH was markedly reduced after a single exposure of UMR-106 cells to maximal concentrations of ET-1 (-38%) or ET-3 (-41%). Conversely, ETs were unable to modify basal cAMP concentrations. We have also evaluated cellular growth in UMR-106 cells either by cell counting or by [^{3}H]thymidine incorporation. Cells were exposed to different concentrations of ET-3 and the peptide was added daily for 1 to 6 days. A chronic treatment of UMR-106 cells with ET-3, under serum free conditions, produced a significant, concentration-dependent (0.1 nM-100 nM), inhibition of cell proliferation. Maximal response observed after treatment with 10 nM ET-3 gave a 34% reduction of basal [^{3}H]thymidine incorporation and a 24% decrease in the number of cells per well. Based on these results ET-3 may act as a modulator of osteoblastic function and may participate, at least in part, to the processes of bone remodelling.

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229P AN INVESTIGATION OF EXPERIMENTAL ARTERIOPATHY IN RAT HIND LIMB BY *IN VIVO* ^{31}P NMR SPECTROSCOPY: EFFECT OF PROPIONYL-L-CARNITINE

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In the present work we apply the *in vivo* ^{31}P NMR spectroscopy technique in order to characterize the changes on muscle energy metabolism in a rat model of experimental peripheral arteriopathy. The damage evolution was monitored over time on the same individuals and the effectiveness of propionyl-l-carnitine (PLC) treatment was evaluated.
Two groups of rats, respectively to be treated with PLC (T) and to be observed as a reference (NT), underwent to sodium laurate (Na lau) injection through the femoral arteries in both the hind limbs (Corsico *et al.*). A third group (sham-operated, SO) was injected with the vehicle (ethanol 0.5% aqueous solution) only. Starting on the next day, the T group was treated orally (120 mg/Kg/die b.w.) with PLC. ^{31}P NMR measurements on the calves have been performed before either the Na lau or the vehicle injection and 3, 10, 16, and 23 days on a Otsuka Vivospec 4.7 T spectrometer using a 4 cm surface coil.
The resonance signals of a normal rat hind limbs are assigned as follows: phosphomonoesters (PME) (+6.94 ppm), inorganic phosphate (Pi) (+5.16 ppm), phosphocreatine (PCr) (0.00 ppm) and γ, α and β phosphate groups of ATP (-2.43, -7.71 and -16.27 ppm). Their levels, expressed as % of the total phosphorus content are as follows (mean± SD): PME 3.6±1.2, Pi 7.4±1.1, PCr 40.9±1.4, γ-ATP 17.0±1.7, α-ATP 15.9±1.7, β-ATP 15.2±1.8.
Na lau injection causes a dramatic increase of the Pi levels on the third day both in the NT and T groups (respectively 426% and 362% of control values), followed by a decreasing trend. The PLC treated animals show a significative difference versus the NT group at 3, 10 and 16 days. Conversely, the PCr levels are dramatically reduced in both groups (29% and 35% of the control level respectively in the NT and T group) on the third day, with a recovery trend in the following days. The Pi/PCr ratios for the NT and T groups have been calculated and their means significatively differ on the 3rd, 10th and 16th day ($p<0.05$). These values lie on two hyperboles which horizontal asymptote is coincident with the control value. These results shows a role of PLC in the reduction of the muscular and/or vascular damage inducted by Na lau. The Pi levels increase and the PCr levels decrease on the 3rd day is consistent with the necrosis of the muscular fibers induced by the Na lau injection in the rat calf with the consequent microcirculation damage (Corsico *et al.*). A recovery trend toward the pre-induction values is present in both T and NT groups at 10, 16 and 23 day. PLC treated animals show a faster recovery trend toward the control values than no-treated ones. This effect is discussed in terms of the drug activity mechanism either on necrosis or on the enhancement of metabolic recovery.

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230P CALCIUM ANTAGONISTS AND AORTA CONTRACTILITY IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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There is evidence showing an altered vascular reactivity to various stimuli in hypertensive animals and human subjects (Triggle & Laher, 1985; Lograno & colleagues, 1989). The major observation indicates that hypertension is characterized by increased sensitivity to constrictor agonists and decreased responsiveness to dilator agents; however the results of different studies are highly variable and depend upon the species and the vascular bed from which the vessels are isolated. The molecular mechanisms underlying the increased contractility of vascular smooth muscles from hypertensive animals are so far not completely understood. A modified calcium influx through L-type calcium channels has been hypothesized as one of the factors involved. Along this line, the aim of the present study was to investigate, by using the "laminar flow" superfusion technique (Ferreira & De Souza-Costa, 1976), the effects of two calcium antagonists, verapamil and nifedipine, on the mechanical activity produced by potassium chloride (20 mM- 80 mM) and phenylephrine (10^{-8} M - 10^{-4} M) in isolated aortae from adult (6-month old) normotensive Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHR). Moreover, the density of L-type calcium channels on the same tissue was assessed by radioreceptor binding experiments using [^{3}H] D888 and [^{3}H] PN 200-110 as selective ligands. Saturation binding data were analyzed using the non linear least-squares curve-fitting program LIGAND (Munson & Rodbard, 1980).
In normotensive rats, the maximal contraction produced by potassium chloride (80 mM) was completely inhibited by the two calcium antagonists (10^{-9} M - 10^{-5} M). However, the maximal contraction produced by phenylephrine (10^{-5} M) was inhibited to a lesser extent (about 70%). In hypertensive rats, the inhibitory action of verapamil and nifedipine on potassium chloride-produced contraction wasn't significantly different in comparison to normotensive rats. Surprisingly, the maximal response to phenylephrine (10^{-5} M) was potentiated by verapamil (30±8 % by 10^{-8} M verapamil) and weakly inhibited by high concentrations of nifedipine (6.5±3 % and 29±6 % by 10^{-7} M and 10^{-6} M nifedipine respectively) (values are means± S.D. of 5-6 experiments).
Scatchard analysis of specific [^{3}H] D888 binding revealed a single high affinity binding site in both WKY and SH rats. The calculated Kd (nM) and Bmax (pmoles mg protein^{-1}) were as follows: WKY Kd 0.2±0.015, Bmax 7±0.9; SHR Kd 22.4±3, Bmax 55±6 (values are means ± S.D. of 3 experiments). Preliminary radioreceptor binding results using the selective ligand for dihydropyridine receptor, labeled PN 200-110, seem to confirm different density and affinity of binding sites in SH rats with respect to WKY rats.
From our results, it could be hypothesized that an altered calcium influx through voltage-operated calcium channels might be involved in the changes of vascular reactivity observed in SHR.

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231P PHENTOLAMINE PROTECTS ATRIAL ACTIVITY FROM HYPOXIA-INDUCED DEPRESSION

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Hypoxia induces impairment of myocardial function such as decrease of cardiac contractility, alterations of membrane potential and metabolic changes. α- and β-Adrenoceptor antagonists have been shown to be effective in protecting myocardium against hypoxia and ischaemia (Nayler et al., 1986; Takeo et al., 1988; Tanonaka et al., 1989). Although it seems certain that the effects of sympathomimetic agents on the mammalian heart are mediated mainly by activation of β-adrenoceptor, α-adrenoceptor has also been demonstrated to play a role in pathological conditions. However, the cardioprotective effect of these agents has not been well established. The aim of this study was to determine *in vitro* the effects of phentolamine , an α-adrenoceptor antagonist, on hypoxia-induced modification of cardiac function. Our experiments were carried out on isolated atrium, a relatively simple preparation without vascular and humoral influences, useful for investigation of the relations among functional parameters and energy metabolism. Results are expressed as mean values ± sem of 7-9 experiments. The effects of hypoxia (PO_2= 180±6 mmHg) alone and in the presence of 50 µM phentolamine were examined in isolated atria from 6-month-old rats. In spontaneously beating atria 30 min after hypoxia contractile tension decreased to about 80% of controls without variation of atrial rate. In the meantime, a drastic increase in diastolic tension was also observed. Hypoxia (30 min) did not change ATP, ADP, AMP and creatine phosphate levels, while a significant increase in lactic acid content was observed (control value: 43.2±5.5 nmol/mg protein, to 79.9±6.8 nmol/mg protein). During normoxia (PO_2= 370±9 mmHg), 50 µM phentolamine (20 min) increased contractile tension (+31±4%) and decreased frequency (-29±2%). During hypoxia, phentolamine countered both the decrease in contractile tension and the increase in diastolic tension. Phentolamine *per se* did not change adenine nucleotides, creatine phosphate and lactic acid at atrial tissue levels. Neither after 30 min of hypoxia phentolamine modified ATP, ADP, AMP, creatine phosphate and lactic acid contents. However, a slightly increase of adenylate energy charge (E= ATP+ 0.5ADP/ ATP+ ADP+ AMP) was observed: control value= 0.90±0.01, to 0.89±0.004; $p<0.05$). The present data indicate that during hypoxia phentolamine partially prevents the hypoxia-induced inhibition of atrial function without increasing energy demand.

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232P A NEW ANTIAGGREGATING AGENT, CHF 2206, AS POTENTIAL NO-DONOR

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It has recently been observed that some furoxans generate nitric oxide (NO) following chemical reaction with mercapto groups of low molecular weight thiols and proteins (Feelisch et al., 1992). We have focused our attention on a new 3,4-disubstituted furoxan, CHF 2206 (4-phenyl-3-phenylsulfonylfuroxan), which induces vascular relaxation through a mechanism independent of endothelium integrity (Ferioli et al., 1993). The aim of this study was to extend the observations in favour of a pharmacological profile of CHF 2206 similar to the one described for NO-donating prodrugs or NO-mimetics.

When pre-incubated (1 min) with human plasma rich platelets (PRP), CHF 2206 reduced the platelet aggregation induced by the threshold aggregating concentration of collagen (0.5-1 µg/ml), ADP (1.36 - 2.40 µM) and PAF (0.1-1 µM); the IC_{50} values were 0.378±0.010, 0.115±0.0186 and 0.132±0.0129 µM respectively (mean ± S.E., n = 5). In the same experimental conditions, CHF 2206 in the concentration range of 0.1 to 10 µM produced a 1.10 to 4.2 fold increase in the platelet guanosine 3':5'-cyclic monophosphate (cGMP) levels (control level was 1.19±0.11 pmol/10^9 platelets, mean ± S.E., n = 5). The rise in cGMP was not altered in the presence of the aggregating agents previously mentioned. In contrast, adenosine 3':5'-cyclic monophosphate (cAMP) levels did not increase after addition of the test compound. The addition of 10 µM oxyhaemoglobin (oxyHb), a substance known to bind NO and destroy its biological activity (Radomski et al., 1987) to PRP stimulated with PAF, ADP or collagen 1 min before CHF 2206, reduced the antiaggregating effect of the compound by 7-10 fold. This effect was completely abolished in the presence of oxyHb 30 µM.
OxyHb markedly inhibited also the CHF 2206-induced increase in cGMP levels (85±8% and 66±5% inhibition at 1 and 3 µM of CHF 2206 with 10 µM oxyHb; full inhibition was observed with 30 µM oxyHb). 3-isobutyl-1-methyl-xantine (IBMX) 5 µM concentration, which did not directly affect platelet aggregation, potentiated the antiaggregating activity of the test compound (2-3 fold at 0.1 µM of CHF 2206).

In conclusion, these results show that CHF 2206, in line with the general profile of NO-donating prodrugs or NO-mimetic drugs, exerts a potent antiaggregating activity at least in part through stimulation of platelet guanylate cyclase (GC). As far as the mechanism of GC stimulation is concerned, the efficient scavenging effect of oxyHb suggests that the compound could release NO.

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233P INHIBITION OF EXCITATORY NON-ADRENERGIC, NON-CHOLINERGIC NEUROTRANSMISSION IN GUINEA PIG BRONCHI *IN VITRO* BY 5-HT AND ITS POSSIBLE MECHANISM

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Electrical field stimulation (EFS) of guinea pig bronchi, *in vitro*, evokes an excitatory non-adrenergic non-cholinergic (e-NANC) neural bronchoconstriction mediated by the release of tachykinins from sensory nerves. 5-Hydroxytryptamine (5-HT) is an important inflammatory mediator and 5-HT receptors have been localised to sensory nerve terminals. We have investigated whether 5-HT modulates e-NANC responses in guinea pig bronchi *in vitro*, the receptor responsible and two possible mechanisms for this modulation: that 5-HT opens charybdotoxin (ChTx)-sensitive Ca^{2+}-activated K^+ channel (K^+_{Ca}; Stretton *et al.*, 1992), or that 5-HT stimulates nitric oxide formation (NO; Bogers *et al.*, 1991).

Male Dunkin-Hartley guinea pigs (300-550g) were sacrificed, main and hilar bronchi were removed and isometric tension recordings were made of EFS-induced e-NANC responses (Stretton *et al.*, 1992). The effect of 5-HT and selective 5-HT receptor agonists was studied on e-NANC responses and on responses to exogenously applied substance P (SP; 10nM-10μM). After preliminary experiments, the 5-HT_2 antagonist ketanserin (1μM) was added prior to each experiment to prevent the direct bronchoconstrictor effect of 5-HT. 5-HT antagonists, ChTx and the NO synthase inhibitor L-N^G-nitroarginine methyl ester (L-NAME) were added 10min prior to commencement of EFS.

5-HT (0.1-100μM) produced concentration-dependent inhibition of e-NANC responses with 50.9±5% (n=5, $p<0.01$) inhibition at 100μM. Ketanserin (1μM), the $5\text{-HT}_{3/4}$ antagonist ICS 205-930 (1μM) and the adrenoceptor antagonists phentolamine (1μM) and ICI 118551 (0.1μM) had no significant effect on this inhibition. The 5-HT_1 antagonist methiothepin (0.1μM) blocked the inhibition evoked by 5-HT (0.1-100μM), methiothepin (1nM-10μM) produced a concentration-dependent inhibition of the effect of 5-HT (10μM) with an IC_{50} of 81nM. ChTx (0.1-30nM) produced a concentration-dependent inhibition of the effect of 5-HT (10μM) producing complete inhibition of the response at 30nM. L-NAME (100μM) was without effect on the inhibition produced by 5-HT (10μM). All antagonists were without effect on e-NANC responses alone. Of the selective agonists tested the most potent was 5-carboxamidotryptamine (5-CT) which produced a concentration-dependent inhibition of e-NANC responses with an EC_{50} of 0.13μM and a maximum inhibition of 51.1±4.7% (n=5, $p<0.01$) at 100μM. The concentration of agonist causing 25% inhibition of the e-NANC response (IC_{25};μM) was calculated giving a rank order of potency : 5-CT (0.13) > 5-HT (0.32) >> 8-hydroxy-DPAT (14) > α-methyl-5HT (80) > sumatriptan = 2-methyl-5HT =5-methoxytryptamine (all >100). 5-HT (10μM) had no effect on cumulative concentration-response curves to exogenous SP.

These results suggest that 5-HT inhibits e-NANC responses in guinea pig bronchi, *in vitro*, via a prejunctional mechanism. The selective attenuation of this inhibition by methiothepin and the rank order of potency of the selective 5-HT receptor agonists suggest this inhibition is via activation of a 5-HT_1-like receptor. The inhibition of this effect by ChTx but not by L-NAME suggests 5-HT is acting via a K^+_{Ca} channel to cause inhibition of e-NANC responses and not by releasing NO.

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234P EFFECT OF N-FORMYL-METHIONYL-LEUCYL-PHENYLALANINE AND PHORBOL MYRISTATE ACETATE ON AIRWAY CELL INFILTRATION IN GUINEA PIGS

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Activated leukocytes are recognized as playing a role in the development of airway inflammation in asthma. These cells release mediators that are responsible for the inflammatory process. "In vitro", the chemotactic peptide N-formyl-methionyl-leucyl-phenylalanine (FMLP) and the activator of the intracellular protein kinase C, phorbol myristate acetate (PMA), activate leukocytes which produce and release proinflammatory substances (Sun, et al., 1989; Shute, et al., 1990; Ward, et al., 1983). As little information is available concerning the "in vivo" actions of these stimuli, we investigated the effect of FMLP and PMA on intratracheal treated guinea pigs, by evaluating leukocyte recruitment in bronchoalveolar lavage (BAL). FMLP and PMA at 10 μg/animal and their vehicle (DMSO 0.1% in saline) were instilled in a volume of 100 μl in the trachea of lightly anesthetized (pentobarbital 30 mg/kg i.p.) male HD guinea pigs (350-400 g). After 4 and 18 hours, BAL was performed and the number of total cells, eosinophils and neutrophils was determined. PMA induced an increase in total cell number (18 h) and a significant increase in neutrophils (4 and 18 h). No effect on eosinophils was observed at either time. FMLP did not modify the total cell number either 4 h or 18 h after treatment, but it did modify the cellular composition of the BAL fluid: neutrophils significantly increased at 4 h and 18 h, while the number of eosinophils increased only at 4 h and returned to the basal values at 18 h.

Table Airway cell infiltration induced by PMA and FMLP intratracheally dosed in guinea pigs (n = 4-6). The results are expressed as mean ± s.e.m. * $P<0.05$.

Treatment	Time (h)	Total cells	Neutrophils		Eosinophils	
		cells x 10^6	cells x 10^6	% vs total cells	cells x 10^6	% vs total cells
None	-	6.9 ± 0.3	0.01 ± 0.01	0.2 ± 0.1%	0.64 ± 0.17	9.1 ± 2.0%
DMSO 0.1%	4	9.6 ± 0.5	0.02 ± 0.01	0.3 ± 0.1%	0.72 ± 0.15	7.5 ± 1.5%
PMA 10 μg	4	6.8 ± 0.6	0.89 ± 0.22*	12.9 ± 2.4%	0.68 ± 0.19	9.7 ± 1.9%
FMLP 10 μg	4	8.3 ± 0.9	0.98 ± 0.68*	10.9 ± 6.5%	1.67 ± 0.66*	19.9 ± 7.5%
DMSO 0.1%	18	7.4 ± 0.2	0.19 ± 0.05	2.5 ± 0.7%	0.68 ± 0.07	9.3 ± 1.1%
PMA 10 μg	18	12.4 ± 1.9*	3.6 ± 1.4*	26.3 ± 5.2%	0.65 ± 0.10	5.4 ± 0.7%
FMLP 10 μg	18	7.4 ± 0.8	0.79 ± 0.35*	9.3 ± 3.5%	0.76 ± 0.11	10.3 ± 1.1%

These results suggest that PMA and FMLP elicit an inflammatory response "in vivo" as shown by the modification in the BAL leukocyte pattern in guinea pigs: PMA recruits only neutrophils, FMLP recruits both neutrophils 4 and 18 h and eosinophils 4 h after treatment. The different recruitment patterns possibly reflect differences in the respective mechanisms of action of the two stimuli.

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235P DESENSITIZATION TO β AGONIST RESPONSES IN HUMAN LUNG MAST CELLS (HLMC)

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Recent epidemiological studies have suggested a correlation between reliance on ß agonists and an excess of asthma deaths (Grainger *et al.*, 1991; Spitzer *et al.*, 1992) One possible hypothesis is that tolerance to ß agonists may develop with continued use. Tolerance to the bronchodilator effects of ß agonists is generally thought to be clinically-irrelevant. However, ß agonists may owe a proportion of their anti-asthma effects to non-bronchodilator properties such as the inhibition of the release of mediators from HLMC. Should tolerance develop selectively to the non-bronchodilator effects of ß agonists, this could have important consequences.

Mast cells were isolated from physically and enzymatically treated human lung tissue. HLMC were incubated with the ß agonist isoprenaline (ISO; 10 μM) for 14 h, washed three times and then challenged with anti-IgE (0.3%) in the presence or absence of ISO (10 μM). This pretreatment did not affect control histamine release (HR) but did cause a reduction (mean 67±5% reduction, n=17) in the inhibition of HR by ISO. The ISO-induced functional desensitization was time-dependent (maximal by 12-14 h of incubation) and statistically significant ($p<0.05$) levels of desensitization were observed at ISO concentrations of 0.1 to 10 μM (n=4). Additional ß agonists, fenoterol, salbutamol and terbutaline all induced qualitatively similar functional desensitizations to that seen with ISO (n=4). The ß receptor anatagonist, propranolol (1 μM), if coincubated with ISO (10 μM), protected against the subsequent refractoriness of HLMC to ISO (n=4). The inhibitory activities of alternative adenylate cyclase activators, prostaglandin E_2 (30 μM) and forskolin (3 μM), which inhibited IgE-mediated HR from untreated HLMC by 60±8% and 55±9% respectively, were not affected by a prolonged (14 h) incubation treatment with ISO (10 μM; n=4).

These results indicate that prolonged exposure of HLMC to ß agonists induces a 'homologous' functional desensitization. Should similar processes be operative *in vivo*, as has been suggested by a recent clinical study (O'Connor *et al.*, 1992), then the use of high dose ß agonists in asthma may lead to a worsening of airways inflammation due to a diminished ability of ß agonists to inhibit the release of mediators from HLMC.

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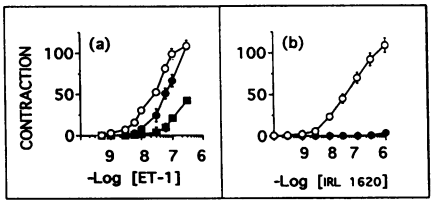
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236P ET_B RECEPTOR SUBTYPES MEDIATE THE CONTRACTION OF ISOLATED GUINEA-PIG UPPER BRONCHI

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In the guinea-pig airways *in vitro* and *in vivo*, endothelins produce bronchoconstriction by multiple mechanisms (see Battistini et al., 1992) but it is not clear which receptor subtypes mediate these effects. However, BQ-123 does not antagonize these effects, so they are not ET_A (Hay, 1992; Noguchi et al., 1993). Here, we have used as agonists ET-1 (ET_A/ET_B receptor non-selective) and IRL 1620 (ET_B receptor selective), and as antagonists BQ-123 and FR-139317 (both ET_A receptor selective), and PD 145065 (ET_A/ET_B non-selective; Doherty et al., 1993), to characterize the endothelin receptors in the guinea-pig upper bronchi (GPUB).

Male Dunkin-Hartley guinea-pigs (350-450 g) were killed by cervical dislocation and rapidly bled. The upper bronchi were excised, placed in Krebs-Henseleit buffer solution, trimmed of connective tissue and fat, and cut into spirals. The tissues were then mounted separately in 20 ml organ baths and connected to auxotonic force-displacement transducers under tensions of 1.5 g. Tissues were equilibrated for 1 hour (with washing every 15 min) in gassed (95% O_2: 5% CO_2) Krebs-Hensenleit solution (37°C, pH 7.4). Contractions are expressed as % of maximum histamine (5 μM) contraction. BQ-123 (10 μM), FR-139317 (10 μM) or PD 145065 (10, 100 μM) were added to the baths 20 min before ET-1 or IRL 1620.



ET-1 (n=8) or IRL 1620 (n=8) caused similar contractions of the GPUB (threshold, 10^{-9} M; contractions at 3 x 10^{-9} M: ET-1, 111.3±10.2%; IRL 1620, 108.9±8.6%). BQ-123 or FR 139317 did not attenuate ET-1-induced contractions (n=4-6). PD-145065 (10 μM; n=4) shifted to the right the ET-1 concentration-response (Fig.1a). PD 145065(100 μM; n=3) greatly attenuated contractions induced by ET-1 at 3 x 10^{-9} M (control, 111.3 ± 10.2%; + PD 145065, 42.7±0.3%). In the presence of PD 145065 (10 μM; n=6), IRL 1620 (up to 10^{-6} M) failed to induce contractions (Fig. 1b).

Fig. 1 (a) Contractions to ET-1; control (O); PD 145065 (10 μM; •); PD 145065 (100 μM;)
(b) Contractions to IRL 1620; control (O); PD 145065 (10 μM; •)

Thus, in the GPUB, ET-1 and IRL 1620 are equally active in producing contractions. BQ-123 and FR-139317 do not block ET-1 induced bronchoconstriction. Conversly, PD-145065 significantly attenuated contractions induced by ET-1 and totally blocked those to IRL 1620. Thus, in the GPUB, ET_B receptors mediate contraction.

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237P EFFECT OF ANTIASTHMA DRUGS ON MICROVASCULAR LEAKAGE INDUCED BY PLATELET ACTIVATING FACTOR IN RAT AIRWAYS

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PAF (Platelet Activating Factor) is a potential mediator in asthma: it induces bronchoconstriction and bronchial hyperresponsiveness, recruits inflammatory cells and elicits plasma exudation. The latter effect is probably the result of a direct action on the endothelial cells of airway postcapillary venules (Evans, et al., 1987). We studied the effect of dexamethasone, salbutamol, aminophylline, cromakalim, nedocromil, disodiumcromoglycate and phenydone on PAF-induced plasma exudation (Boschetto, et al., 1991) in the larynx, trachea and main bronchi of male Sprague Dawley rats (250-300 g). We used Evans blue dye (30 mg/kg i.v.) as a marker. At 5 min, C-16 PAF (Novabiochem) 500 ng/kg i.v. in urethane (1.25 g/kg i.p.) anesthetized rats (n = 26) had significantly increased Evans blue dye content in the various airway tracts: in larynx from 6.4 ± 0.6 ng dye/mg tissue in controls to 18 ± 2.6, in trachea from 13.5 ± 0.9 to 44.6 ± 2.5 and in main bronchi from 43.1 ± 2.6 to 106.9 ± 6.7 ng/mg. The activities of the aforementioned drugs on plasma exudation (n= 8-9 rats) are reported in the Table below:

DRUGS		% Reduction of Evans blue dye content		
	Pretreatment Time	Larynx	Trachea	Main Bronchi
Dexamethasone 7.5 mg/kg p.o.	7 h	71	61	56
Salbutamol 5 mg/kg p.o.	30 min	58	52	43
Aminophylline 100 mg/kg p.o.	2 h	11	13	12
Cromakalim 0.2 mg/kg i.v.	1 min	0	0	0
Nedocromil 5 mg/kg i.v.	1 min	0	21	0
Disodiumcromoglycate 100 mg/kg i.m.	2 h	0	10	0
Phenydone 100 mg/kg p.o.	1 h	0	0	0

Our results show that dexamethasone proved to be active also with an administration route (p.o.) and pretreatment time (7 h) different from those previously described (Boschetto, et al., 1991). Dexamethasone and salbutamol inhibited PAF-induced plasma exudation probably via direct actions on postcapillary venule endothelial cells as it was suggested by Boschetto, et al. (1991) and Persson, et al. (1982). Since our results show that the K^+ activator cromakalin and the inhibitor of cyclo and lipoxygenase phenidone are inactive, we would tend to exclude that the PAF-induced plasma exudation is sustained by both modulation by K^+ channels and an involvement of arachidonic acid metabolites. Aminophylline, nedocromil and disodiumcromoglycate resulted inactive in our experimental conditions. In conclusion, our data suggest that drugs acting on the endothelial cells of postcapillary venules, such as dexamethasone and salbutamol, can reduce microvascular leakage induced by PAF in rat airways.

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238P CORRELATION OF CYCLIC AMP ACCUMULATION AND RELAXANT ACTIONS OF SALMETEROL IN BOVINE TRACHEAL SMOOTH MUSCLE: COMPARISON WITH SALBUTAMOL

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Salmeterol (SALM) is a selective and long-acting β_2-adrenoceptor agonist developed for the treatment of bronchial asthma (Ball et al., 1991; Ullman & Svedmyr, 1988). Previous studies have suggested that SALM causes airway smooth muscle relaxation which is slow in onset and occurs with a lower efficacy, but greater potency compared to salbutamol (SALB) (Ball et al., 1991; Dougall et al., 1991). Here, we have examined the anti-spasmogenic properties of SALM and SALB, and also compared the cyclic AMP stimulatory actions with the ability of these agents to relax spasmogen-induced tone in bovine tracheal smooth muscle (BTSM) strips.

BTSM strips (10 x 2 x 2 mm) were suspended under resting tension in Krebs-Henseleit buffer at 37°C. Strips were pre-exposed to SALM (0.1-1000nM) or SALB (0.1-1000 nM) before constructing concentration-effect curves for either methacholine (MCh) or histamine (HIST). Neither β-adrenoceptor agonist inhibited tone induced by a maximally effective concentration of MCh (10 μM), but both shifted the concentration-effect curve to the right. At 100 nM, each agent caused an approximate 5-fold increase in EC_{50} (control, 127 (42-393); SALM, 672 (462-978); SALB, 714 (300-1688); mean (95% confidence limit) n=8). In contrast, SALM and SALB affected both the maximal response and shifted the HIST-induced contraction curves to the right: a maximally-effective concentration of SALM (100 nM) caused a 43.7 ± 8.0% (n=8) suppression of tone induced by 1 mM HIST, whilst SALB (100 nM) caused a greater inhibition (71.4 ± 8.8%).

The ability of SALM and SALB to relax MCh (500 nM)-induced tone was studied in parallel with experiments to assess changes in cyclic AMP levels. BTSM strips were exposed to β_2-adrenoceptor agonist for varying times and then rapidly frozen using a liquid nitrogen-cooled clamping device. Paired strips were processed for assay of cyclic AMP and results expressed as pmol/mg protein. An increase in cyclic AMP accumulation above basal levels (5.0 ± 0.4) was observed 10 min after SALM (100 nM) challenge (7.0 ± 0.3; $P<0.01$) and reached a maximum increase (8.8 ± 0.9) at 40 min. In contrast, SALB (1000 nM) caused a prompt increase in cyclic AMP which was maximal (12.6 ± 0.7) within 5 min of agonist addition. Consistent with these data, SALM caused a slowly developing relaxation of MCh-induced tone (maximal inhibition, 21.6 ± 4.3% at 45 min), whilst SALB caused a greater and more rapid relaxant effect (maximal inhibition, 79.6 ± 13.9% at 6 min).

We conclude that there is a close temporal correlation between the relaxant and cyclic AMP accumulation responses to SALM and SALB in BTSM. Furthermore, the anti-spasmogenic activity of SALB is similar to, or greater than, that of SALM. This is in contrast to the previously described studies on the spasmolytic activities of these agents in isolated guinea-pig trachealis (Ball et al., 1991).

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239P ANTIBRONCHOSPASTIC ACTIVITY OF RISPENZEPINE HCl, A NEW SELECTIVE ANTIMUSCARINIC COMPOUND

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The parasympathetic system plays an important role in the regulation of airway tone and reflex bronchoconstriction. Different subtypes of muscarinic receptors have been identified recently, suggesting that selective antimuscarinic compounds might have less side-effects with improved clinical usefulness. Indeed the classic non-selective antimuscarinic ipratropium Br (IB) is widely used in the treatment of bronchoconstriction in asthma and chronic bronchitis, even if its lack of selectivity might limit its effectiveness. Rispenzepine HCl (DF594.HCl) (±)11-(N-methyl-nipecotyl)-6,11-dihydro-5-oxo-5H-pyrido[2,3-*b*][1,5]benzodiazepine hydrochloride is a new antimuscarinic compound which preferentially interacts with M_1-M_3 muscarinic receptors. Therefore, we characterized its antibronchospastic activity in different experimental models, in comparison with IB.

The effects of DF594.HCl were evaluated either in conscious or anaesthetized guinea-pigs challenged with acetylcholine (Ach), measuring changes in the collapse time or in pulmonary inflation pressure, respectively. In some anaesthetized animals a cholinergic bronchoconstriction was also induced by electrical stimulation of the vagus nerves. Heart rate was monitored in some experiments through pulse pressure signal.
DF594.HCl administered orally displayed a potent antagonistic activity against Ach induced bronchoconstriction in conscious animals with a ID_{50} of 3.6 mg/kg. The ID_{50} calculated for IB was 59.2 mg/kg p.o.. In this model, DF594.HCl administered by aerosol retained a significant antibronchospastic activity, even if by this route the compound was 2.5 times less potent than IB (ID_{50}: 214.5 and 86.5 µg/ml x 5 min aerosol, for DF594.HCl and IB, respectively). In anaesthetized guinea-pigs, DF594.HCl dose dependently inhibited Ach induced bronchoconstriction (ID_{50} 59.7 µg/kg i.v.) with no significant effect on Ach induced bradycardia. IB was about 100 times more potent than DF594.HCl in this test (ID_{50} 0.5 µg/kg i.v.) but also significantly affected heart rate. In contrast to the results obtained against exogenous administered Ach, DF594.HCl showed an antibronchospastic efficacy similar to IB against vagally induced bronchoconstriction. In contrast, DF594.HCl only slightly reduced electrically stimulated bradycardia, whereas at equieffective antibronchospastic doses IB markedly affected heart rate.

These results indicate that DF594.HCl possesses a clear cut and selective antimuscarinic activity at the bronchopulmonary level. The effectiveness of this compound, as compared to IB, is particularly evident against vagally induced bronchoconstriction, a more physiological model where an important role in the final response is also played by ganglionic M_1 and presynaptic M_2 receptors. Therefore, our data clearly point out that an M_1-M_2 selective antimuscarinic compound might display a better pharmacological profile, being effective against a pathophysiological bronchoconstriction and being devoid of cardiac side-effects. These pharmacological characteristics outline the potential therapeutic usefulness of DF594.HCl as a bronchodilator in asthma and chronic bronchitis.

240P *IN VITRO* PHARMACOLOGICAL PROFILE OF RISPENZEPINE HCl, A NEW SELECTIVE ANTIMUSCARINIC COMPOUND

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Different muscarinic receptor subtypes have been identified and pharmacologically characterized. Within the respiratory system, ganglionic M_1 and smooth muscle M_3 receptors mediate the parasympathetic bronchoconstriction, whereas presynaptic M_2 receptors support a negative feed-back regulation of acetylcholine (Ach) release. Rispenzepine HCl (±)11-(N-methyl-nipecotyl)-6,11-dihydro-5-oxo-5H-pyrido [2,3-b][1,5] benzodiazepine hydrochloride (DF594.HCl), a new compound synthetized and developed by Dompe', has been selected from a series of pyridobenzodiazepinone derivatives and its pharmacological profile characterized in "in vitro" studies in comparison with the M_1-selective antimuscarinic Pirenzepine HCl (P).
Preliminary binding experiments revealed that DF594.HCl bound specifically to muscarinic receptors being devoid, even at a high concentration, of significant interaction with other non-muscarinic receptors. A potentiation of nitrendipine specific binding was however observed.The interaction of DF594.HCl with different muscarinic receptors was evaluated either in rat tissues or in membranes prepared from transfected CHO cells, expressing human m_1 to m_5 receptors, using binding techniques. In rat tissue (vs ^{3}H-QNB) homogenates DF594.HCl bound preferentially to M_1, M_3 receptors with a potency for M_1 subtype similar to that of P and a higher M_3 receptor interaction (Table). In cloned cells, (vs. ^{3}H-NMS) the affinity of DF594.HCl for the muscarinic receptors was comparable with that obtained in rat tissues, even if its selectivity was less pronounced. Unlike P, DF594.HCl was able to distinguish significantly between m_2 and m_3 subtype cloned receptors (Table). The antimuscarinic activity of DF594.HCl has been studied also in different guinea-pig isolated organs. DF594.HCl similarly reduced the contractions induced by Ach in tracheal and lung parenchymal strips. A good antimuscarinic effect of DF594.HCl was also displayed on Ach-induced contractions in guinea-pig ileum.

These data, indicate that DF594.HCl is a potent antimuscarinic compound characterized by a selective interaction with M_1, M_3 receptors. These properties candidate DF594.HCl as an antibronchospastic drug with potentially improved pharmacological profile.

	Ki (nM)							
	M_1 (cerebral cortex)	M_2 (heart)	M_3 (submandibular gland)	m_1	m_2	m_3 CHO cloned cells	m_4	m_5
Rispenzepine HCl	88.8±13.8	547±86	82±7	37.7±1.8	205.8±85	79.6±4.1	120.9±37.9	17.3±0.6
Pirenzepine HCl	96.3±9.2	1149±83	150±10	41.0±0.5	368.8±3	346.8±12	67.4±3.9	116±47

241P MEVALONATE INDUCED RECOVERY OF pH REGULATION IN SIMVASTATIN TREATED TRANSFORMED FIBROBLASTS IS DEPENDENT ON SULPHYDRYL GROUPS

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The Na^+/H^+ antiport is an important regulator of intracellular pH (pH_{in}) and volume, and is activated during cellular proliferation. Inhibition of HMG CoA reductase by simvastatin in human lymphoblasts is associated with inhibition of cell proliferation and pH_{in} regulation by the Na^+/H^+ antiport (Ng & Davies, 1991). The regulation of pH_{in} in an adherent cell line, the SV40 virus transformed MRC5 fibroblasts (MRC5 SV1 TG1), was investigated using microfluorometry with the fluorophore BCECF (2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein). Incubation of cells with 5 mg/l simvastatin for 24 h led to a fall in pH_{in} (from mean $\pm$ s.e.mean 7.21 $\pm$ 0.02 to 6.99 $\pm$ 0.02, $P<0.001$), inhibition of Na^+/H^+ antiport activity (from 37.6 $\pm$ 1.0 to 21.0 $\pm$ 0.6 mM/min, $P<0.001$) and rounding of cell shape. Incubation of simvastatin treated cells with 1 mM mevalonate led to reversal of the changes in cell shape, pH_{in} and Na^+/H^+ antiport activity within 2 h. This effect of mevalonate was not associated with a change in cellular cholesterol content. Mevalonate reversed these simvastatin induced changes in the presence of cycloheximide or tunicamycin, indicating that neither protein synthesis nor N-linked protein glycosylation was necessary for this effect of mevalonate. Since the activity of the Na^+/H^+ antiport was dependent on protein kinase C and calmodulin, the effects of staurosporine and W7 (N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide) were investigated. Neither inhibitor affected the mevalonate reversal of simvastatin induced changes in pH_{in} regulation, cell shape or Na^+/H^+ antiport activity.

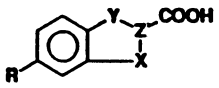
Mevalonate addition would lead to the synthesis of isoprenoids, some of which are associated with protein isoprenylation. This process occurs on cysteine residues. Addition of two recently described inhibitors of protein isoprenylation, namely limonene (5 mM) or farnesol (0.1 mM), did not prevent the reversal of simvastatin induced changes by mevalonate. However, treatment of simvastatin treated cells with 50 µM N-ethylmaleimide (NEM) completely prevented the mevalonate reversal of simvastatin induced changes in pH_{in}, Na^+/H^+ antiport activity and cell rounding. Concurrent treatment of cells with NEM and di-thiothreitol (1 mM) restored the mevalonate reversal of simvastatin induced changes. The effect of mevalonate on simvasatin induced pH_{in} regulation was therefore dependent on sulphydryl groups, and one possibility is protein isoprenylation on cysteine residues.

Ng, L. L. & Davies, J.E. (1991) Am. J. Physiol. 261 (Cell Physiol. 30), C780-C786

242P EFFECT OF NEWLY SYNTHESIZED RIGID CLOFIBRIC ACID DERIVATIVES ON CHLORIDE CHANNEL CONDUCTANCE OF RAT SKELETAL MUSCLE

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Recently, by using chiral analogues of clofibric acid, we have shown that the absolute configuration of the molecule strongly influences the chloride conductance (G_{Cl}) of mammalian skeletal muscle. We demonstrated that the S-(-) enantiomers of a series of 2-(p-chlorophenoxy) isobutyric acid (CPIB) chiral analogues produce a block of G_{Cl} (IC_{50} = 10-20µM), whereas their antipodes, at low concentrations (1-10µM), are able to increase it (De Luca *et al.*, 1992). In order to better evaluate the structure-activity relationship between these compounds and chloride channels, and in particular the importance of the distance between functional groups and the position of the substituents, we synthesized a new series of chiral and achiral CPIB analogues with rigid structure, and we resolved the chiral ones into corresponding enantiomers (Figure 1).



A) X=Z=C(sp^2)

1) Y= NH R= Cl
2) Y= O R= H

B) Z=C(sp^3), Y=O, R=Cl

3) X = $(CH_2)_2$
4) X = CH_2 - CO
5) X = CH_2

Figure 1

The effects of these derivatives on G_{Cl} and excitability characteristics of rat Extensor Digitorum Longus (EDL) muscle fibers were evaluated in vitro at 30°C by using the two intracellular microelectrode technique. Our results show that both compounds A1 and A2 produced a block of G_{Cl}. A1 at 20µM reduced G_{Cl} from a control value of 3448±280 µS/cm^2 (n=8) to 2703±289 µS/cm^2 (n=15). Similarly A2, at the same concentration, produced about a 25% reduction of G_{Cl}. For both compounds 20µM was almost the maximally effective concentration, since no further decrease of G_{Cl} was observed upon increase of concentration. Also, both compounds A1 and A2 did not alter the excitability characteristcs of EDL muscles. On the other hand, all of the derivatives of group B in the racemic forms and the enantiomers of compound B5, tested in the dose range of 1-300 µM, did not produce any significant effect on G_{Cl}. However, these compounds determined a reduction of the resting membrane potential, with an increase of the delay between onset of current pulse and initiation of action potential (latency) and a decrease of the firing capability of fibers. These changes, that were significant for the racemic mixture of compound B3 (10-233µM) and for the levo-rotatory antipode of compound B5 (10-46µM), might be due to the interaction of these compounds with channels other than chloride such as potassium and sodium. The homologue of the compound B3 having the carboxylic group in position 3 of the 4H-2,3-dihydro-1-benzopyran structure significantly decreased G_{Cl} by 20% at 20µM. A significant reduction of G_{Cl} (34%) was also observed with 200µM of the homologue with the carboxilic group in position 4. Our findings show that CPIB analogues with rigid structure have a low potency on G_{Cl} possibly for the remarkable influence of the distance between chlorine atom and carboxylic group, for optimal interaction with the stereospecific binding site.

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243P *IN VITRO* CARDIOVASCULAR EFFECTS OF TOXIN I FROM *DENDROASPIS POLYLEPIS POLYLEPIS*

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Dendrotoxins from green and black mambas have been shown to increase neuronal excitability and facilitate synaptic transmission by inhibition of voltage-dependent potassium currents. For example, the dendrotoxin homologue toxin I from black mamba *(Dendroaspis polylepis polylepis)* venom selectively inhibits a component of the fast-activating, slowly-inactivating potassium conductance at the frog node of Ranvier (Benoit & Dubois, 1986). We have examined the actions of toxin I in four cardiovascular preparations.

1. Rat hearts were perfused in the Langendorff mode at a constant pressure of 50mmHg. Left ventricular pressure (LVP) was monitored from an intraventricular balloon, from which left ventricular dP/dt and rate were determined. Coronary flow was measured by an electromagnetic flowmeter or by measuring minute volumes. Responses to infusions of 3μM toxin I were recorded. *2*. Isolated rat atria were suspended in Krebs, gassed with 95%O_2/5%CO_2 at 37°C at a tension of 0.5g. Left atrial preparations were paced at a rate of 5Hz with square wave pulses of 5V, 5msec duration. Right atrial rate was monitored with a Lectromed rate meter. Responses to 10μM toxin I were measured. *3*. 3mm ring preparations of sheep epicardial coronary artery were suspended between two stainless steel hooks in Krebs gassed with 95%O_2/5%CO_2 at 37°C at a tension determined from their length/tension relationship (Jamieson et al, 1988). Tissues were contracted with 10mM phenylephrine and when the tissue response had reached a plateau, the effect of a bolus dose or cumulative doses of toxin I were determined. *4*. Spirally cut sheep epicardial coronary artery was loaded with ^{86}Rb by incubating in 10μCi ml^{-1} ^{86}Rb in Krebs for two hours at 37°C. The tension was adjusted to 1g and changes in tension were recorded isometrically. The tissue was then superfused with Krebs at 37°C at 2ml min^{-1} and 30sec fractions of perfusate were collected. The contractile response to a 10mM infusion of phenylephrine was recorded and when the response had reached a plateau, the effect of a 3μM infusion of toxin I was determined. At the end of the experiment radioactivity in each fraction and in the tissue was determined by scintillation counting. A plot of desaturation and rate of Rb efflux was drawn.

In the perfused rat heart 3μM toxin I caused a 23.0±3.4% (n=5) reduction in flow with a concomitant drop in LVP of 26.4±5.3% and variable increase in ventricular rate (8.0±3.4%). 10μM toxin I produced a 22.0±4.5% (n=4) increase in right atrial rate and no effect on left atrial tension. 3μM toxin I had no effect on the tension of isolated sheep coronary artery rings. However, when added after 10mM phenylephrine, it produced a dose-related increase in tension which was half-maximal at 1μM. 3μM toxin I caused an increase in tension of 90.2±20.7% (n=19). 10mM phenylephrine caused an increase in tension in the superfused sheep coronary artery of approximately 0.5g which was potentiated by 3μM toxin I. Concomitantly with the increase in tension, phenylephrine caused an increase in the rate of Rb efflux. This effect was also potentiated by toxin I, which is contrary to the expected result. In conclusion, toxin I was shown to increase vascular smooth muscle tension and atrial rate and to have an effect on potassium transport.

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244P EFFECTS OF CHRONIC TREATMENT WITH HMG-CoA REDUCTASE INHIBITORS ON ELECTRICAL PARAMETERS AND EXCITATION-CONTRACTION COUPLING OF RAT SKELETAL MUSCLE FIBERS

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Clinical evidence indicates a potential for skeletal muscle toxicity after therapy with 3-hydroxy-3-methyl glutaryl coenzyme A (HMG CoA) reductase inhibitors (HMGRIs) (Smith *et al.*, 1991). However, 6 months treatment with pravastatin (0.1-20 mg/Kg daily) did not produce any alteration of electromyographic activity or chloride conductance (GCl) or other electrical parameters of rat skeletal muscle fibers (Pierno *et al.*, 1992). The hydrophilic nature of pravastatin could justify the lack of effect on skeletal muscle; indeed radioactivity measurements demonstrated that this drug is selectively taken up by the liver (Komai *et al.*, 1992). Then we evaluated the effects of a pravastatin treatment on rat skeletal muscle in comparison with those of simvastatin, a HMGRI with hydrophobic structure. Pravastatin (100 mg/Kg) and simvastatin (5, 10 and 50 mg/Kg) were administered daily for 2 months by an oesophageal cannula. During the treatment the electromyographic activity, recorded *in vivo* at regular intervals, did not reveal signs of myopathy in both pravastatin and simvastatin treated rats. At the end of the treatment the extensor digitorum longus (EDL) muscles were removed from both pravastatin and simvastatin treated rats and the ionic conductances and the excitability characteristics of sarcolemma were recorded *in vitro* at 30° C using a computerized two intracellular microelectrodes technique (Pierno *et al.*, 1992). The effect on the contractile properties was evaluated by studing the excitation-contraction coupling process analysed by measuring the mechanical threshold of the fibers, with the two microelectrode point voltage clamp method (De Luca & Conte Camerino, 1992). Our results showed a reduction of GCl in simvastatin treated rats with an incidence related to the dose; in particular at 50 mg/Kg a significant 18±3 % reduction of GCl was observed in 6 out of 7 rats with respect to control animals. However the potassium conductance (GK) did not change significantly. The rheobase voltage for contraction of EDL muscle fibers from simvastatin treated rats was shifted significantly toward more negative potentials with respect to the untreated fibers. On the other hand in 10 out of 10 pravastatin treated rats, we did not find any significant alteration of GCl; while a not significant increase of GK was observed. Both pravastatin and simvastatin did not alter significantly the excitability parameters and membrane resting potential. Our results suggest that simvastatin for its lipophilic structure penetrates sarcolemma more than pravastatin, thus altering muscle function. In particular simvastatin, similarly to other hypocholesterolaemic drugs which adversely cause a myotonic-like state, decreases GCl, a parameter important to stabilize muscle membrane (Conte Camerino & De Luca, 1989). The mechanism throught which simvastatin decreases GCl needs to be elucidated but alteration of membrane microviscosity could be a likely hypothesis.

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245P EFFECTS OF THE RING-COUPLING METABOLITE OF TYRAMINE, 2,2'-DIHYDROXY-5,5'-BIS(ETHYLAMINO)DIPHENYL (DITYRAMINE), ON RAT ANOCOCCYGEUS SMOOTH MUSCLE

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We have previously shown that rat intestinal peroxidase catalyses the oxidation of tyramine via a ring coupling reaction, forming the corresponding dimer, 2,2'-dihydroxy-5,5'-bis(ethylamino)diphenyl (dityramine) (Valoti *et al.*, 1992). The calculated Vmax was one order of magnitude greater than that of intestinal monoamino oxidase (MAO) while Km values were similar. This suggests that intestinal peroxidase might make a substantial contribution to tyramine metabolism but the implication of this metabolic pathway for biodisposition of tyramine is still unknown as are the properties of the resulting dimer.

The present work investigates the pharmacological activity of dityramine on the rat anococcygeus smooth muscle.

Dityramine was synthesized according to the method described by Gross and Sizer (1959). Individual anococcygeus muscles were mounted under 0.5 g tension in a organ bath chamber containing Krebs solution at 37° C. Tissues were placed between two platinum electrodes and contractions were stimulated using a biphasic pulse of 1 ms duration, at 10 Hz frequency and supramaximal voltage. Under these conditions dityramine inhibited the contractions promoted by field stimulation with a IC_{50} value of $1.4x10^{-5} \pm 0.05$ M (slope -1.2±0.16). The presence of desipramine ($1x10^{-8}$ M) or yohimbine ($1x10^{-8}$ M) in the perfusion solution left dityramine inhibition unaffected. Furthermore dityramine antagonized noradrenaline-induced contractions (EC_{50} $1.2x10^{-6}\pm 0.02$ M and $4.0x10^{-6} \pm 0.03$ M in absence and in presence of $1.4x10^{-5}$ M dityramine, respectively) in a competitive manner .

These preliminary findings indicate that peroxidase metabolism of tyramine leads to a derivative of diminished potency endowed with pharmacological properties opposite to those of the parent compound.

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246P INVESTIGATION INTO THE DIFFERENTIAL ANTAGONISM OF RELAXIN BY GLIBENCLAMIDE *IN VIVO* VS *IN VITRO* IN THE RAT UTERUS

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In the rat uterus glibenclamide (20 mg kg^{-1}) antagonised relaxin *in vivo*, causing a 19x rightward shift of the dose-response curve, suggesting an action of relaxin involving the opening of K_{ATP} (Downing & Hollingsworth, 1991). However, glibenclamide (10 μM) induced a much smaller antagonism of relaxin (2x) in the isolated rat uterus (Hughes *et al.*, 1992). *In vivo* rats had been ovariectomised 24 h previously and inhibition of spontaneous contractions was recorded, primarily from the circular muscle layer. *In vitro* tissues were taken from rats that were pretreated with oestrogen and inhibition of oxytocin-induced spasm of the longitudinal muscle layer was recorded. This study examined whether differences in hormonal pretreatment, spasmogen or muscle layer were responsible for the *in vivo / in vitro* difference in glibenclamide-induced antagonism of relaxin.
Segments of uteri taken from rats pretreated 24 h previously with oestradiol benzoate (100 μg kg^{-1}) [E] or 24 h post-ovariectomy [OVX] were mounted for isometric tension recording, using muscle cut in the longitudinal [LONG] or circular [CIRC] plane. Tissues were incubated in glibenclamide (10 μM) or vehicle for 30 min prior to the construction of a single, cumulative concentration-effect (C/E) curve to relaxin against the following spasms: oxytocin (0.2 nM)-induced [OT]; spontaneous [SPONT]; field-stimulated (4 s train every 60 s, pulse width of 1 ms, 40 V, 32 Hz) [FS]. pD_2 values were compared by paired or unpaired Student's *t* -test (Table 1).

Table 1 pD_2 values for relaxin-induced inhibition of uterine spasm. * P<0.01 (glibenclamide vs vehicle)

Hormonal state	E		OVX	
	vehicle	glibenclamide	vehicle	glibenclamide
CIRC + OT	8.71 ± 0.14	8.70 ± 0.06	(not tested)	
LONG + OT	8.42 ± 0.12	8.13 ± 0.13 *	(not tested)	
LONG + SPONT	(untestable)		9.23 ± 0.10	9.07 ± 0.14
LONG + FS	8.53 ± 0.05	8.37 ± 0.12	only 30% inhibition at 200 nM	

Relaxin was of similar potency in CIRC + OT, LONG + OT and LONG + FS preparations from E-treated rats. In tissues from OVX rats relaxin, in concentrations up to 200 nM, induced a maximum 30 % inhibition of spasm to FS while in tissue from E-treated rats, relaxin was potent vs spasm to FS. Thus, the action of relaxin vs FS appears to be oestrogen-dependent, as for relaxin against OT-induced spasm *in vivo* (Downing & Hollingsworth, 1992) . Glibenclamide produced a small antagonism of relaxin in the LONG + OT preparation but none in the E-treated CIRC + OT or LONG + FS, or in OVX LONG + SPONT preparations. Therefore the data suggests that the ability of glibenclamide to antagonise relaxin *in vivo* but not *in vitro* is not attributable to the altered hormonal conditions, spasmogen or muscle orientation but is attributable to differences in the actions of relaxin and/or glibenclamide.

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247P COMPARISON OF INDAPAMIDE, 5-OH INDAPAMIDE AND HYDROCHLOROTHIAZIDE IN ISOLATED SMALL VESSELS

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Indapamide (IND) is an atypical thiazide-like diuretic which appears to reduce blood pressure by a combined diuretic and direct vascular action (Campbell and Brackman, 1990). 5OH-Indapamide (5OH-IND) has been reported to be one of the principal metabolites of IND found in urine (Nishimura *et al*, 1982). This study examines the acute relaxation of IND, 5OH-IND and the thiazide diuretic hydrochlorothiazide (HTZ) in guinea pig mesenteric arteries. It also compares the effect of incubation with potassium channel blockers on the IND, 5OH-IND and HTZ induced relaxation.

Guinea pig mesenteric arteries (n=34, mean diameter 415±21 μm) were dissected free and mounted on a microvascular myograph (Mulvany & Halpern, 1977). Concentration relaxation curves were constructed for all three drugs following preconstriction with either noradrenaline (NA: 10μM) or potassium depolarising solution (KPSS: containing 118mM K^+). Agonist-induced relaxation curves were also constructed following NA preconstriction and preincubation with either the ATP-sensitive K^+ channel blocker glibenclamide (GLIB: 100μM), the large conductance Ca^{2+} activated K^+ channel blocker charybdotoxin (CHTX: 100nM) or vehicle control.

IND, 5OH-IND and HTZ all caused dose dependent relaxation of guinea pig mesenteric arteries. Preincubation with GLIB and CHTX had no effect on the subsequent contractions to noradrenaline. Table 1 shows the maximal relaxant effect of IND, 5OH-IND and HTZ on isolated vessels following NA and KPSS preconstriction and following incubation with potassium channel blockers.

Table 1: Comparison of IND, 5OH-IND and HTZ induced vasorelaxation

Constrictor	NA	KPSS	NA	NA	NA
Inhibitor	-	-	vehicle	GLIB	CHTX
IND	70±5	67±6	75±6	79±7	73±8
5OH-IND	82±4	6±3*	87±7	41±4**	7±3**
HTZ	74±5	8±5*	79±3	78±6	7±2**

All values are mean percentage relaxations ± S.E.of a maximal (30μM) concentration of agonist. * Relaxations of NA and KPSS constricted vessels are significantly different ($P<0.01$). ** Relaxation significantly different from control ($P<0.01$).

There are differences between the acute relaxation produced by IND and that of 5OH-IND and HTZ. From these experiments we conclude: (1) In these vessels IND-induced relaxation is not mediated by an effect on potassium channels (2) ATP-sensitive K^+ channels appear to be involved in the acute relaxation of 5OH-IND but not HTZ (3) Both 5OH-IND and HTZ induced relaxation are mediated via an effect on Ca^{2+} activated K^+ channels.

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248P DOSE-RELATED RESPONSES TO VASOACTIVE AGENTS IN THE HEPATIC ARTERY AND PORTAL VEIN OF THE ISOLATED DUAL-PERFUSED RABBIT LIVER: EFFECTS OF HYPOXIA

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We have previously demonstrated the presence of cholinergic and purinergic vasodilator receptors in the isolated dual-perfused rabbit liver preparation (Mathie et al 1991, Ralevic et al 1991). These may be important in the regulation of hepatic blood flow and may be involved in the hepatic (HA) hyperaemic 'buffer' response to reductions in portal venous (PV) flow. It has been postulated that hypoxia may be a driving stimulus for the induction of the buffer response. This study was conducted to investigate the effect of hypoxia upon HA and PV responses to the endothelium-mediated vasodilators acetylcholine (ACh) and ATP, and to the direct smooth muscle vasodilators adenosine and sodium nitroprusside (SNP).

The circuit details and additional methodology have been provided elsewhere (Alexander et al 1992). Briefly, 9 livers (95.1 ± 5.5g) from New Zealand White rabbits (2.5-3.5 kg) were perfused with Krebs-Bullbring buffer, oxygenated with 95% O_2 / 5% CO_2 at 37°C through the HA and PV at flow rates of 25 and 75 ml min^{-1} 100g $liver^{-1}$ respectively. This generated perfusion pressures of 166 ± 15 and 3.7 ± 0.6 mmHg following preconstriction with 10^{-6} M noradrenaline. Following an equilibration period of 10-15 minutes, vascular responses to intra-arterial (n=5) and intra-portal (n=4) injections of vasodilators, measured as a transient reduction in perfusion pressure, were obtained. Hypoxia was induced by changing the gas mixture in the perfusate to 95%N_2 / 5% CO_2. Further dose-response curves were then obtained.

Perfusate pO_2 decreased from 503 ± 17 to 48 ± 6.3* mmHg after 2 hours of hypoxic perfusion and was accompanied by a reduction in hepatic oxygen uptake from 0.89 ± 0.05 to 0.06 ± 0.02** ml O_2 min^{-1} 100g $liver^{-1}$. Bile volume production was also decreased from 7.6 ± 1.9 to 4.5 ±1.5* ml h^{-1}. Perfusate pCO_2 and pH remained steady at 30 ± 1 mmHg and 7.30 ± 0.03. No significant differences were measured in ED_{50} values between oxygenated and hypoxic HA responses to HA injections of ATP, adenosine, ACh and SNP although significant attenuation of maximal responses was observed to all substances except ATP*. Paradoxically, ACh and ATP elicited a predominant vasoconstriction in the PV. During hypoxia PV vasodilatations to HA injections of SNP were reduced at the highest dose used (10^{-6}moles)*,while PV vasoconstriction to repeated doses of 3 x 10^{-6}ATP increased after 1 hour of hypoxia*. PV responses to 3 x 10^{-9} moles ACh were also potentiated after the onset of hypoxia* but returned to normoxic values after 2 hours. HA responses to PV injections of these vasodilators gave similar responses. These changes suggest attenuation of vasodilator action during hypoxic perfusion, but aging of the preparation may also be a contributory factor and further studies will report upon this. (*$P< 0.05$,**$P<0.01$,Student's paired t-test. All results show are mean ± s.e.)

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249P DIFFERENTIAL REGULATION OF RAT GASTRIC PEPSINOGEN SECRETION FOLLOWING ENDOGENOUS OR EXOGENOUS ACTIVATION OF MUSCARINIC RECEPTORS

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Vagal cholinergic pathways are thought to stimulate gastric pepsinogen secretion through the direct activation of muscarinic receptors located on chief cells (Hersey, 1987). In addition, luminal acid enhances pepsinogen output indirectly via local cholinergic reflexes (Johnson, 1972). In the present study, an attempt was made to characterize the relative functional importance of both direct and indirect cholinergic control of pepsinogen secretion. Experiments were carried out on anaesthetised male Wistar rats (200 g body weight, 24-h fasted) undergoing continuous perfusion of gastric lumen with 154 mM NaCl and bilateral cervical vagotomy. Both pepsinogen levels (hydrolysis of bovine haemoglobin assay) and acid output (titration to pH 7 with 0.01 N NaOH) were determined in 15-min perfusate fractions. In some animals gastric secretion was evoked by electrical stimulation of the distal end of the left vagus nerve (5 Hz, 0.5 ms, 10 V, 150 min). Omeprazole (30 mg/kg i.p., 90 min before the beginning of experiments) was used to suppress gastric acid output. Significance of differences was evaluated by Student's t test for unpaired data. Vagal stimulation increased both basal pepsinogen output (from 85±17.6 to 434.2±35 μg of pepsin/15 min; $p<0.001$) and acid secretion (from 3.9±1.12 to 25.6±2.5 μEqH^+/15 min; $p<0.001$), these effects being fully prevented by pirenzepine (10 mg/kg i.v.) or omeprazole. In omeprazole-pretreated animals, acid perfusion (0.01 N HCl) of gastric lumen failed to affect basal pepsinogen secretion; however, vagal stimulation in the presence of acid perfusion could increase pepsinogen output (from 88.9±19 to 443±23.2 μg of pepsin/15 min; $p<0.001$), this effect being antagonised by pirenzepine. Bethanechol (0.3, 1 and 3 mg/kg i.v.) stimulated both basal pepsinogen and acid secretions, the maximal effects occurring with 1 mg/kg (from 78±16.3 to 491.9±33.5 μg of pepsin/15 min; $p<0.001$ and from 3.7±0.98 to 26.2±1.4 μEqH^+/15 min; $p<0.001$). Pirenzepine antagonised bethanechol-induced peptic and acid output, whereas omeprazole pretreatment only partially reduced pepsinogen secretion with a decrease in the peak value of 40.2% while abolishing the acid response. The present results suggest that acid secretion plays a major role in driving the excitatory control exerted by endogenous cholinergic pathways on pepsinogen secretion, whereas exogenous agonists can directly stimulate chief cell muscarinic receptors to promote pepsinogen secretion.

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250P THE SELECTIVE TACHYKININ NK_2 RECEPTOR ANTAGONIST CYCLO(MetTrpPheDapLeu) (MEN10,627) INHIBITS STIMULATED INTESTINAL TRANSIT IN RATS

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MEN10,627 or cyclo(MetAspTrpPheDapLeu), a competitive and selective antagonist of the tachykinin NK_2 receptors, was studied "in vivo" on intestinal transit stimulated by different agents. Male CD rats, 220-250 g were used. Fasted animals were injected with MEN10,627 (10-100 μg/kg i.v.) 5 min before [βAla^8]NKA(4-10) (15 μg/kg i.p.), a selective NK_2 receptor agonist, carbachol (10 μg/kg i.p.) or saline. In other experiments reserpine was administered in a dose (5 mg/kg i.p., 24 before) which produced a comparable increase of intestinal transit. The charcoal meal was given immediately after the stimulant and the animals were killed 15 min later. Results were expressed as percent of total length of intestine covered by charcoal front. MEN10,627 inhibited in a dose-dependent manner [βAla^8]NKA(4-10)-induced increase in intestinal transit without affecting the unstimulated transit or that induced by carbachol or reserpine (Tab. 1). As shown in Tab. 1, [βAla^8]NKA(4-10)-induced increase in intestinal transit was unaffected by pretreatment with atropine (100 μg/kg s.c.).

Treatment and dose:	intestinal transit (%)
saline + saline	59±2
saline + [βAla^8]NKA-(4-10) 15 μg/kg i.p.	71±2 *
MEN10,627 50 μg/kg i.v. + [βAla^8]NKA-(4-10) 15 μg/kg i.p.	66±2 *
MEN10,627 100 μg/kg i.v. + [βAla^8]NKA-(4-10) 15 μg/kg i.p.	58±2
atropine 100 μg/kg s.c. + [βAla^8]NKA-(4-10) 15 μg/kg i.p.	68±1 *
saline + carbachol 15 μg/kg i.p.	75±4 *
MEN10,627 100 μg/kg i.v. + carbachol 10 μg/kg i.p.	73±3 *
atropine 100 μg/kg s.c. + carbachol 10 μg/kg i.p.	59±3
saline + reserpine 5 mg/kg i.p.	83±3 **
MEN10,627 100 μg/kg i.v. + reserpine 5 mg/kg i.p.	83±3 **

Table 1: Effect of MEN10,627 or atropine on intestinal transit stimulated by carbachol, [βAla^8]NKA-(4-10) or reserpine. * and ** = $P < 0.05$ and 0.01 as compared to saline+saline group. n = 8-10 for each group.

The same doses of [βAla^8]NKA(4-10) or MEN10,627 used to stimulate intestinal transit or to block it, respectively, were unable to produce a significant change in gastric transit assessed by phenol red method (Taché et al., 1987).
These results show that MEN10,627 selectively inhibits stimulated intestinal transit and indicate MEN10,627 as a valuable tool to assess the role of NK_2 receptor in intestinal motility.

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251P EFFECTS OF 2-*t*-BUTYL-4-METHOXYPHENOL (BHA) ON PLASMALEMMA AND INTRACELLULAR Ca^{2+} TRANSPORT IN GUINEA PIG GASTRIC FUNDUS SMOOTH MUSCLE

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We have previously described the dual activity of certain hindered phenols, namely their calcium antagonist and antiperoxidant properties (Sgaragli *et al.*, 1993). This study attempts to clarify the calcium antagonist action of the lead model compound exhibiting this bifunctionality, 2-*t*-butyl-4-methoxyphenol (BHA). In circular smooth muscle strips isolated from the gastric fundus of the guinea pig, cyclopiazonic acid (CPA), a specific inhibitor of sarcoplasmic reticulum ATPase, induces a prolonged rise in tension (1.330±0.162 g, n=17) in relation to concentration of extracellular Ca^{2+}. Nifedipine antagonized CPA-promoted contraction (-0.027±0.086, n=6), as well as BHA; in addition, BHA also depressed spontaneous tone (pIC_{50}=5.83±0.11, n=8). Tetrodotoxin did not change the response to CPA and BHA. Contrary to the action of nifedipine, Bay K 8644 did not reverse BHA-induced relaxation. Thus another pathway for BHA must be posited as no direct blocking of the L-type Ca^{2+} channels was shown. Secondary evidence that BHA by-passes the Ca^{2+} channels is afforded by its behaviour in the presence of Ba^{2+} and tetraethylammonium (TEA) where these compounds still elicited nifedipine-sensitive phasic contractions. It may be surmised therefore that BHA impedes Ca^{2+} influx via a voltage-mediated process. In fact, compounds such as cromakalim and SIN-1 and sodium nitroprussiate (SNP), activating, respectively, the ATP-dependent K^+-outward current or the late outward current (Cook *et al.*, 1988) exhibit similar effects. Further confirmation of a voltage-mediated process may be seen when smooth muscle strips preincubated with BHA, SIN-1 or SNP fail to contract despite the addition of CPA (0.311±0.096 g, 0.196±0.080 and 0.297±0.063, respectively, n=6, P<0.001 versus CPA alone). In the absence of CPA, TEA reverses the reduction in spontaneous tone occasioned by BHA, while the latter concomitantly suppresses TEA induction of phasic and tonic contractions. Argument against Cl^- channel involvement comes from the finding that 9-anthracene-carboxylic acid - a Cl^- channel blocking drug (Hille, 1984) - left BHA activity unchanged. An indirect evidence for the specificity of these effects comes from the finding that 2,6-di-*t*-butyl-4-methoxyphenol (di-*t*-BHA) did not antagonize CPA-promoted contraction. On the contrary it increased the spontaneous tone of the preparation. To conclude, in accordance with the capacitative Ca^{2+} entry model proposed by Putney (1990) and the observations of Zholos (1991), this study attributes the antispasmogenic effect of BHA on smooth gastric muscle to the activation of K^+ channels and not to a direct blocking of the Ca^{2+} channels.

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252P *IN VITRO* CHARACTERIZATION OF THE NOVEL SELECTIVE NON-PEPTIDE ANTAGONISTS OF TACHYKININ NK_1 AND NK_2 RECEPTORS SR 140333 AND SR 48968

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We investigated the potent non-peptide tachykinin antagonists SR 140333, (S)-1-{2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidin-n-3-yl]ethyl}-4-phenyl-1-azoniabicyclo[2,2,2,]octane,chloride and SR 48968, (S)-N-methyl-N[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl)butyl]benzamide, (Emonds-Alt et al., 1992, 1993) for their ability to relax isolated tissues contracted by substance P (SP), or neurokinin A (NKA), both non-selective agonists, or [Sar^9,$Met(O_2)^{11}$] SP, and [ßAla^8]-NKA, selective for NK_1 and NK_2-receptors respectively. Ileum (GI) from male Crl:(HA)SPF guinea-pigs (300-400 g), duodenum (RD) and esophagus tunica muscularis mucosae (RE) from male Crl:CD®BR rats (225 -250 g) were carefully dissected and mounted in 20 ml organ baths containing warm (32°C, RD or 37°C RE, GI) oxygenated (95% O_2, 5% CO_2) Krebs (GI, RE) or Tyrode (RD) solution plus atropine ($3 \cdot 10^{-6}$M). Tissues were stretched with 2 g (RD) or 1 g (RE, GI), and contractions elicited by cumulative application of tachykinin agonists were recorded isotonically as a function of: histamine (10^{-5}M) maximal effect in GI, their own maximum in RD and RE. Tachykinin agonists showed different potencies (EC_{50}, nM) depending on the tissue: RD, NKA (5) = [ßAla^8]-NKA (5) > SP (50) > [Sar^9,$Met(O_2)^{11}$]-SP (>100); RE: NKA (16) > [ßAla^8]-NKA (23) > SP (>100), [Sar^9,$Met(O_2)^{11}$]-SP (>100); GI, [Sar^9,$Met(O_2)^{11}$]-SP (2) = SP (2) > NKA (16) > [ß-Ala^8]-NKA, (>100). The antagonists were preincubated for 2 h before agonists were added. In GI (containing mainly NK_1-receptors), SR 140333 induced a pseudoirreversible blockade (Kenakin, 1987) of both SP and [Sar^9,$Met(O_2)^{11}$]-SP effects (K_B = 0.03 and 0.01 nM), but did not antagonize (up to 300 nM) the effective tachykinin agonists in RD and RE. In RD (containing mainly NK_2-receptors), SR 48968 shifted the concentration-response lines of both NKA and [ßAla^8]-NKA parallel to the right (pA_2 ~ 9.5 and 9.4 ± 0.06 s.e. mean, slope 1 ± 0.07 s.e. mean). In RE, it non-competitively antagonized [ßAla^8]-NKA or NKA, but not (up to 300 nM) [Sar^9,$Met(O_2)^{11}$]-SP in GI . Neither antagonists had any intrinsic agonist activity up to 1000 nM. 100 nM SR 140603 or SR 48965, the (R)-enantiomers of SR 140333 and SR 48968, did not antagonize the contractions induced by the tachykinin agonists, [Sar^9,$Met(O_2)^{11}$]-SP and [ß-Ala^8]-NKA, on GI and RD respectively. In GI, SR 140333 (0.3 nM) but not SR 48968 (100 nM) completely antagonized contractions induced by capsaicin ($2 \cdot 10^{-6}$M). These results support the selectivity of SR 48968 (NK_2)and of SR 140333 (NK_1) as potent tachykinin antagonists and suggest that RE might contain a NK_2-receptor subtype different from that of RD.

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253P CYCLO(MetTrpPheDapLeu) (MEN10,627): *IN VIVO* EVIDENCE FOR A SELECTIVE ANTAGONISM OF NK_2 RECEPTOR-MEDIATED RESPONSES IN GUINEA-PIG AIRWAYS

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Tachykinins released from sensory nerves exert powerful bronchomotor effects through stimulation of specific receptors located on airways smooth muscles. In particular, tachykinin NK_2 receptors have been described in human bronchial preparation. In view of the above MEN10,627 or cyclo(MetAspTrpPheDapLeu), a selective antagonist of the NK_2 receptors, was studied "in vivo" in urethane (17.5 mmol/kg i.p.)-anaesthetized guinea-pig. Bronchospasm induced by i.v. acetylcholine, capsaicin or selective tachykinin receptor agonists ([Sar^9]SP sulfone or [βAla^8]NKA(4-10) (for NK_1 and NK_2 receptors, respectively) was studied by the method of Konzett & Rossler (1940). MEN10,627 (0.1 μmol/kg i.v.), selectively inhibited NK_2 receptors mediated bronchoconstriction (1.60±0.48 vs 0.24±0.11 kPa, $P<0.01$, n=6) while (±)-CP96,345 (3 μmol/kg i.v.) markedly decreased that induced by [Sar^9]SP sulfone (1.00±0.14 vs 0.11±0.05 kPa, $P<0.01$, n=4). These findings show that both NK_1 and NK_2 receptors mediate bronchoconstriction in guinea-pig airways and that they are independently activated. Capsaicin-induced bronchospasm was inhibited by 1.5 μmol/kg i.v. of atropine (0.88±0.03 vs 0.33±0.03 kPa, $P<0.01$, n=4) or 1 μmol/kg i.v. of MEN10,627 (1.15±0.22 vs 0.51±0.06 kPa, $P<0.01$, n=4), but unaffected by (±)-CP96,345 (3 μmol/kg i.v.). The non cholinergic-response induced by the bilateral electrical stimulation of the vagi (20 Hz, 10 V, 1 msec) after pretreatment with atropine (1.5 μmol/kg i.v.), was reduced by 0.1 μmol/kg i.v. of MEN10,627 (1.80±0.30 vs 1.13±0.20 kPa, $P<0.05$, n=6), 1 μmol/kg i.v. of MEN10,627 (1.90±0.29 vs 0.79±0.16 kPa, $P<0.01$, n=4) or 3 μmol/kg i.v. of (±)-CP96,345 (0.79±0.13 vs 0.37±0.01 kPa, $P<0.05$, n=4), suggesting that activation of both NK_1 and NK_2 receptors participate in this response.
In summary, in anaesthetized guinea pig i.v. capsaicin induced a bronchospastic response through activation of postjunctional NK_2 (but not NK_1) receptors and cholinergic pathways. MEN10,627 potently inhibits the non-cholinergic component of the bronchospasm induced by capsaicin and vagal stimulation and the specific activation of airways NK_2 receptors.

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254P THE SELECTIVE NON-PEPTIDE NK_2-TACHYKININ ANTAGONIST SR 48968 PREVENTS [β-Ala^8]NKA-INDUCED RAT COLONIC CONTRACTIONS

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SR 48968, (S)-N-methyl-N[4-(4-acetylamino-4-phenyl piperidine)-2-(3,4-dichlorophenyl)butyl]benzamide, a competitive and selective antagonist of the neurokinin A (NK_2) receptors (Emonds-Alt *et al.*, 1992), potently and dose-dependently prevented rat defecation stimulated by the NKA agonist [ß-Ala^8]NKA (Croci *et al.*, 1992). In the present study we used a manometric technique to monitor the effects of [ß-Ala^8]NKA on colonic motility in unanesthetized 24 h fasted rats and the ability of SR 48968 to prevent them. Male Crl:CD®BR rats, 220-250 g, were chronically implanted with open-tip catheters (1 mm i.d.) in the lumen of the colon (6 and 11 cm from the ileo-cecal junction) that were perfused with bidistilled water at a flow rate of 0.03 ml/min and connected to transducers for monitoring pressure waves. Rats (five per dose) were given [ß-Ala^8]NKA (80 $\mu gkg^{-1}h^{-1}$, 60 min iv infusion) either 30 min or 18 h after sc SR 48968 or saline. Recordings started 2 h before [ß-Ala^8]NKA and lasted 240 min. [ß-Ala^8]NKA dramatically increased the overall area under the pressure waves (assessed by a computerized system) and frequency of given waves selected according to their amplitude (area: 300% of pre-infusion values; waves: > 15 mmHg, 800%, < 15 mmHg, 30%). SR 48968 (0.5 to 250 μgkg^{-1}) had no action on its own, but when given 30 min before [ß-Ala^8]NKA it reduced its dramatic effects (most probably involving NK_1 receptors as well), which were still prevented to some extent 18 h after the higher doses (10 to 250 μgkg^{-1}) of SR 48968. SR 48968 thus appears to be a potent and long-acting antagonist at rat colonic NK_2 receptors, which are important in mediating large intestine motility whose propulsive nature is suggested by the defecation promoting action of [ß-Ala^8]NKA (Croci *et al.*, 1992).

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255P LIPOPOLYSACCARIDE INHIBITS BASAL AND INTERLEUKIN-1-β–INDUCED ACTH SECRETION IN RAT ANTERIOR PITUITARY CELLS *IN VITRO*

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Bacterial endotoxin or lipopolysaccharide (LPS), derived from lysed bacteria, plays a central role in septic shock. It stimulates monocyte production of interleukin-1 (IL-1), which in turn triggers the acute-phase responses (Kushner, 1982). It has been shown that both LPS and IL-1 are able to activate the hypothalamo-pituitary-adrenal axis, whose secretory products play a pivotal role in the stress responses as well as a modulatory role in the immune responses (Rivier, 1990).
There are controversial data on IL-1 directly stimulating pituitary ACTH secretion (Lumpkin, 1987).
We have tested the activity of LPS on adrenocorticotropic hormone (ACTH) secretion from rat pituitary cells *in vitro*, both basally and after IL-1β stimulation. Anterior pituitaries were obtained from adult male Wistar rats (200 - 250 g.); animals were killed by decapitation around 10 a. m. and the tissue explants placed in a sterile incubation medium. Cell dispersion was done according to Denef *et al.* (1978). Cell harvest was 1.8 - 2.0 x 10^{-6} cells per pituitary. The pituitary cells were plated into plastic 24 - multiwells (approx. 300,000 cells/well) with 1 ml DMEM + 10% newborn calf serum and incubated for 2 - 3 days at 37° C in atmosphere CO_2/air 5/95%; ACTH release was evaluated by RIA and is expressed in ng/ml ± s. e. mean. In short term (1 hour) incubations, LPS, in the dose range 1 - 100 ng/ml, inhibited ACTH release [controls: 2.6 ± 0.15; LPS (1 ng/ml): 2.41 ± 0.16; LPS (10 ng/ml): 2.06 ± 0.19; LPS (100 ng/ml): 1.61 ± 0.15*; (*P<0.05 vs. controls)] while IL-1β significantly stimulated ACTH release [controls: 2.27 ± 0.08; IL-1β (10^{-12} M): 2.94 ± 0.13*; IL-1β (10^{-11} M): 3.13 ± 0.14*; IL-1β (10^{-10} M): 3.30 ± 0.17*; (*P<0.05 vs. controls)]. After 24 hour preincubation with LPS 1 μg/ml, IL-1β-induced ACTH stimulation was lost, while CRH-induced ACTH release was preserved [controls: 1.64 ± 0.07; IL-1β (10^{-12} M): 1.48 ± 0.07; IL-1β (10^{-11} M): 1.66 ± 0.09; IL-1β (10^{-10}M): 1.93 ± 0.11; CRH (10^{-9} M): 3.9 ± 0.39*; (*P<0.05 vs. controls)].

LPS inhibition of basal and IL-1β-induced ACTH release could be responsible for a blunting of the adrenal cortex response that normally occurs in stress states and this could be of relevance in septic shock outcome.

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256P PHARMACOLOGICAL EVALUATION OF THE RECEPTORS MEDIATING THE ACTION OF 5-HT IN THE GUINEA-PIG ISOLATED URINARY BLADDER

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Several studies have reported direct and indirect (nerve-mediated) stimulatory effects of serotonin (5-HT) on detrusor muscle strips in a number of mammals, including humans (Cohen, 1989). Although the guinea-pig urinary bladder is considered resistant to this agent (Cohen & Drey, 1989), we found that isolated detrusor muscle strips from this species were contracted by 5-HT. The present study was designed to investigate the receptor types involved in these 5-HT-mediated contractile responses. Detrusor muscle strips were set up isometrically (tension 0.5 g), in standard Tyrode solution and gassed with 95% O_2 and 5% CO_2 at 37° C. 5-HT (0.1-100 μM) caused an excitatory response consisting mainly in repeated phasic contractions with slight increase in basal tone at the highest concentrations. The EC_{50} for the contractile response was 1.2 ± 0.06 μM. In the presence of tetrodotoxin (0.6 μM) or hyoscine (1 μM), the 5-HT curve was shifted to the right by the same extent (EC_{50}: 8.0 ± 0.9 μM and 8.4 ± 1.9 μM, respectively). This suggests that part of 5-HT-mediated contraction is neurogenic and cholinergic in nature.
Receptors associated with cholinergic nerve stimulation belong, at least in the guinea-pig intestine, to two separate classes (5-HT_3 and 5-HT_4). The 5-HT_4 receptor antagonist DAU 6285 decreased by 50% the sensitivity of detrusor muscle to 5-HT (EC_{50}: 2.6 ± 0.5) at a concentration (1 μM) ten-fold greater than its K_B value at 5-HT_4 receptors (Tonini *et al.*, 1992). The 5-HT_3 receptor antagonist ondansetron (1 μM) was ineffective. By contrast, DAU 6215 (1 μM), another potent 5-HT_3 receptor antagonist (pKi: 8.42) (Turconi *et al.*, 1991) with additional antimuscarinic activity (Baxter & Clarke, 1992) showed moderate antagonism. The resulting EC_{50} value was 4.4 ± 0.7 μM.
In the continuous presence of 1 μM tetrodotoxin, the 5-HT_2 receptor antagonist ketanserin (3, 10 nM) caused a parallel shift to the right of 5-HT curve with an apparent single point pA_2 value of 8.7.
In summary, two 5-HT receptor subtypes appear to be present in the guinea-pig detrusor muscle. Post-junctional 5-HT_2 receptors mediate a direct response to the muscle, while pre-junctional 5-HT receptors different from the 5-HT_3 or 5-HT_4 subtypes are associated with an indirect cholinergic response.

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257P IS THERE A ROLE FOR 5-HT IN MEDIATING URETHRAL RELAXATION?

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Urethral relaxation has been shown to be a non-adrenergic, non-cholinergic (NANC) phenomenon (Klarskov *et al.*, 1983). Recently, data has been published implicating nitric oxide as the NANC transmitter (Persson & Andersson, 1992). However, we have demonstrated the existence of another NANC inhibitory innervation. Since 5-HT can produce urethral relaxation we have attempted to elucidate whether it has a role in mediating this non-nitrergic, NANC response.

Strips of circular smooth muscle 1x1x8 mm were dissected from urethras of female pigs killed at an abattoir. The strips were mounted under a 1g loading tension in 0.2 ml organ baths continually perfused with warmed Krebs' solution (35-37°C). Tension was measured isometrically. 5-HT produced concentration-dependent relaxations of the urethral smooth muscle, an effect maximal at 30μM (data from 4 pigs (N) and 18 strips (n)) with an estimated EC_{50} value of 1μM. Field stimulation of intrinsic nerves over a range of frequencies (1-60 Hz), using a 5 second train of pulses (0.2 ms pulse width, 20V) produced an initial phasic relaxation due to nitric oxide followed by an adrenergic, cholinergic mediated contraction and then a final more prolonged relaxation. In order to examine the latter phase in isolation the preparations were perfused in Krebs' solution containing N-nitro-L-arginine (10μM), atropine (1μM) and guanethidine (3μM) to remove the nitrergic, cholinergic and adrenergic components respectively. Antagonists selective for $5\text{-}HT_{1\text{-}4}$ receptors were utilised in an attempt to attenuate the relaxation response to nerve stimulation. They were also used to try and identify the receptor sub-type through which applied 5-HT mediates its inhibition. The $5\text{-}HT_1$ receptor antagonist metitepine (10-100nM) produced a rightward shift in the concentration response curve to 5-HT (pA_2 =8.1), but did not inhibit the nerve mediated response. The $5\text{-}HT_{2\&3}$ receptor antagonists ketanserin and MDL72222 (10μM) were also ineffective in inhibiting the relaxation responses to nerve stimulation or applied 5-HT. However, the $5\text{-}HT_{3/4}$ antagonist tropisetron (ICS 205-930), at a concentration (10μM) effective on $5\text{-}HT_4$ receptors, produced a rightward shift in the frequency response curve but with a depression of maximum (N=4;n=24) and produced a similar shift in the concentration response curve to 5-HT (16.9±4.7% reduction in response to EC_{50}). The $5\text{-}HT_4$ selective antagonist SDZ 205-557 (10-100nM) however, produced no significant shift in the response curve to either nerve stimulation (N=4;n=18) or 5-HT (N=2;n=9) suggesting that the effect observed with tropisetron was most likely due to a non-specific action given the high concentration used.

In conclusion, it has been shown that 5-HT can mimic the response to electrical field stimulation in producing a marked relaxation of the urethral smooth muscle. The receptor by which it mediates this effect would appear to be of the $5\text{-}HT_1$ subtype. However, nerve mediated relaxations were unaffected by any of the 5-HT antagonists. Thus it may be concluded that although 5-HT receptors are present on urethral smooth muscle, 5-HT would not appear to be the transmitter responsible for mediating the nerve evoked relaxation in this tissue.

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258P N-ETHYLMALEIMIDE EFFECTS ON ADENOSINE RECEPTOR FUNCTION IN THE GUINEA-PIG CEREBRAL CORTEX

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In intact rat hippocampal slices, pretreatment with N-ethylmaleimide (NEM) has a selective effect on certain adenosine receptor responses. Thus NEM pretreatment blocks A_1 receptor inhibition of L-glutamate release (Fredholm *et al.*, 1986), and A_1 receptor inhibition of forskolin-stimulated cAMP generation, but fails to alter the A_{2B} adenosine receptor-stimulated cAMP generation (Fredholm *et al.*, 1985). In this report, we have investigated the influence of NEM on the activity of adenosine receptors in guinea-pig cerebral cortical slices.

Assays for cAMP generation and phosphoinositide turnover were carried out on at least three separate occasions as previously described (Alexander *et al.*, 1989). Slices were treated with 100 μM NEM for 10 minutes, prior to incubation with [^{3}H]inositol, or for the last 10 minutes of incubation with [^{3}H]adenine.

The basal accumulation of [^{3}H]cAMP in [^{3}H]adenine pre-labelled guinea-pig cerebral cortical slices was unchanged after pretreatment with 100 μM NEM (control 0.09 ± 0.03; 0.18 ± 0.04 % conversion from total [^{3}H]adenine nucleotides). The maximal response to the A_{2B} adenosine receptor agonist 5'-N-ethylcarboxamidoadenosine was reduced after NEM treatment (control 2.71 ± 0.27; NEM 0.85 ± 0.12 % conversion, $P < 0.01$ - Students' t-test), without significantly altering the EC_{50} value (control 4.1 ± 1.0; NEM 6.4 ± 2.0 μM). The response to 30 μM forskolin was not significantly altered by NEM (control 2.66 ± 0.59; NEM 1.48 ± 0.24 % conversion). However, both the maximal inhibition of the forskolin response by the A_1 adenosine receptor agonist N^6-cyclopentyladenosine was significantly altered by NEM (control 83 ± 5; NEM 35 ± 2 % inhibition, $P < 0.01$) as was the IC_{50} value (control 15 ± 2; NEM 4 ± 1 nM, $P < 0.01$).

Basal accumulation of [^{3}H]inositol phosphates was unaffected by pretreatment with NEM (control 557 ± 66; NEM 780 ± 135 dpm). The response to 1 mM histamine was also unaffected (control 2608 ± 410; NEM 2216 ± 230 dpm). Neither the EC_{50} value (control 460 ± 210; NEM 218 ± 36 nM), nor the maximal response (control 277 ± 21; NEM 257 ± 26 %) of the potentation of the histamine response by N^6-cyclopentyladenosine was altered.

Thus, NEM reduces both A_1 and A_{2B} adenosine receptor responses, without altering the adenosine receptor potentiation of histamine-stimulated phosphoinositide turnover. These results indicate the non-specific nature of NEM, and suggest that the action of this agent on adenosine receptors may be species-specific.

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259P EFFECTS OF EXTRACELLULAR ATP AND RELATED ANALOGUES ON THE PROLIFERATION OF RAT CULTURED ASTROCYTES

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Modifications in adenylate cyclase, phospholipases and protein kinase C (PKC) activities have long been related to the proliferation of cultured cells, including astrocytes. Growth factors and endogenous substances, released from cells, were invoked as signals of cell proliferation. Among these substances, purines (P) can be included. In astrocytes P_1 receptor stimulation modulates changes in cAMP levels (Murphy et al., 1991) while P_{2Y} sites mediate the activation of phospholipase C and intracellular calcium mobilization (Bruner&Murphy, 1990). Agonists for P receptors were assayed as mitogen agents in different cell types and ATP seems to act as a modulatory co-factor of proper mitogens (Huang et al., 1991; Van Daele et al., 1991). In cultured glial cells the stimulation of P_{2Y} sites increased P release and activated PKC system (our previous results) as well as provoking a selective release of eicosanoids (Murphy et al., 1991). In this study we attempted to evaluate, in dissociated primary cultures of rat astrocytes, changes of proliferation following P_{2Y} sites stimulation and the possible involvement of eicosanoids in such a mechanism. Cell proliferation was measured by [^{3}H]-thymidine incorporation, serial cell counts and flow cytometric analysis; P in the culture medium were identified by h.p.l.c. analysis and eicosanoid production was assayed by specific RIA. In control at the 10th day of culture [^{3}H]-thymidine incorporation was 1240.9±140.6 cpm/μg of protein, the total cell number per dish was $1.7 \times 10^6 \pm 0.12$ with 18.1±0.8% of cells in the S phase of cell cycle. Exogenous ATP (0.25-1 mM) and 0.1 mM 2methylthioATP inhibited astrocyte proliferation (-35.3±3.2% and -32.7±2.8% of control respectively; n=6) after 16-24 h of treatment. H.p.l.c. analysis of culture medium samples performed 4-16 h after 500 μM ATP adminstration showed an almost complete breakdown of ATP to related nucleosides and the A_1 selective antagonist 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) reversed the ATP-induced inhibition of astrocyte proliferation. These data suggest the involvement of A_1 receptors to account for ATP-induced effect. Experiments are under way to investigate an eventual role of A_2 receptors in this mechanism. On the contrary, a non degradable analogue of ATP such as adenosine beta, gamma-imidotriphosphate (AMP-PNP) enhanced cell proliferation (+25.4±1.9% of control; n=5). The total inhibition of eicosanoid synthesis achieved by a prolonged treatment (3 days) with 1 μM dexamethasone inhibited the AMP-PNP induced stimulatory effect on proliferation. On the whole, our findings seem to support the hypothesis that in primary cultured astrocytes P_{2Y} receptor sites may modulate cell proliferation, probably through an involvement of eicosanoids pathway.

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260P PHARMACOLOGY OF 2-HEXYNYL-NECA, A NEW A_2 ADENOSINE RECEPTOR AGONIST

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Recently, Cristalli et al., (1992) have described the synthesis of a series of 2-alkynyl derivatives of adenosine 5'-N-ethyluronamide which possess selective A_2 agonist properties. The pharmacological profile of one of these compounds, 2-hexynyl-5'-N-ethyl-carboxamidoadenosine (2-HE-NECA) was studied using both radioligand binding and functional assays. Affinities for A_1 and A_2 receptors were determined using [3H] CHA and [3H] CGS 21680 as selective radioligands. Functional activity at A_2 receptors was determined in vascular tissues and platelets by measuring relaxation and antiaggregatory properties. Interaction with A_1 receptors was assessed in rat atrial preparations. Systolic blood pressure (SPB) and heart rate (HR) were recorded by tail-cuff method in conscious spontaneously hypertensive rats (SHRs). Results are summarized in the Table.

	[a]BINDING Ki (nM)		[b]CARDIOVASCULAR TISSUES ED_{50} (nM)			[a]PLATELET AGGREGATION IC_{50} (nM)		SBP ED_{30} (mg/Kg ip)
	Rat Brain		Rat	Rat	Bovine	Human	Rabbit	SHRs
	A_1	Striatum A_2	Atria	Aorta	Coronaries			
2-HE-NECA	130 (116-145)	2.2 (1.9-2.6)	>10 μM	596 (244-1460)	569 (189-1711)	100 (40-150)	70 (30-150)	0.005 (0.001-0.015)
CGS 21680	569 (511-634)	11.0 (9.4-12.9)	>100 μM	115 (53-251)	66 (33-130)	1090 (440-2740)	2160 (1620-3620)	0.107 (0.038-0.211)
NECA	10.4 (9.4-11.6)	7.8 (6.6-9.1)	55 (38-78)	394 (209-742)	170 (133-217)	490 (320-770)	210 (160-280)	0.010 (0.001-0.062)

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[b]Conti A. et al., Naunyn Smiedeberg's Arch. Pharmacol. 1993; 348:108-112

The data indicate that 2-HE-NECA has high affinity and selectivity for A_2 receptors as observed from binding and functional studies. It is more potent than CGS 21680 in inhibiting platelet aggregation and induces dose-dependent decrease in SBP without affecting heart rate. These findings provide evidence that 2-HE-NECA has a pharmacological profile both in vitro and in vivo compatible with selectivity for the A_2 receptor.

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261P CHARACTERIZATION OF A_2 ADENOSINE RECEPTOR BINDING SITES IN HUMAN PLATELETS USING [^{3}H]CGS 21680

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The recent availability of the selective adenosine A2 agonist CGS 21680 (2-[p-(2-carboxyethyl)-phenethylamino]-5'-N-ethylcarboxamidoadenosine) (Jarvis et al., 1989) has provided an important pharmacological tool for the characterization of adenosine receptor subtypes in both central nervous system and peripheral tissues. As far as we know, this is the first study in which the human platelet adenosine receptor has been characterized using the A2 selective radioligand [3H]CGS 21680. The study on temperature dependence of [3H]CGS 21680 binding to human platelet membranes exhibited the highest specific binding at 0°C with an optimal pH between 7.0-7.4 . Specific binding (65%) was saturable, reversible and dependent upon protein concentration. Using increasing concentrations of radioligand (0.06-7.5 uM), saturation experiments revealed a single class of binding sites with Kd and Bmax values of 1.4 uM and 5.9 pM/mg of protein, respectively. Different adenosine agonists competed for the binding of [3H]CGS 21680 (50 nM) to human platelet membranes showing a rank order of potency consistent with the typical adenosine A2 receptor: 2-hexynyl-5'-N-ethylcarboxamidoadenosine (2-hexynyl-NECA) > CGS 21680 = NECA > 5'-N-cyclopropylcarboxamido-adenosine (CPCA) > 2-phenylaminoadenosine (CV 1808) > R(-)-N6-2-phenylisopropyladenosine (R-PIA) > 2-chloro-N6-cyclopentyladenosine (CCPA) > S-PIA > N6-cyclohexyladenosine (CHA). The 2-hexynyl-NECA, a new potent and selective A2 agonist (Cristalli et al., 1992), was the most effective compound with affinity in the nanomolar range (IC50 = 70 nM). Functional studies on adenylate cyclase stimulation and platelet aggregation inhibition exhibited a rank order of potency similar to that observed in binding experiments, and 2-hexynyl-NECA was the most active compound (EC50 = 41 nM and IC50 = 100 nM, respectively). All these data support the hypothesis that [3H]CGS 21680 labels the A2 receptor of human platelets. However, the A1 adenosine agonists R-PIA, S-PIA, CHA and CCPA which are known to stimulate adenylate cyclase and inhibit platelet aggregation in the uM range, displaced [3H]CGS 21680 only at mM concentrations. Thus, further studies are needed in order to elucidate the characteristics of the human platelet A2 adenosine receptor.

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262P BINDING TO ADENOSINE RECEPTORS IN THE RAT BRAIN IN THE KINDLING MODEL OF EPILEPSY

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The term "kindling" refers to the phenomenon in which repeated administration of an initially subconvulsant electrical stimulus results in progressive intensification of seizure activity, culminating in generalized tonic-clonic seizures. Adenosine and adenosine analogues have potent anticonvulsant effects on various models of seizures, including kindling (Chin, 1989). Furthermore, adenosine levels in the brain are dramatically elevated following seizures (Chin, 1989). In the present study, binding of the adenosine receptors in the kindling model of epilepsy has been investigated.

Male Sprague-Dawley rats (300 g) were stereotaxically implanted (under halothane anesthesia) with a bipolar electrode in the right amygdala, allowed to recover for one week after surgery, and stimulated once daily with a single 1 sec train of bipolar pulses (1 msec, 60 Hz, 100 μA above afterdischarge threshold). Animals were sacrified by decapitation 5-7 days after reaching the kindling criteria (3 consecutive generalized tonic-clonic convulsions). Saturation binding experiments to A_1 adenosine receptors were performed in cortical and hippocampal membranes from control, sham-operated and kindled rats using as radioligand [^{3}H]-N^6-cyclohexladenosine (CHA) in the concentration range of 0.1-10 nM. Affinity (K_d) and density (B_{max}) values for A_1 adenosine receptors, as labelled by [^{3}H]-CHA, are reported in Table 1.

Group	Cortex		Hippocampus	
	K_d	B_{max}	K_d	B_{max}
control	1.51 ± 0.10	655 ± 25	2.00 ± 0.04	800 ± 15
sham-operated	1.50 ± 0.12	600 ± 28	1.83 ± 0.08	828 ± 14
kindled	1.61 ± 0.16	596 ± 22	1.30 ± 0.14*	822 ± 10

Table 1. K_d (nM) and B_{max} (fmol/mg protein) values of A_1 adenosine receptors (mean ± s.e.mean of 4 experiments, 3 animals each). *Significantly different from control and sham-operated values, $P < 0.02$.

The present data suggest an increased affinity of adenosine to A_1 receptors in the hippocampus of kindled rats. This phenomenon may play a role in the anticonvulsant effects of adenosine analogues in the kindling model, and in the hypothesized action of adenosine as an endogenous anticonvulsant (Dragunow, 1985). Further studies are presently under way to elucidate the functional relevance of this observation in terms of modulation of the glutamatergic signal and to characterize the binding to A_2 adenosine receptors.

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263P PURINE-MEDIATED RELAXATION IN ISOLATED AORTA FROM HYPERCHOLESTEROLAEMIC YOSHIDA RATS

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Purines are involved in many receptor-mediated effects in a variety of tissues. The receptors mediating responses to purines have been categorized as P_1- and P_2-purinoceptors. On the basis of agonist potency rank order, those receptors sensitive to adenosine are termed P_1 and those sensitive to ATP P_2 (Burnstock, 1978, 1985). Pittsburgh Yoshida (YOS) rat is an inbred hypercholesterolemic strain which has been recently become available (Fantappiè et al. 1992) and could represent an interesting model of endogenous dyslipidaemia. The aim of the present study was to investigate the vasodilator effect of adenosine and ATP both at endothelial and smooth muscle level in isolated aorta from YOS rat, in order to elucidate which purinoceptors are involved in the vascular purine-mediated relaxation of this animal model. Data were obtained from *in vitro* isolated aortic rings of hypercholesterolemic YOS and normocholesterolemic Brown Norway (BN) 6-month-old rats. The descending thoracic aorta was removed, cleaned of the adjacent tissue and cut into transverse rings 3 mm wide. Chains of 4 rings, tied by silk thread, were suspended in tissue baths containing modified Krebs-bicarbonate solution gassed with a mixture of 95% O_2 - 5% CO_2, pH 7.4, at 37°C and equilibrated at a resting tension of 15 mN. In some aortic preparations endothelium was deliberately removed by gently rubbing the intimal surface with a pipe cleaner. For relaxation studies, adenosine and ATP were cumulatively added to aortic rings precontracted with EC_{50} of noradrenaline. In the aortic preparations deprived of endothelium, ATP (1μM-1mM)-induced relaxation was significantly reduced but not abolished in vessels from both BN (-67%±8% *vs* -18%±5% at 100 μM, m ± sem; n=5; $p<0.001$) and YOS rats (-57%±12% *vs* -8%±2% at 100 μM, m ± sem; n=5; $p<0.01$), indicating a dual relaxant effect located at endothelial and smooth muscle level. Endothelium-dependent relaxation is mediated by the well-known P_{2y}-purinoceptor, whilst muscular relaxation could be mediated by P_1- or P_2-purinoceptors. The muscular component of ATP-induced relaxation was significantly impaired in YOS rat compared with BN (-37%±4% *vs* -67%±7% at 1 mM, m ± sem; n=5; $p<0.01$). In order to characterize the purinergic relaxation in rat aorta, also the relaxant effects of adenosine were studied. Both in the presence and in the absence of endothelium, the relaxant effect of adenosine was similar, indicating that the aortic relaxation induced by adenosine is not an endothelium-dependent phenomenon. The relaxant effect of adenosine was significantly impaired in YOS vs BN rats (-20%±3% *vs* 47%±8% at 100 μM, m ± sem; n=5; $p<0.05$). The maximal effect was similar both in YOS and BN rats (90%±13% *vs* 108%±11% at 1 mM, m ± sem; n=5; p N.S.) and not affected by the presence of 8-phenyltheophilline (10 μM), suggesting that the purinergic muscular relaxation is not a P_1-mediated effect. We can also exclude that the purine-induced effect is mediated by P_2-purinoceptor since adenosine is more potent than ATP. Therefore we suggest that purine-induced relaxation at smooth muscle level is mediated by a non-P_1-non-P_2-purinoceptor; this effect appears to be impaired by persistent hypercholesterolemia in YOS rat.

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264P PHARMACOLOGICAL CHARACTERIZATION OF P2x AND P2y PURINOCEPTORS IN RAT BLADDER SMOOTH MUSCLE AND EPITHELIUM

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The activation of P2x and P2y receptors by adenosine-5' triphosphate (ATP) in smooth muscle tissues has been prevalently associated with contraction and relaxation, respectively (Burnstock & Kennedy, 1985). We have studied the effects of ATP on the resting tone of urinary bladder preparations, which have been previously demonstrated to exclusively respond to ATP with a weak and short contraction (Bhat et al., 1989). In rat urinary bladder strips, even at resting tone, 1 mM ATP induced a biphasic response characterized by a rapid and transient contraction (0.76±0.06 mN/mg of dried tissue, n=36), followed by a slower sustained relaxation (0.10±0.02 mN/mg of dried tissue, n=36) . This later phase, which has not been described before at resting tone, could not be attributed to breakdown of ATP to adenosine as, neither the adenosine uptake inhibitor nitro-benzyl-tio-inosine (NBTI) nor the adenosine receptor antagonist 8-phenyl-theophylline (8-PT) significantly affected ATP-induced relaxation. Conversely, NBTI significantly potentiated the relaxation induced on resting tone by exogenously added adenosine (100 µM) (response was 2.5±0.3 fold higher than control response, n=10, $P<0.05$). The activation of P2x receptors was directly responsible for the ATP-induced contractile phase, since these receptors could be desensitized by the P2x selective agonist αβ-methyl-ATP (residual response was 29±4 % of initial ATP-evoked contraction, n=10, $P<0.01$). P2x purinoceptor-mediated contraction was only partially antagonized by 10^{-4} M suramin (residual contraction was 53±10% of control response, n=5, $P<0.01$), whereas P2y purinoceptor-mediated relaxation was not affected by this antagonist. The two opposite responses induced by ATP on rat urinary bladder smooth muscle were differently modified by the presence of intact epithelium in the preparations. Removal of epithelium did not affect ATP-elicited contraction, whereas ATP-induced relaxation was significantly augmented (0.29±0.03 with respect to 0.10±0.02 mN/mg of dried tissue, n=36, $P<0.001$). These data suggest that rat urinary bladder expresses both P2x and P2y purinoceptors; suramin only partially antagonized P2x purinoceptor- and did not affect P2y purinoceptor-mediated responses. Moreover, P2y purinoceptor-mediated relaxant response is modulated by excitatory factor/s released from epithelial cells (Brown, 1980; Pinna, 1992).

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265P INVOLVEMENT OF G PROTEINS IN AGONIST-INDUCED OPENING OF RECEPTOR-OPERATED CHANNELS IN VASCULAR SMOOTH MUSCLE CELLS

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Contractile agonists adenosine triphosphate (ATP), 5-hydroxytryptamine (5HT), and angiotensin II (AII) open channels in vascular smooth muscle cells which allow mono- and divalent cations including Ca^{2+} to enter the cell (Benham et al., 1987, Hughes & Bolton 1991). However the mechanism by which receptor activation is linked to channel opening is unclear. We have examined the involvement of G proteins using pertussis toxin(PTx), GDP-β-S and GTP-γ-S in single vascular smooth muscle cells.

Single cells were isolated enzymatically from rabbit ear arteries as previously described (Benham & Bolton 1986) and studied by the whole cell voltage clamp technique. Supramaximal concentrations of agonists were applied to cells by pressure ejection.

ATP, 5HT and AII evoked transient inward currents when they were applied to cells held at -60mV using a NaCl-containing intracellular solution in physiological saline (Table 1). In cells isolated from arteries pretreated with 0.5μg/ml PTx for 16 hours, 5HT- and AII-induced currents were almost completely abolished, whereas ATP-induced currents were not significantly reduced (Table 1). PTx treated cells that did not respond to either 5HT or AII did respond to ATP.

Table 1: Comparison of ionic currents in control and PTx treated cells

Agonist	Peak inward current in control cells (pA)	Peak inward current in PTx treated cells (pA)
ATP	403.0 ± 102.0 (4)	156.0 ± 32.0 (5)
5HT	171.6 ± 25.6 (4)	2.3 ± 0.6 (3)**
AII	236.0 ± 57.5 (3)	11.6 ± 8.6 (3)*

Results are means ± s.e.means of (n) observations. *=p<0.05, **=p<0.01 by Mann Whitney U test.

Inclusion of 10mM GDP-β-S, a non-hydrolysable GDP analogue, in the intracellular solution blocked responses to all three agonists (n=4). Inclusion of 300μM GTP-γ-S, a non-hydrolysable GTP analogue, induced a noisy sustained cationic current (103 ± 41pA n=3) at -60mV holding potential.

These results suggest that opening of receptor-operated channels by ATP, 5HT and AII is linked to receptor activation by their coupling to G proteins. However the G proteins coupled to 5HT and AII receptors are sensitive to PTx whereas the G protein coupled to the ATP receptor is relatively insensitive to PTx.

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266P VIP MAY CONTRIBUTE TO NEUROGENIC RELAXATION IN THE SHEEP MIDDLE CEREBRAL ARTERY

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Inhibitory neurotransmission in the sheep middle cerebral artery appears to involve a nitrergic component (Matthew et al,1992). The aim of this study was to examine whether vasoactive intestinal peptide (VIP) is also involved in the relaxant response using anti-serum to VIP and the peptidase α-chymotrypsin. Their effects on the relaxant responses produced both by exogenous VIP and electrical field stimulation (EFS) were measured. Middle cerebral artery rings were suspended in Krebs-Henseleit solution at 37°C. Vessels were pre-contracted with near maximal concentrations of 5-hydroxytryptamine or U-46619. The preparations had no functional endothelium as demonstrated by lack of relaxation of the pre-contracted artery on addition of acetylcholine (1 and 10μM). The data are expressed as the the mean±s.e.mean of the % inhibition of tone. Statistical analysis was carried out using Student's t-test with Bonferroni correction ($P<0.05$). Exogenous VIP (Sigma) produced a cumulative relaxation at 20, 50 and 200nM. At 200nM VIP, the relaxation was significantly inhibited by VIP anti-serum (Cambridge Research Biochemicals, 1:600; 45min) from 72±5% to 32±8%(n=6) and by α-chymotrypsin ($0.5Uml^{-1}$; 30min) from 67±7% to 30±6%(n=6). These results show that VIP anti-serum and α-chymotrypsin effectively inhibit VIP. In the presence of guanethidine (20μM) and atropine (1μM), EFS produced a neurogenic relaxation in pre-contracted rings. EFS was performed with parallel silver wire electrodes. A single pulse or trains of stimuli (2-60 pulses, frequency=1-4Hz, 10-100μs pulse width at supramaximal voltage) were delivered every 10 min.

Table 1 The effects of VIP anti-serum and α-chymotrypsin on the EFS induced relaxation after 20 and 40 min incubation.

	Control	20min	40min	n	
VIP anti-serum (1:600)	42±4%	26±6% *	31±5% *	6	(* Sig. different from control)
Vehicle control (NaN_3, 0.01%)	34±3%	39±4%	39±4%	5	
α-chymotrypsin ($0.5-2Uml^{-1}$)	38±4%	36±7%	40±5%	6	
Time control	41±5%	46±5%	46±3%	5	

VIP anti-serum significantly reduced neurogenic relaxation (Table 1) suggesting that VIP may be involved in modulating inhibitory neurotransmission in the sheep middle cerebral artery. The lack of inhibition by α-chymotrypsin may be due to its inability to affect a neurotransmitter which would be present for a short time at high concentration.

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267P LR-B/057: A NOVEL ORALLY ACTIVE AT_1 RECEPTOR ANTAGONIST

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LR-B/057(4-butyl-1-[(2-carboxyphenyl)methyl]-2-methyl-5-[[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl]pyrimidin-6-one) belongs to a series of novel 4-pyrimidinones which are potent and specific Angiotensin II (AII) receptor antagonists. Here we report the in vitro effects of LR-B/057 in rat, bovine and rabbit tissues and its in vivo effects in the rat, using the methods described in the references reported for each experimental model.

In radioligand competition binding studies, LR-B/057 inhibited the binding of [^{3}H]AII to rat adrenal cortex membranes (containing mainly the AT_1 receptors, Chiu et al, 1988), with a Ki of 3±1 nM (n =5) and did not interact with the AT_2 receptors (bovine cerebellar cortex membranes, Renzetti et al, 1993) up to 10^{-5} M . The affinity of LR-B/057 for AT_1 receptors was found to be markedly dependent on the presence of bovine serum albumin (BSA) in the assay buffer: the addition of 0.2% w/v of BSA resulted in a rightward displacement of the inhibition curve with a threefold decrease of its affinity. In saturation binding experiments LR-B/057 (10^{-8} M) decreased the Bmax of [^{3}H]AII from 1020 to 400 fmol/mg protein, without significantly affecting the K_D. In dissociation experiments LR-B/057 did not affect the rate of dissociation of [^{3}H]AII from AT_1 receptors.

In rabbit aorta strips (Wong et al, 1989) , LR-B/057 (10^{-8}-10^{-5} M, n ≥ 3 for each concentration) caused non-parallel shifts to the right of the dose-response curve to AIIreducing the maximal response by 40-50%. The calculated pK_B value was 9.6. In contrast losartan, the prototype of this new class of drugs, behaved as a competitive antagonist with a pA_2 of 8.2. The inhibitory effect of LR-B/057 on the AII induced response was still observed after 120 min of tissue washing, suggesting a slow dissociation of the antagonist from the receptor. LR-B/057, up to 10^{-5} M, did not change the concentration-contractile response curve for KCl and phenylephrine.

In conscious rats (Wong et al, 1990), LR-B/057 (2-6 µmol/kg p.o., n = 3 to 5) dose-dependently antagonized the pressor responses to AII , resulting slightly more potent than losartan and showing a considerably shorter onset of action (1 vs 3.5 h).

In conscious renal hypertensive rats (complete ligation of one renal artery 6 days prior to the experiments, Cangiano et al, 1979) LR-B/057 was significantly more potent than losartan (ED_{50} = 0.37 vs. 0.78 µmol/kg i.v., n = 5 to 9).

In conclusion LR-B/057 is a new non-peptide Angiotensin II receptor antagonist displaying potent and specific AII antagonistic properties and a good oral absorption. As far as the nature of the antagonism, the results suggest that LR-B/057 may be an unsurmountable or a slowly reversible antagonist at AT_1 receptors.

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268P DILATOR PROSTANOID RECEPTORS ON THE RABBIT DUCTUS ARTERIOSUS

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The ductus arteriosus is a shunt blood vessel, extending between the aorta and pulmonary artery, which is patent in fetal and early neonatal life and usually closes, in the human, within 48 hours after delivery. Both PGE_2 and PGI_2 relax the pre-contracted ductus: PGE_2 is apparently 1000 times more potent than PGI_2 (Clyman et al, 1978). The aims of the present study were (a) to identify the prostanoid EP receptor sub-type mediating the dilator effect of PGE_2 and (b) to identify whether the ductus has prostanoid IP receptors. Fetal New Zealand White rabbits were delivered at 28 days (term 31 days) gestation by Caesarean section following sacrifice of the mother. They were decapitated prior to the onset of respiration and rings of ductus arteriosus were isolated *in vitro*. Cumulative relaxation response curves (RRCs) were obtained from the vessel pre-contracted with a combination of indomethacin (1µM), oxygen (100-110 mmHg) and potassium (25mM). A standard RRC was obtained to PGE_2 and test agonists were compared with this initial response by the equipotent concentration ratio (ECR= EC_{50} test agonist divided by EC_{50} PGE_2, mean pEC_{50} PGE_2 9.44 [95% CI 9.39 to 9.49], n=44) and efficacy (PGE_2 =100%). Concurrent time controls demonstrated the reproducibility of the PGE_2 RRC. Antagonists were screened using a high concentration and where an effect was observed data obtained for a Schild plot.

Agonist	n	ECR (95% CI)	Efficacy % (95% CI)	Antagonist vs PGE_2	n	pA2 (95% CI)	Slope (95% CI)
Misoprostol (EP2/3)	8	146 (73 to 217)	99 (97 to 101)	AH6809 (EP1)	11	no effect 10µM	
GR63799X (EP3)	8	685 (427-944)	87 (79.8 to 94.9)	SC19220 (EP1)	11	no effect 30µM	
AH13205 (EP2)	8	>10000	21 (14 to 29)	AH23848B (EP4)	12	4.91 (4.36 to 5.51)	1.45 (1.13 to 1.78)
Sulprostone (EP1/3)	12	>10000	0.5 (-9 to 10)	AH13205 (see below)	7	4.85 (3.98 to 5.96)	1.01 (0.52 to 1.49)
Cicaprost (IP)	8	110 (80 to 140)	99 (96 to 103)				

Summarising the above, the selective EP1 antagonists AH6809 and SC19220 had no effect on RRCs to PGE_2. Of a range of agonists with activity at EP1-3 receptors, none was equipotent with PGE_2. The selective EP4 antagonist, AH23848B (Louttit et al 1992) caused a non-parallel rightward shift in the PGE_2 RRC and the slope of the Schild plot was greater than 1.0. AH23848B (30µM) also caused a decrease in the pEC_{50} to cicaprost (mean -0.19 [95%CI -0.002 to -0.38]) which was less than its effect on PGE_2 ($p<0.05$). These data suggest that AH23848B blocks the ductal EP receptor but also has an additional non-specific effect. The selective EP2 agonist, AH13205, while only a weak dilator of the ductus, caused a parallel shift in the PGE_2 RRC. The Schild plot of these data was consistent with competitive antagonism of the ductal EP receptor and AH13205 had no effect on cicaprost. This suggests that the vessel also has a population of IP receptors. The ductal EP receptor is, therefore, antagonised by AH23848B and AH13205. In conclusion, the fetal rabbit ductus arteriosus has two populations of dilator prostanoid receptors: 'EP4-like' and IP.

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269P INVOLVEMENT OF PROTEIN KINASE C IN CYTOKINE-INDUCED ANGIOGENESIS IN THE RAT

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Cytokines such as basic fibroblast growth factor (bFGF) and tumour necrosis factor (TNF-α) are potent inducers of angiogenesis (Hu *et al.*, 1993). However, their mode of action has not been clearly defined. Earlier studies with tumour-promoting phorbol esters suggest that protein kinase C (PKC) plays a role in angiogenesis *in vivo* (Morris *et al.*, 1988). Recently nitric oxide (NO) is implicated in several distinct signalling pathways in blood vessels. Here we used a highly potent and specific PKC inhibitor calphostin C (CAL, Kobayashi, *et al.*, 1989) and a nitric oxide synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA), to test if these two second messengers mediate or modulate the angiogenic activity of bFGF and TNF-α *in vivo*.

Sterile polyether sponges with attached cannulae were implanted subcutaneously in male Wistar rats (180-200g) and neovascularisation was assessed by using ^{133}Xe clearance to measure blood flow through the implants every two days over a period of 14 days. Test substances (50 μl) were applied to the sponges through the cannulae daily 24h before ^{133}Xe measurement. The effects of test substances on angiogenesis were confirmed histologically.

Daily doses of 100 ng bFGF caused an intense neovascularisation. This bFGF-induced neovascular response was inhibited in a dose-dependent manner by co-administration of CAL. The 6 min ^{133}Xe clearance value obtained on Day 6, 8, 10, 12 for the control group were 14.2±1.9, 19.6±0.7, 28.2±0.8, 36.1±1.3 % (n = 8) and bFGF increased it to 23.4±1.9, 30.9±0.8, 37.1±1.1, 41.8±1.2 %, at the respective time point (n = 8, $P < 0.01$), while the corresponding values for CAL (4 μg day^{-1}) treatment group were 14.1±1.0, 21.0±0.4, 27.4±0.5, 34.9±0.6 (n = 12, $P < 0.01$). Histological studies showed the cellularity and vascularity in the bFGF-treated sponges were profoundly suppressed by CAL. Daily co-administration of 0.4 μg CAL partially inhibited the bFGF-induced neovascular response, but 0.04 μg CAL was ineffective. Parallel studies showed that the neovascular response elicited by 50 ng TNF-α was also blocked by daily co-administration of 4 μg CAL.

In the absence of exogenous cytokines, the sponge-induced neovascularisation was inhibited by daily treatment with 4 μg CAL. The 6 min ^{133}Xe clearance values obtained on Day 10, 12, 14 for control group and treated groups were 28.2±0.8, 36.1±1.3, 40.7±1.0 % (n = 8) and 23.1±1.0, 26.1±1.5, 27.9±0.9 % respectively (n = 6, $P < 0.01$). Histological studies showed that both cellular infiltration and neovascularisation were significantly inhibited by CAL as compared to controls.

Parallel studies showed that bFGF-, TNF-α- and basal sponge-induced neovascular responses were not influenced by L-NMMA (1 x 100 μg day^{-1}, 1 x 1,000 μg day^{-1} or 2 x 1,000 μg day^{-1}). In contrast, a recent report showed that L-NMMA increased both collagenous protein biosynthesis and vascular density of the chick embryo chorioallantoic membrane (Pipili-Synetos *et al.*, 1993).

These results suggest that bFGF- or TNF-α-induced angiogenesis in the rat sponge model may be mediated in part through activation of PKC, but not NO. Further work is in progress to elucidate the modulatory role of NO in angiogenesis.

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270P ANTIATHEROSCLEROTIC ACTIVITY OF LACIDIPINE IN CHOLESTEROL-FED HAMSTERS

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The effect of the long-acting calcium antagonist lacidipine (L) on atherosclerosis has been determined in hyperlipidemic male hamsters.

Control hamsters fed chow containing 2% cholesterol and 5% butter (w/w) were compared to chow baseline animals, and to hamsters receiving different dosages of L (0.3 ; 1.0 ; 3.0 mg/Kg by oral gavage once a day) plus the atherogenic diet (n= 40 each group).

During the study, animals were sacrified at different times,14-20-24 weeks after starting of treatment then total and high density lipoproteins cholesterol (HDL) and triglycerides were measured and low density lipoproteins (LDL) cholesterol computed; aortic arch and thoracic aorta were examined "en face" after Oil red O staining and lesions were quantitated.In addition, ultrastructural examination was performed.

Marked increases in total plasma cholesterol , LDL and triglycerides (from 2.0±0.1 mM/l, 0.7±0.1 mM/l, 1.5±0.2 mM/l, to 24.1± 4.9 mM/l, 14.4± 4.9 mM/l and 16.4± 6.2 mM/l (mean±S.D.) respectively, n=13) were measured in control fat-fed hamsters after 14 weeks and did not significantly increase during the study. No significant differences among groups receiving the atherogenic diet were detected.

A fatty streak area was observed in the aortic arch of the majority of hamsters fed the atherogenic diet from week 14 and the lesion progressed in size and severity with time (2.7± 0.96 % of total arch area by week 24). In contrast, animals treated with lacidipine showed a significant reduction of the lesion area compared to hyperlipidemic controls in week 24 (67% at 0.3 mg/Kg; 41% at 1.0 mg/Kg and 70% at 3.0 mg/Kg).In the thoracic aorta lipid deposits were noted from week 14 and a reduction in incidence and degree of lesion was highly significant ($p<0.05$, Duncan's test) for hyperlipidemic hamsters treated with lacidipine in week 24 compared to the untreated ones (85% at 0.3 mg/Kg; 78% at 1.0 mg/Kg and 97% at 3.0 mg/Kg).

Ultrastructural examination characterized the early changes in the aorta as an involvement of intima and media smooth muscle cells.The study also showed a clear interference upon treatment with L in endothelial ultrastructural changes, macrophage invasion, and medial smooth muscle cell migration.

In conclusion, these results further support the antiatherogenic properties of lacidipine obtained in other animal models of atherosclerosis (Bernini *et al.*, 1993).

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271P MECHANISM OF UNIMPAIRED ENDOTHELIAL ATP-INDUCED AORTIC RELAXATION IN ISOLATED AORTA FROM HYPERCHOLESTEROLEMIC RABBITS

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Selective impairment of muscarinic endothelium-dependent vascular relaxation has been found subsequently to experimental endothelial damage such as mechanical de-endothelialization or diet-induced hypercholesterolemia (Bossaller et al., 1987; De Meyer et al., 1991). Prolonged hyperlipidemic status on genetic basis in Watanabe (WHHL) rabbit has been found not to affect the relaxant response to ATP (Ragazzi et al., 1989; Chinellato et al., 1991). The receptors mediating responses to purines have been categorized as P_1- and P_2-purinoceptors; P_{2y}-purinoceptor, located at endothelium level, has been found to mediate the vascular relaxant response to ATP (Burnstock & Kennedy, 1985). The aim of the present study was to investigate the effect of ATP on the endothelial level in isolated aorta from hypercholesterolemic Watanabe rabbit, in order to elucidate the role of purinoceptors in a condition of prolonged endothelial damage. Experiments were carried out on isolated aorta from WHHL rabbits 10-11 months old, in comparison to normocholesterolemic New Zealand (NZ) age-matched rabbits. The descending thoracic aorta was removed, cleaned of adjacent tissue, cut into transverse rings 3 mm thick. In some aortic preparations endothelium was deliberately removed by gently rubbing the intimal surface with a pipe cleaner. Acetylcholine, ATP and 2-methylthio-ATP were cumulatively added to aortic rings precontracted with EC_{50} of noradrenaline. Morphological examination of aortas was conducted by means of scanning and transmission electron microscopy. Morphological data indicated that most of the endothelial layer is present in 10-month-old WHHL rabbit aorta, although plaques were observed, particularly close to the entry of intercostal arteries. Functional data showed that endothelium-dependent relaxation produced by acetylcholine was significantly reduced in WHHL rabbit aorta in comparison to NZ (-59%±4% *vs* -29%±3% at 1 μM, m ± sem; n=6; $p<0.001$). The potent agonist at endothelial P_{2y}-purinoceptors, 2-methylthio-ATP, did not induce any relaxation in WHHL aorta, indicating a defect in endothelial purinergic P_{2y}-purinoceptor. Despite this, ATP-induced relaxation was not reduced in WHHL aorta, when compared to controls (-53%±3% *vs* -47%±5%, m ± sem; n=6), even after mechanical de-endothelialization (-25%±4% *vs* -24%±6%, m ± sem; n=6). Since ATP relaxes vessels *via* P_{2y}-receptor, maintained endothelial-dependent ATP activity in WHHL rabbit aorta could be mediated by a receptor different from the classical P_{2y}-purinoceptor. In conclusion, the impaired endothelium-dependent relaxation to acetylcholine and 2-methylthio-ATP but not to ATP suggests selective alteration of endothelial cells during the evolution of atherosclerosis.

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272P EFFECT OF CALCIUM ANTAGONISTS IN ISOLATED AORTA FROM HYPERCHOLESTEROLEMIC YOSHIDA RATS

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It is well established that atherosclerosis induces serious vascular diseases. Experimental studies have shown that the pathological conditions may also lead to alterations in vascular responsiveness to various drugs (Jayakody et al., 1986; Ragazzi et al., 1989). Calcium and persistent hyperlipidemia play a crucial role in the pathogenesis of cardiovascular damage in atherosclerosis. Calcium antagonists are widely used in the management of pathologies involving vascular tone alterations. Pittsburgh Yoshida (YOS) rat is an inbred hypercholesterolemic strain which has been recently become available (Fantappiè et al., 1992) and could represent an interesting model of endogenous dyslipidemia. The aim of the present study was to investigate the vasodilator properties of the calcium antagonists verapamil and nifedipine, in isolated aortic rings precontracted with two different vasoconstrictor agents (norepinephrine, NE; potassium chloride, KCl). Aortic preparations were obtained from hypercholesterolemic YOS and normocholesterolemic Brown Norway (BN) 2-month-old rats. The descending thoracic aorta preparations (chains of 4 rings) were suspended in tissue baths (30 ml) and equilibrated at a resting tension of 15 mN, gassed with 95% O_2 - 5% CO_2, pH 7.4, at 37°C. For relaxation studies, the calcium antagonists were cumulatively added to aortic rings precontracted with EC_{50} of contracting agents. Nifedipine and verapamil (1nM to 1μM) induced vascular relaxation in aortic rings precontracted with NE or KCl in hypercholesterolemic and control rat strains. The relaxant effect induced by nifedipine and verapamil was similar in the two groups of animals both on NE- (for nifedipine: -38%±6% in YOS and -39%±5% in BN, m ± sem, n=6; for verapamil: -25%±9% in YOS and -37%±5% in BN, m ± sem, n=6, at 30 nM) and KCl- (for nifedipine: -87%±8% in YOS and -83%±4% in BN, m ± sem; for verapamil: -21%±4% in YOS and -29%±5% in BN, m ± sem, at 30 nM) precontracted aortic preparations. The nifedipine-induced relaxation was maximal on KCl-precontracted aortic rings while the effect obtained on NE-precontracted preparations was significantly lower both in YOS and BN rats ($p<0.01$). No difference was observed in the verapamil-induced relaxation (30 nM), because the potency of verapamil was much lower than that of nifedipine. KCl induces vascular contraction by depolarizing smooth muscle cells, leading to a Ca^{2+} entry into the cell. On the other hand, NE induces vasoconstriction independently on cell depolarization, acting mainly on Ca^{2+}-release from intracellular stores. Calcium antagonists act by selective blockade of extracellular Ca^{2+} influx into the cell. The different mechanism of action of both contractile drugs can therefore explain the differences in the vasodilator activity of both calcium antagonists. Also in YOS and BN rats, the characteristic rank order of potency for calcium antagonists (nifedipine>verapamil) was observed. In conclusion, the present data indicate that hypercholesterolemia, in Yoshida rat strain, does not affect vascular relaxation induced by calcium antagonists.

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273P EFFECT OF β-ADRENERGIC DRUGS ON MACROPHAGE AND VASCULAR RECEPTORS IN NORMOTENSIVE AND HYPERTENSIVE RATS

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The existence of B-adrenergic receptors for neurotransmitters on immune cells has been clearly demonstrated (Ader et al., 1990). Receptor binding studies have suggested that exposure of macrophages to isoproterenol results in decrease of phagocytic activity (Abrass et al., 1985). In this respect, it is known that stress and elevated levels of circulating catecholamines produce immunosuppression and, moreover, hypertensive rats exhibit reduced (30% less than normotensive rats) phagocytic capacity during the development of hypertension (Serio et al., 1992). On the other hand, it is known that reduction in vascular B-adrenoceptor responsiveness occurs in hypertension (Fujimoto et al., 1987). On these grounds, we have evaluated the effect of B-adrenergic drugs on phagocytosis of C. albicans by splenic macrophages in spontaneously hypertensive rats (SHR) (n=8) and in control normotensive Wistar-Kyoto rats (WKY) (n=8), at age of 16 weeks. Splenic macrophages were preincubated with isoproterenol alone and with isoproterenol and propranolol and then phagocytosis was evaluated. Moreover, relaxant effect of isoproterenol was determined in phenylephrine precontracted thoracic aorta strips from SHR (n=8) compared with age-matched control normotensive WKY rats (n=8).
Results show that in WKY rats splenic macrophages treated with isoproterenol alone (from 5x10-8 to 5x10-5 M) exhibited a significant ($P < 0.01$) and dose-dependent decrease (up to 30%) in phagocytic activity; while addition of propranolol (5x10-5 M) reversed (up to 55%) the suppressive effect of isoproterenol. On the contrary, isoproterenol and propranolol were uneffective to modulate phagocytosis in SHR. At vascular level, the relaxation of thoracic aorta strips from SHR in response to isoproterenol (10-4, 2x10-4 M) was significantly decreased ($P < 0.01$) as compared with that of age-matched WKY rats.
These findings suggest that SHR with established hypertension exhibit changes not only in vascular, but also in macrophage B-adrenoceptor responsiveness. Furthermore, isoproterenol incapacity to modulate "in vitro" phagocytosis in SHR may be associated with decrease in B-adrenoceptor functionality that occurs in hypertension. In conclusion, impairment of phagocytosis observed in hypertensive rats may be explained by reduced B-receptor responsiveness.

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274P AGEING AND VASCULAR EFFECTS OF ET-1

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The endothelins are a recently discovered family of potent contractile peptides produced by endothelial cells (Yanagisawa *et al.*,1988). These peptides have been suggested to play an important role in the pathogenesis of hypertension, myocardial infarction, cardiogenic shock, and so on. Advancing age also produces a number of significant changes in the cardiovascular system (Docherty, 1990). The mechanism for age-related vascular changes may involve alterations in the responsiveness of vascular smooth muscle and/or altered function of the endothelium. The present study was therefore designed to examine whether age could alter the ET-1, noradrenaline (NA) and KCl responses in aortic rings from rats of different strains and ages. Thoracic aorta rings from spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats 2-4 (young), 6-8 (adult) and 20-25 (old) months old were used. The concentration-response curves for the contractile effect of ET-1, NA and KCl were determined by cumulative addition of ET-1 from 100 pM to 300 nM, of NA from 1 nM to 10 μM and of KCl from 10 mM to 100 mM. EC_{50} values were interpolated visually from hand-fitted concentration-response curves. The sensitivity of the tissues to the agonists was expressed in terms of the pD_2 (- log EC_{50}) values. Tissue responsiveness was expressed as the maximum effect obtained (Emax) in grams.

Our findings demonstrate that ET-1 is a more potent vasoconstrictor than NA and that this potency remains unchanged throughout the ages and the pathology studied. In contrast, the pD_2 of NA decreases with old age in SHR (young 7.79±0.2, old 6.92±0.2; $P<0.05$) and WKY ((young 8.46±0.3, old 7.25±0.2; $P<0.01$) rats. However, there were no changes in the pD_2 values for ET-1 and NA between WKY and SHR rats at the ages studied. The ET-1, NA and KCl Emax in adult SHR rats (respectively 0.94±0.1, 0.78±0.1, 1.07±0.2 g±s.e. mean) was significantly lower than in the age-matched WKY animals (respectively 1.69±0.07, 1.77±0.2, 1.78±0.3 g±s.e. mean). The old age (animals of 20-25 months) reduced the ET-1, NA and KCl Emax in both SHR and WKY rats; such a decrease develops in an early phase in hypertensive animals, since it is already apparent in tissues from adult animals. In conclusion, our findings suggest that pathological conditions, such as arterial hypertension, could influence the vascular response to ET-1, NA and KCl in some periods of the rat life; in old age such influence seems to disappear. Moreover, old age can induce a significant decrease of rat vascular reactivity to vasoconstrictor agents probably involving alterations in the responsiveness of vascular smooth muscle.

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275P THE ELECTROCARDIOGRAM FROM MICE AS A MODEL TO STUDY ANTHRACYCLINE CARDIOTOXICITY

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The electrocardiogram (ECG) from the rat is a commonly used parameter for investigating cardiotoxic anthracyclines such as doxorubicin (DXR) (Jensen et al., 1984). However in this and many other species DXR also causes a nephrotic syndrome which parallels the incidence of myocardial lesions (Fajardo et al., 1980).
In the mouse DXR induces the same myocardial lesions as in the rat but with no nephrotoxicity and this species has therefore been suggested as a preferred alternative to the rat (de Jong et al., 1990). However the mouse ECG has not been tested previously as a possible predictor of cardiotoxicity.
In order to test this 2 groups of female Balb C mice (n=6) were given 1 and 3 mg/kg DXR i.p. 3 times weekly for a period of 4 weeks (a total of 12 and 36 mg/kg). A third group served as a control and received sterile water (10 ml/kg). The largest dose-schedule chosen has previously been proven to cause severe cardiomyopathy in mice (Klugmann et al., 1981).
During the study the clinical status and weight gain were recorded continuously and the ECG once weekly by s.c. needle electrodes, C-R leads, in light ether anesthesia. As the length of the QαT and SαT intervals of the ECG are reported to be sensitive predictors for cardiotoxity in the rat these intervals were measured in the mice in a blind fashion.
The mice from the high dose DXR-group (3 mg/kg) showed marked clinical signs of toxicity (sedation and piloerection) and two animals died after 3 weeks medication. The group body weight after 4 weeks was lower than that of the control group (20.3±0.3 vs 26.8±1.9g, mean ± s.e. mean, $p<0.05$, Dunnett's test).
The SαT-interval of the ECG which is the most sensitive marker of cardiotoxicity in the rat (Danesi et al., 1986) showed a maximum increase of 89% compared to the control mice (3.4±1.2 vs 1.8±0.4 ms). Also the QαT intervals were markedly prolonged as compared to the control (4.8±1.2 vs 1.6±1.0 ms; 200% increase). However only the latter difference reached statistical significance at the 5% level (one-sided Dunnett's test).
The 1 mg/kg group did not show any clinical signs of toxicity nor were the ECG intervals prolonged.
At the post-mortem examination no indication of nephrotoxicity was observed in the 2 DXR-treated groups evaluated by the relative organ weights and kidney histology.
The observed DXR-induced ECG-changes in the mouse were qualitatively similar to those previously observed in the rat (Rasmussen et al., 1989). However a larger within group variation was observed in the mouse. In order to obtain statistical significance ($2\alpha = 0.05$, $\beta = 0.2$) of the observed prolongations of both the QαT- and SαT-intervals in the mouse it was calculated that a group size of 9-14 animals would be necessary.
Nevertheless it is concluded that the ECG in mice may be a realistic alternative to the rat for assessing anthracycline induced cardiotoxicity.

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276P INHIBITION OF THROMBIN-INDUCED PLATELET ACCUMULATION IN THE CRANIAL VASCULATURE OF THE RABBIT

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Intracarotid (i.c.) injection of thrombin (Thr) induces a sustained accumulation of platelets in the cranial vasculature of the rabbit (May et al.,1990). Platelet accumulation is prevented by treatment with the anti-thrombin agent desulphato-hirudin 1 min prior to Thr (May et al.,1992). In the present study we have investigated several agents which can interfere with the action of Thr to assess their effects on cranial platelet accumulation in vivo.

Circulating ^{111}In-labelled platelets were continuously monitored in the cranium of anaesthetised NZW rabbits as described previously (May et al.,1990). Responses to i.c. injection of Thr (90U kg^{-1} bolus) were expressed as peak % change from baseline levels and presented as mean ± s.e. mean values. The significance of differences was assessed by analysis of variance followed by a modified t-test. Platelet accumulation (-3.7 ± 3.8%; n=3) in animals treated i.c. with Hirulog (Hir; 5mg kg^{-1} bolus) 1 min prior to Thr was significantly ($p<0.01$) less than in controls pretreated with saline (70.1 ± 6.6%; n=3). Doses of Hir as low as 0.2mg kg^{-1} were also effective. Unfractionated heparin (Hep),Fragmin (Frag) and D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) were administered via i.c. infusions for 1 h commencing 30 min prior to injection of Thr. Hep (5,10 or 20U kg^{-1} bolus + 0.75,1.5 or 3U $kg^{-1}min^{-1}$ infusion respectively) produced dose-related reductions in accumulation from control values (78.4 ± 7.4%; n=12) to 29.2 ± 5.3% (n=4; $P<0.01$) with the highest dose. Frag (2.4, 4.8 or 9.6U kg^{-1} min^{-1}) reduced accumulation from control levels (78 ± 10.4%; n=9) to 18 ± 3.2% (n=3; $p<0.01$) with 4.8Ukg^{-1}. 9.6Ukg^{-1} had significantly ($p<0.05$) less inhibitory effect (40.4 ± 4.9%; n=4) than 4.8Ukg^{-1}. PPACK (0.25,0.5 or 1 μg kg^{-1} min^{-1}) decreased accumulation in a dose-related manner from 72.9 ± 12.3% (n=7) to 14.5 ± 7.3% (n=3; $p<0.05$) with the highest dose.

Our results indicate that thrombin-induced cranial platelet accumulation can be reduced by agents acting via anti-thrombin III/ vessel wall charge alteration as well as by thrombin inactivators.

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277P A VASOACTIVE SUBSTANCE WITH A MOLECULAR WT <3,000, RELEASED DURING HYPOXIC PERFUSION OF ISOLATED RAT LUNGS, CONSTRICTS ISOLATED RAT PULMONARY BUT NOT MESENTERIC RESISTANCE ARTERIES

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Despite extensive research the mechanism underlying acute hypoxic vasoconstriction remains unclear. However, recent evidence has suggested that an endothelium-derived vasoconstrictor may, at least in part, mediate the contractile response to hypoxia in isolated small pulmonary arteries (Agrawal et al. 1992). In preliminary experiments we have found that hypoxic perfusion of isolated rat lungs induces a biphasic increase in perfusion pressure, which has a similar time course to the hypoxia-induced biphasic constriction reported in isolated rat pulmonary arteries (Agrawal et al. 1992). We have therefore attempted to isolate any vasoactive substances which may be released during the hypoxic perfusion of isolated rat lungs.

Male Wistar rats (300-350g) were anaesthetised with ether, and the heart and lungs rapidly excised. The main pulmonary artery was cannulated and the lungs were suspended in a humidified chamber at 37°C. The lungs were then perfused with a "normoxic" physiological salt solution (PSS) containing (in mM): NaCl 118; $NaHCO_3$ 24; $MgSO_4$ 1; NaH_2PO_4 0.435; glucose 5.56; Na-pyruvate 5; $CaCl_2$ 1.8; and KCl 4 (gassed at source with $5\%O_2/5\%CO_2/90\%N_2$ (PO_2=46±1mmHg, pH=7.41±0.02, 37°C) at a flow rate of 15ml/min, for an hour before the induction of hypoxia. Perfusion pressure was monitored continuously via a pressure transducer (Bell and Howell type 4-422) from a side port of the pulmonary artery cannula. Hypoxia was induced for one hour by gassing the PSS at source with $5\%CO_2/95\%N_2$ (PO_2=15±3mmHg). The hypoxic perfusate was collected and passed through a Sep-Pak C_{18} cartridge (Millipore). Any substances loaded onto the cartridge were then eluted in 3 steps by washing the cartridge with 30%, 50%, and 100% acetonitrile (ACN). These fractions were then dried under N_2 and the resultant powder re-dissolved in 500µl sterile H_2O. These fractions were subsequently assayed for vasoconstrictor activity on isolated pulmonary and mesenteric resistance vessels (internal diameter 300-600µm) mounted on a small vessel myograph as previously described (Leach et al. 1992).

No constrictor activity was observed in either the 0-30%ACN, or the 50-100%ACN fractions in either pulmonary or mesenteric vessels. The 30-50%ACN fraction was found to cause a dose-dependant constriction of small pulmonary arteries with a maximum effect approximately 30% of that produced by 75mM KCl (n=5). Upon washing these vessels relaxed almost immediately. In contrast this fraction had little effect upon small mesenteric arteries, with a maximum effect approximately 3-5% of that produced by 75mM KCl. This constrictor activity was retained after the fraction was heated to 97°C for 10 mins, and also if the sample was passed through a Microcon 3 concentrator (Amicon, allows only substances with molecular weights less than 3,000 to pass through).

These results suggest that a pulmonary-selective vasoconstrictor, with molecular weight less than 3,000, is released during hypoxic perfusion of isolated rat lungs, although it is possible that the release of this factor is an artifact of perfusion rather than a hypoxia-induced release. This substance is unlikely to be an endothelin, since the constriction observed in these experiments was readily reversed upon washing, whereas the constrictor effects of endothelin-1 and endothelin-3 persist in these vessels after washing (10nM, n=4). Further experiments are required to elicit the chemical nature of this substance(s), and its possible role in acute hypoxic pulmonary vasoconstriction.

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278P POSSIBLE ROLE OF MAGNESIUM IN MYOCARDIAL ISCHEMIA/REPERFUSION INJURY AND IN THE CARDIOPROTECTIVE EFFECT OF PRECONDITIONING

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In several animal species one or more brief episodes of myocardial ischemia (preconditioning) activate adaptive changes rapidly leading to increased myocardial tolerance to a subsequent ischemic insult (Reimer et al., 1990). Considerable derangement of myocardial calcium homeostasis occurs during ischemia (Forman et al., 1993); changes in intracellular pH (pH_i) and increased intracellular magnesium concentration ($[Mg^{2+}]_i$) could be involved in its development. To assess the potential role of these alterations in the mechanism of the protective effect of preconditioning, Langendorff-perfused rat hearts were subjected to 30 min of normothermic ischemia and 20 min of reflow. For preconditioned hearts (PC) these were preceded by four periods of 2 min of ischemia, separated by 3 min of reperfusion. Intracellular high energy phosphate levels (phosphocreatine, ATP), pH_i and $[Mg^{2+}]_i$ were determined on PC and control (C) preparations by ^{31}P-NMR spectroscopy; on the same preparation the contractile performance was also evaluated before, during and after the prolonged period of ischemia. The contractile recovery at the end of the reperfusion period was significantly higher in PC hearts (50±12% of preischemic value, mean±s.e.m., n=8) as compared to C (5±5%, n=8). No significant differences in pH_i and high energy phosphate levels were observed between PC and C preparations. However, after 20 min of reperfusion $[Mg^{2+}]_i$ was found to be significantly lower in the PC group than in C. Statistical significance was assessed by Student's *t* test.

	before prolonged ischemia		after 20 min reflow	
	pH_i	$[Mg^{2+}]_i$ µM	pH_i	$[Mg^{2+}]_i$ µM
Controls	7.07 ± 0.02	284 ± 37	6.98 ± 0.04	817 ± 37
Preconditioned	6.94 ± 0.04	302 ± 40	6.96 ± 0.02	425 ± 46* ($p<0.001$ vs C)

Since intracellular Mg^{2+} can interfere with calcium-dependent processes in the myocardium (Meissner & Henderson, 1987), we suggest that increases in $[Mg^{2+}]_i$ may contribute to ischemia/reperfusion injury and that the improvement in functional recovery observed in PC hearts following a prolonged period of ischemia may be due to a more efficient intracellular ion homeostasis.

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279P POLYMORPHONUCLEOCYTE (PMN) INFILTRATION AFTER RENAL ISCHAEMIA/REPERFUSION (I/R) *IN VIVO*: ROLE OF ADHESION MOLECULES

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We have assessed the role of adhesion molecules in PMN infiltration (as assessed by tissue myeloperoxidase (MPO) activity) in an acute rat renal model of I/R using murine IgG_1 monoclonal antibodies (MoAbs) against rat ICAM-1 and CD18 of β_2-integrins (Tamatani & Miyasaki, 1990).

Male Wistar rats (350-450g) were anaesthetized (Sagatal, 60mg/kg i.p.) and maintained at 37°C. Heparin (200iu/Kg i.v.) was administered 10 min before the left kidney was made ischaemic by occlusion of the pedicle for 45 min followed by R for 0.5, 1, 3 or 6h. The right kidney served as a sham-operated control. Kidneys were then removed and the tissue MPO activity determined photometrically using 3,3,5,5-tetramethylbenzidine (TMB, Suzuki *et al*, 1983) as substrate. The TMB assay of MPO derived from rat blood PMNs was 3-fold more sensitive ($p<0.05$, n=6) than an assay using o-dianisidine described by Bradley et al (1982). MoAbs or the parent murine IgG_1 as control were administered 20μg/kg i.v. both 10 min before renal pedicle occlusion and at the onset of R.

Following 45 min renal pedicle occlusion and 0.5, 1, 3 or 6h of reperfusion, MPO activity in the I/R kidney was 0.23±0.12 (N.S., n=6), 1.50±0.44 ($p<0.05$, n=6), 2.10±0.60 ($p<0.05$, n=6) and 2.24±0.90 ($p<0.02$, n=6) iu/g wet weight, respectively. MPO activity at these time points in the sham-operated kidneys were 0, 0, 0.59±0.38 and 0 iu/g wet weight, respectively. Pretreatment with anti-ICAM-1 or anti-CD18 MoAb reduced renal MPO activity after 45 min renal I / 1h R from 1.50±0.44 to 0.34±0.15 and 0.36±.15 iu/g wet weight, respectively ($p<0.05$, n=6). In addition, the parent murine IgG_1 also reduced I/R renal MPO activity to 0.48±0.22 iu/g tissue ($p<0.05$, n=6). MPO activity was not detectable in the sham kidneys of the treated groups. Fluorescence activated cell sorting analysis showed anti-CD18 MoAb binding to rat blood PMNs, while anti-ICAM-1 or parent murine IgG_1 demonstrated no binding to PMNs.

Hence, the efficacy of antibodies against ICAM-1 and the β_2-integrins to reduce rat renal PMN infiltration after I/R *in vivo* would suggest a role for adhesion molecules in this process. However, the results should be interpreted with caution since the parent murine IgG_1 was also found to be effective.

D.W. Laight is a CASE award student with Roche Products Ltd.

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280P MODULATION OF THE SECRETION OF CORTICOSTERONE AND GROWTH HORMONE (GH) *IN VIVO* BY ANTI-LIPOCORTIN 1 ANTISERA AND DEXAMETHASONE

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Our previous studies have shown that lipocortin 1 (LC1) mediates some aspects of the inhibitory actions of dexamethasone on pituitary function in vitro (Taylor et al 1992) and that it impairs the release of the 41-amino acid corticotrophin releasing factor (CRF-41) induced by interleukins (ILs)-1α, 1β, 6 and 8 (Loxley et al 1993). In the present study we have used an in vivo model to examine the influence of dexamethasone and an anti-LC1 antibody (LC1-Ab, raised in sheep) on the neuroendocrine responses to IL-β. Adult male CFY rats (≈200g) were injected subcutaneously on two successive days with either an affinity purified LC1-Ab or a corresponding volume (1ml/kg) of similarly purified non-immune sheep serum (NSS); a third group of rats was handled but not injected (serum free control, SFC). On the following morning, rats from each group were given either dexamethasone (100μg/kg, i.p.) or the saline vehicle (1ml/kg, i.p.) followed 1h 15 min later by either IL-1β (7.5μg/kg i.p., which produced a submaximal rise in serum corticosterone) or the saline vehicle (1ml/kg, i.p.). After a further hour, the rats were decapitated and the serum collected and stored for determination of immunoreactive (ir) corticosterone, growth hormone (GH) and luteinizing hormone (LH). The data (ng/ml, mean ± SEM, n = 5-6) were analysed by Duncan's range test.

In the uninjected (SFC) control group, IL-1β increased the serum concentrations of ir-corticosterone [850 ± 154 vs. 47 ± 20.2 in saline controls, $p<0.001$] and ir-GH [10.15 ± 1.5 vs. 4.8 ± 0.3 in saline controls, $p<0.05$] but failed to influence ir-LH [2.3 ± 0.03 vs. 1.74 ± 0.3 in saline controls, N.S.]. The secretory responses to the cytokine were inhibited by dexamethasone [ir-corticosterone = 5.3 ± 1 vs. 850 ± 154, $p<0.001$; ir-GH = 4.87 ± 0.6 vs. 10.15 ± 1.5, $p<0.05$] which alone lowered the basal serum ir-corticosterone but not ir-GH concentration. Pretreatment of the rats with NSS did not affect the resting concentrations of either ir-corticosterone [20 ± 7.3 vs. 36.4 ± 17.4 in SFC, N.S.] or ir-GH [5.92 ± 0.84 vs. 6.4 ± 1.4 in SFC, N.S.]. It also failed to influence the adrenocortical response to IL-1β [834 ± 66 vs. 850 ± 154 in SFC, N.S.] but effectively prevented the rise in ir-GH provoked by the cytokine [4.97 ± 0.5 vs. 10.2 ± 1.5 in SFC, $p<0.05$]. In contrast, administration of LC1-Ab caused a small rise in the basal corticosterone concentration [82 ±.26 vs. 36.44 ± 17 in NSS controls, $p<0.05$] together with a highly significant increase in serum ir-GH [36 ± 14 vs. 6.4 ± 1.4 in NSS controls, $p<0.01$]. The LC1-Ab did not affect the rise in corticosterone provoked by IL-1β [831 ± 191 vs.850 ± 154 in NSS controls, N.S.]. However, surprisingly, after the injection of IL-1β, the rise in ir-GH provoked by the LC1 Ab was no longer apparent. Thus, the serum ir-GH concentrations were indistiguishable in LC1-Ab [4.3 ± 0.4 vs. 36 ± 14] and NSS [5 ± 0.5] treated rats 1h after the injection of IL-1β. The serum ir-LH concentration was unaffected by pretreatment with either NSS or LC1Ab.

The results provide further evidence to suggest that LC1 contributes to the mechanisms controlling the secretion of corticosterone and GH but not LH. The paradoxical responses to IL-1β in LC1-Ab treated rats warrant further study.

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281P ANTISERA WHICH DISTINGUISH THE "STRESSED" AND "RELAXED" FORMS OF CORTICOSTEROID BINDING GLOBULIN

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Corticosteroid binding globulin (CBG) is a plasma protein that binds endogenous glucocorticoids and plays an important role in the regulation of glucocorticoid dynamics (Daughaday, 1956). CBG is thought to exist in a "stressed" S form and to be cleaved to a "relaxed" R form by the action of leukocyte elastase (LE: Pemberton *et al.*, 1988; Hammond *et al.*, 1990). After the cleavage of S-CBG to R-CBG, the affinity of CBG for glucocorticoid falls by 10-fold. Thus it has been postulated that CBG could act as a "delivery system" for anti-inflammatory steroid (Hammond *et al.*, 1987; Pemberton *et al.*, 1988) with CBG being cleaved by LE at sites of inflammation. This would increase the concentration of "free", biologically-active steroid in a localised area. We have now generated a specific antibody that recognises CBG and used this to devise a system to differentially identify the S and R forms, allowing investigation of this novel hypothesis.

A peptide representing residues common to both the S- and R- forms of human CBG (DTAGSTGV) was synthesised with an N-terminal Cys residue to facilitate conjugation to pre-activated keyhole limpet haemocyanin (Pierce & Warriner (UK) Ltd). New Zealand White rabbits were immunised with the conjugate according to conventional protocols and serum samples taken at one month intervals. Specificity and titre were assessed by ELISA using the peptide as a target, or by SDS-PAGE and western blotting techniques. The ELISA showed the antiserum to be specifically epitope-directed with a titre of 1:8 x 10^4. Human CBG was incubated with LE (Calbiochem-Novabiochem (UK) Ltd) in a 10:1 ratio (w:w) at 37°C for 2h. At various time-points cleavage products were assessed by western blotting analysis using the epitope-directed antiserum and a commercial sheep polyclonal antibody (Biogenesis Ltd).

Untreated CBG appears as a doublet on western blots with apparent molecular weight (MW) of 57 and 60 kilodaltons (kDa): these comprise the S-form of CBG (Hammond *et al.*, 1990) and were recognised by both the epitope-directed and commercial anti-CBG antibodies. Within 5 min of incubation with LE, a further band of MW 55 kDa was detectable and at 120 min the intact material was transformed to this product (>95%) as assessed by Coomassie staining. This corresponds to the R-CBG described by Hammond *et al.* (1990) and was highly antigenic for the epitope-directed antibody, but in contrast, showed little reaction to the sheep antibody. Thus when used in combination, the two antisera can qualitatively distinguish the S- and R- forms of CBG and could be used to investigate the role of CBG as a vehicle for glucocorticoid delivery after interaction with leukocyte elastase.

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282P GLUCOCORTICOID-CYTOKINE INTERACTIONS: TYPE II GLUCOCORTICOID RECEPTOR GENE EXPRESSION IN THE THYMUS IS DOWN-REGULATED BY IMMUNE SYSTEM ACTIVATION

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With the elucidation of a feedback loop between the brain and the immune system, the hypothalamus-pituitary-adrenal (HPA) axis has emerged as a major component in the complex circuitry permitting the dialogue between neuronal and immune cells. In conjunction with their cognate receptors, glucocorticoids exert negative feedback effects on the hypothamus-pituitary-adrenal-immune axis. The effects of corticosteroids on the cells of the immune system are mediated through both soluble cytosolic and nuclear type II GR. In both the brain and the anterior pituitary, one of the many actions of this receptor is its involvement in the feedback regulation of the HPA axis during the stress response. Corticosteroids inhibit the cascade of immune and inflammatory reactions at multiple levels via effects mediated directly on immunocompetent cells, and indirectly via effects on leukocyte migration and release of inflammatory mediators. Activation of the immune system following antigenic or mitogenic challenge triggers the release of soluble immune products, including the cytokines interleukin 1 (IL-1), interleukin 2 (IL-2) and interleukin 6 (IL-6), as well as tumor necrosis factors (TNF) and platelet activating factors (PAF), that act at different levels of the brain-pituitary immune axis as signals of the afferent arm of the immune response (Sternberg, 1989). The cytokines released from activated immunological cells can affect not only the activity of the HPA axis, but also trigger a sympathetic reflex mechanism that contributes to the final dynamic regulation of the immune response (Morale et al. 1992).

One major immune compartment where glucocorticoid exert their effects is the thymus gland, the key organ of the immune system. Recently, we have demonstrated the sex steroidal modulation of type II GR within the rat thymus gland (Peiffer et al. 1991). In order to investigate the mechanism whereby cytokine-induced activation of the HPA during cytokine activation results in immunosuppression, we have assessed changes in steady-state levels of GR mRNA during cytokine activation. For this end, we have used a 2.2 kb probe which corresponds to the 3'-end of the rat GR messenger RNA and identified a 6.5-7.0 kb GR mRNA of the same size of that revealed in hippocampus, liver and anterior pituitary gland. To study changes in GR transcript during immune axis activation animals were immunized with multiple injections of Freund complete adjuvant in BSA, and rats were sacrificed during the ascending and descending phases of the immune response. The present results show that products released during immune system activation have potent effects on type II GR expression within the thymus. The relative abundance of GR mRNA transcript levels, expressed in arbitrary units of the GR mRNA/ß-actin mRNA ratio, passed from 0.8 ± 0.02 in sham controls, to 0.5 ± 0.02, 0.4 ± 0.01, 0.15 ± 0.01, and 0.75 ± 0.03, at 3,7,14 and 22 days post-injection, respectively. Such sharp down-regulation (70 % loss) of the signal observed at 7 and 15 days suggests a major role of type II GR expression in the HPA-immune axis loop.

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283P INHIBITION OF BRADYKININ-STIMULATED PROSTACYCLIN RELEASE FROM ENDOTHELIAL CELLS FOLLOWING CHRONIC EXPOSURE TO SODIUM NITROPRUSSIDE

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Endothelium derived relaxing factor (EDRF) has been suggested to have a role in the feedback control of release from endothelial cells of another vasodilator, prostacyclin (PGI_2). We have previously demonstrated the bradykinin-stimulated release of PGI_2 from cultured endothelial cells to be inhibited by sodium nitroprusside, which mimics the action of EDRF (Kondo *et al.*, 1989), and enhanced by L-N^G-nitroarginine, which inhibits EDRF formation, following acute exposure (Keen *et al.*, 1990). In this study the effect of chronic exposure to sodium nitroprusside on basal and bradykinin-stimulated release of PGI_2 from the bovine endothelial cell line AG4762 has been examined. In addition to nitric oxide, sodium nitroprusside also releases cyanide ions which may cause cell death. We have therefore included sodium thiosulphate in combination with sodium nitroprusside to assess the involvement of these ions in its effect.

AG4762 cells (passage 16-23) were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum and 1.5mM glutamine and plated out onto 15mm multiwells. Confluent cells were treated chronically (18h) with saline, sodium nitroprusside (500μM), sodium thiosulphate (500μM) or sodium nitroprusside (500μM)/sodium thiosulphate (500μM) in complete medium. The cells were then incubated for 10min at 37°C in DMEM/Ham's Nutrient Mixture F-12 (1:1) containing 0-100nM bradykinin. The release of PGI_2 was assessed by radioimmunoassay of the stable hydrolysis product 6-keto-$PGF_{1\alpha}$ (Crossman *et al.*, 1987). Significance was assessed according to the Student's t-test.

Table 1 Bradykinin-stimulated PGI_2 release from AG4762 cells (mean ± s.e.mean, n=6)

Bradykinin (nM)	ng 6-keto-$PGF_{1\alpha}$/mg protein 0	30	100
saline	2.0±0.7	11.1±4.2	10.5±4.2
sodium nitroprusside (500μM)	1.6±0.8	3.6±0.9	3.3±0.8
sodium thiosulphate (500μM)	2.7±1.1	9.5±3.5	10.3±3.5
sodium nitroprusside (500μM)/sodium thiosulphate (500μM)	0.8±0.2	2.6±0.7	3.1±0.9

Basal levels of PGI_2 released were not significantly affected by any pretreatment. Sodium nitroprusside significantly inhibited release of PGI_2 from AG4762 cells in response to bradykinin by ~55%. The inhibition caused by sodium nitroprusside was not restored by sodium thiosulphate and was therefore unlikely to be the result of cell death induced by cyanide ions. These results suggest a reduction in the release of PGI_2 from vascular endothelial cells following prolonged exposure may contribute to the development of tolerance to sodium nitroprusside.

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284P ENDOTHELIN 1 AND HUMAN PRO-ENDOTHELIN 1 INDUCE PROSTACYCLIN RELEASE FROM RAT VAS DEFERENS

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Endothelin 1 (ET-1) is a product of the proteolytic processing of pro-endothelin (Big ET-1) by an Endothelin-Converting Enzyme. The *in vitro* vasoconstrictor activity of Big ET-1 is at least 100-fold less than ET-1, whereas in vivo both peptides show a similarly effective pressor response, indicating that the conversion of Big ET-1 to ET-1 is essential for biological activity. Another well-documented activity of both ET-1 and Big ET-1 is the capacity to induce an increase of prostacyclin (PGI_2) levels in plasma and in the perfusate of some isolated vascular beds in vitro (i.e. renal, mesenteric, pulmonar vascular beds). It was proposed that the induction of PGI_2 release is one of the way for the autoregulation of vasoconstrictor activity of ET-1.

In the current study we have studied the capacity of ET-1 and human Big ET-1 to promote PGI2 release in the rat vas deferens (RVD) and the effect of phosphoramidon (PHO) on both peptides. Briefly, rats were killed by cervical dislocation and the vasa deferentia were excised, cut in 4-5 mm pieces and put into a tube containing Krebs solution. The tubes were maintained at 37° C and oxygenated (95% O_2, 5% CO_2) throughout the experiment. After three washes at 15 min intervals, the tissues were incubated with vehicle or 50 μM PHO for 20 min. After this period Arachidonic acid (AA), ET-1 or Big ET-1 were added in order to obtain a final concentration of 10 μM, 5 nM and 100 nM, respectively. After an additional 20-min incubation, the tissues were removed and weighted and the incubation medium was stored at -80° C until assay. Released PGI_2 was measured by radioimmunoassay, as 6-keto $PGF_{1\alpha}$.

The effects of AA, ET -1 and Big ET-1 on PGI_2 release in RVD are reported in Table 1. Basal PGI_2 release was 188±13 pg/mg tissue (n=10). PHO did not modify significantly PGI_2 release (193±26 pg/mg tissue; n=8). AA, used as positive control, induced a PHO-resistant PGI_2 release. ET-1 induced a marked increase in PGI_2 release from RVD. This stimulation was not affected by pretreatment with PHO. Big ET-1, at 20-fold higher concentration, increased PGI_2 release to the same extent. However, this effect was pratically suppressed by pretreatment with PHO.

These results indicate: a) RVD releases significant amounts of PGI_2. b) Both ET-1 and Big ET-1 are able to stimulate PGI_2 release also from rat was deferens. c) The activity of Big ET-1 is dependent on a PHO-sensitive enzyme.

Moreover, the inhibition by PHO of Big ET-1 induced release (without affecting significantly ET-1 response), reinforces the concept that an enzyme-mediated conversion to ET-1 is required for the biological actions of the pro-endothelin.

Table 1: Effects of AA, ET-1 and Big-ET-1 on PGI_2 release:

	PHO (50 μM)	PGI_2 (pg/mg tissue over baseline)
AA		
	-	144±36
	+	123±30
ET-1		
	-	109±7.1
	+	131±22
Big ET-1		
	-	145±42
	+	15±5.7*

* $p<0.001$ vs (-) PHO; non-paired Student's t test (n=8-12)

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285P EVIDENCE FOR LIGAND-MEDIATED INTERNALIZATION OF PROSTACYCLIN RECEPTOR IN HUMAN PLATELETS

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The possible internalization of prostacyclin (PGI_2) receptor was investigated in human washed platelets by means of binding kinetic studies at different temperatures and by means of cell washes with acid buffer. When cells, labelled by 10 nM ^{3}H-Iloprost, were incubated at 37°C, steady-state was reached in 40 min. Adding an excess unlabelled iloprost results in a time-dependent loss of radioactivity; however platelets maintained at 37°C lost 71±4% of cell associated radioactivity, while cell shifted to 4°C lost only 53±1.5% of the same parameter. The remaining 18% was lost upon shifting the platelets to 37°C, suggesting that the shift of platelets to 4°C prevents the dissociation of the internalized component of the receptor. These results were confirmed using a method that involved removal of cell surface binding by acid washes (acid wash removed ^{3}H-Iloprost 51% and 24% of the total in cells incubated at 4°C and 37°C respectively).

In order to investigate the fate of the internalized receptor, platelets were preincubated at 37°C and at 4°C with and without 10 nM cold ligand, than subjected to subcellular fractionation followed by binding of 10 nM ^{3}H-Iloprost to each subcellular fraction. Cells preincubated at 37°C without ligand display 45.7±1.06% of the total radioactivity in the membrane fraction, while platelets preincubated at the same temperature with 10 nM Iloprost show 13.3±1.73% in membranes and 86.7±7.18% in cytosol. No differences were observed when cells were preincubated at 4°C where 61±2.03% of the total radioactivity is associated with the membrane fraction and 39±0.95% with the cytosolic fraction. These results indicate that internalization is mediated by the interaction of the ligand with the receptor and that the receptor is translocated from the plasma membrane fraction to a lighter or soluble fraction.

The process is dependent on the concentration of the ligand, as when platelets were preincubated with different concentration of cold iloprost a dose-dependent loss of surface specific radioactivity was observed. Internalization of prostacyclin receptor is a time-dependent process as well, reaching the maximal extent in 120 min, and is dependent on cell energy. Indeed, it is prevented by 2-deoxy-d-glucose, a competitive substrate blocking the entry of sugars into the glycolytic pathway and by 2,4 dinitrophenol, a de-coupling agent. In order to investigate the pathway followed by the ligand after its internalization, we performed HPLC studies which show that internalization of the ligand receptor complex does not bring about any ligand metabolism.

It is concluded that PGI_2 receptor desensitization in human platelets involves internalization by an energy-dependent process.

286P INHIBITORY EFFECT OF ILOPROST ON INTERNAL CALCIUM CONCENTRATION IN α-THROMBIN ACTIVATED HUMAN PLATELETS

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The synthetic prostacyclin analogue iloprost is a very potent inhibitor of platelet aggregation in vitro (Stürzebecher & Losert, 1987). Iloprost specifically interacts with the prostacyclin platelet receptor and activates adenylyl cyclase. Long pre-exposure to prostacyclin stable analogues such as iloprost induces a decrease in iloprost-mediated cyclic AMP production (Alt et al., 1986) and iloprost binding sites without reduction of the binding affinity (Jaschonek et al., 1988), suggesting a receptor-mediated desensitization. The aim of our study is to characterise a) the effect of iloprost on internal calcium concentration ($[Ca^{2+}]i$) at rest and after α-thrombin activation in human platelets and b) the effect of iloprost on $[Ca^{2+}]i$ at rest and after α-thrombin activation in iloprost pre-exposed human platelets.
Platelet rich plasma (PRP) was prepared from human healthy volunteers' blood and incubated with 3 μM Fura-2-AM. Platelets were washed and suspended in Hepes buffer containing 100U/l apyrase and 10 μM indomethacin. $[Ca^{2+}]_i$ was calculated according to Pollock and Rink, (1986). Iloprost was added to platelet suspension 2 min before α-thrombin. In some experiments, PRP was incubated with 35 ng/ml iloprost or iloprost solvent for 3 h; platelets were then washed twice and iloprost was added 2 min before α-thrombin.
Basal $[Ca^{2+}]i$ was 133±3 nM (n=52), 131±6 nM (n=25) and 140±11 nM (n=5) in normal platelets, in iloprost-pre-exposed platelets and in iloprost solvent pre-exposed platelets respectively. The increase in $[Ca^{2+}]i$ induced by α-thrombin was concentration-dependently inhibited by iloprost (IC_{50}=0.42 ng/ml). Exposure to iloprost (35 ng/ml for 3 h) induced a statistically significant shift of the iloprost inhibitory curve to the right (IC_{50}=1.78 ng/ml) without reduction of the maximal inhibitory effect, which was reached at 30 ng/ml.
Our data show that a) iloprost inhibits α-thrombin induced increase in $[Ca^{2+}]i$ in human platelets; b) in iloprost exposed platelets a desensitized state is obtained; c) the desensitization is overcome by increasing iloprost concentration. These results suggest possible interactions between prostacyclin stable analogues and other drugs and/or physiopathological conditions modulating internal calcium homeostasis.

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287P DIFFERENTIAL ROLE OF PLATELETS AND OF CYCLOOXYGENASE METABOLITES ON PAF-INDUCED PULMONARY VASCULAR HYPERTENSION AND BRONCHOCONSTRICTION IN GUINEA PIG

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PAF is a potent activator of platelet aggregation and causes both acute bronchoconstrictive and pulmonary vascular hypertensive responses. The mechanisms by which these effects are mediated are still being debated and discrepancies exist between *in vivo* and *in vitro* results (Goldstein *et al.*, 1991; Munoz *et al.*, 1991). To clarify further the role of platelets and of cyclooxygenase metabolites on both bronchial and pulmonary vascular effects of PAF, we studied the pulmonary responses to PAF on the guinea pig heart-lung preparation (HLP) that occupies an intermediate position between the *in vitro* and the *in vivo* situations.

HLPs were performed on male Dunkin Hartley guinea pigs, according to the previously described method (Bedetti *et al.*,1989). The preparations were perfused with homologous blood (BL) or with a medium devoid of platelets, consisting of washed red blood cells suspended in a modified Tyrode solution (RBC). PAF (2-96 ng) was administered by bolus injection into the venous cannula. The effects of indomethacin (10 μg/ml in the perfusing fluid) on the responses to PAF were studied in two separate groups of experiments. Statistical differences between dose-response curves were calculated by covariance analysis and the potency-ratios were estimated according to parallel line or slope ratio assays (Finney, 1952).

Administration of PAF produced an increase in ventilation overflow and a rise in pulmonary arterial pressure. In all experimental groups, dose-response relationships were estimated for the bronchoconstrictor and the pulmonary hypertensive responses. In HLPs perfused with RBC, both bronchial and pulmonary vascular responses to all doses of PAF were diminished to the same extent (46 and 47%, respectively, according to potency-ratio assay) when compared with the preparations perfused with BL. In HLPs perfused with BL to which indomethacin was added, a slight reduction (23%; confidence limits 8-35) of the bronchoconstriction induced by PAF was observed. On the contrary, a strong reduction (90%; confidence limits 68-100) of the pulmonary hypertensive responses to PAF was observed in the presence of the cyclooxygenase inhibitor. In the preparations perfused with RBC containing indomethacin, the pulmonary hypertensive effects of PAF were almost completely abolished, whereas a dose-response curve for bronchoconstrictor responses was still obtained.

In conclusion, the present results show that both bronchial and pulmonary vascular responses induced by PAF in the guinea pig are partially dependent on the presence of platelets. Furthermore, these data provide evidence that the pulmonary hypertensive effects of PAF are totally dependent on the formation of cyclooxygenase metabolites, which however are only partially responsible for the bronchoconstrictor responses. This supports the hypothesis that different mechanisms regulate the reactivity to PAF at the bronchial and pulmonary vascular level.

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288P FATTY ACID COMPOSITION AND ACTIVATION OF INOSITOL PHOSPHATE AND ARACHIDONIC ACID METABOLISM BY PAF IN ASTROGLIAL AND IN NEUROBLASTOMA SK-N-BE CELLS

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The activation of phospholipases leads to the formation of products such as Platelet-Activating Factor (PAF) and arachidonic acid (AA) metabolites, which are important mediators of physiopathological events in the CNS.
The aim of our study was first to characterize the lipid composition of astroglial and neuroblastoma SK-N-BE cells. Secondly, to study the inositol phosphate and arachidonic acid pathways generated after phospholipase activation by PAF. Astroglial cells were obtained from 1 day old rat brain cortex (Booher and Sensenbrenner, 1972), SK-N-BE cells were differentiated with 10μM retinoic acid and compared with the undifferentiated ones. The inositol phosphate pathway was evaluated by incubating the cells with myo-[^{3}H]inositol for 18 hours. After PAF stimulation, the supernatants were extracted and analyzed by HPLC connected with a detector for the evaluation of labeled inositol phosphates. In astroglial cells maximal production of inositol phosphates and formation of AA-cycloxygenase metabolites was obtained at PAF concentration 10^{-8}M for 1 min stimulation (Fig.1),whereas in SK-N-BE inositol phosphate production and AA metabolism were less active.

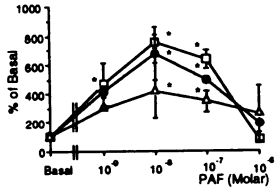


Fig.1 . —□— IP_1 ; —●— IP_2 ; —△— IP_3 .

Inositol phosphate formation after incubation of labeled astroglial cells with different concentrations of PAF.Values, expressed as percentage of basal values are the averages ± S.E. of data obtained from three different experiments. * Statistically different from basal value according to Dunnett's test.

These cells also differently incorporated [^{3}H]-AA and [^{14}C]-docosahexaenoic acid (DHA) over a 48 h period, in phospholipid classes separated by HPLC, connected on line with a radiodetector. In astroglial cells AA was mainly incorporated in diacyl-phosphatidyl-choline (PC) and in phosphatidyl inositol (PI), whereas DHA was mainly incorporated in alkyl-acyl-PC, phosphatidyl serine (PS) and ethanolamine (PE). In SK-N-BE cells, AA levels were partly dependent upon differentiation: in undifferentiated cells AA was 7.5 %, and after differentiation it reached 14 %, suggesting either greater incorporation of the FA in membranes highly enriched with AA, formed after differentiation, or greater formation of AA from precursors ($p<0.025$). It is concluded that astroglial cells differentially incorporated labeled AA and DHA. PAF activates the inositol phosphate and arachidonic acid pathways.

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289P NEUROPEPTIDERGIC ACTIVATION OF SENSITIZED GUINEA-PIG ALVEOLAR MACROPHAGES

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Several studies indicate that alveolar macrophages (AMs) play a key role in the inflammatory response associated with bronchial asthma (Rankin, 1989) and suggest neuropeptides as important mediators of bronchoconstriction and airway hyperresponsiveness (Barnes, 1991). We previously showed that mammalian tachykinins dose-dependently activate guinea-pig AMs by interacting with NK-2 receptor mainly (Brunelleschi *et al.*, 1990) and that AMs isolated from sensitized guinea-pigs show an increased responsiveness to NK-2 receptor stimulation, but not to the bacterial peptide FMLP (N-formylmethionyl-leucyl-phenylalanine) (Brunelleschi *et al.*, 1992). The present study was undertaken to evaluate whether or not this enhanced sensitivity was specific for NK-2 receptor stimulation and to assess the biochemical mechanisms involved in this varied responsiveness. AMs were harvested from ovalbumin-sensitized guinea- pigs and purified by adhesion as previously described (Brunelleschi *et al.*, 1992). Superoxide anion (O_2^-) production was determined spectrophotometrically at 37° C by measuring superoxide dismutase (SOD)-inhibitable cytochrome C reduction and was expressed as nmoles cytochrome C reduced / 10^6 AMs. Dose-response curves for substance P (SP) and neurokinin A (NKA) were shifted to the left (one and three orders of magnitude, respectively) in AMs isolated from sensitized animals, with no variation in maximal effects, while the one for [β-Ala^8]-NKA (4-10), a selective NK-2 receptor agonist, was moved leftwards (about four orders of magnitude) and maximal effects were significantly enhanced. No significant variations were observed by measuring O_2^- generation evoked by the calcium ionophore A23187 or the inflammatory mediator PAF (Platelet Activating Factor) in AMs from control or sensitized guinea-pigs. On the contrary, PMA 10 ng/ml induced a larger O_2^- production in AMs isolated from sensitized animals (35 ± 2 vs 22 ± 2 nmoles cytochrome C reduced / 10^6 AMs, n=3) H7, a non-selective protein kinase C inhibitor, was more potent in inhibiting PMA-evoked O_2^- production in control than in sensitized AMs (48 ± 2 % vs 25 ± 3 % inhibition, n=3). Changes in [3H]PDB (phorbol-12,13-dibutyrate) binding have been reported to parallel the changes in protein kinase C activity in macrophages (Ito *et al.* 1988). Preliminary results indicate that NKA 0.1 μM promotes [^{3}H]PDB binding to control AMs (10 ± 3 %, n=3) and seems to be more potent in sensitized AMs (28 ± 2 %, n=3), while FMLP 0.1 μM potentiates [^{3}H]PDB binding to a similar extent in both control or sensitized AMs. These results suggest that a varied regulation of protein kinase C activity may be involved in the enhanced responsiveness to NK-2 receptor stimulation in sensitized AMs.

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290P SYNERGISTIC EFFECT OF RECOMBINANT HUMAN EPIDERMAL GROWTH FACTOR AND DES-ARG^9 BRADYKININ, ON RAT CROTON OIL-INDUCED GRANULATION TISSUE CONTRACTION

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During the course of wound healing granulation tissue forms, the contraction of which is a prerequisite for normal wound closure. Using the rat croton oil-induced air pouch we have previously demonstrated that the contractile response of granulation tissue to endothelin-1 (ET-1) is dependent on the stage of differentiation of the tissue and reaches a peak at day 21. Furthermore this corresponds to the ultrastructural development of myofibroblasts (Appleton *et al.*, 1992). In experimental models of wound healing, epidermal growth factor has been shown to stimulate granulation tissue formation (Laato *et al.*, 1986). In this study we have investigated the possible interactions between recombinant human epidermal growth factor (rhEGF) and a variety of vasoactive mediators on granulation tissue contraction.

Male Wistar rats (150±10g) were subcutaneously injected with 20ml of air and 1ml of 1% croton oil. The granulation tissue was allowed to develop for 21 days. The granulation tissue was excised and paired strips of tissue prepared (each approximately 200mg) and mounted under 1g resting tension in organ baths containing aerated Krebs solution (37°C). Cumulative dose response curves to a variety of agonists were performed with or without prior incubation with 100ngml^{-1} rhEGF for 30 minutes. The concentration of rhEGF used was the lowest concentration which alone did not elicite a contractile response. The agonists tested were ET-1, $PGF_{2\alpha}$, 5-HT, substance P, adrenalin, α-thrombin, AgII, arg^8-vasopressin, bradykinin and des-arg^9 bradykinin. Statistical analysis was performed using Wilcoxon matched pairs test. Of the vasoactive agonists tested only des-arg^9 bradykinin had a synergistic effect with rhEGF $P<0.05$, $1x10^{-7}$M; $P<0.05$, $3x10^{-7}$M; $P<0.01$, $1x10^{-6}$M. No synergistic effect was observed with the other agents tested, as shown below. Results stated are for the highest concentration of each agonist and are expressed as mean ± S.E.M for n=8 seperate determinations.

Agonist	Conc.	Control Tension (mg)	rhEGF Tension (mg)	Agonist	Conc.	Control Tension (mg)	rhEGF Tension (mg)
$PGF_{2\alpha}$	$3x10^{-5}$M	229.3±103.9	346.7±18.7	AgII	$3x10^{-6}$M	61.3±22.7	42.7±31.4
5-HT	$1x10^{-4}$M	50.7±9.6	42.7±11.6	Vasopressin	$1x10^{-6}$M	461.3±2.7	343.7±62.7
Substance P	$1x10^{-6}$M	0	0	Bradykinin	$1x10^{-6}$M	218±35.9	178.7±58.6
Adrenalin	$1x10^{-4}$M	66.7±23.2	49.3±12.7	Des-arg^9 brad	$1x10^{-6}$M	571±85	825±146 ($P<0.01$)
α-thrombin	$1x10^{-7}$M	0	0	ET-1	$1x10^{-7}$M	440±33.1	465±22.3

These results suggest that the beneficial effects of EGF in wound healing, may in part be attributed to its synergistic action on granulation tissue contraction.

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291P NITRIC OXIDE IS AN IMPORTANT MEDIATOR OF THE EXAGGERATED RELEASE OF PROSTAGLANDIN E_2 IN RABBIT HYDRONEPHROTIC KIDNEY

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The rabbit hydronephrotic kidney (HNK) is a model of renal inflammation characterised by a marked increase in arachidonic acid metabolism resulting in an exaggerated release of prostaglandin E_2. Nitric oxide (NO) released from L-arginine (L-Arg) by the inducible form of nitric oxide synthase (NOS) is produced by several cells in the inflamed kidney (Moncada et al., 1992). We recently reported that NO directly activates the constitutive and inducible forms of cyclooxygenase (COX; Salvemini et al., 1993). We have now evaluated the ability of NO to activate PGE_2 release in the HNK model.

Unilateral obstruction was carried out in male New Zealand white rabbits as described previously (Nishikawa et al., 1977). Three days after ureteral obstruction the renal arteries were cannulated, the kidneys excised and perfused at 10ml/min with oxygenated ($95\%O_2/5\%$ CO_2) Krebs-Henseleit medium at 37°C. Bradykinin (BK) was injected as a bolus (1μg) intraarterially every hour from 0 to 6h of perfusion to stimulate PGE_2 release from the kidney. The venous effluent was collected for 3 min both before and after every BK injection and assayed for PGE_2 production by radioimmunoassay and for nitrites (NO_2^-) by the diaminonapthalene assay (Misko et al., 1993).

BK stimulation revealed a time-dependent increase in PGE_2 release (473±143, 539±121, 880±143 and 3069±946 pg/ml/min at 0, 1, 3 and 6h, n=8) with no change in basal release (77pg/ml/min). However, in the absence of BK, we observed a time-dependent release of NO_2^- (38±8, 60±7, 150±4 and 180±10 pmol nitrites/30μl at 0, 1, 3 and 6h, n=8). The potent anti-inflammatory steroid, dexamethasone (10μM, n=5) abolished the release of PGE_2 and NO_2^- consistent with the observation that dexamethasone selectively inhibits the inducible forms of both NOS (Moncada et al., 1992) and COX (Masferrer et al., 1992). The increase in PGE_2 or NO_2^- was inhibitable by cycloheximide (5μg/ml, n=5) suggesting a requirement for new protein synthesis. N^G-monomethyl-L-arginine (100μM, n=4) blocked NO_2^- release and attenuated the BK-induced PGE_2 release by at least 50%; these effects were reversed by coinfusion with L-Arg but not D-Arg (100μM, n=4). L-citrulline (100μM) formed during the formation of NO by L-Arg, did not modify PGE_2 by BK (n=8).

These results suggest that the endogenous release of NO in the HNK activates an induced cyclooxygenase activity resulting in an increased release of proinflammatory prostaglandins. A nitric oxide driven prostaglandin release further highlights the importance of these mediators in an inflammatory setting where both enzymes are present.

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292P EFFECTS OF COPPER ON PROTEOGLYCAN SYNTHESIS IN PORCINE CARTILAGE-SYNOVIUM CO-CULTURES

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A large body of evidence underlines the role of copper in the pathology of inflammation. The therapeutic value of copper compounds and of copper-containing superoxide dismutase (SOD) has been thoroughly investigated and the results implicate copper as an exogenous anti-inflammatory agent. Additional evidence is provided by the observation that the concentration of endogenous copper significantly changes during the course of many inflammatory processes, both acute or chronic (Milanino *et al.*, 1991). However, the mechanism by which this metal plays its role is far from clear, especially in the case of chronic processes.

In an attempt to gain insight into the mechanism involved, we have investigated the effects of copper in an *in vitro* model of rheumatoid joint inflammation, in which pig articular cartilage explants were co-cultured in direct contact with minced porcine synovium. In rheumatoid arthritis, a chronic inflammatory disease, the articular cartilage is infiltrated by activated synovial cells which, via release of inflammatory cytokines, eventually destroy the joint. During this destructive process, a disturbance of the extracellular matrix equilibrium occurs, resulting in inhibition of proteoglycan (PG) synthesis and increased PG degradation. In the present study PG synthesis was measured by the incorporation of labelled sulphate into PG glycosaminoglycan side-chains. Articular cartilage and synovium were obtained by dissection of the metacarpal-phalangeal joints from pig. All cultures were carried out in serum-free DMEM containing antibiotics. After 24 h the medium was changed and the cultures were incubated for a further 2 h with 0.74 MBq/ml of [^{35}S]-sulphate. After papain digestion (2 h at 65°C), the PG synthesis rate was determined as the $^{35}SO_4^{2-}$ incorporation, expressed as CPM/mg dry weight of the cartilage. Preliminary experiments were performed to assess the toxicity of copper on (i) protein synthesis and (ii) PG synthesis at a range of concentrations. From these data a non-toxic concentration of copper was selected. We have demonstrated that copper sulphate (or chloride, data not shown) added to the medium at concentration of 10 μM was able to directly increase PG synthesis in cartilage cultured along and was able to reduce the inhibition of PG synthesis observed in cartilage co-cultured with synovium. Because of the high variability of response in each group of explants, these results were confirmed by several repeated experiments. A representative experiment is shown in the Figure 1 clearly indicating the significant ($p<0.01$) recovery of PG synthesis in copper-supplemented co-culture. Studies by others have shown that copper may play an important role in the homeostasis of cytokines (Scuderi, 1990). Since it is established that pig synovial cells secrete IL-1 into culture medium and IL-1 inhibits PG synthesis, our results suggest that copper might effect synthesis and/or secretion of IL-1 by the synovial tissue in the co-culture system. Furthermore, stimulation of PG synthesis in cartilage cultured alone or in the presence of synovium indicates the therapeutic efficacy of copper in maintaining cartilage integrity during the inflammatory process.

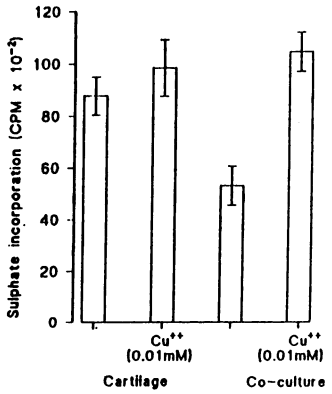


Fig.1: Values are mean ± S.E.M.

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293P RELATIVE IMPORTANCE OF DOPAMINE NEURONAL UPTAKE AND D_2 AUTORECEPTORS IN THE NUCLEUS ACCUMBENS AND CAUDATE PUTAMEN OF THE RAT

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Fast cyclic voltammetry (FCV) was used to compare the contribution of D_2 autoreceptors and dopamine (DA) neuronal uptake to the regulation of electrically evoked DA overflow in the nucleus accumbens (NAc) and caudate putamen (CPu) in rat brain slices. The method used has been reported previously (Trout & Kruk, 1992). Slices were stimulated with either a single pulse (1p; 0.1ms, 20V), or 4p/10Hz, or 20p/20Hz at 2.5 minute intervals, under control conditions and in the presence of drugs. Each slice was superfused with either sulpiride or nomifensin, followed by nomifensin and sulpiride together. The results are expressed as ratios of peak DA overflow following 1p or trains of stimuli, relative to 1p stimulation under control conditions.

Table 1. Effect of nomifensin and sulpiride on electrically evoked DA overflow in the NAc and CPu

	NAc			CPu		
	1p:1p	4p/10Hz:1p	20p/20Hz:1p	1p:1p	4p/10Hz:1p	20p/20Hz:1p
Control	1	1.52±0.1	3.99±0.4	1	1.47±0.1	2.6±0.3
Sulpiride (1μM)	0.99±0.05	1.74±0.2	8.24±1.7##	1.02±0.06	1.61±0.1	3.82±0.6#
Control	1	1.64±0.1	4.66±0.4	1	1.45±0.1	2.29±0.3
Nomifensin (1μM)	3.02±0.3	5.43±0.5	13.12±1.1	3.83±0.6	7.07±1.4	13.31±2.1
Nom(1μM)+Sulp(1μM)	3.66±0.5	8.03±0.8*	20.16±2.1*	4.1±0.7	8.06±1.4	15.77±2.9

Values are a mean ± s.e.mean; n=4 ;# $p<0.05$ ##$p<0.001$ (Cont v Sulp); * $p<0.001$ (Nom v Nom+Sulp),(Students paired t-test)

At 20p/20Hz stimulation, sulpiride potentiated DA overflow in both the CPu and NAc, indicating that D_2 autoreceptors are activated under these conditions. Nomifensin potentiated DA overflow in both regions under all stimulation conditions; addition of sulpiride further increased DA overflow in the NAc but not in the CPu. This indicates that D_2 autoreceptors regulate DA overflow in the presence of a DA uptake inhibitor in the NAc but not in the CPu, and D_2 autoreceptors may be functionally more important for regulating extracellular DA in the NAc than in the CPu.

Trout, S.J. & Kruk, Z.L. (1992) *Br. J. Pharmacol.*, **106**, 452 - 458

294P DIFFERENCES IN DOPAMINE EFFLUX WITH BURSTING PATTERNS OF ELECTRICAL STIMULATION IN BRAIN SLICES OF RAT NUCLEUS ACCUMBENS, MONITORED USING FAST CYCLIC VOLTAMMETRY

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Electrical stimulations applied as short bursts of pulses *in vivo*, have been reported to evoke more dopamine (DA) overflow than the same number of pulses applied at a single frequency (Gonon, 1988). These observations may be significant, since Grace (1991) suggested that phasic and tonic release of dopamine may be differentially regulated. This study investigates the relationship of pattern of electrical stimulation to total dopamine release using a brain slice preparation and fast cyclic voltammetry. The method has been described in detail (Trout & Kruk, 1992). The tissue was stimulated with 20 pulses (20p) applied in a 4 s period every 5 minutes, but in three different patterns: [1] as a regularly-spaced train at 5 Hz, [2] as two bursts of 10p at 10, 20 or 50 Hz (separated by an interval of 2 s), or [3] as four bursts of 5p at 10, 20 or 50 Hz at 1 s intervals. Thus, in each case, 20 pulses were applied within a 4 s period, giving a mean frequency of 5 Hz. The area under the curve of the DA release profile was measured and expressed as a fraction of the area of a 1p stimulus under the same conditions, to standardise results between tissues.

Table 1. Areas under the evoked efflux profile (dopamine concentration with time) for a 20 pulse stimulus, relative to the area of a single pulse stimulus applied in the same tissue. Number of observations = number of brain slices.

Burst parameters	5 Hz	10 Hz	20 Hz	50 Hz
1 x 20 Pulses	3.75 ± 0.37	-	-	-
2 x 10 Pulses	-	4.53 ± 0.50	6.32 ± 0.90 *	6.59 ± 1.29
4 x 5 Pulses	-	4.14 ± 0.36	4.75 ± 0.54	4.80 ± 0.89

Data expressed as mean ± s.e.mean. $n = 5$. * $P < 0.05$, significantly different from regular 20 pulse train at 5 Hz.

DA efflux following two bursts of 10p at 20 Hz was significantly greater than following 20p at 5 Hz. To establish if endogenous DA acting at autoreceptors contributes to this effect, experiments were made in the presence of 1 μM metoclopramide. In the presence of the antagonist, there was no significant difference between efflux evoked by two bursts of 10p at 20 Hz (11.35 ± 2.91) and 20p at 5 Hz (10.61 ± 1.47). This suggests that autoinhibition is at least partly responsible for the regularly spaced 5 Hz train being less efficient at evoking DA release than two bursts of 10p at 20 Hz, two seconds apart.

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295P EFFECTS OF SELECTIVE UPTAKE INHIBITORS ON MONOAMINE EFFLUX IN RAT LATERAL GENICULATE BRAIN SLICES MEASURED USING FAST CYCLIC VOLTAMMETRY

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Fast cyclic voltammetry at carbon fibre microelectrodes (FCV) has been used to monitor stimulated monoamine efflux *in vivo* and *in vitro* (Stamford, 1990). In the present report we used the technique in slices of the ventral lateral geniculate nucleus (LGN) and examined the effects of three selective amine uptake blockers on the efflux and uptake of monoamine.

Slices (350μm) of LGN were prepared from male Wistar rats (100-150g) and superfused with oxygenated artificial cerebrospinal fluid (ACSF) at 32°C at 1.0 ml/min. Monoamine efflux was evoked using trains of electrical stimulation (20 pulses, 0.2 ms, 10 mA, 100 Hz, every 10 min) at bipolar stimulating electrodes and monitored using FCV as previously described (Palij & Stamford, 1992). Experiments lasted for 2½ h and consisted of 15 stimulations. Drugs were added to the ACSF after the third train (S_3).

Electrical stimulation evoked the release of a substance with a voltammogram indistinguishable from that of 5-hydroxytryptamine (5HT) but distinct from those produced by the catecholamines, noradrenaline (NA) and dopamine (DA). Peak amine efflux was equivalent to 21.60± 1.87 nM 5HT (mean ± s.e.m., n=15) and decayed with a half-life ($t_{½}$) of 1798 ± 150 ms (n=16). The efflux and $t_{½}$ were stable for the duration of the experiments. Table 1 shows the effects of the three selective uptake blockers upon stimulated monoamine efflux and uptake half-life ($t_{½}$): Paroxetine (selective for 5HT uptake), desipramine (NA) and vanoxerine (DA).

Drug (μM)	Controls	Paroxetine (0.1)	Desipramine (0.05)	Vanoxerine (0.5)
Efflux	*91±6.6*	*196±23.2 **	*75.4±5.8*	*109.8±11.1*
Uptake $t_{½}$	*103.8±8*	*648±101 **	*166±29.6*	*123.3±12*

*S_{13-15} as percentage of S_{1-3} (mean ± s.e.m., n=4-5). *P<0.05 ,vs all other groups. (Duncan's Multiple Range Test).*

Paroxetine significantly increased stimulated amine efflux and slowed reuptake. Desipramine, at a concentration that potentiates NA efflux (Palij & Stamford, 1992) had no effect on amine efflux but slowed reuptake slightly although not significantly. Vanoxerine, at a concentration that potentiates DA efflux (Hafizi et al, 1992) had no effect. The selective enhancement of efflux by paroxetine but not desipramine or vanoxerine is strong evidence that the released amine is a substrate for synaptic 5HT uptake. The results are consistent with the released substance being 5HT although the modest effect of desipramine on uptake half-life suggests that some released 5HT may be taken up by NA uptake. The data suggests that FCV can monitor 5HT release and uptake in slices of the LGN.

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296P A COMPARISON OF THE EFFECT OF IDAZOXAN ON NORADRENALINE RELEASE IN FREELY-MOVING AND PARTIALLY RESTRAINED RATS

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Central α_2-autoreceptors play an important role in the regulation of synaptic noradrenaline (NA) levels. By blocking these receptors α_2-adrenoceptor antagonists, such as idazoxan (IDX), facilitate NA release (Abercrombie *et al*, 1988). A number of other non-pharmacological factors (e.g. stress) also alter NA release. Since this is likely to result in a change in tone at the autoreceptor, it is possible that the response to α_2-antagonists may be altered. Using the 2-deoxyglucose (2DG) technique, we have previously shown that IDX depresses glucose use in certain brain structures, including the frontal cortex (French *et al*, 1992). This method requires that rats undergo anaesthesia, surgery and partial restraint. The aim of this study was to determine whether these factors alter NA release and, if so, whether this affects the response to IDX. For this purpose, we have used *in vivo* microdialysis to compare the effect of IDX on NA release in freely-moving rats with rats subjected to the techniques of the 2DG method.

Dialysis probes were implanted into the frontal cortex of male Wistar rats (270-310g). Following a 48h recovery period, dialysates were collected every 15min and analysed for NA using HPLC-ECD. In all rats, a stable baseline was established before further manipulation. For the freely-moving groups (n=4 per group), rats were given either IDX (3mgkg^{-1} s.c.) or saline and the response was monitored over the subsequent 75min. For the 2DG procedure groups (n=5 per group), rats were anaesthetised (to allow femoral artery cannulation), partially restrained and allowed to recover for 2.75h. At the end of this period, rats received either IDX or saline. Dialysates were collected continuously throughout the experiment, up to 75min post-injection. Statistical analysis of the raw data was performed using a oneway ANOVA for repeated measures.

In freely-moving animals, IDX significantly elevated basal NA release [F(5)=5.5; p<0.005]. A 2-fold increase in release occurred within 15min and was maintained for 1h post-injection (basal: 2.8±0.4pg/15min; 30min post-IDX: 5.7±0.6pg/15min). In the 2DG procedure groups, the techniques (anaesthesia and surgery, followed by partial restraint) significantly increased NA release [F(12)=8.46; p<0.0001]. Administration of IDX further elevated NA release [F(5)=9.7; p<0.0001], the profile of this response being similar to that of the freely-moving group (basal: 3.2±1.0 pg/15min; pre-IDX: 4.2±1.2 pg/15min; 30 min post-IDX: 8.4±2.1 pg/15min). Saline had no significant effect on NA release in either group. These results show that the techniques inherent to the 2DG method elevate basal NA release but that the magnitude of the response to IDX is similar to that observed in freely-moving rats.

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297P CORRELATION BETWEEN 1-METHYL-4-PHENYLPYRIDINIUM ION (MPP+) LEVELS AND MPTP-INDUCED NEUROCHEMICAL CHANGES IN THE RAT BRAINSTEM

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The neuronal antioxidant system (ascorbic acid, glutathione) may play a role in limiting MPTP neurotoxicity (Desole et al., 1993). MPTP selectively damages the nigrostriatal system, but impairs also the functioning of other central neurochemical systems, including the noradrenergic (Hallman et al., 1984) and serotonergic (Chiueh et al., 1984).

Levels of noradrenaline (NA), 5-HT, 5-HIAA, ascorbic acid (AA), dehydroascorbic acid (DHAA), uric acid (UA) and MPP+ were determined by HPLC in the brainstem of young male Wistar rats, after single injections of MPTP, according to the method previously described (Desole *et al.*, 1993) (Table 1).

Table 1 - Levels of MPP+, NA and UA (pmol/mg protein, mean ± S.D.), 5-HIAA/5-HT and DHAA/AA ratios (mean± S.D.) in the brainstem of 3 month old rats given MPTP 35 mg/kg i.p. (n= 8/group).

	Controls	Sacrifice after MPTP				ANOVA (P)
		1h	3h	8h	24h	
MPP+	--	354.5±0.133	128.8±39.3	49.1±21.7	19.6±5.07	
NA	53.0±7.21	31.5±5.00*	37.2±3.00*	38.4±4.13	50.6±4.11	<0.000001
UA	33.9±2.46	61.9±17.2*	41.0±3.25*	33.4±3.28	30.0±2.48	<0.000001
5-HIAA/ 5-HT ratio	0.730±0.07	0.238±0.03*	0.543±0.08*	0.571±0.08*	0.655±0.08	<0.000001
DHAA/AA ratio	0.101±0.01	0.081±0.04	0.116±0.03	0.137±0.05*	0.136±0.4*	<0.02

(*) P<0.05 vs controls. Pearson's correlation coefficient between: a) individual levels of MPP+ vs: NA, r=-0.712, P<0.00001; UA, r= -0.941, P< 0.00001; 5-HIAA/5-HT ratio values, r= -0.811, P<0.000001; DHAA/AA ratio values, r=-0.491, P<0.02; b) individual DHAA/AA ratio values vs: 5-HIAA/5-HT values, r= +0.399, P<0.03; NA levels, r = +0.318, P = 0.075.

We conclude that in the rat brainstem MPP+ levels well correlate with the increase in AA oxidation and the changes in NA and UA levels and 5-HIAA/5-HT ratio. *(Supported by MURST 40% and 60%).*

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298P MODULATION OF FOOD INTAKE BY α_2-ADRENOCEPTOR ANTAGONISTS AND I_2-IMIDAZOLINE DRUGS IN RATS: LSL 60101 AS A NOVEL AND SELECTIVE LIGAND FOR I_2-IMIDAZOLINE SITES

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Central monoaminergic neurotransmitters (NA; 5-HT) and receptors (α_2-adrenoceptors, α_2R; 5-HT_{1A}-receptors) have been implicated in the control of food intake in various animal species. Recently, the α_2R antagonist idazoxan has been shown to induce an unexpected hyperphagic effect in freely-feeding rats during the daylight phase, a central effect apparently not shared by other more selective α_2R antagonists (e.g. 2-methoxy idazoxan or RX821002) and which has been linked to the high affinity of idazoxan for the newly discovered non-adrenoceptor idazoxan binding sites or I_2-imidazoline sites (I_2R) (Jackson et al., 1991). The purpose of this study was to re-assess the effects of idazoxan and RX821002 on food intake under various experimental conditions and in comparison with the novel I_2R ligand LSL 60101 to further evaluate the involvement of I_2R in feeding behaviour in male Sprague-Dawley rats. In receptor binding studies, LSL 60101 (2-(2-benzofuranyl) imidazole hydrochloride) had a lower affinity than idazoxan for brain I_2R (Ki: 350 nM versus 14 nM) but a much higher I_2R/α_2R selectivity (Ki ratio: 280 versus 0.8). During the light period (minimal food intake) and as expected, idazoxan (10 mg/kg, i.p.) but also the more selective or structurally diverse α_2R antagonists RX821002 (3 mg/kg, i.p.) and yohimbine (10 mg/kg, i.p.) induced significant (at least P<0.05) increases in food intake over 4 h (6.4±0.7, 3.7±0.7 and 14.6±2.4 g/kg rat weight, respectively; n=6-14) compared to freely-feeding control rats (0.7±0.2 g/kg rat weight; n=14). LSL 60101 also produced in a dose-related manner (3-30 mg/kg, i.p.) a significant increase in food intake over the 4 h of the study (30 mg/kg: 13.1±1.4 g/kg rat weight, P<0.05, n=14). Food intake over 24 h was unaffected by these drugs. In contrast, during the dark period (maximal food intake), idazoxan and LSL 60101 did not induce hyperphagia over 4 h (34±4 and 25±5 g/kg rat weight, respectively, n=6-7) compared to control rats (30±1 g/kg rat weight). Similarly but during the light period, idazoxan and LSL 60101 did not modify food intake in food-deprived (20 h) rats (63±3 and 65±4 g/kg rat weight, respectively, n=10) compared to control rats (67±4 g/kg rat weight, n=10). In another series of experiments the relevance of I_2R in feeding behaviour during the light period was evaluated after the preferential alkylation of brain α_2R with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 1 mg/kg, i.p., 6 h) (Miralles et al., 1993). In EEDQ-treated rats the hyperphagic effects induced by idazoxan (5.2±0.9 g/kg rat weight, n=18), RX821002 (1.8±0.8 g/kg rat weight, n=12) and yohimbine (9.8±0.9 g/kg rat weight, n=11) were significantly attenuated (20-50%, P<0.005). In contrast, EEDQ treatment did not alter the increase in food intake induced by LSL 60101 (15.2±2.5 g/kg rat weight, n=11). These results further suggest that α_2R antagonists (directly or through the 5-HT system) and I_2R ligands modulate feeding behaviour in rats. While idazoxan-induced feeding may involve both α_2R and I_2R, the hyperphagic effect induced by LSL 60101 appears to involve activity only at I_2R.

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299P COMPARATIVE TISSUE CONCENTRATIONS OF HISTAMINE AND NORADRENALINE IN MAST CELL-DEFICIENT (W/W^V) AND NORMAL CONGENEIC (+/+) MICE

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To examine whether a relationship exists in the tissue distribution of two amines we quantitated these amines in various organs of the genotype mice, using an HPLC. Histamine (H) and noradrenaline (NA) were determined with a fluorescent (Vohra, 1985) and an EC detector (Chaudhry and Vohra, 1985), respectively. Also, mast cells were counted under a microscope after staining with toluidine, Victoria or Astra blue (see Pearse, 1968). The results summarized in the Table show that various organ weights in the two strains were essentially the same.

TABLE. Noradrenaline and Histamine Content and Organ Wet Weights of Tissues of Congeneic +/+ and $WBB6F_1/J\text{-}W/W^V$ Mice

Organs	Organ Weights (mg) +/+	Organ Weights (mg) W/W^V	Noradrenaline Content(μg/G) +/+	Noradrenaline Content(μg/G) W/W^V	%B	Histamine Content(μg/G) +/+	Histamine Content(μg/G) W/W^V	%B
Heart	207 ± 9	200 ± 10	0.66 ± 0.06	0.74 ± 0.03	112	3.00 ± 0.31	1.60 ± 0.17^	53
Brain	460 ± 7	430 ± 13	0.55 ± 0.06	0.57 ± 0.04	104	0.86 ± 0.09	0.62 ± 0.04^	72
External Ear	117 ± 5	109 ± 7	0.09 ± 0.01	0.09 ± 0.01	100	58.00 ± 8.90	2.00 ± 0.16^	3
Spleen	114 ± 7	110 ± 24	1.10 ± 0.12	0.88 ± 0.13	80	3.10 ± 0.49	1.50 ± 0.19^	48
Lung	211 ± 18	198 ± 10	0.17 ± 0.03	0.12 ± 0.13	71	1.50 ± 0.21	0.64 ± 0.05^	43
Aorta	25 ± 2	27 ± 5	0.61 ± 0.23	0.50 ± 0.27	82	5.60 ± 0.88	0.97 ± 0.08^	17
Vas Deferens	21 ± 2	20 ± 1	10.30 ± 1.00	11.10 ± 1.25	108	14.10 ± 2.70	0.84 ± 0.33^	6
Seminal Vesicle	104 ± 6	95 ± 4	0.91 ± 0.11	1.09 ± 0.09	120	5.80 ± 1.10	0.47 ± 0.07^	8

Values are mean ± SE based on 8 mice in each group; whole body weights of +/+ and W/W^V were 32.9±1.0 and 31.0±0.8g, respectively.
^ Values significantly different from those of the congeneic +/+ controls ($P<0.05$).
B Represents values of W/W^V as % of +/+.

NA concentrations were also identical in all of the organs examined of both genotypes. As expected, H concentrations were significantly lower in the organs of W/W^V than those of +/+ mice. While the ears, vas deferens and seminal vesicles of W/W^V had ~ 3-10% as much H as those of +/+ mice, the brain, heart, lung and spleen of the W/W^V contained ~40-70% as much H as those of the +/+. The ears of W/W^V mice contained <1% as many mast cells as did those of +/+ mice. No mast cells were detectable, however, in other tissues of W/W^V mice. Thus, the tissue concentration of H and NA do not correlate in either genotype and either there is a variable amount of non-mast H in different organs or the organs of W/W^V mice contain atypical mast cells which do not stain with the above dyes. (Supported by MRC Canada.)

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300P FURTHER EVIDENCE FOR THE INDUCTION OF NON-ENDOTHELIAL NO-SYNTHASE IN THE ATHEROSCLEROTIC RABBIT AORTA

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Atherosclerotic lesions reduce the endothelium-dependent relaxations in experimental animal models and in humans (Verbeuren, et al., 1986 ; see Verbeuren, 1991). The contractile responses to 5-hydroxytryptamine (5-HT) are enhanced in experimental atherosclerosis (Henry and Yokoyama, 1980), however those to other vasoconstrictors such as alpha-adrenoceptor agonists are reduced (Verbeuren et al., 1986). We recently reported that hypoxia causes an endothelium-independent contraction in the atherosclerotic rabbit aorta by inhibition of nitric oxide (NO)-synthase (Simonet et al., 1993). The present experiments were designed to further study this non-endothelial NO-synthase.

Rabbits were fed a control or a cholesterol-rich (0.3 %) diet for 30 weeks. After anesthesia (sodium pentobarbital 30 mg/kg i.v.) rings with or without endothelium (E) of thoracic aortas were prepared for isometric tension recording in organ chambers filled with oxygenated Krebs-Ringer solution at 37°C. In some experiments, the aortic tissues were incubated for 15 hours prior to experimentation in solution containing interleukin 1β (IL-1β ; 30 U/ml). In atherosclerotic aortas with and without E, the NO-synthase inhibitor nitro-L-arginine (L-NNA ; 100 μM) causes contractions (0.49 ± 0.09 g and 0.49 ± 15 g respectively ; n=12) that were not observed in control aortas. L-NNA did not alter the contractions to noradrenaline (0.03 μM) in control aortas and only augmented those to 5-HT (0.3 μM) in control aortas with E. In atherosclerotic aortas with and without E, L-NNA markedly enhanced the contractions to both noradrenaline (0.1 μM) and 5-HT (0.1 μM) (by 89 ± 26 % and 90 ± 33 % in aortas with E and by 76 ± 21 % and 101 ± 20 % in aortas without E respectively, n=5). Both L-NNA (1-100 μM) and mono-methyl-L-arginine (L-NMMA ; 1-100 μM) dose-dependently augmented the contractions to noradrenaline in atherosclerotic aortas with and without E with similar potency and these effects were reversed by L-arginine (1 mM). In control aortas, treatment with IL-1β caused marked reductions in the contractile responses to noradrenaline (-42 ± 4 % ; n=4) ; in the atherosclerotic aortas, IL-1β did not further reduce the responses to noradrenaline (+23 ± 12 %).

The data obtained with the NO-synthase inhibitors offer support for our previous suggestion (Simonet et al., 1993) that NO-synthase has been induced in non-endothelial cells of atherosclerotic rabbit aortas. This concept, is reinforced by the observation that IL-1β, which induces NO-synthase in control vascular smooth muscle cells (Schini et al., 1991) and in control rabbit aortas, is no longer actif in the atherosclerotic aortas. The NO derived from this inducible NO-synthase may be at the origin of the reduced contractions to e.g. alpha-adrenoceptor agonists in atherosclerosis.

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301P ENDOTHELIUM-DERIVED NITRIC OXIDE ATTENUATES THE INHERENT TONE IN ISOLATED PULMONARY ARTERIES FROM RATS WITH HYPOXIC PULMONARY HYPERTENSION

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Isolated pulmonary artery preparations from rats with chronic hypoxic pulmonary hypertension (PH), unlike preparations from normal rats, appear to have inherent tone (Wanstall & O'Donnell, 1992). A previous study provided indirect evidence that this tone may be attenuated by the endothelium (Wanstall *et al.*, 1992). The aims of this study were to quantify the inherent tone and investigate, directly, the influence of the endothelium on this tone.

Male Wistar rats were housed in room air (control rats) or in 10% oxygen (hypoxic rats) for 2 weeks. The hypoxic rats had elevated pulmonary artery pressures, 30±1.5 mmHg cf. 17±2.1 in controls (mean±s.e.mean; n=14; P<0.05). Inherent tone in ring preparations of main pulmonary artery (with and without endothelium; confirmed with acetylcholine) was quantified as the relaxation, from resting baseline, produced by replacing the physiological salt solution (PSS) with Ca^{++}-free PSS containing 2 mM EGTA. Tone was seen in preparations from hypoxic, but not control, rats and was greater in the absence than in the presence of the endothelium. In separate preparations, with endothelium, the effects of the nitric oxide (NO) synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 0.1-100 μM) were examined. Contractions to L-NAME were seen only in preparations from hypoxic rats. Contractions to 80 mM K^+, obtained in all preparations, were smaller in hypoxic rats than in controls, possibly because the preparations were already partially contracted due to the inherent tone. All values in Table 1 are force/cross sectional area (mN/mm^2).

Table 1		Inherent tone	Max contraction to L-NAME	K^+ contraction
Control rats	+ endothelium	0.2±0.2 (n=5)	0.8±0.4 (n=4)	30.7±2.3 (n=9)
	- endothelium	0.7±0.2 (n=5)		26.3±3.3 (n=5)
Hypoxic rats	+ endothelium	9.1±1.2 (n=5)#	7.4±0.7 (n=4)#	16.8±1.9 (n=9)#
	- endothelium	14.5±1.9 (n=5)*#		8.9±1.0 (n=5)*#

* P < 0.05 - endothelium vs. + endothelium. # P < 0.05 Hypoxic rats vs. Control rats

Thus the inherent tone in main pulmonary arteries from rats with hypoxic PH is attenuated, but not eliminated, by an endothelium-derived factor that is probably NO. This *in vitro* finding is compatible with the suggestion that, in rats with hypoxic PH, there may be continuous release of NO from the pulmonary vascular endothelium *in vivo* (Wanstall *et al.*, 1992). If the inherent tone in the pulmonary vascular smooth muscle contributes to the elevated pulmonary artery pressure in these rats, the PH is likely to be exacerbated if endothelial cell function is impaired.

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302P EFFECT OF L-ARGININE AND ENDOTOXIN ON RESPONSES TO NORADRENALINE IN RAT ISOLATED MESENTERIC RESISTANCE ARTERIES

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Endotoxin induces nitric oxide synthase in vascular smooth muscle and endothelium, leading to modification of vasoconstrictor responses. However, little is known of its effects on the resistance vasculature *in vitro*. In this study we have investigated the effect of endotoxin and L-Arginine on constrictor responses to noradrenaline in rat isolated mesenteric resistance arteries.

Male Wistar rats (225-300g) were killed by cervical dislocation and the mesentery removed into ice-cold buffer. Resistance arteries (internal diameter 263-403 μm) were dissected and mounted on a small vessel myograph (Mulvany & Halpern, 1977) in physiological saline (PSS). For each artery a concentration response curve to noradrenaline was constructed (50nM-7μM). Subsequently, 3 further concentration responses to noradrenaline, each separated by 1 hr, were performed in either PSS alone (control), 100ng/ml endotoxin, 10μg/ml endotoxin, 10^{-4}M L-Arginine, and 10^{-4}M L-Arginine/100ng endotoxin. After the fourth concentration response curve the NO synthase inhibitor N^{ω}-Nitro-L-Arginine Methyl Ester (L-NAME) was added to the bathing medium (10^{-4}M without L-Arginine and $2x10^{-4}$M with L-Arginine) and left for 10 minutes after which a final concentration response curve to noradrenaline in the presence of L-NAME was carried out. These responses were performed over 6 hr. The effect of endotoxin and L-Arginine on responses to noradrenaline were compared with the initial concentration response of each group by paired t-test.

The arteries bathed in PSS alone showed no change in sensitivity [pEC_{50} 5.92 ± 0.05 (mean ± s.e. mean)] or maximum response [77.8% ± 2.1% (shown as % of response to 5μM noradrenaline in 125mMK+)] to noradrenaline over the time course of the experiment. L-NAME increased sensitivity (pEC_{50} 6.48 ± 0.16 p<0.01 paired t-test) to noradrenaline but did not affect the maximum response. Endotoxin at either 100ng/ml or 10μg/ml did not affect the vasoconstrictor responses to noradrenaline or the effect of L-NAME. L-Arginine slightly depressed the maximum responses on the 3rd and 4th noradrenaline concentration responses (75.7% ± 2.45 and 76.4% ± 3.4% respectively) and this was significantly different from the maximum of the initial concentration response (86.0% ± 3.4% P<0.05 paired t-test). This was reversed back to the initial maximum by L-NAME. Endotoxin and L-Arginine depressed the maximum responses in a similar fashion to L-Arginine alone.

This study does not provide evidence for induction of nitric oxide synthase *in vitro* by endotoxin. The reduction of the maximum response to L-Arginine may not be mediated by the nitric oxide pathway as inhibition of the constitutive nitric oxide pathway leads to a change in sensitivity not a reduction in maximum response.

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303P ACUTE ADMINISTRATION OF METHYLGUANIDINE IN THE RAT PRODUCES AN INCREASE IN BLOOD PRESSURE WHICH IS ABOLISHED BY L-ARGININE

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The role of the endothelial cell as an important source of mediators which influence vascular reactivity has been established. An important component of endothelial products is nitric oxide (NO) that accounts for the endothelium-derived relaxing factor activity. NO is formed from one of the nitrogen atoms of the guanidinic group of L-arginine by a cytosolic enzyme (Palmer et al. 1988). This enzyme can be inhibited by certain derivatives of L-arginine with a modified guanidinic group such as N^G-nitro-L-arginine methyl ester (L-NAME; Moncada et al., 1991). Hypertension is one of the symptoms that occurs in renal insufficiency (Kim et al., 1975). In this pathology a retention of nitrogen-containing products derived from protein catabolism, such as methylguanidine (MG) is observed. We have shown that MG is able to reduce endothelium-derived NO release induced *in vitro* by acetylcholine and calcium ionophore A23187 on rabbit aorta rings (Pinto et al. in press). Here, we show the effect of MG on the blood pressure of rat anaesthetised with urethane (1 g kg^{-1}, i.p.).

Intravenous injections of MG, 1, 3, 10 and 30 mg kg^{-1}, induced a dose-related increase in mean arterial pressure (110.7±5.5 mmHg basal value). The increase was of 4.1±1.3%, 7.2±0.5%, 12.1±1.4% and 15.5±2.6% (n=8) respectively. Blood pressure was also increased after the same doses of L-NAME (9.7±3.2%, 21.5±4.4%, 33.9±5.9% and 37.9±6.9 (n=5) respectively). Doses of L-arginine 30 and 150 mg kg^{-1} i.v. given after MG 30 mg kg^{-1} produced an immediate and dose-dependent fall in blood pressure reducing the MG effect to 11.1±2.2% and to 8.6±2.0% respectively. In contrast, administration of D-arginine (30-150 mg kg^{-1}) did not produce any variation of MG-induced increase in blood pressure.

These results confirm *in vivo* what we observed *in vitro* (Pinto et al., 1993) and suggest that products of protein catabolism, such as MG, can modulate the L-arginine-NO pathway. This finding may have important implications in patients with uraemia. Indeed, some of these patients develop hypertension as secondary complication (Kim et al., 1975) a syndrome that has been related to reduced NO formation (Moncada et al., 1991).

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304P EFFECTS OF INHIBITION OF NITRIC OXIDE SYNTHASE ON RELAXATIONS TO ACETYLCHOLINE IN RAT MESENTERIC ARTERIES

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In recent years, it has become evident that the vascular endothelium plays an important role in the local control of blood flow. Endothelium-dependent relaxations (EDR) involve at least one substance termed endothelium-derived relaxant factor (EDRF), which may be nitric oxide, although other factors may be involved such as endothelium-derived hyperpolarising factor (EDHF). There is debate as to whether EDRF and EDHF are one and the same (see Garland & McPherson,1992). We have investigated EDR in rat isolated mesenteric arteries.

Rat isolated mesenteric arteries of diameter 250-350 μm were mounted in a small vessel myograph for the investigation of EDR to acetylcholine (ACh) in tissues pre-contracted with submaximal concentrations of noradrenaline (NA) (10 μM), KCl (40 mM) or the thromboxane mimetic U-46619 (10 μM). Interactions with the nitric oxide synthase inhibitor N^G-monomethyl-L-arginine (L-NMMA) were investigated.

ACh produced concentration-dependent relaxations, but the maximum relaxation depended on the contractile agent. L-NMMA (100 μM) inhibited EDR no matter which contractile agent was used, although there were variable effects on potency (concentration of ACh producing 50% of maximum relaxation) and maximum relaxation to ACh (Table 1).

Table 1. Effect of L-NMMA on potency (-log EC_{50}) and maximum relaxation (%) to ACh.

Constrictor	contraction (post veh)	contraction (post L-NMMA)		Vehicle	L-NMMA
KCl	0.51±0.09g	0.73±0.13g	potency of ACh	7.10±0.20	6.53±0.14*
			maximum relaxation	57.5±5.8%	40.5±7.9
NA	1.14±0.09g	1.55±0.12g*	potency of ACh	7.58±0.09	6.59±0.08*
			maximum relaxation	87.0±2.3%	57.1±12.9%*
U-46619	1.17±0.15g	1.41±0.16g*	potency of ACh	7.28±0.26	6.55±0.16*
			maximum relaxation	59.4±7.8%	26.4±5.8%*

Results are mean ± s.e. mean from at least 5 experiments. *P<0.05, Student's t-test.

These results are consistent with the involvement of only one relaxant factor in EDR of rat mesenteric arteries to ACh, since L-NMMA was effective against relaxations whether or not the membrane was depolarised with KCl to prevent effects of EDHF. This relaxant factor is probably nitric oxide.
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305P ROLE OF NITRIC OXIDE IN GASTROPROTECTION INDUCED BY COPPER-CHELATING DRUGS

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It has been proposed that the maintenance of gastric mucosal integrity is regulated by the interaction between local mediators such as sensory neuropeptides, nitric oxide (NO) and prostaglandins (Whittle et al., 1990). A role for sensory neuropeptides has been deduced from the findings that chronic pretreatment with capsaicin, which destroys primary sensory neurones, and deplets their neuropeptide content, greatly increases gastric mucosal damage induced by various pro-ulcerogenic agents. Conversely, acute intragastric capsaicin administration, which stimulates the neuronal release of sensory neuropeptides, protects the mucosa against damage without altering the ex-vivo formation of prostaglandins (Holzer et al., 1990). The primary mechanism of afferent nerve-mediated protection seems to be an increase in local blood flow. The possible role of the potent endogenous vasodilator, nitric oxide, in capsaicin-induced gastric mucosal protection has been studied, and the results indicate that it has a mediator role (Peskar et al., 1991).
CuNSN, a bis (2-benzimidazolyl)thiother complex with copper, has been shown to prevent the formation of acute gastric mucosal lesion induced by acetylsalicylic acid and ethanol, and this has been explained in part by the stimulation of prostaglandin synthesis (Franco et al, 1992). In the present study we have investigated the role of NO in CuNSN protection from ethanol-induced gastric damage. For this purpose we have used the inhibitor of NO biosynthesis, N^G-nitro-L-arginine (L-NNA) as well as L- or D-arginine. We utilized 8 male Sprague-Dawley rats (200-220 g) for each group. Two groups of rats received intravenous injection of L-NNA 40 mg/kg or saline (2 ml/kg). Other two groups received immediately before L-NNA either L-arginine or D-arginine (100 mg/kg i.v.). After 30 min 1.5 ml of absolute ethanol was instilled into the stomach of all animals. Additional 3 groups of animals received orally, 10 min after pretreatment with L-NNA or the combination of L- or D-arginine plus L-NNA, CuNSN suspended in 0.25% carboxymethylcellulose (100 mg/kg). A futher group received saline and CuNSN (100 mg/kg). Thirty min after the administration of CuNSN, 1.5 ml of 100% ethanol was instilled into the stomach. All the rats were killed 5 min after the instillation of ethanol, the stomachs were removed, opened and a lesion index (l.i.) of gross mucosal damage was determined. Gastric mucosal damage caused by ethanol (l.i. 37.62 $\pm$10.12) was significantly increased by i.v. administration of L-NNA (l.i. 52.25$\pm$13.82 $P<0.05$). The effect of L-NNA was completely antagonized by the administration of L-arginine (l.i. 35.28$\pm$8.18) while D-arginine did not cause a reduction in the damage (l.i. 50.00 $\pm$11.01). Treatment with CuNSN has shown significant protection (l.i. 12.83 $\pm$4.05 $P<0.001$) against damage produce by ethanol. This protection was not reversed by L-NNA (l.i. 15.28$\pm$3.98) and was significant ($P<0.001$) as compared to the corresponding control group. The combination of L-NNA plus L-arginine potentiates this protection (l.i. 5.50$\pm$4.15 $P<0.001$) that was not evident after the combination of L-NNA plus D-arginine (l.i. 18.14$\pm$7.52 $P<0.001$). These results suggest that NO is partially involved in CuNSN-induced gastroprotection.

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306P EFFECT OF PLATELET-DERIVED GROWTH FACTOR ISOFORMS ON INTRACELLULAR Ca^{2+} IN HUMAN VASCULAR SMOOTH MUSCLE CELLS

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Platelet-derived growth factor (PDGF) is one of the most potent mitogens for vascular smooth muscle cells (VSMC). It is a dimeric protein which exists in three isoforms, designated AA, AB and BB. There are important differences in biochemical and proliferative responses to the isoforms. Recent studies in cultured rat VSMC have shown that PDGF-AB and PDGF-BB have approximately equivalent effects on cellular DNA synthesis, while the AA isoform is virtually inactive (Kondo et al, 1993). The active isoforms both stimulate calcium entry, while PDGF-AA does not. We have investigated the effects of PDGF isoforms on intracellular calcium responses in cultured human VSMC.

Short segments of human saphenous vein were obtained at coronary bypass surgery. VSMC were grown from explants of the media, as described by Chamley-Campbell et al (1979), and were used for experiments after the third passage. Cell monolayers were serum - deprived for 48h, loaded with 2μM fura 2/AM for 90 min at 37°C, briefly trypsinised and then resuspended in a HEPES-buffered Tyrode's solution. Measurements were made in a PTI Deltascan spectrofluorimeter with excitation wavelengths of 340 nm and 380 nm and emission of 510 nm. Intracellular calcium was estimated as described by Grynkiewicz et al (1985).

PDGF-AA (5 ng/ml) caused no detectable increase in intracellular calcium (Ca_i). Equal concentrations of the other isoforms produced increases to a median level of 163nM (range 145-205nM) from a basal level of 92nM (range 86-151nM, n=3). The rise in Ca_i was attenuated by removal of external calcium and by 2mM nickel chloride. Calcium channel blockers were not tested since these cells do not express voltage-operated calcium channels under these experimental conditions.

The present findings are therefore consistent with a link between calcium influx and the mitogenic activity of PDGF. This has been supported by preliminary observations in our Department on the effects of PDGF isoforms on human VSMC growth. Further characterisation of the calcium channels involved will lead to better insight into the control of human VSMC proliferation and could have implications for therapeutic approaches to controlling inappropriate VSMC growth in myointimal hyperplasia and atherosclerosis.

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307P IMMUNOCYTOCHEMICAL DETECTION OF SALMFamide-LIKE IMMUNOREACTIVITY IN THE NERVOUS SYSTEM OF THE NEMATODE *HAEMONCHUS CONTORTUS*

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The neuropeptide Gly-Phe-Asn-Ser-Ala-Leu-Met-Phe-NH_2, named SALMFamide-1 (S1) has been identified in the nervous system of species belonging to the phylum Echinodermata (Elphick *et al.*, 1991). FMRFamide like immunoreactivity has been demonstrated in *Ascaris* motorneurones (Davenport *et al.*, 1988; Sithigorngul *et al.*, 1990) where it may be colocalised with either GABA or ACh, the inhibitory and excitatory neuromuscular transmitters, respectively. Recently, we have shown SALMFamide immunoreactivity in the *Ascaris* nervous system and here we demonstrate immunoreactivity to SALMFamide in the nematode *Haemonchus contortus* and compare this with our findings in *Ascaris*.

We used whole mount and longitudinal sections of *Haemonchus contortus*. The sections were labelled with rabbit polyclonal anti-SALMFamide (primary antibody) and rhodamine conjugated goat anti-rabbit (secondary antibody). The primary antibody was used at a dilution of 1:400 for the *Haemonchus* experiments and at a range of dilutions (1:250-1:32000) for the *Ascaris* experiments. The secondary antibody was used at a dilution of 1:40.

In *Ascaris*, SALMFamide-like immunoreactivity was detected in both head and muscle preparations. In whole mount head preparations, SALMFamide immunoreactivity was detected at both dorsal and ventral ganglia, the nerve ring, lateral and sub-lateral line (n=5, 1:4000), as well as commissures (e.g. deirid commissures, n=6, 1:8000). Nerves processes were also stained in the pharyngeal muscle (n=6, 1:2000). In the *Haemonchus contortus* preparations SALMFamide like immunoreactivity was detected in the whole mount head and tail sections where the major ganglia and nerve cords were stained (n=2). The nerve rings and lateral lines were also stained (n=2). The results from the longitudinal sections mirrored those obtained with the whole mount preparations.

SALMFamide like immunoreactivity has been observed in the nervous system of two nematode species, namely *Ascaris suum* and *Haemonchus contortus* using an antibody directed against an echinoderm neuropeptide. These initial experiments have confirmed the presence of SALMF-amide like substances in *Haemonchus contortus*, a relatively unexplored parasite, and have shown that the immunoreactivity mirrors that obtained in a well characterised nematode, *Ascaris suum.*

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308P CLONING OF A HUMAN trkB GENE AND DISTRIBUTION IN HUMAN BRAIN BY *IN SITU* HYBRIDIZATION

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The neurotrophins are a group of structurally related proteins each of which has a specific distribution and promotes the survival of a discrete population of peripheral and central neurons. One of these, brain derived neurotrophic factor (BDNF) has been shown to promote the survival and neurite outgrowth of certain placodal and neural-crest derived sensory neurons and is highly expressed in CNS tissue. It has been shown to be able to support the survival of cholinergic basal forebrain cells and dopaminergic substantia nigra cells in culture. Several receptors have recently been identified which bind neurotrophic proteins. BDNF binds to the common low affinity neurotrophin receptor, $p75^{NGFR}$. In addition it binds to a tyrosine kinase receptor $p145^{trkB}$ and to a truncated form of this receptor which lacks the catalytic domain, $p95^{trkB}$, both of these appear to be products of the *trk*B gene (Klein et al., 1990; Middlemas et al., 1991). We have cloned and sequenced a human *trk*B gene and describe here its distribution in human brain.

A human hippocampal λ ZAPII cDNA library was screened for *trk*B hybridising clones. Eight positive clones were identified and sequenced, two of which were found to represent full length cDNA clones for the truncated human *trk*B receptor. A 1200bp PstI fragment, to the extracellular domain, was random prime labelled with ^{35}S-dATP and used to determine the distribution of *trk*B mRNA in human foetal brain. Sections were cut on a cryostat (20μm) and hybridised overnight at 42°C with denatured probe in hybridization buffer containing 50% formamide. Slides were washed overnight at room temperature in buffer containing 50% formamide. Sections were dehydrated in ethanol and placed on autoradiography film or dipped in emulsion (Amersham, LM-1).

mRNA for *trkB* was found to be expressed in cerebral cortex, hippocampus and the caudate and putamen and the cells lining the ventricles. Knowledge of the distribution of mRNA for *trk*B will allow us to determine which neurons will respond to BDNF.

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309P EXPRESSION OF THE α AND β SUBUNITS OF G_S AND G_i PROTEINS IN HUMAN ATRIUM

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We have recently shown that there is no difference in the ratio of $G_s\alpha$ to $G_i\alpha 2$ mRNA in right atrial appendages (RAA) from patients taking β_1-adrenergic receptor (β_1AR)-selective blocker treatment, as compared to those not taking β-blockers; however, we found slightly more mRNA for each individual Gα subunit in the β_1-blocked atria (Jia et al., 1993). In the present studies, we have examined in further patients the expression of $G_s\alpha$ and $G_i\alpha_2$ as a proportion of an abundant housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and determined the expression of three β subunits of G proteins in human atrium. We also investigated whether there are effects of treatment with β_1AR-blocking drugs on expression of either the α or the β subunits of G proteins.

The expression of Gβ1 , Gβ2 and Gβ3 subunits in human RAA was determined initially by reverse transcription and amplification of the cDNA (RT / PCR). For ECL Northern blot analysis of each G-protein subunit, the specific Gβ cRNA probes consisted primarily of corresponding 3'-untranslated regions unique to each Gβ variant. The cRNA probes for $G_s\alpha$ and $G_i\alpha 2$ were as previously described. Both the sense $G_i\alpha 2$ cRNA transcribed from cloned $G_i\alpha 2$ cDNA and GAPDH mRNA served as internal controls to correct for possible differences in the recovery of intact mRNA from each sample. The sense $G_i\alpha 2$ cRNA was used as a standard on each film. Blots were quantified by laser scanning. Results were expressed as integrated pixel volumes, and submitted to statistical analysis by unpaired Student's t test.

The RT / PCR revealed bands of predicted size for all three Gβ subunits, and their identity was confirmed by Southern blot analysis with an independent oligonucleotide probe. Hybridisation at high stringency with the digoxigenin-labelled Gβ1 cRNA probe detected a message of about 3.4 Kb, whereas the Gβ2 cRNA probe hybridised specifically to a mRNA transcript of 1.8 Kb. The cRNA probe encoding Gβ3 revealed a 1.9-Kb mRNA in human atrium. The mRNA level of Gβ1 was found to be 1626.40 ± 580.00 (Mean ± SD) in β_1AR-blocker treated (n = 6) and 1170.80 ± 250.68 in non-βAR-blocker treated human RAA (n = 6), respectively. There was no significant difference between the two groups studied in the Gβ1 mRNA level ($p > 0.05$). Levels of Gβ2 and Gβ3 mRNA are currently under investigation. The mRNA level of $G_s\alpha$ was, again, not significantly higher in β_1-blocked human RAA (n = 12) than those in non-β-blocked RAA (n = 12) (1.9-Kb mRNA: 2207.70 ± 1359.79 vs 1590.01 ± 1143.35, $p > 0.05$; 1.7-Kb mRNA: 830.35 ± 512.28 vs 494.30 ± 337.07, $p > 0.05$), nor was there a significant difference in $G_i\alpha 2$ mRNA level in β_1-blocked RAA (1032.02 ± 246.70 vs 1007.56 ± 260.47, or 0.66 ± 0.18 vs 0.71 ± 0.35 ng $G_i\alpha 2$ mRNA / μg total RNA, $p > 0.05$, n = 12). The mRNA level of GAPDH used as the internal control was similar between the two groups (1.45-Kb mRNA: 7812.17 ± 1969.25 vs 7181.22 ± 2959.84, $p > 0.05$). Moreover, the ratio of both 1.9-Kb $G_s\alpha$ mRNA / 1.7-Kb $G_s\alpha$ mRNA (2.76 ± 0.94 vs 3.36 ± 1.61, $p > 0.05$,) and 1.9-Kb $G_s\alpha$ mRNA / 2.3-Kb $G_i\alpha 2$ mRNA (2.30 ± 1.52 vs 2.07 ± 2.05, $p > 0.05$) in β_1-blocked RAA (n = 12) were almost identical to that in non-β-blocked RAA (n = 12).

The results show that there is no significant change in gene expression of $G_s\alpha$ and $G_i\alpha 2$ proteins in patients taking β_1AR-blocker treatment. Three Gβ subunits were found in human atrium; quantification of the Gβ1 mRNA again revealed no effect of previous β_1-blocker treatment. Therefore, the increased sensitivity following β-blockade of receptors coupled to G_s (Hall et al., 1990) is unlikely to be due to altered G-protein expression.

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310P EFFECT OF LIFE-LONG HYPOCALORIC DIET ON AGE-RELATED CHANGES IN NEUROTRANSMITTER RECEPTORS

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Evidences for age-dependent alterations of CNS receptors and learning and memory decline have been reported by several groups. Recently Algeri et al. (1991) have characterized a model of hypocaloric diet in the rats. In this diet 50% of lipids and 35% of carbohydrate were replaced with vegetable fibers. Aged (24 months) rats, kept on hypocaloric diet from the age of three weeks, had a better cognitive behaviour than aged animals kept with standard diet. We studied if the hypocaloric diet could prevent the well-know decrease in neurotrasmitter receptors i.e.: dopaminergic receptors DA_1 and DA_2, cholinergic muscarinic receptors M_1 and M_2, serotonin $5HT_1$ subtype receptors and glutamate receptors, using the autoradiography binding tecnique. Three to four animals per each group were used.DA_1 and DA_2 receptors markedly decreased in several brain areas of old rat brain mainteined at standard diet; the greater decrease was observed in the anterior and central part of caudate-putamen (DA_1 receptors: young 135±31, old 99±19 fmol/mg tissue, $p < 0.02$ Anova . DA_2 receptors: young 42±13, old 28±10 fmol/mg tissue, $p < 0.003$ Anova). Muscarinic receptors showed small but significant decreases in cortical regions (i.e. ant. cingulate cortex : young 428±46, old 316±26 fmol/mg tissue, $p < 0.0001$ Anova. Front. cortex, medial, motor area: young 366±47, old 254±23 fmol/mg tissue, $p < 0.0001$ Anova) and in the striatum (young 736±90, old 454±53 fmol/ mg tissue, $p < 0.0001$ Anova) while a large number of regions showed no effect of aging. Interestingly, the decrese of specific muscarinic receptors binding in old rats was found in the anterior but not in the posterior cortical areas. Among $5HT_1$ serotonin receptors subtypes only $5HT_{1B}$ receptors in substantia nigra exibited an age dependent decrease in density (young 177±28, old 88±40 fmol/mg tissue, $p < 0.02$ Anova). The kainate sensitive glutamate receptors were decreased in the CA3 hippocampus of old rats (young 1.11+0.41, old 0.68+0.14 normalized binding values; i.e. ratio between optical density of area examined over optical density of the whole brain slice. $p < 0.047$ Anova).The hypocaloric diet was not able to prevent the decrease of DA_1, DA_2, muscarinic and $5HT_{1B}$ receptors induced by aging. Significant effect of diet was seen only for the kainate sensitive binding in the hippocampus (young rats 1.11+0.41, old rats hypocaloric diet 0.91+0.14 normalized binding value). The data will be discussed on the light of behavioural results obtained in parallel group of animals.

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311P THE INFLUENCE OF DENBUFYLLINE ON CORTICOTROPHIN RELEASING FACTOR (CRF-41) RELEASE FROM THE HYPOTHALAMUS *IN VITRO*

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The alkylxanthine denbufylline, a type IV phosphodiesterase (PDE) inhibitor and adenosine A_1 receptor antagonist, stimulates the hypothalamo-pituitary-adrenocortical (HPA) axis when given intraperitoneally in the rat. In order to identify its site and molecular mode of action, we have used an in vitro model (Buckingham & Hodges 1979) to examine the influence of denbufylline on the release of CRF-41 from isolated hypothalami in the presence and absence of the adenylate cyclase activator, forskolin, and to compare its effects with those of rolipram (a type IV PDE inhibitor with negligible adenosine receptor blocking activity) and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX, a selective adenosine A_1 receptor antagonist). Parallel measurements of the tissue cAMP contents were also made and, in addition, a preliminary radioligand binding study was performed to measure the adenosine A_1 binding sites in the hypothalamus. CRF-41 and cAMP were measured by radioimmunoassay and the results expressed as pg/ml and pmol/mg tissue respectively; values represent mean ± SEM, n = 4-6 with control (basal/vehicle) values shown in parenthesis. Statistical significance was determined by Mann Whitney U test; as both the rate of basal peptide release and the basal cAMP content varied between experiments, statistical comparisons were made only within experiments.

Rolipram (0.1-100μM) and denbufylline (10μM-1mM) both caused significant increases in CRF-41 release from the isolated hypothalami but the effects of the latter were considerably more marked [rolipram (10μM) = 20 ± 1.6 (vs. 10 ± 4) $p<0.05$; denbufylline (1mM) = 34 ± 5 (vs. 6 ± 1) $p<0.01$]. The adenylate cyclase activator, forskolin (100μM) also produced significant increases in CRF-41 release [97 ± 11 (vs. 43 ± 6) $p<0.01$] and its effects were additive with those of denbufylline (1mM) [basal = 8 ± 0.9; denbufylline = 42 ± 3 ($p<0.01$); forskolin = 14 ± 1 ($p<0.05$); denbufylline + forskolin = 50 ± 2 ($p<0.01$)] and rolipram (10μM) [basal =17±0.8; rolipram = 23 ± 1($p<0.01$); forskolin = 21 ± 1.1 ($p<0.05$); rolipram + forskolin = 29 ± 2.3 ($p<0.01$)]. The alterations in CRF-41 release provoked by rolipram (10μM) and forskolin (100μM) were accompanied by parallel increases in the tissue cAMP content [basal=0.43±0.05; rolipram = 0.6 ± 0.05 ($p<0.05$); forskolin = 2.4 ± 0.18 ($p<0.005$); rolipram + forskolin = 3.3 ± 0.4 ($p<0.005$)]. By contrast, denbufylline (1mM) alone had no significant effect on cAMP content although it potentiated the rise in cAMP content provoked by forskolin (100μM) [basal = 1.4 ± 0.16; denbufylline = 1.6 ± 0.12; forskolin = 3.0 ± 0.2 ($p<0.005$); denbufylline + forskolin = 4.5 ± 0.18 ($p<0.005$)].

The adenosine receptor antagonist, DPCPX (0.1-10nM), initiated significant increases in CRF-41 release [1nM = 34 ± 6 (vs. 18 ± 1) $p<0.05$] while a stable adenosine analogue selective for the A_1 adenosine receptor, N6-cyclohexyladenosine, CHA (1-100nM), produced a small but significant inhibition of CRF-41 release [100nM = 6 ± 1 (vs. 10 ± 1) $p<0.05$]. Saturation analysis using ^{3}H-DPCPX as a ligand demonstrated the presence of specific A_1 receptor binding sites (Kd 0.7nM) in the hypothalamus (Bmax = 367 fmol/mg protein).

The results suggest that the adenosine A_1 receptors mediate an inhibition of CRF-41 release from the hypothalamus and that the stimulatory actions of denbufylline on CRF-41 release are due primarily to blockade of these receptors rather than inhibition of type IV PDE.

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312P EFFECT OF POTASSIUM CHANNEL BLOCKERS ON ACETYLCHOLINE RELEASE FROM RAT HIPPOCAMPUS *IN VITRO*

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Toxin I, a dendrotoxin homologue, derived from the venom of the black mamba snake and two bee venoms, MCDP (mast cell degranulating peptide) and apamin, have been shown to block voltage dependent (toxin I and MCDP) and Ca^{2+} activated (apamin) K^+ channels in excitable cells, both in the peripheral and central nervous systems (for review see Strong, 1990). Toxin I has been shown to increase the release of GABA, glutamate and noradrenaline from CNS preparations (Schweitz et al., 1990; Tibbs et al, 1989; Ennis & Minchin, 1993). The present study was designed to investigate the effects of these toxins on electrically-evoked [^{3}H]-ACh release from rat hippocampus *in-vitro* compared with that of the non-selective agent 4-aminopyridine (4AP).

Rat hippocampal slices (0.25 x 0.25 x 2mm) were preloaded with [^{3}H]-choline for 30 min at 37°C, rinsed and 35μl aliquots were placed in each of 20 chambers of a Brandel "Superfusion 2000" system. The slices were superfused at a rate of 0.4 ml/min with oxygenated Kreb's Buffer containing hemicholinium (10μM). Fractions of the superfusate were collected every 4 min and 48 (S_1) and 68 (S_2) min later the slices were stimulated electrically for 4 min via platinum electrodes with bipolar square wave pulses of 24 mA at a frequency of 5 Hz. The tritium remaining in the tissue and that in the superfusate fractions was measured by liquid scintillation counting. The results were expressed as fractional release and the effects on basal release as the ratio of the fractions immediately prior to S_1 and S_2 as B_2/B_1 and that induced by electrical stimulation as S_2/S_1.

The control B_2/B_1 ratio was 0.94± 0.04 and the control S_2/S_1 ratio was 0.91± 0.07 (n = 11). The effects of the potassium channel blockers on basal and evoked release are shown in Table 1.

Table 1: Effect of potassium channel blockers on basal and electrically-evoked [^{3}H]-ACh release expressed as % control.

	control	4AP (10μM)	toxin I (100nM)	apamin (100nM)	MCDP (100nM)
Basal	100 ± 1.2%	103 ± 9%	105 ± 3.2%	96 ± 3.1%	100 ± 3.0%
Evoked	100 ± 3.4%	177 ± 18%*	111 ± 16%	111 ± 7.2%	111± 8%.0

*$p< 0.01$.cf controls Mann-Whitney U test, 2, tailed. ($n\geq4$).

The data suggest that the voltage dependent K^+ channels that are blocked by toxin I or MCDP or the calcium-activated K^+ channels activated by apamin are not involved in the mechanism of ACh release under these conditions although the less selective compound 4AP did enhance ACh release. It is possible that blockade of more than one type of potassium channel may be necessary to modulate ACh release.

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313P INDIVIDUAL SENSITIVITY TO DRUGS OF ABUSE AS DEFINED THROUGH A FREE CHOICE PARADIGM, RELATED TO DISTINCT LEVELS OF CENTRAL DOPAMINERGIC ACTIVITY

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Individual sensitivity to psychotropic drugs such as drugs of abuse could be seen as an essential topic in modern neuropsychopharmacology in the attempt to define central targets for new therapies. It has been recently observed that animals manifest spontaneously different behaviours when submitted to identical environments or experimental procedures (i.e. low or high locomotor reactivity to novel enviroment) (Piazza et al., 1990). We have therefore investigated the possible existence of a relationship between putative individual sensitivity to psychotropic drugs and consumption of drugs of abuse such as cocaine or alcohol in naive adult male rats submitted to a free choice paradigm. Voltammetric analyses were subsequently performed in order to investigate the activity of dopamine in the nucleus accumbens of these animals. Naive male Sprague - Dawley rats (250g) were exposed to a free choice paradigm, i.e. rats were free to drink from a bottle containing water and a bottle containing ethanol 10%v/v (ETOH) or cocaine . Five different concentrations of cocaine solutions were tested within 5 weeks: 0.1; 0.25; 0.50; 1.0; 2.0 mg/ml, one per week for each animal. Cocaine preferring (7 rats vs 3 rats drinking mainly water) or ETOH preferring (4 rats drinking 2.4mg/kg (±0.3 s.e.m.) ETOH daily vs 6 rats drinking mainly water) animals were selected. It appeared that the 0.1mg/ml cocaine solution was not discriminative and that the cocaine preferring animals manifested individual preference for specific drug concentrations with a predilection for the 0.5mg/ml formulation. Since the experiments were performed in a free choice paradigm (bottle of water vs bottle of drug) and the daily total liquid intake was similar in all the cocaine, ETOH or water preferring rats (approx. 25ml), one can argue that individual sensitivities may be the driving mechanism of the different drinking behaviours. In addition, the spontaneous preference exhibited by the rats between various concentrations of cocaine solution supports the concept of differences even in a homogeneous population of naive rats.

In support of the free choice "behavioural" model as less constrictive and more representative of the human situation, data obtained by means of the technique of *ex vivo* differential pulse voltammetry (Hassoni et al., 1990) indicate biochemical differences between distinct behaving (cocaine or water preferring) rats. In particular the levels of DOPAC, a metabolite of dopamine measured in the nucleus accumbens were similar in rats drinking water (34 ± 7µM, n=3 + s.e.m.) and rats preferring water but forced to drink cocaine (37 ± 11µM, n=3). In contrast these levels were higher in rats choosing cocaine in the free choice drinking test (47 ± 8µM, n=3 ± s.e.m.). These data seem not to be directly related to the known pharmacological action of cocaine on the dopaminergic system (Pulvirenti & Koob, 1990) as the intake of the drug was approx. 2.5mg/hour for the free-choice rats drinking cocaine vs 6.5mg/hour for the forced-cocaine rats. Since the dopaminergic system is a major neural substrate mediating the acquisition of psychostimulant self-administration and the nucleus accumbens is a critical region for the mediation of the effects of psychostimulants (Pulvirenti & Koob, 1990), our results may further support the hypothesis that individual propensity for drug consumption may be related to differences in the activity of this neural system in this brain area. Finally the spontaneous selection of the drug of abuse associated with distinctive biochemical data support the free choice approach in behavioural studies which may reflect more closely the "normal" human conditions.

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314P THE EFFECT OF NALTRINDOLE ON RESTRAINT INDUCED ANTINOCICEPTION IN MICE

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It is well established that stress can induce antinociceptive activities in experimental animals. The characteristics of stress-induced antinociception depend upon the type and duration of the stress and on the method by which antinociception is assessed (Kelly, 1982). Recently, we have reported that naloxone blocks restraint-induced antinociception in the female but not in the male mice (Wong, 1992). The present study was undertaken to investigate the effect of blocking the opioid δ receptors with naltrindole on restraint-induced antinociceptive activity in both male and female mice.

Male and female CFLP mice weighing between 20-25g were used. The animals were restrained individually in a cylindrical tube with holes along the whole length for ventilation (Wong & Bentley, 1979). Each animal would have only a limited degree of movement inside the tube. The animals were restrained for one hour then tested for latency response to hot plate test at 55°C, at 0, 10, 20, 40 and 60 min post-restraint. Naltrindole hydrochloride was given s.c. either 15 min before restraint or immediately after restraint for one hour. Control animals received saline injection 10ml kg^{-1} at the corresponding time. Mean hot plate response times were analyzed and compared with the analysis of variance.

Restraint for one hour induced significant antinociceptive activity in mice (10.61±0.74 s versus 4.47±0.30 s in controls for male mice and 10.63±0.82 s versus 4.80±0.33 s in controls for female animals). The antinociceptive activity was significant throughout the one hour period of observation. In the male animals, prior administration of naltrindole (0.5 - 2.0mg kg^{-1} s.c.) 15 min before restraint for 60 min did not affect the degree of antinociceptive activity induced by restraint. In addition, naltrindole administered s.c. immediately after restraint for one hour also did not affect the degree of antinociceptive activity in male mice. However, for the female mice naltrindole administered s.c. before restraint dose-dependently suppressed the antinociceptive activity induced by restraint. In the group receiving naltrindole 2.0mg kg^{-1} s.c., the hot plate response time was 6.42±0.77 s versus 10.63±0.82 s in saline controls ($P<0.01$). Naltrindole when administered after restraint also reversed the antinociceptive activity. The response time was 7.19±0.50 s versus 10.21±0.58 s for saline controls ($P<0.05$) when measured 10 min after drug injection. It is concluded that restraint can induce antinociceptive activity in mice, however, different mechanisms may be involved in the antinociception observed. In male mice the endogenous opioid systems do not seem to play a significant role in restraint-induced antinociception, while for female animals blockade of opioid δ receptors would greatly diminish the antinociception observed after restraint.

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315P EPILEPTOGENIC PROPERTIES OF SOME LIMPHOKINES MICROINFUSED IN RAT BRAIN

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Brain damage occurring during epilepsy is often associated with astrocytic proliferation as part of the natural repair processes and this may be mediated through interleukin-1 produced in response to injury (Giulian *et al.*, 1988). Since the effects of interferons and interleukins on epilepsy are not clearly studied, we planned to characterize the central electrocortical (ECoG) effects of some limphokines in rats. The intracerebroventricular injection of interleukin-1ß (8, 12 and 13.35 pmol, *n*= 18), interleukin-2 (13.35, 16 and 24 pmol, *n*= 24), interleukin-3 (16 and 24 pmol, *n*= 12) or tumor necrosis factor-alpha (1.14 and 2.28 pmol, *n*= 11) induced ECoG spiking activity which was associated with wet dog shake episodes or more frequently with episodes of frozen immobility. Interleukin-6 injected intracerebroventricularly (8, 12 and 24 pmol, *n*= 18) did not induce behavioural or ECoG epileptic phenomena. The volume of injection was 0.5 - 2.0 µl. ECoG epileptic discharges occurring after interleukin injection were characterized by sharp waves, spikes waves, single or polyspikes. ECoG epileptic discharge started within 1-3 min from the infusion and lasted from 22 ± 4 to 81 ± 15 min depending on the dose. Much lower doses of interleukin-1ß (1.35 and 2.67 pmol, *n*= 12), interleukin-2 (1.35 and 2.67 pmol, *n*= 16) or tumor necrosis factor-alpha (0.14 and 0.28 pmol, *n*= 12) were able to induce similar epileptic phenomena, when microinjected into the dorsal hippocampus. Epileptic ECoG discharges started within 15-60 s from the injection and lasted from 20 ± 6 to 92 ± 23 min depending on the dose. A pretreatment with anti-interleukin-1ß monoclonal receptor antibodies (10 ng) was able to antagonize the epileptogenic effects of interleukin-1ß (*n*= 8) whilst a pretreatment with anti-interleukin-2 monoclonal receptor antibodies (10 ng) was unable to prevent the epileptogenic effects of interleukin-2 (*n*= 11). The present data show that abnormal concentration of interleukin may participate to the onset and maintenance of epileptogenic phenomena.

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316P MEN 10,627, A POTENT POLYCYCLIC PEPTIDE ANTAGONIST AT TACHYKININ NK_2 RECEPTORS

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MEN 10,627, or cyclo(MetAspTrpPheDapLeu), is a novel polycyclic peptide possessing an extremely rigid conformation developed from the cyclic hexapeptide NK_2 receptor antagonist L659,877 [cyclo(MetGlnTrpPheGlyLeu)] (McKnight et al.,1991). The activity of MEN 10,627 was evaluated "in vitro" on several bioassays for the NK_1 receptor (guinea-pig ileum, GPI), NK_2 receptors (rabbit pulmonary artery, RPA; hamster trachea, HT; rat vas deferens, RVD and guinea-pig gallbladder, GPG) and NK_3 receptor (rat portal vein, RPV) using methods described previously (Patacchini et al., 1989; Maggi et al., 1990; Patacchini & Maggi, 1992). MEN 10,627 was compared with the NK_2 receptor antagonists L659,877 and the non-peptide compound SR 48968 (Emonds-Alt et al., 1992). The antagonists under study (15 min incubation) were tested against SPOMe (GPI), NKA (RPA, HT, RVD and GPG) or [Arg^0]NKB (RPV) as agonists, in the presence of peptidase inhibitors (thiorphan, captopril and bestatin 1 µM each). Results, expressed as pK_B values ± S.E. of the mean (n = 9-12), are reported below.

Table 1

Antagonist	GPI	RPA	HT	RVD	GPG	RPV
MEN 10,627	6.1 ± 0.1	8.1 ± 0.05	10.1 ± 0.1	8.7 ± 0.1	7.9 ± 0.1	inactive*
L 659,877	5.6 ± 0.1	6.7 ± 0.1	7.9 ± 0.1	7.9 ± 0.1	6.3 ± 0.1	5.4 ± 0.1
SR 48968	inactive**	9.6 ± 0.1	8.5 ± 0.1	8.3 ± 0.1	Not competitive	inactive**

(*): inactive at 1 µM and (**) 3 µM.

MEN 10,627 exerts a potent and competitive antagonism toward NK_2 receptor-mediated responses in all preparations examined and displays a high degree of selectivity for the NK_2 receptors, as shown by the poor interaction with the NK_1 and NK_3 receptors. MEN 10,627 is the most potent NK_2 receptor antagonist thus far described in the HT. As compared to L 659,877, the modifications inserted in MEN 10,627 led to an increase in absolute potency at NK_2 receptors, without modifying significantly the affinity for NK_1 or NK_3 receptors. As compared to SR 48968, MEN 10,627 is more potent in the HT and RVD and less potent in the RPA.

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317P EFFECT OF MEN 10,627, A NOVEL POTENT NK_2 TACHYKININ RECEPTOR ANTAGONIST, ON ISOLATED HUMAN PREPARATIONS AND IN ANAESTHETIZED RATS

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The activity of MEN 10,627 or cyclo(MetAspTrpPheDapLeu), a novel polycyclic peptide antagonist of tachykinin NK_2 receptors, has been evaluated in vitro on human preparations (human urinary bladder, HUB; human colon HC; human ileum HI) as compared to SR 48,968 (Emonds-Alt et al., 1992), MEN 10,376 and L 659,877 (McKnight et al., 1991).
MEN 10,627 behaved as a potent (see pK_B values in the Table 1) and competitive antagonist in HUB, HC and HI: slope of Schild plot was -1.01 (-1.30, -0.72 c.l.), -0.99 (-1.70, -0.29 c.l.) and -0.86 (-1.55, -0.17 c.l.) respectively.
MEN 10,627 (10 μM), did not influence the contractile response to carbachol in the isolated preparations examined.

Table 1

Antagonist	HUB	HC	HI
MEN 10,627	8.55 ± 0.03	8.95 ± 0.13	8.33 ± 0.07
SR 48,968	8.36 ± 0.08	8.52 ± 0.12	7.81 ± 0.07
MEN 10,376	7.00 ± 0.07	7.34 ± 0.03	6.93 ± 0.05
L 659,877	7.10 ± 0.17	7.00 ± 0.10	6.83 ± 0.12

Each value in the Table is mean pK_B (negative logarithm of antagonist dissociation constant) ±S.E.M. of 6-9 determinations. As agonists were used neurokinin A for HUB and [βAla^8]neurokinin A (4-10) for HC and HI. Contact time for the antagonists was 15 min. Experiments have been performed in the presence of captopril, bestatin and thiorphan (1 μM each).

In urethane-anaesthetized rats (see Maggi et al., 1991), MEN 10,627 (3-30 nmol/Kg i.v.) showed high potency in inhibiting the contractions of the urinary bladder produced by i.v. administration of the selective NK_2 agonist [βAla^8]neurokinin A (4-10) (1 nmol/Kg) without affecting those produced by the selective NK-1 agonist [Sar^9]substance P sulfone (1 nmol/Kg) nor the hypotension and salivary secretion produced by the latter agonist. The inhibitory effect of MEN 10,627 (30 nmol/Kg) against the NK_2 mediated bladder contraction lasted more than 3 hours.
In conclusion MEN 10,627 is a potent, selective and competitive antagonist of the tachykinin NK_2 receptor both in human isolated smooth muscle preparations and in vivo in anaesthetized rats. MEN 10,627 possesses the highest antagonist potency among the compounds tested.

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McKnight, A.T., Maguire, J.J., Elliott, N.J., Fletcher, A.E., Foster, A.C., *et al.* (1991) *Br. J. Pharmacol.* 104, 355-360.

318P INCREASE IN PRESSURE IN THE BILIOPANCREATIC DUCT CAUSES NOCICEPTIVE BLOOD PRESSURE REFLEXES VIA CAPSAICIN-SENSITIVE AFFERENTS IN THE RAT

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Diseases of the pancreas which cause obstruction of the biliary or pancreatic duct system are associated with severe pain. In order to investigate whether capsaicin-sensitive afferent neurones transmit the nociceptive information, blood pressure reflexes in response to electrical stimulation of the biliopancreatic duct and to increases in intraluminal pressure were determined in rats anaesthetized i.p. with pentobarbitone sodium and phenobarbitone sodium (40 mg/kg each). Blood pressure was monitored in a carotid artery.

Electrical stimulation of neurones in the biliopancreatic duct was carried out at 30 V, 3 ms, 50 Hz for 20 s using bipolar platinum electrodes placed beneath the duct about 1 cm proximal from the duodenum. The stimulation was repeated twice at intervals of 5 min. Fifteen to 30 min after the i.p., i.v., or s.c. administration of drugs the stimulation procedure was resumed. In untreated animals the electrical stimulation caused small rises in blood pressure (by 3 ± 3 mmHg). After phentolamine (7 μmol/kg, i.p.) this response was changed to pronounced and reproducible depressor reflexes of −11 ± 3 mmHg. Twenty min after s.c. treatment with capsaicin (30 mg/kg) the reflex was abolished. Capsaicin treatment had no significant effect on the initial pressor effects of electrical stimulation before phentolamine. The depressor responses in phentolamine-treated rats may be classified as pain responses since they were abolished by morphine (1 μmol/kg, i.v.). The inhibition by morphine was reversed by naloxone (3 μmol/kg, i.v.). Naloxone given before morphine did not affect the depressor reflex but prevented the inhibitory action of subsequently applied morphine.

To investigate the effects of pressure increases in the biliopancreatic duct system, the duct was cannulated near the duodenum. At intervals of 5 min, 300 μl of a 154 mM solution of NaCl were injected via the cannula in a retrograde direction within 15 s. The cannula was opened again 20 s later to relieve the pressure. This procedure was carried out at the same time intervals as the electrical stimulations discribed above. During the periods of increased intraductal pressure blood pressure fell by 8 ± 4 mmHg. This effect was significantly augmented by phentolamine (to −16 ± 3 mmHg). No change in blood pressure occurred when the intraductal pressure was increased 30 min after the administration of morphine. The depressor reflex was, at least partly, restored to −9 ± 3 mmHg by naloxone. Acute administration of capsaicin (Holzer, 1991) abolished the depressor responses seen before or after phentolamine treatment. Rats which had been treated with capsaicin as neonates, showed somewhat smaller blood pressure responses under basal conditions (−3 ± 1 mmHg). In these animals, phentolamine was unable to augment this response.

In summary, the hypotensive response both to electrical stimulation of nerve fibres in the biliopancreatic duct and to increases in intraluminal pressure constitutes a pain-related reflexe since it is abolished by morphine and restored by naloxone. The afferent part of the reflex is mediated by nerve fibres sensitive to capsaicin.

Holzer, P. (1991) *Pharmacol. Rev.*, 43, 143–201.

319P EFFECT OF GTP CONCENTRATION ON AGONIST AFFINITY ESTIMATES OBTAINED USING THE OPERATIONAL MODEL

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According to the steady state ternary complex model (Mackay, 1990), for agonists that act through G protein coupled receptors, the level of GTP in the system should affect estimates of apparent agonist affinities. We previously reported (Malkhandi and Keen, 1992) the effect of altering GTP concentration on agonist apparent affinities estimated according to the receptor inactivation technique (Furchgott, 1966). This study investigates the effect of GTP levels on agonist apparent affinities estimated according to the operational model (Black et al., 1985), and compares the results with those obtained from Furchgott analysis. We have used NCB-20 cells which express prostacyclin receptors (IP receptors) coupled to the stimulation of adenylyl cyclase. Pretreatment of these cells with iloprost, an IP receptor agonist, causes a reduction in the number of IP receptors without altering other components of the receptor/effector system (Kelly et al., 1990).

Confluent NCB-20 cells were incubated overnight in the absence or presence of 0.01, 0.1 or 1μM iloprost, washed three times in phosphate buffered saline and stored at -80^{o}C. Adenylyl cyclase activity and [^{3}H]-iloprost binding in cell homogenates were assessed as described previously (Kelly et al., 1990). Iloprost dose-response curves in homogenates of control or pretreated cells were obtained at nominal assay concentrations of 0, 10 and 100μM GTP. Estimates of Ka were obtained at each GTP concentration by fitting the data with the operational model using BMDP. Results were compared using Student's t-test with $P<0.05$ taken to be significant.
The apparent Ka of iloprost obtained from binding studies in the presence of 100μM GppNHp was $\approx$200nM (log Ka -6.68$\pm$0.54, mean$\pm$s.e.mean n=3). At 100μM GTP, the iloprost apparent Ka estimated according to the operational model was $\approx$220nM (log Ka -6.66$\pm$0.19, mean$\pm$s.e.mean n=5); Furchgott analysis of the same data gave a Ka estimate of $\approx$110nM (log Ka -6.96$\pm$0.19, mean$\pm$s.e.mean n=5), the two values being significantly different. At 10μM GTP the Ka estimated according to the operational model was $\approx$270nM (log Ka -6.57$\pm$0.26, mean$\pm$s.e.mean n=5), Furchgott analysis of these data gave a significantly different Ka estimate of $\approx$68nM (log Ka -7.17$\pm$0.25, mean$\pm$s.e.mean n=5). In the absence of GTP the Ka estimated according to the operational model was $\approx$467nM (log Ka -6.33$\pm$0.15, mean$\pm$s.e.mean n=4), Furchgott analysis of these data gave a Ka estimate of $\approx$282nM (log Ka -6.55$\pm$0.24, mean$\pm$s.e.mean n=4); these values were not significantly different. Using either the operational method or the Furchgott technique, the mean Ka at 100μM GTP was not significantly different from that obtained at 10μM GTP, however, with both techniques, the Ka obtained in the absence of GTP was significantly different from that obtained at 100μM GTP.

At 100 and 10μM GTP, the operational and Furchgott techniques gave significantly different estimates of apparent affinity. When either technique was used, the apparent affinity estimated at 100μM GTP was significantly different from that obtained in the absence of GTP. These results show that altering GTP concentration may affect agonist apparent affinity estimates obtained according to either the operational model or the receptor inactivation techniques and tend to support the steady state ternary complex model.

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320P EVALUATION OF CHROMOSOMAL DAMAGE BY N-METHYL-N'-NITRO-N-NITROSOGUANIDINE (MNNG) IN YEAST USING PULSED FIELD GEL ELECTROPHORESIS

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We report data showing a novel possible utilization of the Pulsed Field Gel Electrophoresis (PFGE) technique (Schwartz & Cantor 1984), namely the possibility of evaluating the chromosomal damaging potential of mutagenic drugs in eukaryotic organisms. Cells of the yeast *Candida albicans* (CA6 strain) were treated with the mutagenic drug MNNG (0.15 mg per 10^8 cells/ml for 90 min. at 37°C), and metabolic auxotrophic variants were isolated. Under such conditions about 5 to 10% of cells survive treatment and of these, only 1 to 2 % give rise to nutritional mutants. By using standard CHEF and TAFE methodologies of PFGE, the electrophoretic karyotypes of the variants were compared to those of non-drug-treated cells or drug-treated cells retaining the prototrophic pattern. We found that the appearance of metabolic variants following MNNG mutagenesis was consistently accompanied by evident chromosomal alterations. Six out of seven MNNG mutants so far tested presented evident aberrations. Such alterations appear to be mostly deletions generating smaller chromosomes. In contrast, no alterations were detected in the karyotypes of a comparable number of drug-treated prototrophic yeast lines. Moreover, by resorting to a more sensitive hybridization technique, we were able to define the origin of the aberrant chromosome in relationship to the parental karyotype. These data seem to suggest that PFGE in low eukaryotic cells might be a valuable screening test to assess the genotoxic potential (in term of chromosome damaging capability) of mutagenic drugs. Under our experimental conditions this may be the result of the particular susceptibility of *Candida albicans* to drug-induced mutagenesis combined with the remarkable discriminative power of PFGE in the yeast range of chromosomal dimensions.

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321P BINDING OF GENTAMICIN AND TOBRAMYCIN BY RAT PERITONEAL MAST CELLS

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The aminoglycoside antibiotics are frequenty used in the clinical management of Gram negative infections. These drugs are polyaminated and thus are polycationic at physiological pH; because of their polar nature, aminoglycosides are largely excluded from most cells. However, these drugs interact with anionic phospholipids of the brush border membrane at the apical side of renal tubular cells, and are subsequently transported and accumulated by these cells. The direct consequence of this accumulation is an acute nephrotoxicity (Sastrasinh *et al.*, 1982). Also the anthracycline antibiotic adriamycin, one of the most useful antitumor agent, binds specifically with anionic phospholipids on the plasma membrane, and this interaction is believed to play an important role in the antitumor activity and in the cardiotoxicity of this drug (de Wolf *et al.*, 1991).

In our laboratory we have shown that adriamycin has a very high affinity for rat peritoneal mast cells in comparison with many other normal or neoplastic cells. Indeed, in mast cells adriamycin induces a significant non cytotoxic histamine release, and, as a consequence of the exocytotic process, a granular binding site is externalized for which adriamycin possesses an extraordinary affinity (Decorti *et al.*, 1991). The aim of our study was therefore to verify if, similarly to adriamycin, two aminoglycosides, gentamicin and tobramycin, were able to bind to the plasma and granular binding sites in mast cells.

Purified rat peritoneal mast cells, incubated in quadruplicate at 37 °C for predetermined experimental time were used. The amount of histamine released and adriamycin concentrations were measured by fluorimetric methods; gentamicin and tobramycin were determined by fluorescence polarization immunoassay using the TDX FLX (Abbott). Cells were also processed for epifluorescence microscopy.

A 10 min incubation of mast cells with gentamicin and tobramycin (0.1-10 mM) did not induce any significant histamine release; accordingly, almost no aminoglycoside was detectable in mast cells. However, when cells were treated with adriamycin (100 µg/ml) or compound 48/80 (5 µg/ml) and the aminoglycosides, an important histamine release was induced and, as a consequence of the exocytotic process, a significant amount of both gentamicin and tobramycin was measured in mast cells. On the other hand, these aminoglycosides were also able to displace adriamycin from its plasma and granular binding sites. The microscopical examination revealed that, in cells treated with adriamycin alone, an intense fluorescence was present in cytoplasmic granules; whereas mast cells pretreated with the aminoglycosides showed hardly any fluorescence.

We conclude that aminoglycosides are effective competitive inhibitors of adriamycin accumulation but, in spite of a more favourable concentration gradient, they are taken up almost one order of magnitude less than adriamycin. A possible explanation could be that on the mast cell membrane a carrier molecule exists on which a recognition site can form a complex both with aminoglycosides and adriamycin, the complex with the aminoglycosides being more stable than that with adriamycin. Aminoglycosides can be good competitors and less good transported species than adriamycin.

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322P KINFIT II: A NONLINEAR LEAST SQUARES PROGRAM FOR ANALYSIS OF KINETIC BINDING DATA

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We have developed a versatile computer program designed to analyze association and dissociation time-courses for ligand binding experiments simultaneously. The program KINFIT II uses a weighted nonlinear least squares (WNLLS) algorithm implemented as a series of subroutines in MATLAB (Moler *et al.*, 1987), an interactive program for scientific numeric calculations.

Our program uses the exact mathematical solution of the kinetic binding system in the case of two ligands binding to any number of classes of binding sites for both the association and dissociation curves. The differential equation describing the association and dissociation binding curves have an analytical solution when there is a single class of binding sites and a single ligand (Rodbard & Weiss, 1973). In the case of multiple classes of binding sites and when considering both the labeled and the unlabeled ligand, one must use a system of two different differential equations. We, therefore, used a Gear's 2^{nd} and 3^{rd} order formula to integrate a stiff system of ordinary differential equations. The minimization routine makes use of a Gauss-Newton algorithm as modified by Levenberg and Marquardt (Marquardt, 1963) and estimates the required derivatives numerically.

The program KINFIT II was designed to analyze families of kinetic curves performed at different "hot" ligand concentrations. In fact, given that association time-courses are dependent on the total ligand concentration, multiple kinetic curves that are performed at different ligand concentrations, must be analyzed simultaneously in order to characterize complex receptor systems. Our algorithm can also perform the analysis of dissociation curves that have been started with different "cold" ligand concentrations or using different dilution ratios.

We have also inferred some basic characteristics of kinetic binding experiments. We have demonstrated that a ligand concentration at least equal to the K_d value is necessary to estimate k_{on}, k_{off} and B_{max} values precisely and that precision and accuracy in the parameter estimates decrease proportionally to the receptor occupancy. In the presence of a single class of binding sites, equilibrium and kinetic experiments might each be used to obtain separate estimates of K_d and B_{max}. With two classes of binding sites, kinetic experiments can be used to characterized subclasses of sites even when available ligands are poorly selective. This is a situation where equilibrium binding experiment might fail to recognise the presence of binding site heterogeneity. Moreover, kinetic experiments have the power to distinguish between two different binding sites that are characterized by an equal equilibrium dissociation constant, but different kinetic constants.

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323P ADENOSINE A_1 BINDING SITES IN RAT SMOOTH MUSCLE PREPARATIONS

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Investigation of the peripheral effects of adenosine have mostly used functional studies and very few successful binding studies for adenosine receptors in smooth muscle have been reported. As part of a project to study the development of purinoceptors in a variety of peripheral tissues, we have developed radioligand binding studies for A_1-receptors using [^{3}H]-DPCPX (1,3-dipropyl-8-cyclopentylxanthine) as a binding ligand. Adult Wistar albino rats (200-250g) were killed by cervical dislocation and tissues (vas deferens, bladder, duodenum, colon longitudinal muscle and muscularis mucosae) were rapidly removed and placed in 50mM Tris HCl buffer, pH 7.4 at 0°C. All tissues were cleared of connective tissue, roughly chopped, homogenised, centifuged at 1000g for 20 min at 0°C, and the supernatants recentrifuged at 50,000g for 20 min. The pellets were resuspended in Tris HCl and incubated with adenosine deaminase (5U ml^{-1}) at 25°C for 30 min prior to binding studies. Saturation assays (in triplicate) were carried out using [^{3}H]-DPCPX (0.05-10nM) for 30 min at various temperatures (colon, 25°C; duodenum, 0°C; vas deferens and bladder, 35°C) and non-specific binding determined using 1μM CPA (N^6-cyclopentyladenosine). Assays were terminated by the addition of ice-cold 50mM Tris HCl followed by vacuum filtration through Whatman GF/B filters with three 2.5 ml washes using a Brandel cell harvester. Radioactivity was determined by scintillation counting after solubilization with 20% Triton X100 in toluene. Competition studies were carried out using 1nM [^{3}H]-DPCPX against 10-12 concentrations of displacing ligands.

Table 1. Binding affinity (K_D) and capacity (B_{max}) for [^{3}H]-DPCPX in various isolated tissues

	Colon muscularis mucosae	Colon longitudinal muscle	Duodenum	Vas deferens
B_{max} (fmol mg^{-1} protein)	430 ± 35	2950 ± 700	388 ± 40	433 ± 122
K_D (nM)	0.84 ±0.15	1.18 ± 0.47	1.59 ± 0.18	0.93 ± 0.17

Values are the mean ± s. e. mean of 3-9 measurements derived from Scatchard analysis of the saturation data.

Specific, saturable, single site binding of [^{3}H]-DPCPX was observed in colon longitudinal muscle and in muscularis mucosae, duodenum and vas deferens (Table 1) which we have previously shown to contain A_1-receptors (Nicholls et al., 1992; Bailey et al., 1992; Hourani et al., 1993). Binding was displaced in a dose dependent manner by adenosine receptor ligands with a potency order of DPCPX> CPA> N^6-phenylisopropyladenosine (R-PIA)> 5'-N-ethylcarboxamidoadenosine (NECA), indicative of binding to A_1-receptors. No binding was detected in urinary bladder which we have shown to contain A_2-receptors (Nicholls et al.,1992), but surprisingly the highest density of A_1-receptors was found in the longitudinal muscle of the colon in which previous functional studies have only demonstrated the presence of A_2-receptors (Bailey & Hourani, 1992).

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324P COMPUTER ANALYSIS OF A POSSIBLE MODEL FOR CYSTEINYL LEUKOTRIENE RECEPTORS IN HUMAN LUNG MEMBRANES

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Cysteinyl leukotrienes (cysteinyl-LTs), previously known as Slow Reacting Substance of Anaphylaxis (SRS-A), are potent constrictors of bronchial airways (Hanna *et al.*, 1981) and have been demonstrated to induce mucosal edema and secretion of bronchial mucus. Therefore, they have been suggested to play a significant role as mediators of immediate hypersensitivity reactions such as asthma (Samuelsson, 1983). It is, therefore, of crucial importance to identify and characterize a model for cys-leukotriene receptor(s) (pLT receptors) in human lung parenchyma for the development of new and selective antagonists which might represent potential drugs for the pharmacological treatment of asthma.

The approach we decided to follow is characterized by the use of computer modeling of ligand-binding data to test different biological models of increasing complexity. ^{3}H-LTD_4, ^{3}H-LTC_4 and the potent antagonist ^{3}H-ICI 198,615 were used as labeled ligands. Self- and cross-displacement curves were constructed using the three corresponding unlabeled compounds and several other unlabeled ligands, both agonists and antagonists. The present studies indicate heterogeneity of binding sites for LTD_4 in membranes from human lung parenchyma, a high affinity-low capacity class of sites (K_d=0,13 nM; B_{max}=0.007 pmol/mg prot) and a low affinity-high capacity class of sites (K_d=4.2 μM; B_{max}=21 pmol/mg prot). The specific leukotriene antagonist ICI 198,615 (K_{d1}=0.027 nM; K_{d2}=337 nM) as well as the agonist LTE_4 (K_{d1}=40 nM; K_{d2}=24.9 μM) recognize the same two classes of binding sites. A similar heterogeneity is found for LTC_4 binding sites (K_{d1}=0.14 nM; K_{d2}=1.3 μM), but these sites do not seem to be exactly the same identified for the previous compounds. Indeed, cross-displacement does not take place between LTC_4 and ICI 198,615.

To pharmacologically characterize the above mentioned classes of leukotrienes binding sites, we have performed a series of heterologous displacement curves using a series of chemically different leukotriene antagonists such as Ro 24-5913, SKF 104353 and its enantiomer SKF 104373, FPL55712 and LY171883. The computer assisted analysis indicates that they all seem to recognize a homogeneous class of binding sites, but not necessarily the same one. In fact, the most potent antagonists of this series, Ro 24-5913 (K_d=25 nM) and SKF 104353 (K_d=3.7 nM) interact with the high affinity-low capacity binding site, while SKF 104373 (K_d=6.3 μM), FPL55712 (K_d=7.3 μM) and LY171883 (K_d=15.9 μM) seem to interact with the low affinity-high capacity binding site, both recognized by ^{3}H-LTD_4 and ^{3}H-ICI 198,615. Whether both the identified binding sites are of biological importance remains to be established.

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325P RESPONSE TO ConA OF LYMPHOCYTES HARVESTED FROM IN VIVO TREATED MICE OR *IN VITRO* CHALLENGED WITH LYSOZYME

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Lysozyme (hen egg-white lysozyme) is capable of controlling tumor growth with a number of experimental tumors (Sava et al., 1988). The antineoplastic activity of plasma samples obtained from lysozyme treated mice (Sava et al., 1989), the effects on spleen (Ceschia et al., 1988) and the improved response of splenocytes to ConA (Sava et al., 1992) indicate that lysozyme behaves like a biological response modifier that, after oral administration elicits host responses against tumors and particularly against tumor metastases. Correspondingly, in a phase-II clinical study with a group of patients with impaired lymphocyte subsets and/or functions, lysozyme significantly modified CD3+, CD4+ and CD8+ cells (Cartei et al., 1992).

With the present investigation we examine the effects of lysozyme given p.os at 100mg/Kg/day for 10 consecutive days on the pattern of response of lymphocytes from different sites. The study is focused on the examination of the mitogenic activity of ConA in lymphocyte subsets, *in vitro* selected with IL-2, obtained from intact mice and from hosts carrying i.m. implants of MCa mammary carcinoma. Similar studies were also performed on lymphocytes freshly removed from the test organ, with or after removal of adherent mononuclear cells using lysozyme at 25-250μg/ml. The results indicate that lysozyme a) increases the response of all lymphocyte subsets with the exception of those harvested from the gut associated lymphatic tissue (GALT) (thymus 3.49 vs 1.44; TIL 1.03 vs 0.54; spleen 19.96 vs 4.50; blood 1.03 vs 0.89 and GALT 0.48 vs 0.50) and b) raises the tumor dependent reduction of responses to ConA of splenocytes to the basal values of intact mice (80.6% vs 58.7% of untreated controls). On GALT, lysozyme causes a marked reduction of the number of the masses of lymphatic tissue placed under the basal lamina but not Peyer's patches (reduction by 36.6±11.8, $p<0.05$, t-test). This result might explain the lack of response to ConA of lymphocytes obtained from this tissue. These data support the view that the oral administration of lysozyme to tumor bearing mice promotes the recovery of lymphocyte responses slowed down by tumor growth. This effect has probably to be attributed to a mobilization of lymphocytes from GALT that bring the information sistemically to other lymphatic tissues; this data stress the observation that lysozyme absorption to circulation is not a pre-requisite for effectiveness on tumor growth and on metastasis formation.

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326P SPECIES VARIABILITY IN THE STEREOSELECTIVE *N*-OXIDATION OF *N*-ETHYL-*N*-METHYLANILINE

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N-Oxidation frequently represents a major route of metabolism of aliphatic, alicyclic and *N,N*-dialkylarylamines and is believed to be mediated by the flavin-containing monooxygenase (FMO) enzyme system (Damani, 1988). In order to investigate the stereoselectivity of the tissue and species specific isoforms of FMO we have examined the *in vitro* metabolic *N*-oxidation of *N*-ethyl-*N*-methylaniline (EMA). Such tertiary amines are prochiral but the corresponding *N*-oxide metabolites exist in two enantiomeric forms. Data are presented here with respect to species variability in the stereoselectivity of *N*-oxidation of EMA. Incubations were performed using hepatic microsomal tissue derived from male Wistar rats, LACA mice, DBA/2Ha mice, New Zealand White rabbits and Dunkin Hartley guinea-pigs (n=3 in each case) in the presence of appropriate cofactors (37°C; pH 7.4). Initial incubations were performed to determine the maximum linear time period for *N*-oxide formation with a fixed substrate concentration (1mM) and microsomal tissue content (equivalent to 0.5 g original tissue). The linear time period was then used for subsequent incubations where substrate concentration was varied. Quantitative analysis of the samples was by reverse-phase HPLC and chiral analysis of the *N*-oxide metabolite by use of a Chiralcel OD HPLC chiral stationary-phase. Sample preparation was by solid-phase extraction of the analytes from the microsomal matrix (Hadley *et al*, 1993).

The major microsomal metabolites of EMA were *N*-ethylaniline, *N*-methylaniline and EMA *N*-oxide in each species. Both enantiomers of the EMA *N*-oxide were found in all species with the (-)-*S*-isomer predominating in each case. The calculated enantiomeric excesses (e.e.) were found to vary between species (Table 1), but within a species only small differences were seen in e.e. on varying incubation time, microsomal protein content and substrate concentration. Apparent Km values for a single species were the same for the formation of both enantiomers of the *N*-oxide, whereas the Vmax values differed (Table 1).

Table 1. Stereoselectivity in the *in vitro* metabolic *N*-oxidation of EMA.

Species	*e.e. (% with respect to *S*)	Km (1 x 10^{-4} M)		Vmax (nmoles/mg protein/min)	
		R	*S*	*R*	*S*
Rat	56 ± 3.1	0.90	0.89	0.39	1.38
LACA Mouse	52 ± 1.2	1.22	1.22	0.55	1.76
DBA/2Ha Mouse	47 ± 3.0	1.22	1.22	0.44	1.20
Rabbit	68 ± 0.7	2.35	2.36	0.55	2.82
Guinea-Pig	39 ± 3.0	2.64	2.64	1.00	2.33

*e.e. [(-)-(*S*) % - (+)-(*R*) % (mean ± sd)] calculated with respect to the number of points in the concentration range (n = 6 - 8).

The results indicate for the first time that stereoselective *N*-oxidation of EMA varies between species, resulting in a rank order of e.e. of rabbit > rat ~ LACA mouse ~ DBA/2Ha mouse > guinea-pig. The possibility exists that within a species *N*-oxidation is mediated by a single isoform of FMO; the enantiomeric composition of the product being determined by the flexibility of the prochiral substrate in the enzyme active site, which appears to vary between species.

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327P EFFECT OF *SAMBUCUS NIGRA* L ANTHOCYANINS ON BRAIN GLUTATHIONE CONTENT IN SELENIUM-DEFICIENT RATS

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The most important functional seleno-protein described in man and animals is selenium-dependent glutathione peroxidase (Se-GSH-Px). This enzyme is responsible for protecting the cell from peroxidative damage (one of the crucial components in the pathogenesis of several degenerative processes). Furthermore, its activity (also in the brain) is subjected to several influences, including dietary selenium (Beckett et al., 1989).
Flavonoids constitute a group of naturally occurring benzo-γ-pyrone derivatives which have been found to possess various biological properties; many of these actions are correlated to their antioxidant activity. Among flavonoids, anthocyanins and their aglycons (anthocyanidins) are known to act as cytoprotective agents, very likely because of their ability to scavenge free radicals (Morazzoni & Malandrino, 1988). The present study was undertaken to investigate, in the rat, the protective effects of a standardized extract of *Sambucus nigra* L. corresponding to 40.96% anthocyanins (IdB 1455) against dietary selenium (Se) deficiency-induced changes in cerebral levels of glutathione (GSH) and ascorbic acid, which are useful parameters for the detection of free radical activity.

Male Sprague Dawley rats (weighing 250-270 g at the beginning of the experiments) were used; they were housed in controlled conditions and tap water was available ad libitum. For 6 weeks, the animals were fed a rodent diet not supplemented with Se; only a group of rats (controls Se+) received the same diet supplemented with Se. During this period, IdB 1455 (30, 50 mg/Kg) was given by gastric gavage, 3 times a week. A group of rats fed the diet not supplemented with Se (controls Se-) and controls Se+ received the same volume of the vehicle alone (5% arabic gum suspension). Twentyfour hours after the last treatment, the rats were killed and their brain immediately removed. Brain GSH levels were measured according to a modification of the method described by Kudo et al. (1990); cerebral levels of ascorbic acid were evaluated as described by Lyrer et al. (1991). Cerebral GSH levels (expressed as mean ± S.D., n = 10) decreased from 7.27 ± 0.89 μg/mg protein in controls Se+ to 5.39 ± 0.802 μg/mg protein in controls Se- ($P < 0.05$ calculated by Anova Tukey test), without modifications in brain ascorbate concentrations. This change was prevented by administration of IdB 1455. Thus, one can speculate that the protective effect of IdB 1455 on brain GSH depletion induced by Se deficiency is related to anthocyanin capability to scavenge free radicals. To confirm this hypothesis, IdB 1455 inhibitory effects on non-enzymatic lipid peroxidation were evaluated "in vitro" in a peroxidizing model formed by linoleic acid and Fe2+ (Valenzuela et al., 1986). The addition of IdB 1455 to the peroxidizing system elicited a concentration-dependent, long-lasting inhibition of linoleate peroxidation. These results indicate that IdB 1455 is endowed with a significant antioxidant activity preserving brain from GSH depletion induced by Se-deficient diet in rats.

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328P THE UPTAKE OF HISTAMINE BY RAT WHITE ADIPOSE TISSUE

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The wide variety in histamine action and its related mechanisms has been extensively investigated since this amine was first isolated from various mammalian tissues (Best, 1927). It is now clear that histamine regulates many physiological and pathophysiological functions through at least three types of membrane receptors (Hill, 1990). Recently, we observed that this amine can modulate lipolysis, one of the most important functions of white adipose tissue, acting through two types of receptors: the H_1 which inhibits and, apparently, the H_2 which stimulates lipolysis (Raimondi et al., 1992). Moreover, we found that histamine is present in white adipose tissue where it is mainly associated to mast cell population, but seems also to be localized within adipose cells (Raimondi et al., 1992). Even if rat white adipose tissue is not provided with the classical histaminase activity (diamine oxidase), histamine metabolism is guaranteed by the presence of semicarbazide-sensitive amine oxidase activity, which probably concurs with N-methyl transferase in the control of histamine levels (Raimondi et al., 1991). To further characterize the relationship between histamine and white adipose tissue, we investigated the possible uptake system for histamine in this tissue.
Small white adipose tissue pieces (weighing about 10 mg) or white mature adipocytes (prepared according to Rodbell, 1964) were preincubated in Krebs-bicarbonate buffer (pH = 7.4) at 37 °C in a humidified atmosphere of 95% O_2 and 5% CO_2 in the presence of 0.1 mM semicarbazide and 1 mM pargyline to inhibit histamine metabolism. Various concentrations of [^{3}H]-histamine were then added and kept in contact with the tissue pieces or adipocytes for different times. When ouabain (1 mM) or histamine receptor antagonists were used, they were preincubated for 30 min before the addition of [^{3}H]-histamine. When histamine receptor agonists were used, they were added simultaneously with [^{3}H]-histamine. [^{3}H]-histamine was taken up by white adipose tissue and isolated white adipocytes in a time, concentration and temperature dependent manner. The rate of uptake was linear over the first 10 min, so that we determined the apparent kinetic constants of this system which were the following: Km = 11.71 ± 0.39 μM; Vmax = 0.156 ± 0.030 nmoles/ g of tissue/ min for white adipose tissue (n = 5); Km = 1.99 ± 0.46 μM; Vmax = 0.024 ± 0.018 nmoles/ mg of proteins/ min for white mature adipocytes (n = 10). This process was inhibited by 1 mM ouabain both in white adipose tissue (49.6 ± 4.8% of inhibition) and in isolated white adipocytes (44.5 ± 10% of inhibition). Histamine uptake in isolated white adipocytes was unaffected by 1 μM pyrilamine and 100 μM 2-methyl histamine, an antagonist and an agonist of histamine H_1 receptors respectively, whereas 100 μM 4-methyl histamine and dimaprit (H_2 agonists) and 1 μM cimetidine and ranitidine (H_2 antagonists) maximally inhibited this process respectively of 38 ± 14 %, 47 ± 10 %, 51 ± 7 %, 45 ± 8 % (n = 5, $p < 0.05$). These results indicate that histamine is taken up by white adipose tissue and isolated white adipocytes by an active transport system, which could probably be associated with an H_2 histamine receptor. The mechanism by which the H_2 receptor is associated with the transport system remains to be elucidated.

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329P ARE THE PROTECTIVE EFFECTS OF PHENTOLAMINE AGAINST HYPOXIA DEPENDENT ON α-BLOCKADE?

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Phentolamine is generally considered to be a competitive α_1- and α_2-adrenoceptor antagonist without any agonist activity. However, authors suggested that phentolamine has agonist-like activity at rabbit presynaptic receptors in cardiovascular system (Broadhurst et al., 1983). Moreover, recent studies have shown that phentolamine can antagonize the effects of cromakalim, an ATP-dependent K^+ channel opener, in different models (McPherson & Angus 1990; Schwietert et al., 1992) by an action unrelated to the blockade of α-adrenoceptors. The aim of this study was to compare the effect of phentolamine with prazosine (a selective α_1-adrenoceptor antagonist) and idazoxan (a selective α_2-adrenoceptor antagonist) on hypoxia-induced modifications of atrial function. All experiments were carried out on isolated rat atria. Hypoxia was obtained by bubbling the organ bath solution with 20% O_2- 5% CO_2- 75% N_2 (PO_2= 180±6 mm Hg) and maintained for 30 min. Results are expressed as mean values ± sem of 8-10 experiments. Hypoxia induced a great decrease of contractile tension (-83±2%), an increase of diastolic tension (+3.4±0.7 mN) without significant variation of atrial rate. In normoxia (PO_2= 370±9 mm Hg), after 20 min of incubation with 10μM prazosin a decrease of both contractile tension (-8.6±4.4%) and frequency (-22.5±5.6%) was observed. In the presence of prazosin, hypoxia-induced variations of atrial function were similar to those obtained with hypoxia alone. Idazoxan (100μM), after 20 min of incubation, decreased contractile tension (-12.8±3.1%) and slightly reduced atrial rate (-5.8±1.9%). Idazoxan did not counter the effects of hypoxia on isolated atria. In view of the fact that the two α-antagonists, prazosin and idazoxan, were unable to influence the effects of hypoxia, we studied also the action of mixed prazosin and idazoxan against hypoxia. After incubation in normoxia of mixed drugs (20 min), both contractile tension and atrial rate increased (+65.8±9.6% and +33.5±8%, respectively). In the presence of both α-antagonists (10μM prazosin plus 100μM idazoxan), the effects of hypoxia did not change. Thus, the action of prazosin plus idazoxan was different from that of phentolamine. Further, we studied the effect of 10 μM phentolamine on hypoxic atria from reserpinized rats. In this condition, phentolamine was unable to contrast the hypoxia-induced variations. In conclusion, the present data indicate that the protective effect of phentolamine on atrial function during hypoxia does not depend on α-adrenoceptor antagonism (α_1 or α_2) but is related to catecholamine levels.

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330P THE PERCUTANEOUS ABSORPTION OF NICOTINE USING THE RABBIT ISOLATED EAR PREPARATION

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The bioavailability of many drug preparations applied to the skin can be tested *in vitro* using diffusion cells. The release rate *in vitro* is dependent on the lipophilicity of the drug, its diffusion characteristics and partitioning of the drug between the drug preparation and the skin barrier. Most studies *in vitro* assume that the skin is a passive barrier and ignore any potential contribution to bioavailability from enzyme processes. Nicotine is often administered topically as an anti-smoking aid and we have studied its percutaneous absorption and metabolism when applied topically to the isolated perfused rabbit ear.

New Zealand white rabbits were heparinised, anaesthetised with pentobarbitone and the main ear artery cannulated. After removal, the ears were continuously perfused with Tyrode alone or with Tyrode containing either rabbit plasma or dialysed egg albumin. The perfusate was recirculated after aeration and warming to 37°C. Aliquots (1ml) were removed at various times and assayed by GLC for nicotine and two of its metabolites, nicotine N-oxide, and cotinine as previously described (Keysell & Davies,1985). Lactate dehydrogenase (LDH) activity, a measure of tissue damage was measured photometrically (Vassault 1983). After perfusing at 1.3 ml min^{-1} for 240 min the ears became oedematous, their weight increasing by 85%, 63% and 12% for Tyrode, Tyrode with 30% plasma and Tyrode with albumin (70mg ml^{-1}) respectively. Similarly, LDH activity was measured at 2170 mU, 330 mU and 490 mU ml^{-1} respectively.
The viability of the preparation to respond to vasoconstrictor agents was assessed by infusing noradrenaline, NA, (60μg) at the end of the experiment. A transient vasoconstriction to NA was obtained in ears perfused with Tyrode alone, but constriction persisted on average for 5 min or 38 min when Tyrode containing plasma or albumin was used. Thus, viability of the ears was maintained most readily in ears perfused with Tyrode containing albumin and this perfusion fluid was used in all subsequent studies.

Geliperm discs (21 mm diameter) containing nicotine base (9.5mg), were applied to the inner surface of ears preparations perfused at a rate of 0.54ml min^{-1}. The percutaneous transport of nicotine into the perfusate showed a lag time of 30 min, followed by a linear increase in concentration to give a total amount of 1.55 mg of base transported in 6 h. The amounts of metabolites measured at the end of the experiment were nicotine N-oxide 95 μg and cotinine 120 μg. This study suggests that the microsomal cytochrome P450 and the flavoprotein, amine oxidase, contribute to the metabolism of nicotine during its percutaneous absorption *in vivo*.

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331P ANTINOCICEPTIVE ACTION OF GLYCINAMIDE

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Analgesics are subdivided into two main groups according to their ability to relieve mild, moderate or severe pain. Narcotic (opiod) analgesics are used to control moderate and severe pain but their employment is limited because of the severity of their side effects. Non-steroid anti-inflammatory drugs (NSAIDs) are widely used against inflammatory diseases. Although their mechanism of action is thought to be mainly peripheral (Vane, 1971), they are also employed as general analgesics, even when inflammation plays a minor role in the pathophysiology of pain. Besides their pharmacological properties, NSAIDs exhibit some considerable undesired effects.

The antinociceptive properties of glycine are contradictory because it is devoid of any analgesic activity but it can increase the antinociceptive action (a.a.) of morphine (Carrara *et al.*, 1990). Furthermore, the latter effect is hampered because the non essential aminoacid glycine does not cross the brain-blood barrier with ease. This study was performed in order: 1) to evaluate the a.a. of glycinamide (Glyam), a supposed glycine pro-drug endowed with higher liposolubility; 2) to compare its pharmacological action with those of narcotics and NSAIDs; 3) to study whether Glyam can modify the a.a. of these drugs; and 4) to evaluate the liability of Glyam to cause gastrointestinal side-effects.

Glyam was 2.6 times less active than acetylsalicylic acid in the mice phenyl-*p*-quinone writhing test (ED_{50} = 83±14 mg/kg p.o.). Lower doses of Glyam (25-50 mg/kg p.o.) significantly increased the anti-writhing action of NSAIDs and morphine. Furthermore, Glyam failed either to produce in mice any detectable thermal antinociceptive activity (hot-plate) or to displace [^{3}H]-naloxone from crude synaptic membrane of rat brain. Nevertheless, Glyam decreased, in a significant manner, the thermal antinociception of morphine. It differed from standard NSAIDs because the doses that produced anti-writhing action did not show any ulcerogenic liability (UD_{50} > 2 g/kg). The ulcerogenic index (UD_{50}/ED_{50}) was close to 10 for the reference NSAIDs (indomethacin, naproxen, ibuprofen and acetylsalicylic acid), whereas was higher than 25 for Glyam. The above results allow distinguish Glyam from both the opiate and NSAID families. Furthermore, the p.o. co-administration of Glyam and a NSAID could provide the desired analgesic effects using lower doses of NSAIDs, so minimizing the side effects of this class of drugs.

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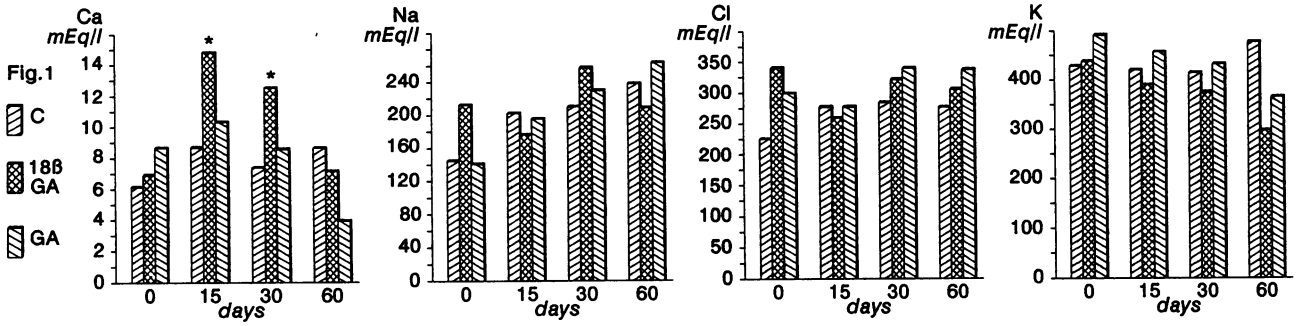
332P CYCLIC AMP MAY BE INVOLVED IN 18β GLYCYRRHETINIC ACID-INDUCED HYPERTENSION

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In a recent work (Rossi *et al* (1992)) we showed that glycyrrhizin (GA) and 18ß glycyrrhetinic (18ß GA) at 30 and 15 mg/kg/die per os for 30 days cause significant increases in systolic pressure and a significant decrease in diuresis in normotensive rats. The effect was transitory in GA-treated animals, but grew steadily from 15th day of treatment in 18ß GA-treated animals. This led us to pinpoint the diastereoisomer 18ß GA as the molecule largely responsible for bouts of hypertension (already reported in clinical studies of particularly sensitive patients). In order to ascertain its mechanism of action, we conducted further tests on three other groups of normotensive male SD rats (mean weight 300 ± 20g) divided into a) controls b) rats treated with 30 mg/kg/die/os of GA and c) rats treated with 15 mg/kg/die/os of 18ß GA. As well as recording pressor and diuretic effects, we used a selective potentiometric technique to assay the electrolytes Na^+, K, ^+Cl, $^-Ca^+$ and P^+ in samples of urine taken from rats housed in single metabolic cages for 24h with water ad libitum and normal laboratory chow. Assays were carried out at the beginning of treatment (t=0), on the 15th and 30th day and again 1 month from the end of treatment (t=60).

The results confirm the previous data regarding pressor values and urine retention and also show that, in the 18ß GA-treated animals, there is a massive and significant elimination of the calcium ion from the urine (Fig.1, *P<0.05) with accompanying hypertension. This would suggest that there is a considerable increase in the number of L-type channels specific to the calcium ion, which are in turn activated intracellularly by AMPc.

This interesting hypothesis clearly requires fuller investigation in order to clarify the role played by 18ß GA in bouts of hypertension. However, it must be stressed that, at the doses used, suspension of treatment leads to a complete reversal of the effects observed (Fig.1).



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333P SPECIFIC INHIBITION OF L-CARNITINE UPTAKE INTO PRIMAY RAT CORTICAL CULTURES BY GABA AND GABA UPTAKE BLOCKERS

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L-carnitine is involved in the transport of acyl groups into mitochondria for β-oxidation. In the brain, L-carnitine is found to be differentially distributed with the highest level of carnitine being reported in the cerebellum and lowest in hippocampus and pons (Shug et al., 1982). The levels of L-carnitine are probably controlled by the plasma levels and active uptake processes. Experiments were carried out using primary cells prepared from rat fetal cortex (Virmani et al., 1993a). The cell density was one million cells per well at the start of the culture and experiments were carried out at 2 weeks of age when the cells were highly differentiated and sending out numerous processes. The cells were washed three times with 2 ml of Krebs-bicarbonate buffer and preincubated with 5 min with this buffer. This was then replaced with the same warmed buffer containing the 14C-labelled carnitine (100 μM) and uptake allowed to occur at 37°C for 15 min. After the incubation the supernatant was removed and the cells were washed three times with 2 ml of ice-cold Krebs buffer. We have shown before that the uptake of L-carnitine into cultured rat cortical neurones was temperature dependent, as well as potently inhibited by factors affecting the Na gradient (0.5 mM ouabain and Na free medium) (Virmani et al., 1993b). It was less dependent on the blockade of energy by NaCN (1mM) or rotenone (1.25 μM). In addition, we found that there was competition of L-carnitine uptake by molecules resembling its structure, e.g. D-carnitine, acetyl-L-carnitine (ALC) and gamma aminobutyric acid (GABA). GABA was the most potent inhibitor of L-carnitine uptake and at 100 μM its effects were already maximal (53 % inhibition). In further studies we have found that specific GABA uptake blocker cis-4-hydroxynipecotic and nipecotic acid also inhibit L-carnitine uptake, at 50 μM they caused a 50 % and 56 % reduction respectively, in L-carnitine uptake compared to control. In contrast, the $GABA_A$ receptor agonists, isonipecotic acid and isoguvacine, at similar concentrations (50 μM), did not inhibit the uptake of the L-carnitine to the same extent (3 % and 11 % respectively). The results indicate that the uptake of L-carnitine by rat cortical neurones occurs in part by a process that can be potently inhibited by GABA and GABA uptake blockers.

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334P AN INTERACTIVE COMPUTER-BASED TUTORIAL PACKAGE TO TEACH NERVE BIOPHYSICS TO UNDERGRADUATE STUDENTS

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Interactive, computer-based learning materials offer a flexible and effective approach to undergraduate studies and a number of computer simulations of experiments in physiology and pharmacology have been demonstrated to the Society in recent years (e.g. Dewhurst *et al*, 1992a,b;). Computer-based tutorials may be equally effective learning aids in undergraduate pharmacology (Hollingsworth & Foster, 1991; Ogg & Stevenson, 1993) and offer a student-centred alternative to staff-intensive small group tutorials. Here we present details of a program designed to teach the biophysical properties of nerve cells to undergraduate students from a range of biomedical science courses.

The program, which is contained on a single diskette, is written in C (Borland, Turbo C++) to run on any IBM-compatible microcomputer with a graphics adaptor (VGA, EGA, Hercules). A combination of text and high-resolution, colour graphics (with some animation) is used to guide students through the principles of ion movement across membranes, ion channels and the resting membrane potential, the genesis of, and illustration of conductance changes during, the action potential, threshold of excitation and refractory period, propagation of the action potential and the cable properties of the nerve. The material is based on the properties of the giant axon of the squid and the program is designed to be used with a companion program which simulates this preparation and allows students to investigate the effects of changing various ion concentrations (in intracellular and extracellular fluids) on the resting and action potential and to simulate voltage clamp experiments.

The tutorial is highly interactive and allows students to use data generated from theoretical models to test the importance of different ions in giving rise to the resting membrane potential and action potential. The section on cable properties covers the local circuit theory of propagation and explains the resistive and capacitative characteristics of the axon.

It is estimated that the program will occupy students for 3-4 hours of intensive study and is structured such that, in each section, students progress, in a linear fashion, from relatively simple through to quite complex material, making the package suitable for a range of student abilities.

This work was supported by The Lord Dowding Fund (NAVS).

Dewhurst, D.G., Overfield, J., & Williams, A.D. (1992a) *Brit. J. Pharmac.* **106**, 144P.
Dewhurst, D.G., Leathard, H., Higman, M. & Ullyott, R.T. (1992b) *Brit. J. Pharmac.* **106**, 143P.
Hollingsworth, M. & Foster, R. (1991) *Brit. J. Pharmac.* **102**, 216P.
Ogg, G.D. & Stevenson, I.H. (1993) *Brit. J. Pharmac.* **108**, 311P.

333P SPECIFIC INHIBITION OF L-CARNITINE UPTAKE INTO PRIMAY RAT CORTICAL CULTURES BY GABA AND GABA UPTAKE BLOCKERS

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L-carnitine is involved in the transport of acyl groups into mitochondria for β-oxidation. In the brain, L-carnitine is found to be differentially distributed with the highest level of carnitine being reported in the cerebellum and lowest in hippocampus and pons (Shug et al., 1982). The levels of L-carnitine are probably controlled by the plasma levels and active uptake processes. Experiments were carried out using primary cells prepared from rat fetal cortex (Virmani et al., 1993a). The cell density was one million cells per well at the start of the culture and experiments were carried out at 2 weeks of age when the cells were highly differentiated and sending out numerous processes. The cells were washed three times with 2 ml of Krebs-bicarbonate buffer and preincubated with 5 min with this buffer. This was then replaced with the same warmed buffer containing the 14C-labelled carnitine (100 μM) and uptake allowed to occur at 37°C for 15 min. After the incubation the supernatant was removed and the cells were washed three times with 2 ml of ice-cold Krebs buffer. We have shown before that the uptake of L-carnitine into cultured rat cortical neurones was temperature dependent, as well as potently inhibited by factors affecting the Na gradient (0.5 mM ouabain and Na free medium) (Virmani et al., 1993b). It was less dependent on the blockade of energy by NaCN (1mM) or rotenone (1.25 μM). In addition, we found that there was competition of L-carnitine uptake by molecules resembling its structure, e.g. D-carnitine, acetyl-L-carnitine (ALC) and gamma aminobutyric acid (GABA). GABA was the most potent inhibitor of L-carnitine uptake and at 100 μM its effects were already maximal (53 % inhibition). In further studies we have found that specific GABA uptake blocker cis-4-hydroxynipecotic and nipecotic acid also inhibit L-carnitine uptake, at 50 μM they caused a 50 % and 56 % reduction respectively, in L-carnitine uptake compared to control. In contrast, the $GABA_A$ receptor agonists, isonipecotic acid and isoguvacine, at similar concentrations (50 μM), did not inhibit the uptake of the L-carnitine to the same extent (3 % and 11 % respectively). The results indicate that the uptake of L-carnitine by rat cortical neurones occurs in part by a process that can be potently inhibited by GABA and GABA uptake blockers.

Shug, A.L. et al. (1982) *Life Sci.* **31**, 2869-2874.
Virmani, M.A. et al. (1993a) *Pharmacol. Res.* **27**, 57-59.
Virmani, M.A. et al. (1993b) *Neuropsychopharmacology* **9**, 144S, Abs 322.

334P AN INTERACTIVE COMPUTER-BASED TUTORIAL PACKAGE TO TEACH NERVE BIOPHYSICS TO UNDERGRADUATE STUDENTS

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The program, which is contained on a single diskette, is written in C (Borland, Turbo C++) to run on any IBM-compatible microcomputer with a graphics adaptor (VGA, EGA, Hercules). A combination of text and high-resolution, colour graphics (with some animation) is used to guide students through the principles of ion movement across membranes, ion channels and the resting membrane potential, the genesis of, and illustration of conductance changes during, the action potential, threshold of excitation and refractory period, propagation of the action potential and the cable properties of the nerve. The material is based on the properties of the giant axon of the squid and the program is designed to be used with a companion program which simulates this preparation and allows students to investigate the effects of changing various ion concentrations (in intracellular and extracellular fluids) on the resting and action potential and to simulate voltage clamp experiments.

The tutorial is highly interactive and allows students to use data generated from theoretical models to test the importance of different ions in giving rise to the resting membrane potential and action potential. The section on cable properties covers the local circuit theory of propagation and explains the resistive and capacitative characteristics of the axon.

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335P HETEROLOGOUS AND HOMOLOGOUS DESENSITIZATION OF HISTAMINE H_1-RECEPTORS IN THE HAMSTER VAS DEFERENS CELL LINE DDT_1MF-2

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336P FLUOROMETRIC DETERMINATION OF ELECTRICALLY EVOKED INCREASE IN $[Ca^{2+}]_i$ IN CULTURED CEREBELLAR GRANULE CELLS

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ORAL COMMUNICATIONS

In oral communications with more than one author, the first author is the one who intended to present the work

1P **Missale C, Boroni F, Losa M, Dal Toso R, Balsari A & Spano PF** Nerve growth factor suppresses tumorigenicity of human prolactinomas

2P **Bowden C, Cheetham SC, Crompton MR, Katona CLE, de Paermentier F, Theodorou AE & Horton RW** Dopamine D_1 and D_2 receptor binding sites in depressed suicide victims

3P **Meucci O, Ventra C, Grimaldi M, Scorziello A, Paolillo M, Apicella A & Schettini G** Lack of alternative mRNA splicing in MMQ prolactin-secreting cell line: expression of a native dopamine D_{2L} receptor isoform

4P **Syers JA, Jenner P, Marsden CD & Hider RC** The iron chelator 1,2-diethyl-3-hydroxy-pyridyn-4-one (CP94) does not protect against MPP^+ toxicity to substantia nigra

5P **Desole MS, Esposito G, Fresu L, Migheli R, Enrico P, Miele M, De Natale G & Miele E** Correlation between 1-methyl-4-phenyl-pyridinium ion (MPP^+) levels and MPTP-induced neurochemical changes in the rat striatum

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7P **Gnanalingham KK, Hunter AJ, Jenner P & Marsden CD** Selective antagonism of the antiparkinsonian effects of benzazepine D_1 dopamine agonists in the MPTP-treated marmoset

8P **Chiamulera C, Tessari M, Valerio E, Reggiani A & Trist DG** The effect of SCH-23390 on food and cocaine self-administration in the rat

9P **De Salvia MA, Roberts AC, Wilkinson LS, Muir JL, Everitt BJ, Robbins TW & Cuomo V** Effects of 6-hydroxydopamine lesions of the prefrontal cortex on the attentional set-shifting ability in monkeys

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12P **Nencini P & Fraioli S** Amphetamine-mediated hyperdipsia is prevented but also reinstated by (-)-norpseudoephedrine

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14P **Spadaro C & Arrigo-Reina R** Clonidine and yohimbine modulate the antinociceptive effect of cholecystokinin in rat

15P **Bax WA, Petterson RWG, Inan T, Bos E & Saxena PR** Heterogeneity of endothelin/sarafotoxin receptors mediating contractions of the human isolated coronary artery

16P **Spampinato S & Ventura C** Dynorphin gene expression and release in the myocardial cell

17P **Guerra L, Cerbai E, Barbieri M, Varani K, Borea PA & Mugelli A** Functioning β_1- and β_2-adrenoceptors coexist in rat cardiomyocytes and are modified by aging

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26P **Kaumann AJ & Sanders L** 5-HT_4 receptor-mediated arrhythmias in isolated human atrium

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28P **Finance O, Manning AS & Chatelain P** SR 33589B, a new amiodarone-like agent: effect on ischaemia-induced ventricular arrhythmias in anaesthetized pigs

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34P **Corsini E, Marinovich M & Galli CL** Differential effects of detergents on interleukin-1 production in murine keratinocyte cell line

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59P **Rose S, Hindmarsh JG & Jenner P** Inhibition of nitric oxide synthase enhances NMDA-evoked dopamine release in rat striatum *in vivo*

60P **Mollace V, Bagetta G & Nisticò G** Evidence that HIV gp 120 glycoprotein activates the inducible isoform of NO synthase in cultured astrocytoma cells

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123P **Perez J, Mori S, Cagnoli C, Zanardi R, Tinelli D, Brunello N, Smeraldi E & Racagni G** Modification of intracellular mechanisms after long term antidepressant and lithium treatment

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