ELECTROPHYSIOLOGICAL PROPERTIES OF THE MESENTERIC ARTERY IN THE HYPERTENSIVE RABBIT

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In the mesenteric artery of rabbits with perinephritis hypertension there is enhanced contractile responsiveness to noradrenaline and increased ^{45}Ca uptake (Hall et al, 1983, 1984).

Rabbits were anaesthetised with pentobarbitone, one kidney wrapped in cellophane and the contralateral kidney removed. Rabbits were used after 8-18 weeks at which time the mean arterial blood pressure of rabbits with wrapped kidneys was 121±4 mmHg and of sham operated rabbits 78±3 mmHg.

Pieces of mesenteric artery 2-3 mm long were supported on parallel stretched wires in Krebs-Henseleit solution at 36°C and a simultaneous recording was made of membrane potential and force. The separation of the wires was set to give circumferential tension in the range 0.3-2.1 g/mm tubular length (equivalent to pressure within a tubular segment of 21-110 mmHg). Increasing tension within this range did not affect the resting membrane potential (RMP), threshold for the initiation of the spike (V_{TS}) or the membrane time constant as calculated from the decay phase of the e.j.p. (τ_{ejp}). It was concluded that the amount of stretch occurring in moderate hypertension does not itself cause notable electrophysiological changes.

The sham and hypertensive arteries had RMP of 71±1 and 68±1 mV, V_{TS} of 50±1 and 48±1 mV and τ_{ejp} of 233±11 and 228±12 ms. It was concluded that the mesenteric artery becomes slightly depolarised with no change in V_{TS} or τ_{ejp} during the development of perinephritis hypertension in the rabbit.

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2P

DIFFERENCES BETWEEN NEGATIVE INOTROPIC EFFECTS OF VERAPAMIL, DILTIAZEM AND KB 944 IN GUINEA PIG PAPILLARY MUSCLE

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The potency of calcium antagonists as negative inotropic agents can be frequency and/or membrane potential dependent (Ehara and Kaufmann, 1978; McDonald et al, 1980). This observation indicates that the various compounds of this pharmacological class do not share the same site of action. The purpose of this communication is to compare the myocardial depressant activity of verapamil, diltiazem and KB 944 on the guinea-pig papillary muscle superfused with either a standard or a depolarizing salt solution.

Right guinea-pig papillary muscles were immersed in a standard physiological salt solution ($CaCl_2$: 1.8 mM), warmed at 30°C and paced at a constant rate (2 Hz). Resting and developped isometric contraction was measured. The resting tension (0.12-0.18 g) was adjusted to give a maximal isometric contraction (0.35-0.5 g). In the depolarizing medium ($CaCl_2$: 3.0 mM) the preparations were paced at 0.2 Hz and exposed to 10 µM of histamine in order to evoke slow action potentials. Under these conditions the resting tension and maximal isometric tension were 0.27-0.30 g and 0.9-1.2 g, respectively. The negative inotropic effects of verapamil, diltiazem and KB 944 were quantified by calculating the concentration of each compound reducing by 50% (IC_{50}) and 75% (IC_{75}) the baseline tension. Slow action potentials were also recorded with glass microelectrodes and the effects of the IC_{50} and IC_{75} concentrations of each compound on dV/dt_{max} (maximal rate of development of action potential: V/s) was determined.

The IC_{50}'s of verapamil, diltiazem and KB 944 in a standard medium were 0.26 ± 0.01 (n=10), 1.7 ± 0.1 (n=7) and 12.1 ± 0.05 (n=7) µM, respectively. These values were 0.21 ± 0.01 (n=10), 0.4 ± 0.01 (n=8) and 2.04 ± 0.12 (n=8) µM under depolarizing conditions. The maximum rate of rise of the slow action potential (dV/dt_{max}) was 32.4 ± 3.2 (n=8), 30.6 ± 2.9 (n=9) and 43.1 ± 5.3 (n=4) V/s under control conditions in the groups used to study verapamil, diltiazem and KB 944, respectively. Verapamil at 0.2 µM (IC_{50}) reduced dV/dt_{max} by 26% and at 0.6 µM (IC_{75}) by 48%. Diltiazem at 0.4 µM (IC_{50}) and 1.0 µM (IC_{75}) decreased dV/dt_{max} by 19 and 40%, respectively. KB 944 at 2.0 µM (IC_{50}) and 5.6 µM (IC_{75}) reduced dV/dt_{max} by 38 and 48%, respectively.

These results indicate that the decreasing order of potency of these agents as negative inotropic agents is verapamil > diltiazem > KB 944. The effects of verapamil are independent of the resting membrane potential since its IC_{50} values in standard and depolarizing mediums were similar. However, the negative inotropic potency of diltiazem and KB 944 are 4.3 and 6 times greater, respectively, under depolarization conditions. Finally, an IC_{75} is required to observe a 50% decrease of dV/dt_{max} by verapamil or KB 944 whereas a higher concentration of diltiazem is necessary to produce this effect.

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Bay K 8644 augments contraction in smooth and cardiac muscle and it has been proposed that it acts by enhancing the opening of membrane Ca channels (Schramm et al, 1983). The development of hypertension is associated with changes in Ca transport (Hall et al, 1984) which may indicate changes in the function of Ca channels.

Rabbits were anaesthetised with pentobarbitone, one kidney wrapped in cellophane and the contralateral kidney removed. Rabbits were used after 2-5 months at which time the mean arterial pressure of the animals with wrapped kidneys was 115±2 mmHg and of the sham operated animals was 79±3 mmHg. Cross-sectional rings about 5 mm long were prepared from the aorta, and incubated in HEPES-buffered Krebs solution at 37°C, bubbled with O_2. After pre-incubation with Bay K 8644 for 25 min, the tissue was loaded with ^{45}Ca 0.5µCi/ml in the presence of an agonist for 10 min, then transferred to HEPES-buffered Krebs solution containing $LaCl_3$ 50mM, kept at 0.5°C for 60 min. Finally, calcium remaining in the tissue was extracted with EDTA 5mM and counted by liquid scintillation.

Basal Ca uptake was 99±6 nM/g tissue in aortic rings from the sham operated rabbits and was increased to 131±17 nM/g tissue in the hypertensives. Ca uptake was stimulated by Bay K 8644 14nM and 56 nM, by KCl 30 mM and by noradrenaline (NA) 50 nM, but these effects were less in the hypertensives than in the shams (Bay K 8644 at 14 nM 16% vs 28%, at 56 nM 27% vs 36%, KCl 92% vs 152%, NA at 50 nM 10% vs 15%). Bay K 8644 augmented the stimulation of Ca uptake produced by KCl or NA, but this effect was also less in the hypertensives (21% augmentation for KCl, 41% augmentation for NA) than in the shams (40% augmentation for KCl, 116% augmentation for NA). These results probably indicate that the Ca channels become less sensitive to Bay K 8644 or/and calcium efflux is increased, and confirm the view that changes in cellular Ca regulatory mechanisms occur during the development of hypertension.

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4P

COMPARATIVE ANTIARRHYTHMIC, ELECTROPHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF BEPRIDIL AND THE CALMODULIN ANTAGONISTS TFP AND W 7

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The antianginal agent, bepridil, also possesses ventricular anti-arrhythmic actions, attributed to inhibition of the fast inward sodium current (reviewed by Marshall et al., 1984). Recently bepridil has also been shown to inhibit calmodulin in several non-cardiac tissues (Itoh et al., 1984; Agre et al., 1984; Lugnier et al., 1984). The aim of the present study was to investigate the potential antiarrhythmic and electrophysiological actions of the calmodulin antagonists, TFP and W7, to ascertain whether calmodulin inhibition could play a role in the antiarrhythmic actions of bepridil. TFP and W7 (2.5-10 mg/kg i.v.) and bepridil (1-5 mg/kg i.v.) were all markedly effective in antagonizing the development of reperfusion-induced ventricular fibrillation and death following 5 minutes of coronary artery occlusion in the anaesthetised rat. TFP and bepridil also reduced the incidence of ventricular tachycardia whilst all 3 drugs reduced its duration. All 3 drugs were also effective in reducing ischaemia-induced arrhythmias produced by 30 minutes of coronary artery ligation. Only bepridil (2-10 μM) induced a marked concentration-dependent decrease in the maximum rate of depolarization (Vmax) of guinea pig papillary muscle (in the absence of a fall in resting membrane potential (RMP)), prolonged action potential duration (APD) and increased the absolute refractory period (ARP). In contrast W7 (5-50 μM) caused only a very modest decrease in Vmax whilst only high concentrations of TFP (40-100 μM) reduced Vmax and this was accompanied by a fall in RMP. Neither TFP nor W7 significantly altered ARP. Phentolamine (0.5-2 mg/kg) did not significantly reduce the incidence of reperfusion-induced arrhythmias suggesting that α-adrenoceptor antagonist properties of TFP or W7 are not important in their anti-arrhythmic effects.

Calmodulin is known to regulate cGMP-specific phosphodiesterase activity (Grain & Appleman, 1978). However, in concentrations which decreased contractile tension by 36% and 48% respectively, both bepridil (50 μM) and TFP (200 μM) failed to increase cGMP levels in rabbit papillary muscles.

Thus although the calmodulin-antagonists, bepridil, TFP and W7 all exerted significant anti-arrhythmic effects, the underlying mechanisms responsible for the effects of the individual drugs may be different.

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REDUCTION OF STIMULUS EVOKED POST - BUT NOT PRESYNAPTIC CALCIUM INFLUX IN RAT HIPPOCAMPUS BY ORGANIC CALCIUM ANTAGONISTS

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Influx of Ca^{++} into neurones via voltage-gated membrane channels is involved postsynaptically in control of cellular excitability and, presynaptically, in mediating transmitter release. Organic Ca^{++} "antagonists", such as verapamil, seem to block somatic and dendritic Ca^{++}-conductances when applied at high concentrations but, generally, have little effect on transmitter release (see Miller and Freedman, 1984). In the present study we have looked at the effects of such agents on both pre and postsynaptic Ca^{++}-influx in area CA1 of the rat hippocampus in vitro.

Hippocampal slices were prepared and maintained in vitro using conventional methods. Pyramidal cells in CA1 were orthodromically activated by stimulation of the Schaffer-commissural fibres in s.radiatum and antidromically activated by stimulation of the alveus. A double-barrelled reference(NaCl)/ion-sensitive electrode was used to record field potentials and concommitant changes in extracellular Ca^{++}, close to s. pyramidale, approximately equidistant between the stimulation sites. Repetitive stimulation (10s, 20Hz, 10-40μA) at either site evoked large decreases in extracellular Ca^{++} (up to 0.7mM). Blockade of synaptic transmission by reduction of Ca^{++} (2mM to 0.2mM) and elevation of Mg^{++} (2 to 4mM) in the perfusion medium abolished the field potentials evoked by orthodromic stimulation but appreciable decreases in extracellular Ca^{++} could still be recorded (up to 10μM). This remaining change in Ca^{++} was attributed to influx into presynaptic terminals. Antidromically evoked field potentials persisted in the low Ca^{++} medium and reductions in extracellular Ca^{++} evoked by this stimulation were easily recorded (up to 30μM). The latter are likely to reflect predominantly postsynaptic Ca^{++}-influx. We tested the effects of 3 Ca^{++}antagonists on alternately evoked pre- and postsynaptic Ca^{++}-influxes by including them in the perfusion medium. The maximum percentage changes (mean ±SEM) in Ca^{++} signals evoked by the drugs are shown in the table:

	VERAPAMIL		NIFEDIPINE		FENDILINE	
	50μM	100μM	5μM	10μM	100μM	200μM
PRE	+6±15	+3±14	-5±1	-9±11	+3±4	-1±4
POST	-13±8	-47±9	-37±9	-52±3	-2±6	-17±6

Thus, generally, the agents tested to varying degrees reduced the postsynaptic Ca^{++}-influx but had little effect on the extracellular loss into presynaptic terminals. Nifedipine was clearly the most potent in this respect.

Recent electrophysiological studies have shown the existence of at least two types of neuronal Ca^{++}currents (Llinas and Yarom, 1981; Carbone and Lux, 1984), a slow, inactivating current and a transient, rapidly inactivating current (Carbone and Lux, 1984). The latter current is not blocked by verapamil, while the former is (Boll and Lux, 1985). The present experiments raise the speculative possibility that presynaptic axon terminals in CA1 possess predominantly the transient type of Ca^{++}- channel.

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6P

THE ACTION OF DILTIAZEM ON THE RELEASE OF RENAL KALLIKREIN FROM NORMAL AND SPONTANEOUSLY HYPERTENSIVE RATS

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Factors controlling the physiological release of renal kallikrein still remain unclear. A number of ions, hormones and transmitters have been reported to influence such release (Mills & Newport, 1980; Lâuar et al, 1982). Previous studies have shown that arginine-vasopressin (AVP) raises cAMP in the kidney (Beck et al, 1971). Both cAMP and cGMP stimulate kallikrein release from the submandibular gland and the omission of calcium inhibits this release (Albano et al, 1976). In fact,the role of calcium as an intracellular messenger for initiating enzyme secretion is well documented (Rasmussen & Barrett, 1984). In the present investigation we have examined the effect of calcium on the release of renal kallikrein from cortical kidney slices of spontaneously hypertensive (SHR) and normal (N) rats by AVP and potassium in the presence and absence of the calcium antagonist, diltiazem.

Kidney cortical segments of either normal Wistar (N) (mean arterial blood pressure 116 ± 3.1 mm/hg, wt 271 ± 0.5 g, n = 23), or hypertensive Okamoto (SHR) (mean arterial blood pressure 174 ± 3.4 mm/Hg, wt 296 ± 6.1 g, n = 17) were cut (100-200 μ) using a hand tissue slicer. Pooled slices weighing 150-250 mg were placed into individual Erhlenmeyer flasks containing 5 ml (Krebs-buffer solution, pH 7.4, O_2 95%, CO_2 5%) and incubated in a shaking water bath (120 strokes/min, 37°C) for 20 min. Changes in potassium were balanced with sodium and calcium with magnesium in modified Krebs-buffer solutions. Tissue kallikrein (amidase activity) was measured in the presence of Soya bean trypsin inhibitor (100 μg/ml) using the chromogenic substrate H-D-val-leu-arg-pNA. All samples were trypsin (12.5 μg/ml) activated to give a measure of total kallikrein.

No difference was observed in the basal release of kallikrein between either strain, nor with diltiazem (10^{-3}, 10^{-5}, 10^{-7}M). In the N Wistar rat AVP 10^{-8} stimulated the release of active kallikrein which was significantly reduced by diltiazem. In contrast, potassium (100 mM) evoked active kallikrein release from N and active and total from SHR kidney slices was not affected by diltiazem. Our results therefore indicate that AVP-evoked release of active kallikrein from N Wistar kidney slices is antagonised by diltiazem. However, AVP does not evoke such a release in the SHR Okamotos, probably because of desensitization of AVP receptors occurring in response to a chronically elevated blood pressure. Our experiments also suggest an action of diltiazem on receptor rather than potassium coupled calcium channels in renal membranes.

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SEROTONIN-2(5- HT_2) RECEPTOR MEDIATED PHOSPHOINOSITIDE BREAKDOWN AND CALCIUM MOBILISATION IN A7r5 SMOOTH MUSCLE CELLS

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Serotonin (5-HT) receptors have been classified functionally into M and D receptors (Gaddum and Picarelli, 1957) and by binding studies into 5-HT1 and 5-HT2 receptors (Peroutka and Snyder, 1979). We have recently shown the great similarity between 5-HT2 and D receptors (Engel et al, 1985). It has been demonstrated in several models that 5-HT2 receptors are linked to the inositol lipid metabolism. Evidence is presented here that phosphatidyl inositol 4,5 bisphosphate breakdown and calcium mobilisation can be stimulated via 5-HT2 receptors in A7r5 smooth muscle cells (Doyle and Rüegg, 1985). In these cells, 5-HT stimulates the rapid and transient production of inositol trisphosphate (InsP3) and inositol 1,4 bisphosphate (InsP2). Inositol 1 phosphate (InsP1)accumulates in a time and concentration dependent manner and is potently inhibited by the 5-HT2 antagonists ketanserin and pirenperone. Antagonists of other 5-HT receptors were almost ineffective. 5-HT stimulated the release of 45Ca++ from preloded A7r5 cells with an EC50 of 26 nM. This effect was competitively antagonised by ketanserin (pA2 = 9.03). The effects of various 5-HT agonists and antagonists on the Ca++ release were compared with their affinity for [3H]ketanserin binding to rat brain cortex.

Table 1:

(apparent pK_D or pEC_{50} values, -log mol/l, mean +/- s.e.mean)	Ca^{++} efflux	$5\text{-}HT_2$ binding
5-HT	7.59+/-0.06	7.13+/-0.34
α-methyl-5-HT	7.31+/-0.10	6.90+/-0.18
5-carboxamidotryptamine	3.45+/-0.42	4.66+/-0.11
8-OH-DPAT*	5.50+/-0.14	5.04+/-0.09
RU 24969*	6.05+/-0.27	6.00+/-0.06
ketanserin	9.34+/-0.18	8.69+/-0.10
pirenperone	9.43+/-0.12	8.76+/-0.06
phentolamine	6.74+/-0.33	6.06+/-0.02
ICS 205-930*	5.62+/-0.73	5.36+/-0.40
(-)21-009*	6.20+/-0.01	5.47+/-0.33

*8-OH-DPAT, (8-hydroxy-2-(di-n-propylamino)-tetralin, 21-009, (4-[ter-butyl-amino-2-hydroxy-propoxy]-indol-2-carbonic-acid isopropylester), RU 24969, (5-methoxy-3-[1,2,3,6-tetrahydropyridin-4-yl]1H-indole), ICS 205-930, ((3α-tropanyl)1H-indole-3-carboxylic-acid ester).

There was an excellent correlation between the effects of 13 structurally different antagonists on 5-HT stimulated Ca++ efflux and their affinity for 5-HT2 receptors ($r = 0.928$, $p = 0.0001$), and the rank orders of affinity or potency of a series of agonists also showed good agreement. Furthermore, the use of selective agonists and antagonists showed 5-HT1A, 1B, 1C or 5-HT M receptors are not involved in the 5-HT stimulated Ca++ mobilisation.

The data suggests that in this cell line, 5-HT acts at 5-HT2 receptors to mobilise intracellular Ca++ via the production of InsP3.

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8P

A PREFERENTIAL ACTIVITY OF ADENOSINE AGAINST INTRACELLULAR CALCIUM IN RAT AORTA

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Contractions of the rat aorta in response to various agents are dependent upon extracellular and/or intracellular stores of calcium (Daniel, 1984). Since the mechanism of the vasodilatory action of adenosine is largely unknown, it was of interest to know whether one or both of these calcium sources were modified by the purine.

Male Wistar rats (250-350g) were killed by cervical dislocation and the aorta rapidly removed into Krebs solution. Helical strips were cut and mounted under 0.8g tension. Drugs were added to the bath in a volume of 0.1ml. Sodium concentrations were reduced in high K^+ to maintain isotonicity. Adenosine was added to the bath when the tension induced by added constricting agents had reached its peak.

The potency of adenosine was dependent on the contracting stimulus. When tissues were contracted with equipotent concentrations of agonist, adenosine produced relaxations in the order prostaglandin $F2\alpha \simeq$ noradrenaline(NA) $> K^+$. In the absence of extracellular calcium, the contractions of $PGF2_\alpha$ and NA were depressed to 26.1% and 28.3% of controls respectively, while a depolarising solution of 60mM K^+ failed to elicit any contraction, indicating that $PGF2\alpha$ and NA are dependent upon both intra- and extra-cellular pools of calcium, whilst K^+ uses only extracellular calcium.

When precontraction with 1μM NA was used throughout and $[Ca^{2+}]_{ext}$ was varied from 2.5mM to zero, the response to adenosine (100μM) increased from 22.5±1.7% relaxation to 46.5±6.7% (n=6); $p<0.05$. Also, in the presence of verapamil (1μM), the response to adenosine increased to 37.8±4.2% (n=6); $p<0.05$. These results are not due to the decreased initial tension since ACh induced relaxations (also expressed as a percentage) did not change with differences in the precontraction peak height.

We have also shown that adenosine increases markedly in potency when acting against a low NA precontraction. Cellular $^{45}Ca^{2+}$ influx in response to NA was measured by a modification of the method of van Breemen et al. (1972). The curve of uptake (in terms of % max uptake) against [NA] was not completely superimposable upon a curve of % max tension against [NA]. At low [NA] there was a disproportionately high generation of tension relative to the Ca^{2+} influx generated. The balance at low [NA] therefore may originate from an intracellular store indicating that at lower (5-100nM) [NA] a greater proportion of the calcium used in contraction is of intracellular origin.

We therefore conclude that adenosine induces relaxation in the rat aorta by an action upon calcium availability and that this action is preferentially directed against intracellular calcium.

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PHARMACOLOGICAL PROFILE OF CICLOXOLONE: A NOVEL ANTI-VIRAL AGENT

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Cicloxolone sodium (CCX), a close analogue of carbenoxolone sodium (CBX), has a potent and broad spectrum of anti-viral activity, both in vivo (Poswillo & Roberts, 1981; Csonka & Tyrrell, 1984) and in vitro (Dargan & Subak-Sharpe, 1985). The present work assesses the anti-inflammatory, analgesic and anti-ulcer activities of CCX in comparison with relevant standard drugs: phenylbutazone (PBZ), aspirin (AS) and ibuprofen (IB).

Table 1 Pharmacological profile of cicloxolone (CCX)

Test and route of drug administration	CCX			Standard			
	Dose ($mg.kg^{-1}$)	Inhibition (%)			Dose ($mg.kg^{-1}$)	Inhibition (%)	
Carrageenin oedema (oral)	100	31	**	PBZ	100	67	***
Adjuvant arthritis (oral)	100	68	***	PBZ	100	61	*
Acetic acid writhing (oral) (a)	200	52	***	AS	200	65	***
Carrageenin hyperalgesia (oral) (b)	10	74	***	IB	10	63	***
Stress erosions (i.p.) (cold + restraint)	60	100	*	CBX	60	40	
Electrocautery ulcer (oral)	15	57	***	CBX	20	45	**
Ethanol necrosis (oral)	70	83	***	CBX	70	79	***

(a) Test in mice, all others in rats, (b) Response: increase in pain threshold. Significance: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

It is clear that CCX has a significant and potent anti-inflammatory and analgesic activity. In contrast to most classical anti-inflammatory and analgesic drugs, CCX also has a marked anti-ulcer action. The effect of CCX (and other triterpenoids) on cell membranes may account, in part, for its anti-viral activity. CCX may have cell membrane stabilizing effects, thus counteracting the release of lysosomal enzymes and other mediators of inflammation, ulceration and pain response (Symons & Parke, 1980; Lewis, 1984). It is known that CBX elevates PGE_2 levels and inhibits the formation of TxA_2 and LTB_4 (Peskar, B.M. Personal Communication). If CCX has a similar mode of action, it may ameliorate inflammation and ulceration by increasing PGE_2 levels and decreasing TxB_2 formation, and its possible inhibitory effect on LTB_4 synthesis may account for its analgesic activity (Levine et al, 1984). Such studies are in progress.

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10P

LINOLEIC ACID INHIBITS THROMBOXANE A_2- INDUCED PHOSPHOINOSITIDE HYDROLYSIS AND Ca^{2+} FLUX IN HUMAN PLATELETS

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Polyunsaturated fatty acids (PUFA) (e.g. Linoleic acid, LA), but not saturated fatty acids (e.g. Stearic acid, SA), inhibit platelet activation induced by receptor directed agonists but not by A23187. PUFA did not alter agonist-receptor combination, but perturbed membrane core lipid order, monitored as a decrease in diphenylhexatriene polarization of intact platelets and platelet membranes (Karnovsky et al, 1982; MacIntyre et al, 1984). Platelet activation results from the synergistic interaction of elevated cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) and 1,2-Diacylglycerol (DG), produced as a consequence of receptor-mediated activation of phospholipase C and subsequent hydrolysis of phosphoinositides (Nishizuka, 1984). To investigate the mechanisms underlying inhibition of platelet activation by PUFA we compared the effects of LA and SA on platelet activation induced by the DG-mimetic, phorbol-12-myristate-13-acetate (PMA) and on phosphoinositide hydrolysis and Ca^{2+} flux induced by the TxA_2-mimetic, U44069.

All studies were performed at 37°C using plasma-free suspensions of human platelets that were pre-incubated (2-5 min) with LA, SA or ethanol (vehicle control). Platelet aggregation was measured photometrically, $[Ca^{2+}]_i$ was assessed using Quin 2, and phosphoinositide hydrolysis was monitored as $[^{32}P]$-phosphatidate (-PtdA) formation (Pollock et al, 1984).

Neither LA ($\leq$100μM) nor SA ($\leq$100μM) inhibited PMA (15 - 90nM)-induced platelet aggregation or altered resting $[Ca^{2+}]_i$ = 114 ± 8nM (mean ± S.E., n = 8), or basal $[^{32}P]$-PtdA. U44069 (10nM - 1μM) elicited aggregation, elevation of $[Ca^{2+}]_i$, to around 500nM, and $[^{32}P]$-PtdA formation, to ~4-fold the basal level. Aggregation, elevation of $[Ca^{2+}]_i$ and $[^{32}P]$-PtdA formation induced by U44069 (300nM) were inhibited, in a concentration-dependent manner by LA (1 - 100μM) but not SA ($\leq$100μM). U44069-induced aggregation and elevation of $[Ca^{2+}]_i$ were abolished by LA, but $[^{32}P]$-PtdA formation was inhibited by a maximum of 60%.

These results indicate that PUFA do not inhibit platelet activation by opposing the actions of DG or elevated $[Ca^{2+}]_i$. Rather, by perturbation of the platelet membrane in specific lipid domains, they suppress the transduction processes, namely phosphoinositide metabolism and associated elevation of $[Ca^{2+}]_i$ and DG formation, that link (TxA_2) receptor occupancy to platelet activation. Recent studies indicate that receptor occupancy is coupled to phospholipase C activation and resultant phosphoinositide hydrolysis via a GTP-binding protein (Joseph, 1985). Whether PUFA impair the activation or the catalytic activity of phospholipase C remains to be determined.

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SK&F 94120, AN INOTROPE-VASODILATOR INHIBITS AGGREGATION AND SECRETION OF HUMAN PLATELETS

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Elevation of cAMP either by stimulation of adenylate cyclase (eg. Haslam et al., 1978; Siegl et al., 1982) or by inhibition of phosphodiesterase (eg. Haslam & Rosson, 1975; Haslam et al., 1978) results in inhibition of platelet responses.

We report here that SK&F 94120 an inotrope-vasodilator agent which is a specific inhibitor of phosphodiesterase type III (Gristwood et al., 1985) inhibits platelet responses to a variety of agonists and modestly increases intracellular cAMP concentrations ([cAMP]), probably but not definitely sufficient to account for the inhibitory effects of the drug.

Aggregation was measured in either a HU aggregometer or simultaneously with ATP secretion in a Coulter Lumi aggrometer. These experiments were performed on human PRP from healthy donors who denied taking aspirin or NSAID's for the previous ten days. cAMP was measured by RIA (N.E.N.) in TCA extracts from human platelets resuspended in Hepes-buffered physiological saline (Hallam et al., 1984). The aggregation or secretion evoked by collagen (5 µg/ml, Hormon-Chemie) were both inhibited by SK&F 94120 with IC_{50}'s of 37 ± 9 (s.e.m.) µm and 34 ± 6 µm. The inhibition produced at a maximum dose of 100µM SK&F 94120 was more complete than that produced by sufficient indomethacin (10 µM) to abolish responses to arachidonic acid, implying an inhibitory effect beyond that due to inhibition of products of the action of cyclooxygenase on arachidonic acid. In PRP pretreated with 100 µM aspirin, SK&F 94120 had marked inhibitory effects on the aggregation evoked by 10 µM of the stable prostaglandin endoperoxide U44069 (Upjohn), the IC_{50} for SK&F 94120 was 1.7 ± 0.5 µM. For inhibition of the residual aggregation in indomethacin treated cells stimulated with collagen (63.5 ± 6% of control) the IC_{50} for SK&F 94120 was 24 ± 5 µM. The inhibitory effect of SK&F 94120 upon ADP-evoked aggregation (in PRP pretreated with 100 µM aspirin) was much less than that seen for the other agonists - only 55% ± 11% at 100 µM SK&F 94120. The reason for this difference is currently being investigated.

Incubation of washed platelets with SK&F 94120 produced a significant concentration dependent increase in [cAMP] with a maximum observed effect at 100 µM giving a 87.1 ± 15% (n=6) increase over control (mean resting [cAMP], 4.7 pmol/10^8 cells).

In conclusion, SK&F 94120 an inotrope-vasodilator selective PDE III inhibitor, inhibits platelet responses even in aspirin or indomethacin treated platelets, at least partly via elevation of [cAMP].

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12P

QUINACRINE INHIBITS VASODILATATION CAUSED BY 5-HT BUT NOT PAPAVERINE IN THE RAT ISOLATED HEART

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In 1980 Furchgott demonstrated that acetylcholine-induced relaxation in isolated blood vessels was dependent on an intact endothelium, and an endothelium-dependent relaxation factor (EDRF) was proposed to exist. This has subsequently been shown to be released in isolated vessels in response to a number of vasoactive agents in addition to acetylcholine. To our knowledge, endothelium-dependent vasodilation has not yet been demonstrated in an intact isolated perfused organ.

Using an isolated perfused rat heart we have studied the effects on coronary flow of optimal doses of : the EDRF agonist, 5-hydroxytryptamine (5HT, $1x10^{-7}M$,), the EDRF antagonist quinacrine ($1x10^{-6}M$,), and papaverine ($5x10^{-6}M$) the smooth muscle relaxant, which acts independently of the endothelium.

Hearts (n=8 in each group) were perfused in the Langendorff mode at a constant pressure of 60 cm H_2O with Krebs-Henseleit bicarbonate buffer (gassed with 95% O_2 + 5% CO_2, pH 7.4, 37°C). After a 15 minute stabilization period, hearts were subjected to the following protocol (Figure 1):-

Perfusion Time (mins)	15	3	5	3	5	3	5	3
Group 1 (5HT)	Drug	5HT	Drug	Quin	Drug	5HT + Quin	Drug	5HT
Group 2 (Pap)	Free	Pap	Free	Quin	Free	Pap + Quin	Free	Pap

Figure 1 - Experimental protocol for both groups. All drugs were solubilized in Krebs buffer. 5HT = 5-Hydroxytryptamine ($1 \times 10^{-7}M$), Pap = Papaverine ($5 \times 10^{-6}M$), Quin = Quinacrine ($1 \times 10^{-6}M$).

Initial exposure (3 min) to either 5HT (Group 1) or papaverine (Group 2) induced a comparable increase in coronary flow (+20.7±2.3% and +22.5±2.4% respectively). During the following 5 min washout period the coronary flow returned to control in both groups. The subsequent 3 min exposure to quinacrine alone had no significant effect on coronary flow in hearts that had been previously exposed to 5HT or papaverine. When hearts were then perfused (3 min) with quinacrine in combination with either 5HT or papaverine, the vasodilatory effect of papaverine was maintained (+18.0±2.0%), but the response to 5HT was totally abolished and a slight vasoconstriction was observed (-2.0±1.7%). This quinacrine-induced loss of responsiveness to 5HT was however reversible since, when hearts were subjected to a 5 min wash-out and exposed to 5HT alone (3 min), there was an increase in coronary flow (+14.4±1.6) which was not significantly different from the initial response to 5HT (+20.7±2.3). The response to papaverine was also restored (+21.4 +3.6%) and was not significantly different from the initial value (+22.5 +2.4%).

If as suggested, quinacrine is a specific antagonist of EDRF (Furchgott 1980), our results provide indirect evidence that EDRF is released in the isolated perfused rat heart.

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IgE-DEPENDENT STIMULATION OF HUMAN ALVEOLAR MACROPHAGES

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The alveolar macrophage is the most abundant cell in the lumen of human airways (Tomioka et al, 1984) and has been shown to release potent inflammatory mediators derived from arachidonic acid metabolism, lysosomal hydrolases and active oxygen species (MacDermot et al, 1984). These cells are exposed to inhaled antigen and we have examined the possibility that they are stimulated by IgE-dependent mechanisms. A positive result would suggest that macrophages are important in antigen-related bronchoconstriction. Cells recovered by bronchoalveolar lavage in 39 patients have been studied. The cells obtained were filtered and washed before culture in 35 mm plastic wells in 2 ml of Dulbecco's modification of Eagle's essential medium for 1 hour at 37°C. Electron microscopy has revealed that >95% of the cells that adhere to the plastic are macrophages; no mast cells have been seen. The cells were then washed before incubation in the presence of 10,000 units of human myeloma IgE, or on 1 occasion with serum of a patient allergic to D. pteronyssinus at 4°C for 1 hour. The cells were washed again, and incubated in the presence of either non-affinity purified rabbit anti-human IgE, affinity purified rabbit anti-human IgE (no activity against IgG), affinity purified goat anti-human IgE (no activity against IgG), mouse monoclonal anti-human IgE or water soluble extract of D. pteronyssinus for 30 minutes at 4°C, and then incubated for selected times at 37°C. Supernatants were then harvested and the amount of thromboxane B_2 measured by radioimmunoassay, $PGF_{2\alpha}$ by GC/MS, LTB_4 by HPLC/RIA, lysosomal hydrolase activity by specific enzymic assay, and active oxygen species production was measured by lucigenin chemiluminescence. The cell protein content was also measured. The amount (mean ± SD) of TXB_2 produced by cells not incubated with anti-sera or antigen was 1.9 ± 1.7 ng/mg cell protein, $PGF_{2\alpha}$ was 2.1 ± 0.4 ng/mg, LTB_4 was 5.4 ± 6.5 ng/mg, glucosaminidase activity was 13.3 ± 13.8 units and chemiluminescence at 10 minutes was 0.15 ± 0.06 mV. All 4 anti-human IgE sera caused a concentration-dependent increase in release of thromboxane B_2. Maximum release was 2.8 ± 1.4, 3.9 ± 3.4, 4.8 ± 2.9, and 2.3 ± 2.4 ng/mg for the 4 anti-sera respectively. There was also a 25% increase in thromboxane produced on challenge with antigen. Prostaglandin $F_{2\alpha}$, LTB_4 and glucosaminidase activity all increased at least 2-fold on stimulation with the anti-sera. There was, however, no increase in lucigenin chemiluminescence with the specific anti-sera. This supports the finding (Joseph et al, 1980) that human alveolar macrophages can be stimulated by an IgE-dependent mechanism, and may therefore be involved in antigen-related asthma.

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14P

EFFECT OF DEXAMETHASONE ON BRADYKININ-INDUCED RELEASE OF EICOSANOIDS FROM GUINEA-PIG INFLAMED LUNGS

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Exposure to high concentrations of oxygen (O_2) for prolonged periods induces a form of acute lung injury termed "oxygen toxicity" which causes non-specific lesions in the lungs, e.g. oedema, alveolar haemorrhage, fibrin deposition, thickening and hyalinisation of alveolar membrane and atelectasis (Clark & Lambertsen, 1971). In some species the initial damage occurs in the vascular endothelium (Klister, et al., 1967). Synthesis of eicosanoids in guinea-pig isolated lungs varies qualitatively and quantitatively with the stimulus employed; for example, arachidonic acid and the calcium ionophore A23187 release more TXB_2 than 6-oxo-$PGF_{1\alpha}$ whereas bradykinin (Bk) releases more 6-oxo-$PGF_{1\alpha}$ than TXB_2, probably because its action is on the pulmonary vascular endothelial cells (Bakhle et al., 1985). We have now examined the effect of exposure to O_2-rich atmospheres in vivo on eicosanoid synthesis in guinea-pig isolated lungs stimulated with Bk.

Male guinea-pigs (350-400g body weight) were exposed to pure O_2 at a pressure of 1 atm and a flow rate of 4 l/min, for periods of 24, 48, 72 and 96h. Control animals breathed laboratory air. The guinea-pigs were anaesthetised and the lungs removed and placed in a heated chamber. The lungs were perfused via the pulmonary artery with oxygenated (95% O_2 - 5% CO_2) Krebs' bicarbonate solution (37°C), pumped at 5 ml. min^{-1}. Bradykinin (0.2 µM) was infused for 5 min and the lung effluent collected and analysed for TXB_2 and 6-oxo-$PGF_{1\alpha}$ by RIA. The concentrations (ng. ml^{-1}) of eicosanoids were (mean ± s.e.) as follows:

O_2 exposure	6-oxo-$PGF_{1\alpha}$	TXB_2	n
0	6.30 ± 0.94	2.39 ± 0.71	19
24h	4.61 ± 0.69	3.00 ± 0.91	9
48h	6.89 ± 1.03	4.50 ± 1.35	10
72h	17.94 ± 2.69**	10.01 ± 3.01**	9
96h	22.33 ± 3.34**	10.29 ± 3.08**	10

The increase observed in the release of eicosanoids after 72h and 96h exposure to O_2 could be due to: (i) increase in phospholipase activity, (ii) decrease in prostaglandin metabolism or (iii) decrease in metabolism of Bk. The basal level of release of eicosanoids from the inflamed lungs was not different from the control lungs and therefore the first possibility seems unlikely. Prostacyclin was not removed from the circulation either by control lungs or by lungs of guinea-pigs exposed to O_2 and removal of PGE_2 was not affected by exposure to O_2. However, Bk metabolism, measured by bioassay was reduced significantly in the inflamed lungs (control lungs: 94 ± 1%, n=8; 96h O_2: 84 ± 3%, n=7), suggesting that this could be the mechanism by which Bk induced greater release of eicosanoids in lungs of animals exposed to O_2.

The release of eicosanoids from guinea-pig isolated lungs stimulated with Bk is insensitive to corticosteroid treatment (Blackwell et al., 1978). However, the release of 6-oxo-$PGF_{1\alpha}$ and TXB_2 induced by Bk in inflamed lungs was reduced significantly by infusion of dexamethasone (5 µg.ml^{-1}, 60 min). Since dexamethasone had no effect on Bk metabolism it is possible that during the inflammatory process Bk reaches different pools of phospholipases which are sensitive to steroids.

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INHIBITION BY EBSELEN OF MACROPHAGE EICOSANOID GENERATION AND LYMPHOCYTE PROLIFERATION IN VITRO

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Ebselen is an organoselenium compound with glutathione peroxidase (GSH-Px)-like activity in vitro (Müller et al, 1984; Wendel et al, 1984), which also inactivates leukotriene B_4 (LTB_4) by isomerisation and at higher concentrations inhibits cyclo-oxygenase and lipoxygenase (Kuhl et al, 1985, Parnham & Kindt, 1984; Safayhi et al, 1985). We report here the inhibition by ebselen of macrophage prostaglandin E_2 (PGE_2) and LTC_4 generation and relate this to inhibition by ebselen of lymphocyte proliferation.

Resident macrophages were obtained by peritoneal lavage of male CBA mice with phosphate buffered saline pH 7.4, resuspended in Medium 199 (+Penicillin/Streptomycin) and incubated at $2x10^6$ cells ml^{-1} with 0.5 mg ml^{-1} opsonized zymosan for 3 h at 37°C under CO_2, as described previously (Parnham & Kindt, 1984). Eicosanoids were extracted from supernatants with Sep-Pak C_{18} mini-columns (Waters) before radioimmunoassay for PGF_2 and LTC_4 using commercial kits (NEN, ^{125}I and ^3H, respectively). Mononuclear cells were obtained from human venous blood and separated by centrifugation with Ficoll-Paque (Pharmacia). $2x10^5$ mononuclear cells were incubated in round-bottomed microculture plates (Greiner) in RPMI with 10% FCS and 50 µmol l^{-1} mercaptoethanol for 72 h at 37°C under 5% CO_2. Concanavalin A (5 µg ConA ml^{-1}, Difco), leukoagglutinin (2 µg LAG ml^{-1}, Pharmacia) or pokeweed mitogen (10 µg PWM ml^{-1}, Gibco) were used for induction of lymphocyte proliferation, determined by incorporation of ^3H-thymidine (Amersham) added for the last 18 h of incubation (0.5 µCi per well). Preincubation of mouse macrophages for 10 min with ebselen resulted in dose-dependent inhibition of PGE_2 (control: 4.16±0.32 ng ml^{-1}; mean±s.e.mean) and LTC_4 release (control: 330±10 ng ml^{-1}) with IC_{50} values of 20 and 3.6 µmol l^{-1}, respectively (n=3-6). Pretreatment of mice 2 h before cell harvest with the GSH synthesis inhibitor phorone (650 mg kg^{-1} i.p. in olive oil) reduced PGF_2 release from 1.26±0.08 ng ml^{-1} to 0.71±0.37 ng ml^{-1} (mean± s.e.mean, n=3), but did not markedly affect the inhibitory response to ebselen in vitro. Ebselen incubated with human PBL for 72 h also dose-dependently inhibited ^3H-thymidine uptake with all three mitogens, most markedly at 32 µmol l^{-1} (-65% for ConA, -66% for LAG and -65% for PWM, n=5). The GSH synthesis inhibitor buthionine sulphoximine (1 mmol l^{-1}) also inhibited lymphocyte proliferation (-89% for ConA, -94% for LAG and -92% for PWM, n=3). However, it is unlikely that ebselen inhibited lymphocyte proliferation by depleting GSH as addition of GSH (30 µmol l^{-1}) together with ebselen (10-32 µmol l^{-1}) could not restore the diminished response. These data indicate that ebselen inhibits both LT and PG release from macrophages and lymphocyte proliferation at similar concs. Neither action appears to be markedly affected by changes in intracellular GSH concentration, though the action of ebselen in the total absence of GSH could not be tested, since under these conditions all responses would be absent. In view of the findings of Dinarello et al (1980) that joint cyclo-oxygenase/lipoxygenase inhibitors inhibit thymocyte proliferation to the macrophage product, interleukin 1, it is possible that ebselen may exert a similar action.

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EFFECTS OF ANTAGONISTS OF 5-HYDROXYTRYPTAMINE ON BLOOD PRESSURE IN THE ANAESTHETISED SHR

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Ketanserin is a potent antagonist of 5-hydroxytryptamine (5-HT) at 5-HT_2 receptors, but it has been suggested that its blood pressure lowering effects are due mainly to alpha_1-adrenoceptor antagonism (Cohen et al., 1983). We have examined the effects of ketanserin and other 5-HT_2 antagonists (cyproheptadine and LY 53857) on blood pressure in the anaesthetised Spontaneously Hypertensive Rat (SHR). LY 53857 is a potent 5-HT_2 antagonist with little affinity for alpha_1-adrenoceptors (Cohen et al., 1983).

Male SHR (250-300 g) were anaesthetised with pentobarbitone sodium or, for pithed rat experiments, with ether. The carotid artery was cannulated for blood pressure recording, and the jugular vein was used for the administration of drugs.

In pithed rats, the alpha_1-adrenoceptor agonist amidephrine produced dose-dependent rises in diastolic blood pressure (DBP) with an ED50 (dose producing 50% of maximum pressor response) of 30.9 µg/kg (95% confidence limits 21.4 - 44.7 µg/kg). Prazosin (0.01 mg/kg) produced a 4.8 fold shift, and ketanserin (1 mg/kg) a 5.8 fold shift, in the potency of amidephrine, but LY 53857 (1 mg/kg) produced no significant shift.

In anaesthetised rats in the presence of prazosin (1 mg/kg) to eliminate alpha_1-adrenoceptor mediated responses, ketanserin, LY 53857 and cyproheptadine (0.1 - 1 mg/kg) all produced further falls in DBP (e.g. LY 53857 1 mg/kg lowered DBP a further 14.8 ± 4.0 mmHg, $n=4$; $P<0.05$ from effects of saline vehicle).

In the absence of prazosin, both ketanserin and LY 53857 (0.1 mg/kg) produced marked falls in DBP (e.g. LY 53857 lowered DBP by 42.0 ± 15.9 mmHg, $n=3$).

It is concluded that, whilst ketanserin can lower DBP by alpha_1-adrenoceptor blockade, it shares with other 5-HT_2 antagonists an ability to lower DBP independent of alpha_1-adrenoceptor blockade. The site of this action remains to be established.

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EFFECTS OF 6-NITROQUIPAZINE ON [^{3}H]-5-HT OVERFLOW IN HYPOTHALAMIC SLICES FROM NORMAL AND PCPA TREATED RATS

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6-Nitroquipazine (DU 24565) is a potent and selective 5HT uptake inhibitor devoid of antagonist properties at the level of the presynaptic 5HT autoreceptor (Classen et al., 1984). In rat hypothalamic slices, 5HT uptake inhibitors such as imipramine, amitriptyline, citalopram or paroxetine did not increase by themselves the electrically-evoked release of [^{3}H]-5HT (Galzin et al., in press). However, 48 h after injection of the tryptophan hydroxylase inhibitor parachlorophenylalanine (PCPA), citalopram significantly enhanced [^{3}H]-5HT overflow (Galzin et al., 1983). In an attempt to further analyze this effect, we decided to compare the effect of 6-nitroquipazine on the electrically-evoked release of [^{3}H]-5HT in hypothalamic slices from normal, acute and chronically PCPA-treated rats.

Rat hypothalamic slices were labelled with [^{3}H]-5HT (creatinine sulphate), and superfused with Krebs solution. Two periods (S_1 and S_2) of electrical stimulation were applied with an interval of 44 min, and 6-nitroquipazine was added 20 min before S_2. The parameters of stimulation were 3 Hz, 2 msec, 20 mA for 2 min. When added 20 min before S_2, 6-nitroquipazine (0.001 - 1 µM) had only small effects on the electrically-evoked release of [^{3}H]-5HT in normal hypothalamic slices (S_2/S_1 at 1 µM was 1.36 $\pm$ 0.10, n = 11, $p < 0.05$, when compared to the control ratio : S_2/S_1 = 1.04 $\pm$ 0.07, n = 14). In rats injected acutely with PCPA (300 mg/kg i.p. in saline, 48 h before the experiment), 6-nitroquipazine (0.001 - 1 µM) increased in a concentration-dependent manner the overflow of [^{3}H]-5HT elicited by electrical stimulation. The maximal effect was 330 % increase at 1 µM, with an EC_{50} of 40 nM. After chronic treatment with PCPA (300 mg/kg i.p. 5 times every 3 days, last injection 48 h before the experiment), the maximal effect of 6-nitroquipazine was of the same order of magnitude than in acute PCPA experiments, but the concentration-effect curve was further shifted to the left, with an EC_{50} of 3.5 nM. The values of fractional release for the spontaneous outflow of radioactivity obtained before S_1 were significantly decreased in hypothalamic slices following pretreatment with PCPA (Sp_1 = 2.23 $\pm$ 0.09 %, n = 14 in normal rats ; Sp_1 = 1.45 $\pm$ 0.07 %, n = 16 in acute PCPA-treated rats and Sp_1 = 1.24 $\pm$ 0.08 %, n = 11 in chronic PCPA treated rats, $p < 0.01$ when compared with control value in normal rats).

To test the possibility that PCPA treatment affects the 5HT transporter at the level of the site of action of 5HT uptake inhibitors, we compared the binding of [^{3}H]-paroxetine, a selective ligand for the 5HT transporter complex (Habert et al., 1985) in cerebral cortex membranes of normal or chronic PCPA treated rats. Chronic PCPA treatment did not modify the Kd or Bmax values of [^{3}H]-paroxetine binding in rat cortical membranes (Kd = 0.051 $\pm$ 0.006 nM, Bmax = 411.7 $\pm$ 26.7 fmoles/mg prot, n = 5 in control rats, not different from the value in PCPA treated rats : Kd = 0.040 $\pm$ 0.004 nM, Bmax = 394.7 $\pm$ 35.7 fmoles/mg prot, n = 5). These results suggest that chronic inhibition of tryptophan hydroxylase by PCPA modifies the properties of the neuronal 5HT transporter, but that the changes do not involve the modulatory site labelled with [^{3}H]-paroxetine. The finding that chronic depletion of endogenous 5HT can modify the effect of selective 5HT uptake inhibitors on the electrically-evoked release of 5HT is of interest, in relation to the role of the 5HT transporter in the pathogenesis of depression and as a site of action for antidepressant drugs which inhibit 5HT uptake.

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THE UPTAKE PROCESSES THAT ALLOW ACCESS OF 5-HYDROXYTRYPTAMINE TO MONOAMINE OXIDASE IN THE ISOLATED PERFUSED HEART OF THE RAT

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Extensive studies have been carried out in perfused lungs on the uptake of 5-HT and its metabolism by monoamine oxidase (MAO) in the pulmonary circulation (Gillis & Pitt, 1982). However, little is known about the dissipation mechanisms available to 5-HT in systemic blood vessels, such as in the coronary circulation. It is known, from data obtained from isolated perfused hearts of rats, that 5-HT can be taken up by extraneuronal uptake in the heart (Grohmann & Trendelenburg, 1984), but the subsequent metabolism of the amine has not been examined. Also, the possible involvement of neuronal uptake and/or uptake into vascular endothelial cells in the dissipation of circulating 5-HT in the heart has not been investigated. In the present study, the relative contribution of these three uptake processes in the dissipation of 5-HT has been examined.

Rats were pretreated with reserpine (1 mg/kg, i.p., 18 h before the experiment) and some were also pretreated with 6-hydroxydopamine (6-OHDA, 50 mg/kg, i.v., 24 h before the experiment). Isolated hearts from the rats were perfused, via the coronary circulation, with Tyrode solution containing 1 μM 5-HT (10 ml $g^{-1}min^{-1}$, 36.5°C, 80 min). In some experiments, cocaine (30 μM), corticosterone (100 μM) or O-methylisoprenaline (OMI, 100 μM) were also included in the Tyrode solution. 5-Hydroxyindoleacetic acid (5-HIAA), which was the only metabolite detected, was assayed in effluent samples by HPLC.

The steady-state rate of appearance of 5-HIAA (calculated from data obtained during the 66th to 80th min of perfusion) was used as a measure of the uptake and metabolism of 5-HT in the heart. In control hearts (no inhibitor), the rate was 421 ± 10.3 pmol $g^{-1}min^{-1}$ (n=10). It was reduced (by 22%) by 6-OHDA pretreatment (328 ± 13.5 pmol $g^{-1}min^{-1}$, n=4), indicating that some 5-HT was taken up and metabolized in the adrenergic neurones. This was not significantly different from the rate observed in the presence of cocaine (305 ± 12.9 pmol $g^{-1}min^{-1}$, n=7). Hence, the effect of cocaine was probably due to inhibition of neuronal uptake of 5-HT and there appeared not to be significant uptake by an alternative cocaine-sensitive process, such as uptake into vascular endothelial cells. A more marked reduction of the rate of 5-HIAA appearance (by about 60%) was obtained if either corticosterone or OMI (extraneuronal uptake inhibitors) was present (176 ± 4.6 pmol $g^{-1}min^{-1}$, n=4, and 150 ± 4.7 pmol $g^{-1}min^{-1}$, n=4, respectively). Thus, most of the 5-HT that is metabolized in the heart is exposed to MAO by extraneuronal uptake. In the presence of both OMI and cocaine, the metabolism of 5-HT was not totally inhibited (by 87%, 5-HIAA rate: 54 ± 7.4 pmol $g^{-1}min^{-1}$, n=3), suggesting that a small amount of 5-HT was exposed to MAO after diffusional entry into cells in the heart.

In conclusion, 5-HT in the coronary perfusate of isolated hearts from rats can be exposed to MAO by both the neuronal and extraneuronal uptake processes used also by catecholamines. The data suggest that it is unlikely that the vascular endothelial cells in the coronary circulation take up and metabolize 5-HT. This is in contrast to the dissipation of 5-HT in the pulmonary circulation, in which 5-HT is metabolized predominantly in the capillary endothelial cells (Gillis & Pitt, 1982).

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SEROTONIN (5-HT) RECOGNITION SITES IN HUMAN BRAIN: EVIDENCE FOR SPECIES DIFFERENCES

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Evidence from functional and radioligand binding studies supports the existence of multiple 5-HT receptors. Gaddum and Picarelli (1957) characterised M and D receptors. Peroutka and Snyder described 5-HT1 and 5-HT2 receptors. Pedigo et al (1981) introduced the concept of 5-HT1A and 1B receptors and we described recently 5-HT1C receptors in the pig choroid plexus (pazos et al, 1984).

Appropriate radioligands were used to label these various 5-HT recognition sites in human post-mortem brain membranes: [3H]ketanserin (Leysen et al, 1982), [3H] 8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)-tetralin, Gozlan et al, 1983), [125I] iodocyanopindolol (ICYP) in the presence of 30 µM isoprenaline (Hoyer et al, 1985) and [3H]mesulergine (Pazos et al, 1984). Binding to human (table 1), pig and rat brain membranes was characterised on the basis of competition experiments performed using various selective agonists and antagonists. [3H]8-OH-DPAT labelled 5-HT1A sites in human cortex and hippocampus which are identical with those of rat and pig cortex. [3H]mesulergine bound to 5-HT1C sites in human choroid plexus that are extremely similar to those found in pig choroid plexus and rat cortex. [3H] ketanserin labelled 5-HT2 receptors in human cortex which are equivalent to those found in pig cortex but different from rat cortex 5-HT2 receptors: several potent 5-HT2 ligands in the rat (e.g. mesulergine, methysergide) showed lower affinities for human and pig 5-HT2 receptors. There was no detectable 5-HT1B binding in human brain membranes with ICYP or [3H]5-HT in contrast to rat but in analogy to pig membranes.

Table 1:	5-HT_{1A} cortex	5-HT_{1C} ch. plexus	5-HT_{2} cortex
	(pK_D values, mean ± s.e.mean, -log mol/l)		
5-HT	8.28±0.07	6.78±0.20	6.76±0.50
5-carboxamidotryptamine	8.91±0.12	5.37±0.22	5.09±0.07
8-OH-DPAT	8.59±0.18	4.40±0.28	-
RU 24969	7.97±0.13	6.27±0.08	-
spiperone	9.39±0.19	6.08±0.30	8.41±0.13
pirenperone	5.82±0.03	7.28±0.20	8.75±0.08
ketanserin	5.47±0.17	6.73±0.10	8.61±0.13
mesulergine	6.39±0.09	8.37±0.30	6.82±0.07
methysergide	-	-	7.34±0.11

*RU 24969 = 5-methoxy-3-[1,2,3,6-tetrahydropyridin-4-yl]-1H-indole. The close similarity between pig and human 5-HT recognition sites suggests that species differences exist which are not related to the use of post-mortem tissue. 5-HT1 receptor subtypes were originally defined in rat preparations, however, the data presented here indicates that this subclassification has to be used with caution in other species, including man.

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THE 5HT AGONIST 8-OH-DPAT ATTENUATES STRESS-INDUCED DEFICITS IN OPEN FIELD ACTIVITY AND FEEDING BEHAVIOUR

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Rats restrained for 2h show deficits in open field activity, food intake and growth 24h later. Adaptation occurs on repeated restraint and is associated with the development of enhanced response to stimulation of postsynaptic 5HT receptors (Kennett er al., 1985). We therefore examined whether the 5HT agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), which elicits feeding in rats (Dourish et al., 1985a) could reverse stress-induced behavioural depression and anorexia. Male Sprague-Dawley rats (200-250 g) were restrained for 2h. At 10.00h (2h after release) 8-OH-DPAT (60 or 1000 μg/kg) or saline were injected s.c. Open field behaviour (5 mins) was scored the next day (10.00 - 12.00h) and 24h food intake and body wt changes measured.

Table 1. Open field activity and food intake in 8-OH-DPAT treated rats.

Parameter	Treatment	Saline Control	(n)	8-OH-DPAT 60μg/kg s.c.	(n)	8-OH-DPAT 1000μg/kg s.c.	(n)
Squares	Control	90 ± 6.1	(13)	92.2 ± 9.1	(10)	82.1 ± 6.7	(10)
Crossed	Stress	21.3 ± 3.3^{+}	(17)	16.5 ± 5.0^{+}	(10)	49.5 ± 6.3**$^{+}$	(10)
24h Food	Control	27.4 ± 0.6	(13)	26.4 ± 0.9	(10)	24.7 ± 0.8	(10)
Intake (g)	Stress	12.6 ± 0.6^{+}	(17)	16.7 ± 0.9**$^{+}$	(10)	16.5 ± 0.5**	(10)
24h Wt	Control	4.1 ± 0.8	(13)	2.6 ± 0.9	(10)	2.5 ± 1.1	(10)
change (g)	Stress	-13.3 ± 0.9^{+}	(17)	-8.0 ± 1.4**$^{+}$	(10)	-9.6 ± 1.0*$^{+}$	(10)

+ $p < 0.01$ from respective unstresssed control. *$p < 0.02$, **$p < 0.01$ from respective saline treated group by Mann Whitney U test (open field) or 2 tailed 't' test (food intake and body wt change) following appropriate ANOVA.

Stress markedly decreased locomotion in the open field and this effect was attenuated by 1000 μg/kg but not by 60 μg/g of 8-OH-DPAT (Table 1). 8-OH-DPAT had no effect on the open field behaviour of unstresssed rats (Table 1). Both food intake and body weight were decreased by stress. This effect was attenuated by both doses of the drug though it had little effect on 24h food intake in controls. This confirms our previous finding that 8-OH-DPAT increased food intake during a 2-4h daytime test but had no effect on 24h intake (Dourish et al., 1985b). The difference in dose dependency of the effects of 8-OH-DPAT on stress-induced anorexia and open field deficits suggests that these effects may be mediated pre- and postsynaptically respectively (Dourish et al., 1958b; Curzon et al., this meeting). Two other 5-HT_{1A} agonists, Buspirone and TVX Q 7821 produced similar effects to 8-OH-DPAT in both these tests. As there are both neurochemical and behavioural parallels between defective adaptation to restraint stress and depressive illness these results may conceivably be relevant to the latter disorder. They may also be relevant to anorexia nervosa.

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NEUROCHEMICAL AND PHARMACOLOGICAL STUDIES OF 8-HYDROXY-2-(di-n-PROPYLAMINO) TETRALIN (8-OH-DPAT)-INDUCED FEEDING

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We have recently shown that low doses of the novel serotonin agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) increase food intake in non-deprived rats (Dourish et al., 1985). As serotonin agonists and releasers generally inhibit feeding (Blundell, 1977) one possible explanation of this paradoxical effect of 8-OH-DPAT is that it occurs via stimulation of serotonin autoreceptors and resultant inhibition of 5-hydroxytryptamine (5HT) synthesis and release. We have therefore examined (a) the effect of a low dose of 8-OH-DPAT on regional brain serotonin metabolism and (b) the effect of the 5HT synthesis inhibitor p-chlorophenylalanine (pCPA) on feeding induced by low doses of 8-OH-DPAT and stereotyped behaviour induced by higher doses of the drug.

Male Sprague Dawley rats were housed in individual plastic cages, maintained on a 12 h light: dark cycle (lights on 06.00 h) and allowed food and water ad-libitum. All behavioural testing was carried out between 10.30 h and 17.30 h during the light phase of the cycle. In the biochemical study, groups of rats were killed 0, 30, 60 and 120 min after an injection of 0.9% NaCl or 8-OH-DPAT (60 μg/kg s.c.). Brains were removed, quickly dissected into regions, frozen on solid CO_2 and stored at -70°. 5HT and 5-hydroxyindoleacetic acid (5HIAA) were determined by high pressure liquid chromatography with electrochemical detection. In the behavioural study, food intake was measured in the home cage 2, 4 and 24 h after injection of 8-OH-DPAT 15, 60, 250, 1000, 4000 μg/kg s.c. or 0.9% NaCl. One week later, the rats were injected daily for 3 days with pCPA (150 mg/kg i.p.). Twenty four hours later they were retested for their feeding response to 8-OH-DPAT as given before pCPA. An additional group of rats given 0.9% NaCl for 3 days served as controls when determining the extent of 5HT depletion by pCPA. In a subsequent behavioural experiment rats were pretreated with either 0.9% NaCl or pCPA as before. 24 h later they were given a single dose of 8-OH-DPAT (250 μg/kg s.c.) and food intake determined over a 2 h period. Behaviour was recorded on videotape and subsequently analysed for frequency, duration and latency of various behaviours of interest using a microcomputer program. The biochemical results showed that a low dose of 8-OH-DPAT (60 μg/kg s.c.) transiently and significantly decreased 5HIAA/5HT, in the cortex ($F = 4.53$, df 3,43, $p < 0.01$), hypothalamus ($F = 6.31$, df 3,43, $p < 0.01$), pons + medulla oblongata ($F = 3.95$, df 3,43, $p < 0.05$) and midbrain ($F = 3.95$, df 3,43, $p < 0.05$) but not in the striatum, septum, or hippocampus. These changes are consistent with decreased 5HT release and metabolism. In the behavioural study,8-OH-DPAT significantly increased food intake 2 h ($F = 6.68$, df 5,24, $p < 0.01$) and 4 h ($F = 2.79$, df 5,24, $p < 0.05$) after injection. The largest increases of feeding at 2 h followed injection of 60 and 250 μg/kg of the drug. In contrast, in pCPA treated rats 8-OH-DPAT dose-dependently decreased feeding 2 h after injection ($F = 7.86$, df 5,24, $p < 0.01$). The pCPA treatment depleted both whole brain 5HT and 5HIAA by 91%. Locomotion and stereotyped behaviour induced by 8-OH-DPAT (250 μg/kg s.c.) were not affected by pCPA.

Taken together these data are consistent with the effects of a high dose (250 μg/kg) of 8-OH-DPAT on locomotion and stereotypy being mediated by postsynaptic 5HT receptors and the effects of a low dose (60 μg/kg) of 8-OH-DPAT on food intake and 5HT metabolism being mediated by 5HT autoreceptors.

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REGIONAL AND PHARMACOLOGICAL CHARACTERISTICS OF MULTIPLE (^{3}H)-5-HT BINDING SITES IN RAT BRAIN MEMBRANES

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We have previously shown that spiperone and mianserin inhibit ^{3}H-5-hydroxytryptamine (5-HT) binding in a complex manner, suggesting the presence of three 5-HT_1 subsites (Blurton and Wood, 1985). We have further examined the effects of mianserin and spiperone on ^{3}H-5-HT binding and report regional and pharmacological differences between the $5HT_1$ subsites.

Binding of ^{3}H-5-HT (2-3nM) to rat brain membranes was performed according to Pedigo et al (1981). Data were analysed by computer-fitted non-linear regression analysis using the Allfit and Ligand programs.

Detailed analysis revealed that spiperone displaced ^{3}H-5-HT binding to cortical membranes from three sites, whereas mianserin displaced 5-HT from only two sites. The inclusion of 250nM mianserin, to block the mianserin-sensitive site, selectively suppressed one of the sites discriminated by spiperone (Table 1). In agreement with other groups (Pazos et al, 1985) these sites were defined as: 5-HT_{1A}, blocked by 1000nM spiperone; 5-HT_{1C}, blocked by 250nM mianserin; 5-HT_{1B}, specific ^{3}H-5-HT binding remaining in the presence of mianserin and spiperone.

Table 1. Inhibition of ^{3}H-5-HT binding by spiperone: effect of mianserin

Spiperone in the presence of	SITE I (1A)		SITE II (IB)		SITE III (IC)	
	IC_{50}	Bmax	IC_{50}	Bmax	IC_{50}	Bmax
Vehicle	23±7	2.9±0.2	86,000±30,000	4.72±.03	529±240	2.11±.33
Mianserin (250nM)	66±14	3.3±0.2	93,000±19,000	3.94±.23	NOT DETECTED	

Results (mean ± SEM) are computer-fitted parameters (IC_{50}, nM; Bmax, pmol/g cortex) from 3 paired experiments using 16 spiperone concentrations.

Regional studies showed that the hippocampus was rich in 1A sites (60-70%), whilst the striatum was rich in 1B (60%) and 1C sites (30%). Drug selectivity for these sites was assessed from approximate IC_{50}'s determined by computer analysis of biphasic displacement curves in striatal and hippocampal membranes exploiting the differential distribution of 5-HT_1 subsites in these tissues (Table 2).

Table 2. Drug potency at 5-HT_1 subsites

Drug	n	IC_{50} (nM) mean ± SEM		
		1A (hippocampus)	1B (striatum)	1C (striatum)
l-propranolol	4	48 ± 9	23 ± 3	13,500 ± 5,100
8-OH-DPAT	4	4.3 ± 1.4	4,800 ± 1,100	97 ± 49
RU 24969	4	6.0 ± 2.3	0.85 ± 0.45	117 ± 48
Mianserin	3	1,100 ± 100	2,700 ± 760	31 ± 9

These results further support the presence of three 5-HT_1 binding sites with different regional distributions and different affinities for various drugs. Thus, 8-OH-DPAT, mianserin, l-propranolol and RU 24969, showed some selectivity for the 1A, 1C and 1B sites respectively. The regional and pharmacological differences described here may be important in elucidating the function of these subsites.

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8-OH-DPAT; 8-hydroxy-2 (Di-n-propylamine) tetralin
RU 24969; 5-methoxy-3 (1,2,3,6-tetrahydropyridin-4-yl)1-H indole

INTERACTION BETWEEN 5-HT UPTAKE INHIBITORS AND PRESYNAPTIC INHIBITORY 5-HT AUTORECEPTORS: COMPARISON OF K^+ AND ELECTRICAL DEPOLARIZATION

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In rat hypothalamic slices labelled with [^{3}H]-5HT, tricyclic antidepressants like imipramine and amitriptyline and non-tricyclic 5HT uptake inhibitors like paroxetine or citalopram antagonized the autoreceptor-mediated inhibition by LSD or 5-methoxytryptamine of the electrically-evoked release of [^{3}H]-5HT (Langer and Moret, 1982 ; Galzin et al., 1983 ; Galzin et al., in press). This interaction between 5HT uptake inhibitors and presynaptic inhibitory autoreceptors was not dependent on the level of endogenous 5HT (Galzin et al., 1983 ; Galzin et al., in press), and it was suggested that a functional link could exist between the 5HT transporter and the presynaptic 5HT autoreceptors. However, it was recently reported that, in synaptosomes from cerebral cortex, the inhibition by LSD of the K^+-evoked release of [^{3}H]-5HT was not affected by citalopram or chlorimipramine (Raiteri et al., 1984). Therefore, we decided to compare the effect of citalopram on the LSD-induced inhibition of [^{3}H]-5HT release elicited by K^+ or electrical stimulation in hypothalamic slices.

Rat hypothalamic slices were labeled with [^{3}H]-5HT (creatinine sulphate) and superfused with Krebs' solution. Two periods (S_1 and S_2) of stimulation were applied with an interval of 44 min. Citalopram, when used, was added to the medium 20 min before S_1, and LSD was added 20 min before S_2. The parameters of stimulation were 3 Hz, 2 msec, 20 mA, 2 min, or 20 mM K^+ for 4 min.

Experimental group	n	Electrical stimulation S_1 (%)	Electrical stimulation S_2/S_1	n	K^+ stimulation S_1 (%)	K^+ stimulation S_2/S_1
Control	8	1.77 ± 0.25	1.05 ± 0.10	8	1.15 ± 0.11	0.81 ± 0.07
LSD 0.1 µM	7	1.92 ± 0.19	0.32 ± 0.07*	8	1.46 ± 0.17	0.46 ± 0.05**
1 µM	7	1.54 ± 0.20	0.19 ± 0.06*	9	1.16 ± 0.10	0.25 ± 0.04**
Citalopram 0.1 µM	4	1.96 ± 0.30	0.98 ± 0.09	10	1.44 ± 0.10	0.77 ± 0.06
+ LSD 0.1 µM	4	1.85 ± 0.28	0.77 ± 0.07	9	1.49 ± 0.16	0.39 ± 0.03*
+ LSD 1 µM	5	1.74 ± 0.21	0.92 ± 0.14	7	1.25 ± 0.13	0.28 ± 0.03*

Shown are mean values ± S.E.M. ; n = number of experiments per group ; * $p < 0.05$; ** $p < 0.01$ when compared to the corresponding control (Duncan test)

Results are summarized in Table 1. The inhibition of [^{3}H]-5HT overflow by LSD was of the same magnitude under both conditions of stimulation. However, while citalopram completely antagonized the inhibitory effect of LSD on the electrically-evoked release of [^{3}H]-5HT, it failed to significantly affect the inhibition by LSD of the K^+-evoked release of [^{3}H]-5HT.

These results suggest that the interaction between uptake inhibitors and presynaptic autoreceptors is dependent on the depolarizing stimuli applied to induce transmitter release. Under the physiological conditions of electrically-induced depolarization, the interaction between inhibition of 5HT uptake and the autoreceptor is clearly observed, while with the tetrodotoxin-resistant release induced by K^+, this interaction is not present.

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ANTAGONISM BY PIMOZIDE OF THE SUPRESSANT EFFECT OF d- AMPHETAMINE ON OPERANT BEHAVIOUR

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Many of the central effects of d-amphetamine can be antagonized by neuroleptic drugs (Kreiskott, 1980). However little is known about the interaction between neuroleptics and d-amphetamine on operant behaviour. In the present experiment we have examined whether the neuroleptic pimozide can antagonize the suppressant effect of d-amphetamine on performance maintained under variable-interval schedules of positive reinforcement.

Twelve female Wistar rats aged approximately five months at the start of the experiment were maintained at 80% of their free-feeding body weights and were trained to press a lever in an operant conditioning chamber using 0.05 ml 0.6 M sucrose as the reinforcer. In Phase I of the experiment, six rats were trained under a variable-interval 300-sec schedule. At least 30 preliminary training sessions were completed before the start of the drug-treatment regimen. Drugs were administered intraperitoneally (2.5 ml/kg). In each drug-treatment session the rats were pre-treated three hours before behavioural testing with either pimozide or its vehicle alone (0.02 M tartaric acid); ten minutes before testing they were treated with either d-amphetamine sulphate or its vehicle alone (distilled water). The doses of pimozide were 0.0625 and 0.125 mg/kg, and those of d-amphetamine sulphate were 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mg/kg. Each pre-treatment/treatment combination was administered to each rat on one occasion, and the order of presentation of the doses was balanced across subjects. In Phase II of the experiment, the rats were trained under the schedule of reinforcement which they had not been exposed to in Phase I, and the entire procedure was repeated. The effect of d-amphetamine was expressed as proportional change in response rate, compared to vehicle control, and the data were analyzed by a three-factor analysis of variance (schedule of reinforcement, pre-treatment, treatment), with repeated measures on all three factors.

d-Amphetamine produced a dose-related suppression of response rate ($F(5,55) = 43.18$; $P < 0.001$), the suppressant effect being greater in the case of the variable-interval 30-sec schedule ($F(1,11) = 4.95$; $P < 0.05$). The effect of d-amphetamine was significantly antagonized by pimozide ($F(2,22) = 6.91$; $P < 0.01$). There was a significant pre-treatment x treatment interaction ($F(10,110) = 2.17$; $P < 0.05$), reflecting a somewhat greater antagonistic effect of pimozide in the case of higher dose levels of d-amphetamine. None of the other interaction terms was statistically significant.

The predominantly suppressant effect of d-amphetamine on variable-interval performance, and the greater effect on performance maintained under the higher frequency of reinforcement, are in agreement with our previous findings (Morley et al, 1985). The antagonism of the effect of d-amphetamine by pimozide is consistent with the interaction between neuroleptics and d-amphetamine in other behavioural test systems (see Kreiskott, 1980), and is also in accord with the suggestion that the suppressant effects of d-amphetamine on operant behaviour may be mediated by an interaction with dopaminergic mechanisms (Robbins et al, 1983).

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EVIDENCE OF PRESYNAPTIC DOPAMINE RECEPTORS IN THE IN SITU PERFUSED RAT KIDNEY

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The vascular regions involved in the blood pressure lowering effects of dopamine receptor agonists such as apomorphine and pergolide in the rat have not been identified. A region of interest is the renal vascular bed, which is known to contain postsynaptic dopamine receptors. Lokhandwala and Steenberg (1984) recently showed in vitro that this vascular bed also contains presynaptic dopamine receptors. To confirm these in vitro data, we studied the effect of local administration of apomorphine and pergolide in the in situ autoperfused rat kidney.

The experiments were done in Wistar rats weighing 320-430 g, anesthetized with pentobarbital. After intravenous administration of heparine (5 mg/kg), in situ autoperfusion of the left kidney was performed using an extracorporeal flow circuit between a carotid artery and an aortic pouch from which the left renal artery was the only outlet. Flow was adjusted at the start of the experiment so that perfusion pressure was equivalent to systemic pressure, and was kept constant during the experiment. The periarterial renal nerves were stimulated (supramaximal voltage, 1 msec, 4 Hz) with a bipolar electrode. The animals were pretreated with atropine (1 mg/kg IV).

Local infusion of apomorphine (1 µg/kg/min for 5 min, n = 6) and of pergolide (1 µg/kg/min for 5 min, n = 6) had no effect on perfusion pressure per se, but reduced the pressor response to electrical stimulation of the renal nerve bundle to resp. 49.8 $\pm$ 4.8 and 54.8 $\pm$ 2.7 %. Similar increases of perfusion pressure produced by locally administered noradrenaline (0.08 - 0.15 µg/kg) were not modified by either apomorphine (n = 6) or pergolide (n = 6). Local infusion (1 µg/kg/min for 5 min) of the α_2-adrenoceptor agonist UK-14,304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline; Cambridge, 1981) did not influence perfusion pressure per se, but reduced the pressor response to stimulation to 56.1 $\pm$ 2.4 % (n = 6).

The inhibitory effect of apomorphine and pergolide on neurogenic vasoconstriction of the renal vascular bed was completely antagonized by local administration of haloperidol (1 µg/kg, both n = 6), but was not influenced by the α_2-adrenoceptor antagonist rauwolscine (100 µg/kg, both n = 6). This dose of rauwolscine antagonized the inhibitory effect of the α_2-adrenoceptor agonist UK-14,304 (n = 6), which was not influenced by haloperidol (n = 6). Local administration of rauwolscine per se doubled the pressor response to stimulation at 4 Hz, haloperidol per se did not modify this response.

The results of this in vivo study are in agreement with the in vitro data of Lokhandwala and Steenberg (1984) and indicate that presynaptic dopamine receptors are present on renal noradrenergic nerve endings in the rat; these dopamine receptors, together with those previously demonstrated on the sympathetic innervation of the vascular bed in the rat hindquarters (Dupont et al., 1985),could be involved in the hypotensive action of apomorphine and pergolide in this species.

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DISPLACEMENT OF ^{3}H-SCH 23390 BINDING FROM HUMAN PUTAMEN D-1 DOPAMINE RECEPTORS BY 1-PHENYL-3-BENZAZEPINES

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7-substituted 1-phenyl-1H-3-benzazepines such as SCH 23390 and SK&F 38393 are compounds with selective affinity for D-1 dopamine receptors (Iorio et al, 1983; Hyttel, 1983; O'Boyle & Waddington, 1984). Comparison of a series of benzazepine analogues for their relative abilities to displace the binding of ^{3}H-piflutixol from rat striatal D-1 receptors has shown that the nature of the substituent at position 7 can importantly influence such affinity (O'Boyle & Waddington, 1985a). In this study we have examined the activities of 3- and/or 7-substituted 1-phenyl benzazepines at human putamen D-1 receptors labelled with ^{3}H-SCH 23390.

Specific binding of 0.2-0.8 nM ^{3}H-SCH 23390 to human putamen membranes was defined by 100 nM piflutixol and showed a typical D-1 receptor profile: the rank order of displacement potencies was flupenthixol>fluphenazine>dopamine with domperidone and sulpiride displacing <50% of specific binding 1 and 10 µM respectively (O'Boyle & Waddington, 1985b). Relative potencies of seven 1-phenyl benzazepine analogues to displace ^{3}H-SCH 23390 binding are shown in the Table.

Benzazepine	Substitution		Ki nM
SK&F 77174	7-H	3-H	898 ± 138
SK&F 38393	7-OH	3-H	735 ± 259
SK&F 83692	7-H	3-CH_3	538 ± 99
SK&F 75670	7-OH	3-CH_3	132 ± 25
SK&F 85257	7-CH_3	3-H	169 ± 84
SCH 23390	7-Cl	3-CH_3	1.3 ± 0.3
SK&F 83566	7-Br	3-CH_3	2.7 ± 1.6

mean ± s.e.mean, n=3-5

Substitution at position 7 of the benzazepine molecule critically influenced affinity for human D-1 receptors over a 500-fold range. The least active compounds had a H- or OH- group at this position. Introduction of 7-CH_3 group produced a 5-fold increase in activity. A 7-halogen (Br or Cl) was associated with a dramatic increase in activity. 3-methylation had someinfluence on affinity; comparison of 7-H-3-H and 7-OH-3-H compounds with their 3-CH_3 counterparts indicates that a 3-methyl substitution increased affinity only 2-5-fold. The rank order of potency of 7-substituents for displacement of specific ^{3}H-SCH 23390 binding was Br = Cl >> CH_3 > H = OH which is similar to that obtained for displacement of ^{3}H-piflutixol binding to rat striatal D-1 receptors (O'Boyle & Waddington, 1985a).

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DOPAMINE D_2 ANTAGONIST-LIKE EFFECTS OF INTRASTRIATAL PERTUSSIS TOXIN IN THE RAT

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Striatal dopamine D_2 receptors are linked in an inhibitory fashion to adenylate cyclase and appear to specifically inhibit cyclic AMP formed following dopamine D_1 activation (Stoof & Kebabian, 1981). Whilst D_1 receptors enhance adenylate cyclase activity probably via the cholera toxin (CT)-sensitive regulatory protein N_s, D_2 receptors may inhibit adenylate cyclase via a pertussis toxin (PT)-sensitive regulatory protein N_i. In this study we have investigated some consequences of direct injection of PT or CT into the rat striatum.

Under halothane anaesthesia, male Sprague-Dawley rats (~200 g) were given unilateral intrastriatal injections of PT (2 µg) or CT (4 µg) at 4 sites (0.5 µg or 1 µg at A 8.5 & 9.5, L 2.5, V 5.0 & 4.5; Konig & Klippel, 1963). BSA was injected into the contralateral striatum to serve as control. Striatal dopamine and DOPAC concentrations were measured by HPLC with electrochemical detection. 24 Hr. following unilateral PT, animals frequently showed a tendency to posture towards the PT injected side, which intensified (sometimes into circling) upon apomorphine administration (2.0 mg/kg, sc). Intrastriatal injection of the D_2 selective antagonist (±)-sulpiride (10 µg) in untreated rats also induced posturing towards the injected side. In contrast, unilateral intrastriatal CT induced spontaneous circling away from the CT-injected side. Intrastriatal PT reduced the striatal dopamine to DOPAC ratio (Table 1), as did intrastriatal CT and (±)-sulpiride (10 µg). These changes reflected increases in DOPAC rather than changes in dopamine concentrations. Unilateral intrastriatal injection of the D_1-selective antagonist SCH 23390 (7-chloro-2,3,4,5-tetrahydro-3-methyl 5-phenyl-1H-(3-benzazepine-7-ol) did not influence dopamine metabolism. 20-24 Hr. prior injection of PT caused a partial reversal of the ability of the D_2-selective agonist RU 24926 (N-n-propyl di-β-(3-hydroxyphenyl)-ethylamine (1 µM) to inhibit cyclic AMP accumulation in striatal slices incubated with 1 µM of the D_1-selective agonist SKF 38393 (2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1H-benzazepine) (60 ± 5% reversal in BSA and 38 ± 8% reversal in PT-treated striata; n = 5 experiments, $P < 0.05$, paired t test).

Table 1 Striatal dopamine:DOPAC ratios; *$P < 0.05$ compared to control side, paired t test.

	PT (24 hr)	CT (24 hr)	(±)-Sulpiride (10 µg;1 hr)	SCH 23390 (5 µg;1 hr)
Control	5.09 ± 0.45	3.63 ± 0.34	5.76 ± 0.65	6.93 ± 0.21
Treated	3.79 ± 0.19*	2.60 ± 0.23*	3.84 ± 0.28*	6.30 ± 0.22

These results indicate that intrastriatal PT evokes some of the behavioural and biochemical consequences of D_2 receptor blockade. This in turn suggests that striatal D_2 receptors may function via N_i to inhibit adenylate cyclase. However, the possibility of non-specific effects should not as yet be discounted.

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A PATCH CLAMP STUDY OF THE CHOLINERGIC BLOCKING ACTION OF HEXAMETHONIUM ON BOVINE CHROMAFFIN CELLS

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The ganglion blocking action of hexamethonium (C_6) has been proposed to depend mainly on a block of the acetylcholine (ACh) ion channel (Ascher et al., 1979). Using the patch clamp technique we have investigated the ACh-antagonist actions of C_6 on cultured bovine chromaffin cells, which possess nicotinic receptors pharmacologically similar to those found in vertebrate autonomic ganglia (Fenwick et al., 1982), in an attempt to determine the mechanism of action of C_6 on these cells.

Bovine chromaffin cells were isolated, cultured and used 1-6 days after plating (Fenwick et al., 1982). The "whole-cell" voltage clamp configuration of the patch clamp technique (Hamill et al., 1981) was employed and the cells were dialysed intracellularly with a pipette solution containing 140 mM CsCl to suppress the various potassium conductances of the cell membrane. Experiments were performed at room temperature (20-22°C) and all results are expressed as the mean ± standard error of the mean.

Local application of ACh by pressure ejection (100 µM at 1.4×10^5 Pa for 20ms) from a modified patch pipette evoked an inward current (440 ± 36pA, n = 35, holding potential -80mV) on all cells tested. Bath application of C_6 (100 nM - 30 µM) caused a concentration-dependent decrease of the ACh-induced current which reached equilibrium within 3-5 minutes and reversed on washout. The IC_{50} for C_6 was approximately 1 µM at a holding potential of -100 mV.

The amplitude of the ACh-induced inward current was linearly related to holding potential over the range -20 mV to -100 mV, increasing with membrane hyperpolarisation. In the presence of C_6 (1-10 µM) the current-voltage relationship of the ACh response deviated from linearity, with the degree of C_6-induced block increasing e-fold for a 24 ± 2.2 mV hyperpolarisation (n = 8). C_6 (30 µM; a concentration which completely blocks the ACh-induced inward currents at -100 mV) had no effect on ACh-induced outward currents at +80 mV. Collectively these results are consistent with C_6 blocking the ACh ion channel. In an attempt to gain information on the site of action of C_6, cells were dialysed intracellularly with a pipette solution containing 1 mM C_6. These cells responded to locally applied ACh with an inward current of 469 ± 146 pA at -80 mV (n = 4), the amplitude of which was linearly related to holding potential (-20 mV to -100 mV). In contrast, subsequent external application of 3 µM C_6 to these cells caused a voltage-dependent block of the ACh-induced current (-20 mV to -100 mV).

A previous study has demonstrated a relatively weak agonist action of (+)-tubocurarine (Lambert et al., 1985) on bovine chromaffin cells. C_6 (1-30 µM) microperfused onto "whole cells" had no direct agonist actions.

In conclusion, the cholinergic antagonist actions of C_6 on bovine chromaffin cells are best explained by C_6 binding, in a voltage-dependent manner, to a site associated with the ACh ion channel, which can be accessed only from the extracellular membrane surface. The study further demonstrates that a weak agonist action is not a universal property of cholinergic antagonists.

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REGIONAL DIFFERENCES IN MUSCARINIC RECEPTOR LINKED INOSITOL PHOSPHOLIPID HYDROLYSIS

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Muscarinic receptor agonists can be divided into two groups based upon their ability to recognise high and low affinity states of the muscarinic receptor (Birdsall et al, 1978). This ability reflects the efficacy of muscarinic agonists in functional assays, including inositol phospholipid (PI) hydrolysis (Fisher et al, 1983). We have examined the ability of muscarinic agents to stimulate PI turnover in tissue slices of rat cerebral cortex, parotid gland and guinea-pig ileum. Tissue slices (350 x 350 μm) were prepared and washed three times in Krebs-bicarbonate buffer, followed by a 30 min preincubation in the presence of [^{3}H]-myo-2-inositol (2μCi) and 10mM lithium. Tissue slices were subsequently incubated in the presence of muscarinic agonists for 45 min. Water soluble inositol-1-phosphate was isolated by anion exchange chromatography (Brown et al, 1984).

Carbachol (1mM) stimulated [^{3}H]-inositol-1-phosphate accumulation in all tissues with a 10-,10-, and 45-fold increase over basal levels in cortex, ileum and parotid respectively. The ability of a variety of muscarinic agents to stimulate PI breakdown is shown in Table 1.

Table 1 Effect of Muscarinic Agonists on PI Breakdown

Agonist	Rat Cerebral Cortex		Guinea-Pig Ileum		Rat Parotid	
	EC_{50} (μM)	Max Response (%)	EC_{50} (μM)	Max Response (%)	EC_{50} (μM)	Max Response (%)
Carbachol	150	100	36	100	5.6	100
Acetylcholine+	34	163	7	117	0.8	100
Muscarine	43	90	12	100	1.4	99
Oxotremorine	(*)	10	0.9	37	0.7	63
Pilocarpine	31	15	37	23	3.4	29
RS 86++	(*)	9	11	24	4.5	42
McN-A-343	(*)	6	10	20		

(*) EC_{50} could not be determined due to low efficacy. (n=2-5)

\+ In presence of 20μM physostigmine

++ (2-ethyl-8-methyl-2,8-diazaspiro-(4,5) decane-1,3-dione hydrobromide.

Results are expressed as a percent of the maximum response to carbachol (1mM), included in all experiments.

These results demonstrate that full agonists exhibit significant differences (≃30 fold) in potency between the tissues examined, all having greatest potency in the parotid gland. In contrast partial agonists such as oxotremorine show smaller differences in potency but display large differences in efficacy between tissues, being most efficacious in the parotid gland.

These results and those recently reported by others (EK and Nahorski, 1986) suggest that the coupling of muscarinic receptors to PI breakdown may differ between tissues and that the receptor reserve may influence agonist potency.

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MODULATION OF (3H)-NA & (3H)-ACH RELEASE BY MUSCARINIC RECEPTORS IN THE RAT, DIFFERENCES BETWEEN OCCIPITAL CORTICAL, STRIATAL & ATRIAL SLICES

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The existence of muscarinic receptor subtypes has been reported at the postsynaptic level (Hammer and Giachetti, 1982), and also for the presynaptic receptors modulating transmitter release in synaptosomal preparations (Marchi and Raiteri, 1985). In the present study, we have evaluated the occurence of presynaptic muscarinic receptors modulating noradrenaline (NA) release in the central and peripheral nervous systems. Slices from rat occipital cortex and atria or corpus striatum were labelled respectively with DL-^{3}H-NA (0.5 µM) or [methyl-^{3}H]choline (0.064 µM). The release of ^{3}H-NA was elicited by electrical stimulation (E.S.) or by tyramine (Kamal et al., 1981), and that of ^{3}H-acetylcholine (ACh) by E.S. (Cantrill et al., 1983). Two periods of E.S., S_1 and S_2, were applied in each case.

Table 1 : Effects of carbachol and McN-A-343 on release of ^{3}H-transmitters

	µM	Cortex (^{3}H-NA)	Atria (^{3}H-NA)	Striatum (^{3}H-ACh)
		Ratio S_2/S_1		
Electrical stimulation		(3 Hz, 2 min)	(5 Hz, 2 min)	(1 Hz, 2 min)
Control	-	1.17 ± 0.05 (35)	1.17 ± 0.08 (30)	0.70 ± 0.05 (7)
Carbachol (S_2)	30	1.25 ± 0.12 (3)	0.68 ± 0.09* (4)	0.06 ± 0.02* (7)
	100	1.34 ± 0.21 (4)	0.21 ± 0.05* (8)	
McN-A-343 (S_2)	30	1.98 ± 0.18* (17)	2.41 ± 0.20* (17)	0.65 ± 0.02 (4)
	100	3.06 ± 0.25* (6)	3.34 ± 0.34* (6)	0.76 ± 0.05 (3)
Tyramine stimulation		(5 µM, 2 min)	(5 µM, 2 min)	
Control	-	0.86 ± 0.19 (4)	0.88 ± 0.06 (4)	-
McN-A-343	100	1.05 ± 0.29 (5)	0.74 ± 0.08 (4)	-

S_1 represents the percent of total tissue radioactivity released by the first period of stimulation (control) and S_2 corresponds to the second one applied 20 min after exposure to drugs. * $p < 0.05$ vs the corresponding control. Values are mean ± S.E.M. from ( ) experiments per group.

The release of ^{3}H-NA by E.S. from the cortex was unaffected by carbachol (Table 1). On the other hand, in atrial slices, carbachol inhibited the electrically-evoked release of ^{3}H-NA (Table 1). In the striatum, carbachol was rather potent at inhibiting ^{3}H-ACh release (Table 1). The inhibitory effects of carbachol were atropine-sensitive. In both cortex and atria, McN-A-343 enhanced the release of ^{3}H-NA by E.S. (Table 1), without affecting the spontaneous tritium efflux. The facilitatory effect of McN-A-343 was not antagonized by atropine (100 nM), pirenzepine (3 µM), secoverine (3 µM), d-tubocurarine (10 µM), or desipramine (3 µM). Yet, McN-A-343 did not modify the calcium-independent tyramine-induced release of ^{3}H-NA from cortex and atria (Table 1). In the striatum, McN-A-343 did not modify the release of ^{3}H-ACh (Table 1).

In conclusion, our results indicate that a) receptors sensitive to McN-A-343 are not involved in the regulation of ^{3}H-ACh release from the rat striatum, b) McN-A-343 facilitates only the calcium-dependent evoked release of ^{3}H-NA from rat cortex and atria, c) the nature of this effect of McN-A-343 remains to be clarified, and d) noradrenergic terminals of the brain differ from those of the heart as far as the presynaptic modulation of transmitter release by muscarinic inhibitory receptors.

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FURTHER EVIDENCE FOR A CHOLINOMIMETIC ACTIVITY OF MINAPRINE

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Minaprine (M) is an atypical antidepressant drug which has been shown to stimulate serotonergic, dopaminergic and cholinergic brain transmissions (Bizière et al., 1985a). In addition, M was proved to be beneficial in the treatment of senile dementia (Passeri et al., 1985). This prompted us to further investigate the cholinergic aspect of M pharmacology. The starting hypothesis was that the lack of dopaminomimetic activity reported for the high doses of M could be due to its cholinomimetic effect at these doses.

Female Swiss CD1 mice (25-30g) and male Wistar rats (200-230g) (Charles River France) were used. The induction of stereotyped behaviour was assessed in rats according to Bizière et al (1984). Atropine was injected i.p. 30 min. before M(30 mg/kg,s.c.) ; arecoline was injected i.p. 15 min. before M(1 mg/kg s.c.). The induction of rotations after direct unilateral injection in the mouse striatum was performed according to Bizière et al. (1985b). Atropine, scopolamine, haloperidol, or vehicle were injected i.p. 30 min. before the intrastriatal injection of either M(30 µg) or oxotremorine (0.01 µg). A + sign indicates ipsilateral rotations and a - sign indicates contralateral rotations.

In rats, M induced stereotypies at a dose of 1 mg/kg s.c. (m±S.E.M. cumulated stereotypy score : 17.9±1.1), an effect which was antagonized by arecoline (5 mg/kg i.p. : 8.6±2.1**) ; apomorphine - induced stereotypies (0.3 mg/kg s.c.) were also diminished by arecoline (Apo. : 15.8±0.9 ; Apo.+arec. : 7.9±0.8**). At 30 mg/kg, M hardly induced any stereotypies (1.5±0.6) ; however, when treated by atropine and M, rats exhibited a clear stereotyped behaviaour (M+atr.0.3 mg/kg : 9.2±1.2** ; M+atr.1 : 14.4±1.0** ; M+atr.3 : 18.9±1.5**).

In mice, the injection of buffer into the striatum elicited a weak ipsilateral turning (m±SEM cumulated nb. of turns/6 min : +1.4±0.2 to+2.8±0.5). Intrastriatally injected oxotremorine induced marked ipsilateral rotations (0.001 µg : +6.8±0.9 ; 0.01 µg : +12.0±2.5) which were blocked by i.p. atropine (oxo. 0.01 µg : +15.8±1.9 ; oxo. +atr.1 mg/kg : +8.0±1.5** ; oxo. +Atr.3 : +4.7±1.0**) or scopolamine (oxo. 0.01 µg : +11.5±0.8 ; oxo. +scop. 1mg/kg : +3.8±1.1**). Carbachol (0.0001-0.1 µg) and arecoline (0.001-1 µg) also induced ipsilateral rotations. M(1 µg) elicited a contralateral turning (-14.4±0.9) whereas it hardly induced any turning when injected at 30 µg (see below). However, mice treated by i.p. atropine or scopolamine before M(30 µg) exhibited marked contralateral rotations (M :-0.6±0.3 ; M+Atr.1 mg/kg : -6.9±1.1** ; M+Atr.3 : -9.2±1.0** . M+Scop. 1 mg/kg : -4.4±1.1** ; M+Scop.3 :-8.6±0.5**). Finally, when pretreated by i.p. haloperidol, mice injected whith M (30 µg) exhibited ipsilateral rotations (M : -1.1±0.3 ; M+hal 0.01 mg/kg : +6.4±1.2 ; M+hal 0.03 : +7.0±0.9). In these conditions, neither atropine, scopolamine nor haloperidol induced turning in buffer injected controls.

These data suggest that a pretreatment with anticholinergic drugs unmasked a dopaminomimetic effect of M at high doses (mice, rats), whereas a pretreatment with a neuroleptic unmasked a cholinergic-type effect of M at these high doses (mice). This brings new evidence for a cholinomimetic effect of M, this effect being counterbalanced by its dopaminomimetic activity in behavioural experiments.

**p < 0.01 vs respective controls (Student's t test).

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MUSCARINIC RECEPTOR-MEDIATED PHOSPHOINOSITIDE METABOLISM IN GUINEA PIG PAROTID GLAND, ILEUM AND CORTEX

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There is now substantial evidence that muscarinic receptor stimulation induces a rapid hydrolysis of phosphoinositides in many tissues (Brown & Brown-Masters, 1984). We have examined this response in guinea-pig cerebral cortex (CX) ileal smooth muscle (IL) and parotid gland (PG) in view of indications of receptor heterogeneity (M_1 and M_2) between such tissues. Measurement of ^{3}H-inositol phosphate (^{3}H-IP) accumulation was made in tissue slices after preincubation with ^{3}H-inositol in the presence of Li+ (5 mM) using methods essentially described by Brown et al. (1984).

Carbachol markedly stimulated ^{3}H-IP in all tissues (20-fold in cortex and parotid gland, 5-fold in ileum), though significant differences were observed in the apparent EC_{50} values for this full agonist (Table 1). Oxotremorine on the other hand, was a partial agonist in each case and although EC_{50} values were similar, maximal responses differed significantly between the three tissues (Table 1). The response to carbachol was potently blocked by atropine with similar affinity in each tissue. The so-called M_1 antagonist, pirenzepine, also inhibited the response though with a slightly higher affinity in cortex (Table 1).

^{3}H-N-Methylscopolamine binding to homogenates of these tissues revealed complex displacement by pirenzepine in cortex best described using computer-assisted curve fitting by a two-site interaction (K_H 17 nM, K_L 274 nM) but by a single low affinity site (446 nM) in ileum and parotid gland.

The results suggest that there may be a different degree of coupling of muscarinic receptors to phospholipase C in view of the close relationship between the apparent EC_{50} value of the full agonist carbachol and the maximal response of the partial agonist oxotremorine between the tissues. Furthermore, the data does not appear to be simply compatible with the concept that M_1 but not M_2 receptors are linked to this enzyme.

Table 1 Apparent EC_{50} and inhibition constants (K_i) for muscarinic agonists and antagonists with phosphoinositide assays in guinea-pig tissues

	EC_{50} (μM)			App. K_i (nM)	
	CARB.	OXO.	OXO. % max. resp.	ATR.	PIR.
PG	3.7 ± 0.5	0.65 ± 0.2	32 ± 2	0.45 ± 0.2	60 ± 16
IL	20.2 ± 4.3	0.95 ± 0.4	18 ± 2	0.34 ± 0.1	33 ± 14
CX	40.1 ± 7.3	0.92 ± 0.3	9 ± 2	0.74 ± 0.1	23 ± 16

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DIFFERENTIAL AFFINITIES OF MUSCARINIC ANTAGONISTS AT ILEAL AND ATRIAL RECEPTORS

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Barlow & Shepherd (1985) have proposed that those receptors present in the atria differ from those in the ileum on the basis of the selective antagonist action of 4 DAMP (4 diphenylacetoxy-N-methylpiperidine methiodide) and its pentamethylene derivative. In addition, it has also been shown that the agonist, ethoxyethyltrimethylammonium (EOE), exhibits a degree of selectivity towards ileal muscarinic receptors in comparison to atrial receptors (Barlow & Weston-Smith, 1985). The aim of the present study was to assess the action of other muscarinic antagonists at ileal and atrial receptors, including those such as pirenzepine and dicyclomine which have been reported to exhibit differences in affinity between cortical and myocardial muscarinic binding sites (Hammer et al, 1980; Kenny et al, 1985)

The methods used have been previously described (Clague et al, 1985). Carbachol was used as the agonist in the antagonist studies and all experiments were conducted at 30°C. Antagonist affinities were determined using the method of Arunlakshana & Schild (1959). The antagonist affinities are shown in Table 1. The majority of compounds examined did not distinguish between ileal and atrial receptors. 4 DAMP and CPPS (cyclohexylphenyl [2-piperidinoethyl] silanol) were more selective for ileal receptors in comparison to atrial receptors. Dicyclomine and oxyphenonium acted as competitive antagonists at atrial receptors only and the affinity for the ileal receptor could not be calculated.

Table 1 Antagonist affinities at guinea-pig ileal and atrial receptors

Antagonist	Ileum	Atria
Pirenzepine	6.8	6.7
Telenzepine	7.9	7.8
4 DAMP	9.0*	7.9
Trihexylphenidyl	8.0	7.5
CPPS	8.9*	7.5
Dicyclomine	N.C.	6.8
Oxyphenonium	N.C.	9.7
Propantheline	9.4	9.2
Isopropamide	8.9	8.6

Values are mean pA_2, sem less than 5%, n = 4 - 6. N.C.= non-competitive action, affinity not calculable. *$p < 0.05$ determined by Student's t-test.

The agonist EOE exhibited a degree of selectivity towards ileal receptors (ileal $EC_{50} = 1.3 \times 10^{-6}$ mol.litre^{-1}; atrial $EC_{50} = 1 \times 10^{-5}$ mol.litre^{-1}). In summary, the majority of antagonists did not distinguish between ileal and atrial receptors. However, the selective action of 4 DAMP, CPPS and EOE is consistent with the concept of ileal and atrial receptors forming two subtypes of the muscarinic receptor (Barlow & Shepherd, 1985).

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THE INTERACTION BETWEEN GALLAMINE AND ATROPINE ON RAT ATRIUM

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The following experiments were undertaken to investigate further the relationship between the antagonism by gallamine of the effects of muscarinic agonists on isolated atria and the allosteric effect produced by gallamine in binding studies (Stockton et al., 1983). The interaction between atropine and gallamine is compared with that expected if both antagonists were competitive (Paton & Rang, 1965).

Isolated whole atria were prepared from Lister male hooded rats (250g) and suspended in a Krebs solution equilibrated with 95% oxygen and 5% carbon-dioxide and maintained at 37°C. Doses of agonist were applied every 5 minutes and left in contact with the tissue for about 20 seconds before being washed out. The percentage decrease in the rate of beating was recorded.

The EC_{50} values for carbachol and muscarine were determined in the absence of antagonist, in the presence of $3x10^{-5}$M gallamine or $1x10^{-7}$M atropine, and in the presence of both antagonists. 'Gallamine plus Atropine' values were obtained following incubation with gallamine alone first. As the concentrations of the antagonists used produced parallel rightward shifts of the log-dose response curves, dose ratios were calculated from the mean log EC_{50} values.

Treatment	Carbachol -log (EC_{50})	(n)	DR	Muscarine -log (EC_{50})	(n)	DR
NO ANTAGONIST	6.15±0.09	(8)		6.03±0.01	(10)	
$3x10^{-5}$M GALLAMINE	4.64±0.05	(11)	33	4.46±0.04	(14)	37
$0.3x10^{-7}$M ATROPINE				4.34±0.12	(9)	49
$1x10^{-7}$M ATROPINE	4.10±0.07	(11)	112	3.98±0.05	(10)	112
$1x10^{-7}$M ATROPINE PLUS	$0.3x10^{-7}$M ATROPINE			3.82±0.07	(9)	162
GALLAMINE PLUS ATROPINE	3.94±0.05	(11)	162	3.73±0.06	(14)	199
ATROPINE PLUS GALLAMINE	3.79±0.07	(11)	229	3.64±0.04	(10)	245

It can be seen from the table above that the combined effects of atropine and gallamine are greater than those expected for two competitive antagonists (144, 148), and appear to depend on the agonist used and on which antagonist the tissue had first been exposed to. These differences can not be attributed to sensitivity changes in the tissue or the size of the combined dose ratio expected because combining two concentrations of atropine ($0.3x10^{-7}$M plus $1x10^{-7}$M) using a similar procedure gave a 'combined' dose ratio of 162 which was similar to that expected (160).

In conclusion, although gallamine and atropine do not appear to interact competitively on rat atrium, the combined dose ratios were greater than expected rather than less as seen by Clark & Mitchelson (1976) on guinea-pig atrium. This cannot be attributed to the species used because the interaction between gallamine and quinuclidinyl benzilate on guinea-pig atria has recently been shown to be similar to the interaction described here (Choo et al., 1985).

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EFFECT OF IVERMECTIN ON PENTYLENETETRAZOL-INDUCED SEIZURES IN MICE

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The activity of the antiparasitic agent ivermectin is mediated through gamma-aminobutyric acid (GABA) (Wang and Pong, 1982). However, there are suggestions that ivermectin does not penetrate the blood-brain barrier (Anderson, 1984). In view of these suggestions and the evidence that associates GABA with protection against seizures (Simmonds, 1981), we decided to investigate the effect of ivermectin on pentylenetetrazol-induced seizures in mice.

Adult mice of either sex (26-30g) were used in the study. Test animals received 20,30 and 40 mg/kg 1% ivermectin (Ivomec, MSD Agvet) i.p., 30 min, 1 h and 2 h before 100 mg/kg of 1% pentylenetetrazol (PTZ) s.c. Controls received PTZ only.

Controls had generalized seizures and died within 12.4 ± 1.43 (s.e. mean) minutes of PTZ administration. Generalized seizures and some deaths occurred in the 20 mg/kg group. In the 30 mg/kg group seizures occurred only in those animals which received the drug 30 min and 1h but not in those which received it 2h before PTZ.

All animals in this group were still alive 10 weeks after the experiment regardless of whether or not they had had seizures.

No seizures were observed in the 40 mg/kg group, however, all the animals were dead 24h after PTZ administration.

In conclusion, ivermectin penetrates the blood-brain barrier and protects mice against PTZ-induced seizures. This effect is both dose - and time-dependent and is probably linked to its action on GABA.

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DECREASE IN $GABA_B$ BINDING IN THE FRONTAL CORTEX OF OLFACTORY BULBECTOMIZED RATS

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Recent evidence implicates GABA synapses both in the etiology of depression and the mechanism of action of antidepressant drugs(AD)(cf. Lloyd et al, 1985). Lesion of the olfactory bulbs in the rat results in a series of behavioural alterations responding selectively to ADs (Leonard, 1984). As repeated administration of ADs of different classes selectively increases $GABA_B$ binding in rat frontal cortex (Pilc & Lloyd, 1984; Lloyd et, 1985),we have examined this in the olfactory bulbectomized rat at a time (2 weeks post lesion) when behavioural alteration are most evident.

Male Wistar rats (250-300 gm) were submitted to olfactory bulbectomy or sham-operations under ether anaesthesia (Garrigou et al, 1981). Fourteen days later the rats were decapitated and the frontal cortex removed, carefully examined for damage and then frozen on dry ice. Membranes were prepared within 48 hrs and $GABA_B$ binding performed according to Pilc & Lloyd(1984).

Region	$GABA_B$ Binding at 10nM ^{3}H-GABA			
	Sham-operated		Olfactory bulbectomized	
	fmol/mg protein	n	fmol/mg protein	n
Frontal Cortex	153.8 ± 9.7	19	70.0 ± 5.3*	20
Occipital Cortex	165.2 ± 11.5	3	122.4 ± 18.5	4
Amygdala	28.0 ± 4.8	3	37.6 ± 6.1	10
Cerebellum	105.4 ± 4.0	3	144.7 ± 13.9	7
Hippocampus	38.1 ± 4.7	3	37.8 ± 4.2	6

n = number of animals ; * $p<0.001$ vs sham-operated (2-tailed t test).

At a concentration of 10 nM ^{3}H-GABA, $GABA_B$ binding was lower in the frontal cortex of olfactory bulbectomized than in sham-operated animals, whereas for other regions no statistically significant differences were observed.Scatchard analysis on pooled membranes showed that the Bmax was lower by 40% in the frontal cortex membranes from bulbectomized rats (532 fmol/mg protein) as compared to sham-operated animals (888 fmol/mg protein) whereas the Kd's were very similar (30.5 nM for bulbectomized and 34.0 nM for sham-operated rats).These results suggest that a deficit of $GABA_B$ synaptic function may play a role in the behavioural deficits following olfactory bulbectomy. This is suppported by the observation that the $GABA_B$ agonist baclofen (Leonard, 1984) and the mixed $GABA_{A+B}$ agonist progabide (Lloyd et al, 1983) reverse the behavioural deficits in this animal model. A GABA synaptic dysfunction may be a more general aspect of behavioural models for depression as altered GABA release occurs in the learned helplessness model (Sherman & Petty, 1982). Furthermore GABAergic mechanisms may be an integral response to AD action as GABA agonists are active in behavioural models of depression and exert clinical antidepressant activity and also ADs of different classes selectively upregulate $GABA_B$ binding to rat frontal cortex membranes (Lloyd et al, 1983, 1985).

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$GABA_B$ RECEPTOR ACTIVATION INHIBITS THE INCREASE IN NERVE TERMINAL Ca^{++} INDUCED BY DEPOLARIZATION

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$GABA_B$ receptors in the mammalian brain are present both pre- and post-synaptically (Bowery et al 1985) and it has been suggested that the receptor at both locations may be associated with membrane K^+ channels (Gähwiler and Brown 1985). However an inhibitory link with Ca^{++} channels is possible (Desarmenien et al 1984; Cherubini and North 1984) although any effect on Ca^{++} movement may result from a change in K^+ conductance (Gähwiler and Brown 1985). Nevertheless a direct effect on Ca^{++} movement cannot be excluded (Deisz and Lux 1985). In an attempt to study this possibility at presynaptic sites we have examined the effects of $GABA_B$ receptor stimulation on the intrasynaptosomal Ca^{++} levels which arise during depolarisation. For this purpose we have chosen the fluorescent Ca^{++} indicator quin-2 developed by Tsien et al (1982), to monitor intracellular Ca^{++}. Nerve terminal fragments (P_2 pellet) were prepared from rat cerebellum and loaded with quin-2 ester according to a modified method of Ashley et al (1984) for purified synaptosomes. The modification was to store the loaded preparations for up to 2 h at 24°C and to measure the fluorescence emissions at the same temperature. This provided greater reproducibility. Measurements were performed using a Perkin-Elmer LS5 fluorimeter. Aliquots of the suspension were pipetted into cuvettes and left for 5 minutes in the fluorimeter before the addition of any drug and the emission signal constantly monitored. Drugs were added using a microsyringe.

The addition of KCl (final concentration 35mM) produced a rise in the fluorescence emission at 492nm which was associated with increased free intracellular Ca^{++}. This response was completely blocked by Cd^{++} (1mM). The prior addition (5-20 s before) of (-)baclofen (1-100μM) reduced the evoked emission signal in a dose dependent manner with a maximum reduction of 51.1% ± 7.0 (s.e.m. n=7). This was not mimicked by (+)baclofen or isoguvacine in the same concentration range. (-)Baclofen had no effect on the basal emission. GABA (10μM) produced the same reduction in the evoked emission as (-) baclofen. The increase in emission produced by KCl could also be reduced significantly by the dihydropyridine and benzothiazepine Ca^{++} antagonists nitrendipine and diltiazem in a dose-dependent manner (0.1-1μM) without any effect on basal emission. The reductions produced by 1μM of each of the Ca^{++} antagonists were 54.6% ± 5.3 (s.e.m. n=4) and 54.7% ± 6.1 (s.e.m. n=3) respectively. The effects of (-)baclofen and the Ca^{++} antagonists were additive. In the presence of a maximally effective concentration of (-)baclofen the Ca^{++} antagonists produced a further reduction in the evoked emission.

These data indicate that $GABA_B$ receptor activation and Ca^{++} antagonists reduce intracellular Ca^{++} through separate mechanisms and suggest that $GABA_B$ receptor activation may directly influence Ca^{++} movement in nerve terminals.

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GABA-DEPENDENT AND GABA-INDEPENDENT EFFECTS OF BENZODIAZEPINES AND ZOLPIDEM

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Pharmacological effects of benzodiazepines (BZs) result from the action on specific BZ sites in the CNS. Although biochemical evidence suggests that these sites are not homogeneous, no conclusive pharmacological evidence for the multiplicity of BZ receptors has been provided. In this report we compare several pharmacological activities of BZs and zolpidem, a new non-BZ hypnotic (Nicholson & Pascoe, 1985; Langer et al, 1985) which binds preferentially to the type I BZ receptor (Arbilla et al, 1985).

Experiments were performed on male CD1 mice weighing 18-25g. Presence of tonic convulsions (pentetrazole, 125 mg/kg, s.c.) or delay to the first convulsion (isoniazide, 800 mg/kg, s.c.) were the end points measured. Locomotor activity was measured in a photocell-equipped activity meter during 10 min (exploratory phase). Myorelaxant activity was evaluated by measuring grip strength in a loaded grid test (Fleury, 1957). Drugs were injected i.p. 30 min before tests.

Drug	ED_{50} (mg/kg, i.p.)			
	Anti-pentetrazole	Anti-isoniazide	Locomotor Activity	Loaded grid
Triazolam	0.005	0.03	0.03	0.015
Flunitrazepam	0.04	0.1	0.20	0.09
Diazepam	0.4	1	1	1
Midazolam	0.68	1.2	1.2	1.1
Zolpidem	9	1.1	1.1	17

All agents antagonized pentetrazole-induced convulsions and increased the delay to the appearance of isoniazide convulsions. Zolpidem was 9 times more potent in delaying isoniazide-induced convulsions than in antagonizing pentetrazole, whereas BZs were 2-6 times more potent against pentetrazole. In terms of the maximum effect in the isoniazide test, zolpidem was most efficacious by producing a maximum delay of 93.9±5.5 min while midazolam, diazepam, triazolam and flunitrazepam produced maximum delays of 67.1±4.8, 70.3±5.9, 75.5±5.9 and 68.3±4.4 min, respectively (vehicle treated rats: 24.3±0.8 min). All drugs also reduced locomotor activity and induced myorelaxation. However, zolpidem was more active in decreasing locomotor activity than in inducing myorelaxation in contrast to BZs which showed similar activities in the two tests. The anticonvulsant and central depressant effects of zolpidem were antagonized by Ro 15-1788.

Comparisons of relative potencies in the four tests showed that myorelaxant activity correlates best with the antagonism of pentetrazole (r=0.997) and that the locomotor suppressant activity correlates best with anti-isoniazide effects (r=0.984). In view of the fact that isoniazide induces convulsions by inhibiting GABA synthesis, it can be suggested that the central depressant effects fo BZs and zolpidem involve BZ sites coupled to the GABA receptor. Conversely, myorelaxant effects may be related to BZ sites whose function does not involve GABA transmission. Further, it may be suggested that the type I BZ receptor is coupled to the GABA receptor.

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^{3}H-ZOLPIDEM: A NOVEL NON BENZODIAZEPINE LIGAND WITH PREFERENTIAL AFFINITY FOR THE BZ_1 RECEPTOR SUBTYPE

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Zolpidem (ZOLP) is a novel hypnotic which is not chemically related to benzodiazepines (BZ), but behaves as an agonist at BZ receptors (Arbilla et al., 1985a). Two GABA-dependent BZ receptor subtypes (BZ_1 and BZ_2) have been described (Braestrup and Nielsen, 1981 ; Lo et al., 1982). Most of the BZs with hypnotic activity in man do not possess selectivity of either receptor subtype, and they have similar affinity for BZ receptors in different brain regions (Sieghart and Schuster, 1984). Therefore, ^{3}H-ZOLP was used as a new ligand to explore its binding characteristics in various regions of the rat central nervous system. Suspensions in buffer (50 mM Tris HCl, 120 mM NaCl, 5 mM KCl, pH 7.4) of crude membrane preparations (0.5 mg of prot/ml) from cerebral cortex, cerebellum or spinal cord were incubated at 0°C for 30 min with 0.1 - 22 nM ^{3}H-ZOLP (60.5 Ci/mmol, J. Allen, J. Parent, A. Tizot and A. Wick, L.E.R.S., Chemistry Department), or 0.1 - 12 nM ^{3}H-diazepam (DIAZ, 72.5 Ci/mmol, N.E.N.). Non specific binding of ^{3}H-ZOLP or ^{3}H-DIAZ was determined in the presence of Ro 15-1788 2 µM or DIAZ 1 µM respectively. Assays were terminated by filtration.

Table 1 : Regional characteristics of specific ^{3}H-ZOLP and ^{3}H-DIAZ binding

Region	Kd (nM)		Bmax (fmol/mg prot)	
	^{3}H-ZOLP	^{3}H-DIAZ	^{3}H-ZOLP	^{3}H-DIAZ
Cerebellum	7.15 ± 0.53 (3)	6.57 ± 0.05 (3)	1064 ± 66 (3)	992 ± 65 (3)
Cortex	7.05 ± 0.51 (5)*	5.34 ± 0.12 (4)	1688 ± 57 (5)**	2189 ± 39 (4)
Spinal cord	N.D.	9.50 ± 1.01 (3)	N.D.	370 ± 42 (3)

Kd : dissociation constant ; Bmax : maximal number of binding sites ; N.D. : not detectable ; Shown are mean ± S.E.M. of ( )$_3$ individual determinations ; ** $p < 0.001$; * $p < 0.02$ vs corresponding values in ^{3}H-DIAZ binding.

Scatchard analysis of saturation data indicate that ^{3}H-ZOLP binds with high affinity to a single class of recognition sites in cerebral cortex and cerebellum. The cerebellum possesses similar number of binding sites for ^{3}H-ZOLP and ^{3}H-DIAZ (Table 1), while in the cortex, the density of binding sites for ^{3}H-ZOLP was significantly lower than that for ^{3}H-DIAZ (Table 1). In the spinal cord, under conditions in which ^{3}H-DIAZ binding is detectable, the binding of ^{3}H-ZOLP is virtually absent (Table 1). The regional densities of ^{3}H-ZOLP binding sites found correlates with the regional distribution of BZ_1 receptors (Braestrup and Nielsen, 1981 ; Watanabe et al., 1985). In addition, ZOLP was shown to be more potent at displacing ^{3}H-DIAZ binding from the cerebellum than from the hippocampus (Arbilla et al., 1985a). In the rat cortex, the inhibition of ^{3}H-ZOLP binding by several BZs is highly correlated with their affinities for ^{3}H-DIAZ binding. In rat cerebral cortex washed membranes, exposure to GABA enhances ^{3}H-ZOLP binding with an EC_{50} of 0.22 µM. The present data are compatible with the view that ^{3}H-ZOLP labels the GABA-BZ receptor complex, having preference for the BZ_1 receptor subtype. Since ZOLP possesses preferential hypnotic properties (Arbilla et al., 1985b ; Langer et al., 1985), the use of ^{3}H-ZOLP as a radioligand may offer a useful tool for the understanding of the mechanism of action of hypnotic drugs acting through BZ receptor subtypes.

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CALCIUM CHANNEL ANTAGONISTS DECREASE THE ETHANOL WITHDRAWAL SYNDROME

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We have studied the effects of three calcium channel antagonists on the convulsive component of the ethanol withdrawal syndrome.

Male Sprague-Dawley rats, 130-170 g, were fed on a liquid diet of Complan, 200 g L^{-1}, containing ethanol (concentration starting at 7% v/v and rising to 15% v/v) for five weeks (Group A) or six weeks (Group B). Water was freely available bu no other food. On the test day the ethanol diet was removed and 20 ml Complan mixture given per animal. The drugs were suspended in Tween 80 (one drop in 10 ml distilled water) and injected by the intraperitoneal route. Controls were given vehicle injections. The treatment schedules were as follows : Controls (Group A) vehicle, on withdrawal and at 3h intervals; nitrendipine, 100 mg kg^{-1} on withdrawal and at 3h intervals; verapamil, 10 mg kg^{-1}, on withdrawal and at 2h intervals; controls (Group B), vehicle, on withdrawal; flunarizine, 40 mg kg^{-1} on withdrawal.

Animals were observed for 7½ h after withdrawal of ethanol and then subjected to an audiogenic stimulus (electric bell, 45s). The incidence of convulsions and the total mortality up to 24h after withdrawal were noted by an observer who did not know the prior drug treatment. (Drug treatment was given only up to 6h after withdrawal). Spontaneous convulsions were mainly of the clonic type (rarely proceeding to tonic) while the responses to the audiogenic stimulus consisted of running fits leading to clonic then tonic convulsions. Some mortality was seen in the control groups before the audiogenic stimulus time was reached.

Treatment	Numbers of rats showing spontaneous convulsions	Total numbers of spontaneous convulsions	Numbers of rats convulsing to bell	Total numbers of rats showing convulsions	Mortality up to 24h
Controls (A)	5/9	5	4/5	8/9	8/9
Nitrendipine	0/9*	0	0/9*	0/9**	0/9**
Verapamil	0/8*	0	5/8	5/8	5/8
Controls (B)	6/8	14	5/5	8/8	8/8
Flunarizine	3/8	5	7/7	8/8	2/8*

**$P < 0.001$, *$P < 0.05$, Fisher's exact test, comparison with concurrent control group (A for nitrendipine and verapamil, B for flunarizine).

Nitrendipine and verapamil significantly decreased the incidence of convulsions and nitrendipine and flunarizine significantly decreased the mortality. The effects seen were not due to alterations in the metabolism of ethanol. At these doses verapamil and flunarizine did not alter the convulsion threshold to pentylenetetrazol (i.v. infusion), while nitrendipine caused only a small increase in this threshold. They showed no sedative actions. We suggest that this type of compound may provide possibilities for a novel therapeutic approach to ethanol withdrawal.

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