EFFECTS OF CNQX ON EXCITATORY AMINO ACID AND SYNAPTIC RESPONSES OF RAT VENTROBASAL THALAMUS NEURONES

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Previous work has shown that excitatory amino acid receptors mediate the responses of ventrobasal thalamus (VB) neurones to stimulation of non-nociceptive somatosensory afferents. Furthermore, use of selective N-methyl-D-aspartate (NMDA) antagonists and broad spectrum excitatory amino acid antagonists indicates that these responses consist of both NMDA and non-NMDA receptor components (Salt, 1987). The novel non-NMDA receptor antagonist, CNQX (6-cyano-2,3-dihydro-7-nitroquinoxaline) (Honoré et.al., 1987), now provides a more direct means of detecting non-NMDA receptor components of synaptic responses. The effects of this antagonist on excitatory amino acid-evoked and VB synaptic responses have therefore been investigated.

Extracellular single neurone recordings were made with multi-barrel iontophoretic electrodes in VB of urethane anaesthetised (1.2g/kg, i.p.) rats (Salt, 1987). The effects of iontophoretically ejected CNQX (4-40nA, 1mM in 50mM NaCl) have been studied on eighteen neurones. On eleven of these neurones, CNQX reduced responses to iontophoretically applied quisqualate by 50%, on average, whilst having little or no effect on responses to N-Methyl-D,L-aspartate (1% average reduction). On the remaining neurones the antagonist reduced responses to both agonists by approximately equal amounts. Six of the eighteen neurones were also tested with kainate, and responses to this agonist were found to be most sensitive to CNQX (mean reduction: 92%). Sensory synaptic responses of VB neurones were evoked by an air jet, of either 10ms or 2000ms duration, directed at the peripheral receptive field. CNQX was found to antagonise responses to both types of stimuli when ejected with currents which were selective for non-NMDA receptors.

These results confirm that CNQX can indeed be used, with care, as a non-NMDA receptor antagonist. Furthermore, they provide direct evidence that there is a non-NMDA receptor component to sensory synaptic responses of rat VB neurones.

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STRYCHNINE-RESISTANT EFFECTS OF GLYCINE ON CENTRAL NEURONES

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Besides the well established role of glycine as a central inhibitory neurotransmitter at strychnine-sensitive receptors, it has recently been observed that glycine facilitates channel opening at the N-methyl-D-aspartate (NMDA) subclass of excitatory amino acid receptor (Johnson & Ascher, 1987). In initial studies on spinal neurones in vivo and cortical neurones in vitro, we were not able to reproduce this observation, a result which is consistent with the presence of extracellular concentrations of glycine high enough to saturate any such glycine receptor. So we decided to test the hypothesis that some NMDA antagonists might act via the glycine site. In particular we were interested in the pyrrolidone, HA-966 (Davies & Watkins, 1972) which is not thought to act at any of the established NMDA antagonist sites.

Using the technique of microelectrophoresis on trigeminal neurones in pentobarbitone anaesthetised rats, and using the grease-sealed cortical wedge preparation, we tested glycine's ability to reverse the action of a series of NMDA antagonists. Responses to quisqualate were used as controls. Using both techniques, the NMDA antagonist action of kynurenate and HA-966 but not that of 2-amino-phosphonovalerate, dextrorphan or ketamine could be reduced by glycine.

On trigeminal neurones in vivo, all the antagonists tested preferentially reduced NMDA responses which were further reduced by glycine. After blocking this inhibitory effect of glycine with strychnine, selective enhancement of NMDA but not quisqualate responses was seen during ejection of kynurenate.

On cortical wedges a similar effect of glycine (31.6uM-1mM) was not blocked by strychnine (50uM) but was mimicked by D-serine (100uM-1mM). D-cycloserine, cycloleucine, DL-isoleucine and D-proline (all 100uM-1mM) failed to show glycine-like activity. In the absence of antagonists, none of these amino acids affected depolarisations evoked by NMDA or quisqualate. The NMDA block by HA-966 (200uM) could be fully reversed by glycine whereas that of kynurenate (200uM) was only partially reversed and the inhibition produced by 50-100uM kynurenate was relatively resistant to glycine. These results suggest that part of kynurenate's action is not via the glycine site.

On epileptiform activity recorded from cortical wedges perfused with magnesium-free medium, glycine (100uM-1mM) occasionally produced a slight enhancement of this bursting behaviour. Only the depression of epileptiform activity by kynurenate (and HA-966) was reversed by glycine.

Our results support Johnson and Ascher's hypothesis that glycine facilitates the action of NMDA, indicate that in more intact preparations the glycine levels are normally sufficient to facilitate synaptic transmission mediated by NMDA receptors, and further demonstrate that kynurenate and HA-966 reduce responses to NMDA by antagonising the effect of endogenous glycine.

E.J. Fletcher is a Wellcome Trust Research Scholar.

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EVIDENCE FOR SYNAPTICALLY ACTIVATED EXCITATORY AMINO ACID RECEPTORS IN SLICES OF RAT NUCLEUS ACCUMBENS

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There is strong biochemical evidence that the nucleus accumbens receives glutamatergic inputs by at least two pathways. These pathways originate from the cerebral cortex (Walaas, 1981) and the hippocampus (Walaas and Fonnum, 1980). Therefore, we have investigated the possible postsynaptic contribution of excitatory amino acid receptors to responses elicited electrically in rat nucleus accumbens in vitro.

Slices of nucleus accumbens (350μM thick) were cut from parasaggital sections of rat brain and placed in a perfusion chamber where each slice was superfused with oxygenated artificial cerebrospinal fluid (aCSF) solution, warmed to 33°C, at a rate of 2ml min^{-1}. The aCSF contained 6.25mM K^+ and normally 2mM Mg^{2+}. Extracellular recordings were made using glass microelectrodes filled with 3M NaCl. Statistical analyses were performed using Student's paired t-test.

Stimulation of the tissue, just ventral to the anterior commissure, evoked an apparent presynaptic fibre volley followed by a negative going population spike superimposed upon a positive going field EPSP. The broad spectrum excitatory amino acid antagonist, kynurenic acid, reduced the amplitude of the population spike in a concentration-dependent manner. The mean ($\pm$ SEM) reduction of a just submaximal population spike was 11.9 $\pm$ 2.2% for 100μM, 50.4 $\pm$ 4.9% for 300μM and 100 $\pm$ 0% for 1mM kynurenate (n = 4 in all cases). The N-methyl-D-aspartate (NMDA) antagonist D(-)-2-aminophosphonovalerate (D(-)AP5) at 30μM did not significantly affect the population spike amplitude ($P > 0.1$; n = 3). Neither kynurenate nor D(-)AP5 affected the presynaptic fibre volley.

Perfusion with Mg^{2+} free-aCSF led to an augmentation of the population spike at submaximal stimulus strengths, which was only partially reversed by 30μM D(-)AP5. The mean maximal response was not significantly augmented by removing the Mg^{2+} ($P > 0.1$; n = 5). Unlike in the hippocampus (Coan and Collingridge, 1987), no additional population spikes were produced in $0Mg^{2+}$ although the "decay" phase of the field EPSP was prolonged for a given response amplitude. The interval to where the EPSP decayed to half maximal amplitude following the population spike was increased from a mean ($\pm$ SEM) value of 4.9 $\pm$ 0.3ms in 2mM Mg^{2+} to 11.2 $\pm$ 1.4ms in 0 Mg^{2+} (n = 5). D(-)AP5 (30μM) reduced this interval in 0 Mg^{2+} to 4.9 $\pm$ 0.9ms (n = 4). This was significant at the 1% level.

Therefore, it appears that excitatory amino acid receptors of the non-NMDA subtype(s) mediate synaptic responses in the nucleus accumbens in vitro. Synaptic activation of NMDA receptors could also be expressed by removal of Mg^{2+} from the tissue perfusate.

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FG9065 AND FG9041 ANTAGONISE RESPONSES TO NMDA VIA AN ACTION AT THE STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR

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Excitatory amino acid receptors have been divided into at least three subtypes based on their selectivity to the agonists N-methyl-D-aspartate (NMDA), kainate and DL-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA). Recent attention has focussed on the allosteric interaction between strychnine-insensitive glycine receptors and the NMDA receptor (Johnson & Ascher, 1987). In these studies, glycine and D-serine were shown selectively to potentiate responses to NMDA. Kynurenate is an antagonist at this site (Kessler et al., 1987; Ascher et al. 1988; our own unpublished observations). We report here that the quinoxalinediones, FG9065 and FG9041 (Drejer & Honoré, 1988), also have antagonist actions at the strychnine-insensitive glycine receptor.

Spinal cords from 2-7 day old Sprague-Dawley rats were removed, hemisected and placed through a greased slot in the central barrier of a two compartment bath (Birch et al., 1988). Both ends of the hemicord were superfused (3ml/min) with Krebs-Ringer of the following composition (mM): KCl 4.7, NaCl 118, $CaCl_2$ 1.3, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11.1, H_2O_2 0.0024%; equilibrated with 95% O_2/5% CO_2, pH 7.4, at room temperature. TTX (30nM) was present throughout. Drugs were applied (3-min pulses) to one side and the resulting depolarisations recorded. Semi-cumulative concentration-response curves to NMDA (3-30μM), AMPA (1-10μM) and kainate (3-30μM) were constructed, repeated, and then antagonists, in the presence or absence of D-serine or glycine, superfused for 15min before redetermination of the agonist concentration-response curve.

D-serine (10^{-8}-10^{-3}M) or glycine (10^{-8}-10^{-4}M) had no effect on the response to NMDA. Superfusion with FG9065 or FG9041 antagonised responses to NMDA. At threshold concentrations, small parallel shifts were seen (FG9041 10^{-5}M, dose-ratio = 2.9±0.9[n=4]; FG9065 $3x10^{-5}$M, dose-ratio = 2.1±0.1[n=4]). Higher concentrations (FG9041 $3x10^{-5}$M-10^{-4}M; FG9065 10^{-4}M-$3x10^{-4}$M) produced non-parallel shifts with a large decrease in maximum response. The effects of FG9065 and FG9041 were fully reversible (2hr washout). Co-superfusion with D-serine ($3x10^{-6}$M-10^{-3}M) dose-dependently prevented the apparent unsurmountable antagonist action of FG9065 and FG9041. A similar effect was observed with glycine 10^{-3}M (strychnine 10^{-4}M present). D-serine ($3x10^{-4}$M) had no effect on the antagonism of NMDA responses by the competitive antagonist CPP (pA2 value [95% C.L.]: 6.0 [5.8-6.2] and 5.9 [5.8-6.1] with and without D-serine (n=5), respectively) or the non-competitive antagonist phencyclidine (10^{-5}M; dose ratios 2.3±0.1 and 3.0±0.2 with and without D-serine (n=4), respectively). Similarly, D-serine (10^{-3}M) did not affect the antagonism by FG9041 of responses to AMPA or kainate; apparent pA_2 values were: FG9041 versus AMPA 6.0 [5.8-6.3] and 6.0 [5.6-6.4]; FG9041 versus kainate 6.0 [5.8-6.3] and 5.9 [5.7-6.1], in the presence and absence of D-serine, respectively (n=4).

The results show that D-serine and glycine selectively prevent the unsurmountable antagonism of NMDA responses by FG9065 and FG9041. The results suggest that FG9065 and FG9041 antagonise responses to NMDA via an allosteric action at the strychnine-insensitive glycine receptor.

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DIFFERENCES IN THE N-METHYL-D-ASPARTATE ANTAGONIST PROFILES OF TWO COMPOUNDS ACTING AT THE GLYCINE MODULATORY SITE

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Glycine markedly potentiates N-methyl-D-aspartate (NMDA) responses by an action at a modulatory site on the NMDA receptor-ionophore complex (Johnson & Ascher, 1987). We have found that two compounds, 7-chloro kynurenic acid (7-Cl KYNA) and HA-966 (1-hydroxy-3-aminopyrrolid-2-one), appear to interact selectively with this site, but have different profiles as NMDA antagonists.

Population depolarisations of rat cortical slices evoked by excitatory amino acids were recorded across a greased gap as previously described (Kemp et al, 1986). Whole cell patch clamp recordings from rat cortical neurones in primary cell culture and the application of drugs by fast perfusion were performed essentially as described by Johnson & Ascher (1987).

On rat cortical slices 7-Cl KYNA produced a clearly non-competitive antagonism of NMDA responses, resulting in a progressive flattening of the NMDA concentration-response curve with increasing concentrations from 10-100μM. At 100μM the antagonism was completely unsurmountable by increasing the NMDA concentration. It could, however, be almost completely reversed by the addition of glycine or D-serine (100μM). In contrast, the antagonism produced by HA-966 had a threshold at 100μM (log concentration-ratio = 0.21 $\pm$ 0.04, mean $\pm$ SEM, n=7) and was maximal at 250μM (mean log concentration-ratio = 0.47 $\pm$ 0.04, n=4). Concentrations of 500 & 1000μM HA-966 produced no further increases in the NMDA concentration-ratio and no obvious flattening of the NMDA concentration-response curve. These NMDA antagonist effects of HA-966 were also fully reversible by glycine and D-serine. In whole cell patch clamp experiments, 7-Cl KYNA (10 and 30μM) completely abolished both the basal and glycine (1μM) potentiated NMDA responses. In contrast, HA-966 (100 and 300μM) had little effect on basal NMDA responses but reduced the potentiation produced by glycine. The effects of both 7-Cl KYNA and HA-966 could be reversed by increasing the glycine concentration.

These results suggest that both 7-Cl KYNA and HA-966 produce their NMDA antagonist effects by an action at the glycine modulatory site. In agreement with this is the finding that both compounds selectively inhibit strychnine-insensitive [3H]-glycine binding to rat brain membranes (Donald et al, this meeting). The differences between the NMDA antagonist profile of HA-966 and 7-Cl KYNA suggest two likely possibilities. One is that HA-966 is a pure antagonist at the glycine site whilst 7-Cl KYNA possesses negative modulatory (inverse-agonist) activity. Alternatively, it is possible that even in the absence of any added glycine some activation of the glycine site occurs and therefore it may be that 7-Cl KYNA is the true antagonist and HA-966 is a weak partial agonist. If the latter is the case, this implies that activation of the glycine site by an agonist is a prerequisite for NMDA receptor activation.

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STEREOSELECTIVITY OF A BICUCULLINE-INSENSITIVE GABA RECEPTOR IN ASCARIS SUUM

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Bicuculline and baclofen insensitive GABA receptors have been described in vertebrate and invertebrate nervous systems (Johnston et al, 1984; Sattelle et al, 1988). We have previously characterised the GABA receptor on the somatic muscle of the nematode Ascaris (Hewitt et al, 1986). It is distinct from mammalian GABA-A and GABA-B receptors as it is not activated by baclofen or inhibited by bicuculline. The results presented here provide further information on the structural requirements and stereoselectivity of this receptor.

Intracellular recordings were made from somatic muscle cells of Ascaris using conventional electrophysiological techniques. A second microelectrode was inserted to inject current pulses and enable determination of membrane conductance. Drugs were applied by perfusion over the preparation. The maximum conductance increase of the cell membrane caused by GABA was 3.2 ± 0.3 μSiemens with an EC_{50} of 26.3 ± 3.5 μM (n=17). The response to a submaximal concentration of GABA did not desensitize.

Table 1. The relative potency of drugs compared to GABA. A point on a parallel portion of each dose-response curve was chosen and the ratio between the concentration of GABA and the concentration of the drug that produced the same response was determined (mean ± SEM, n=5). 4-Me-t-ACA is 4 methyl-transamino-crotonic acid; Values less than unity, compounds less potent than GABA.

DRUG	RELATIVE POTENCY COMPARED TO GABA
GABA	1.00
(S)-(-)-4-ME-t-ACA	0.007 ± 0.0009
(R)-(+)-4-ME-t-ACA	NO EFFECT UP TO 1 mM
(S)-(+)-3-OH-GABA	0.13 ± 0.02
(R)-(-)-3-OH-GABA	0.25 ± 0.02
(S)-(+)-DIHYDROMUSCIMOL	7.53 ± 0.98
(R)-(-)-DIHYDROMUSCIMOL	0.85 ± 0.08

The results indicate that (S)-(+)-dihydromuscimol most closely mimics the active conformation of GABA at this receptor. Except for 3-(OH)-GABA, the stereo-selectivity of the compounds at this site is the same as that at the mammalian GABA-A receptor (Krogsgaard-Larsen, in press).

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GABA POTENTIATES NORADRENALINE-STIMULATED INOSITOL PHOSPHATE FORMATION IN RAT CEREBRAL CORTEX

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GABA, acting on $GABA_B$ receptors, inhibits histamine-induced inositol phosphate formation in rat cerebral cortical slices, but has no effect on the response to carbachol in the same tissue (Crawford & Young, 1988). In contrast, in rat hippocampal slices GABA is reported to stimulate noradrenaline-induced inositol phospholipid breakdown via an action on $GABA_A$ receptors (Corradetti et al, 1987; Rugiero et al, 1987). We describe here the action of GABA on α_1-agonist-induced inositol phosphate formation in rat cerebral cortex.

Responses were measured in cross-chopped slices (350 x 350 μm) of rat (Wistar strain, males, 250-350 g) cerebral cortex in the presence of 10 mM LiCl following 30 min incubation with 0.3 μM [^{3}H]-inositol. α-Agonist action was terminated by addition of perchloric acid and the inositol phosphates were extracted and separated as described previously (Crawford & Young, 1988). GABA was added immediately prior to the α_1-agonist.

GABA potentiated noradrenaline-induced [^{3}H]-inositol monophosphate ([^{3}H]-IP_1) formation with an EC_{50} of *circa* 0.3 mM. The effect on the concentration-response curve to noradrenaline, measured with 2 mM GABA, was primarily to increase the maximum response with a small shift of the EC_{50} to lower concentration. The mean potentiation after 60 min incubation with 100 μM noradrenaline was 65 ± 10% (25 measurements). However, the most striking feature of GABA action was that [^{3}H]-inositol bisphosphate ([^{3}H]-IP_2) formation was potentiated to a greater extent (361 ± 40%, n=14) than that of [^{3}H]-IP_1 or [^{3}H]-IP_3 (96 ± 32%, n=14). The ratio of [^{3}H]-IP_2 to [^{3}H]-IP_1 formed in response to noradrenaline was increased at all times between 2 and 60 min in the presence of 2 mM GABA. Prazosin (1 μM) abolished inositol phosphate formation induced by noradrenaline, both in the presence and absence of GABA. Preliminary measurements with the α_1-selective agonists phenylephrine and methoxamine have shown the same pattern of potentiation by 2 mM GABA as with noradrenaline and again the largest effect is on the formation of [^{3}H]-IP_2.

The nature of the GABA receptor involved in this interaction is not clear. Bicuculline methiodide (100 μM) gave only a 12 ± 4% inhibition (n=3) of the increased [^{3}H]-IP_1 formation in the presence of 2 mM GABA in response to 100 μM noradrenaline and isoguvacine (10 and 100 μM) failed to produce any significant potentiation. (-)-Baclofen, (10 μM) did give a small stimulation of the noradrenaline response, but only 21 ± 10% (n=3).

The mechanism of the GABA potentiation of the response to α_1-agonists is not known, but the enhanced formation of [^{3}H]-IP_2 suggests that an increased level of intracellular Ca^{2+} could be a part of the sequence of events. The uncertain pharmacology of the action of $GABA_A$ is reminiscent of that observed with GABA inhibition of histamine-induced [^{3}H]-IP_1 formation in guinea-pig cerebellum (Crawford et al, 1987).

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EFFECTS OF PUTATIVE ANTAGONISTS ON $GABA_B$ RECEPTOR MODULATION OF RAT CORTICAL cAMP ACCUMULATION

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δ-Aminovaleric acid (δ-AVA) and phaclofen can act as antagonists at central and peripheral $GABA_B$ receptors (see Hunter et al, 1988 for references). We have examined these compounds in $GABA_A$ and $GABA_B$ binding, and as antagonists of the effects of (-)-baclofen on forskolin and isoprenaline stimulation of cAMP levels in cortical prisms. Binding of [^{3}H]-muscimol (10nM) and [^{3}H]-(-)-baclofen (30nM) to rat cortical membranes was assayed. To assay cAMP accumulation, cortical prisms were washed (37°C, 60 min, Krebs Ringer bicarbonate), preincubated for 10 min with (-)-baclofen and other drugs before the addition of forskolin (0.5μM, 20 min) or isoprenaline (1μM, 10 min). cAMP was measured by a protein binding assay. Forskolin experiments were conducted in the presence of IBMX (1mM).

Phaclofen weakly displaced [^{3}H]-(-)-baclofen binding (pIC_{50} 3.64) and displaced < 10% [^{3}H]-muscimol binding at 1mM. δ-AVA was equipotent on [^{3}H]-(-)-baclofen (pIC_{50} 4.93) and [^{3}H]-muscimol binding (pIC_{50} 5.14). (-)-Baclofen inhibited forskolin-stimulated cAMP accumulation (IC_{50} 7.9 ± 4.0μM; n=3) but the effect was not stereospecific; (+)-baclofen: IC_{50} 8.0 ± 4.0μM (n=3). GABA (1mM) mimicked (-)-baclofen. Phaclofen failed to abolish the (-)-baclofen inhibition of the forskolin effect but increased forskolin-stimulated cAMP accumulation. δ-AVA acted as a $GABA_B$ agonist, inhibiting the forskolin effect; an inhibition not additive with (-)-baclofen (Table). (-)-Baclofen potentiated isoprenaline-stimulated cAMP accumulation, an effect antagonised by phaclofen. δ-AVA did not alter the isoprenaline effect, but increased the basal concentration. δ-AVA may have attenuated the (-)-baclofen effect but this is difficult to interpret given the raised basal concentration of cAMP (Table).

In summary, although (-)-baclofen inhibited forskolin-stimulated cAMP accumulation this may not be via $GABA_B$ receptors. δ-AVA but not phaclofen acted as an agonist with respect to this effect. Phaclofen antagonised $GABA_B$ receptors modulating isoprenaline-stimulated cAMP accumulation while δ-AVA may have a weak antagonist action. Behavioural (Hunter et al, 1988) and biochemical studies (Stirling et al, 1988) have indicated that both phaclofen and δ-AVA can act as agonists or antagonists at central $GABA_B$ receptors. We conclude therefore that heterogeneity may exist in central $GABA_B$ receptors.

Table The effects of $GABA_B$ drugs on cAMP biochemistry in rat cortex.

Drug	Basal	Forskolin	(-)baclofen + forskolin	Basal	Isoprenaline	(-)baclofen + isoprenaline
Control	12.2(1.2)	30.2(1.3)	21.0(2.5)*	4.2(0.1)	8.5(0.4)	11.2(1.3)†
Phaclofen	15.9(1.9)	39.4(2.1)*	30.4(2.3)	4.7(0.4)	7.4(0.9)	7.3(0.9)
δ-AVA	11.0(2.3)	20.8(1.4)*	19.2(2.3)*	8.8(1.8)*	13.8(1.8)*	15.2(0.9)*

Values are pmol cAMP/mg protein, mean (SEM) (n=4-8). Concn. used: baclofen 10μM; δ-AVA and phaclofen, 1mM. Different from appropriate control *p 0.01; †p 0.05.

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THE EFFECTS OF THE PUTATIVE $GABA_B$ ANTAGONISTS PHACLOFEN AND δ-AMINOVALERIC ACID ON $GABA_B$ FUNCTION IN VIVO

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Baclofen, a selective $GABA_B$ agonist, has been shown to alter several behavioural responses in vivo, including analgesia, hypothermia, sedation and inhibition of 5-HTP induced head twitch behaviour (Gianutsos and Moore, 1979; Gray et al, 1986, 1987; Hammond and Drower, 1984). The present experiments examined the actions of phaclofen and δ-aminovaleric acid (δ-AVA), two putative $GABA_B$ antagonists (Dutar and Nicoll, 1988; Kerr et al, 1987; Watling and Bristow, 1986), on these responses when administered alone or in combination with baclofen.

Male Lister Hooded rats (200-250g) or C57BL/6J mice (25-30g) obtained from Harlan Olac, U.K., were used. In the mouse we examined rectal temperature and 5-HTP-mediated head twitch (method of Gray et al, 1986, 1987) while rectal temperature, analgesia (latency to tail flick) and sedation (number of rears in an open field) were recorded in the rat. Phaclofen was synthesised at Astra and δ-AVA was obtained from Sigma. They were administered at a dose of 100mg.kg^{-1} i.p. either alone or 20 min prior to (±)-baclofen (5mg.kg^{-1} i.p.). When administered centrally via intracerebral (i.c.v) injection in conscious mice the antagonists were given immediately prior to the peripheral injection of (±)-baclofen (5mg.kg^{-1}). Data analysis was by one-way ANOVA followed by Tukey tests.

Phaclofen (100mg.kg^{-1} i.p.) had no effect on any of the above measures when administered alone other than it caused a decrease in 5-HTP induced head twitches (see Table). It did not reverse any baclofen induced changes. δ-AVA (100mg.kg^{-1} i.p.) had no effect when administered alone on any measure and when administered with baclofen it only reversed baclofen induced analgesia ($F=14.1$, $p<0.01$). When administered by icv injection at a dose of 5μg/mouse phaclofen neither altered rectal temperature nor reversed the hypothermia induced by baclofen. δ-AVA (5μg/mouse) alone had no effect on temperature but antagonised the baclofen-induced hypothermia ($F=14.3$, $p<0.01$). The lack of effect seen with peripheral injections of δ-AVA on temperature and sedation responses may be due either to poor brain penetration or to an inability to reach sufficiently high concentrations in the brain when administered via this route.

The effects of Phaclofen and δ-AVA on 5-HTP induced head twitches in the mouse (mean number + s.e.m).

TREATMENT	SALINE	PHACLOFEN	δ-AVA
Saline	9.0 ± 0.5**	4.3 ± 1.6**	9.0 ± 1.4*
Baclofen (5mg.kg^{-1})	2.8 ± 0.5	1.5 ± 0.6	4.6 ± 0.9

*=$p<0.05$, **=$p<0.01$ compared to control

In conclusion δ-AVA, but not phaclofen, does appear to act as a $GABA_B$ antagonist on baclofen induced hypothermia and analgesia.

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TOLERANCE AND WITHDRAWAL STUDIES WITH DIAZEPAM AND GR38032F IN THE RAT

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The induction of tolerance and subsequent withdrawal effects are a major problem with benzodiazepine therapy (Lader, 1984). Lack of these effects would be of considerable benefit to a new anxiolytic, as would be the ability to combat benzodiazepine withdrawal. We have therefore addressed these problems by inducing diazepam tolerance and withdrawal in the rat and examining the ability of the potential anxiolytic $5HT_3$ receptor antagonist GR38032F (Jones et al., 1988) to affect the withdrawal response. The ability of GR38032F to induce tolerance and withdrawal has also been studied.

Using the social interaction test (SI) (File, 1980; Jones et al., 1988) we have examined the effects of chronically treating rats (male Hooded Lister, 200-250g starting weights, n=8 pairs/group) with either diazepam (Dz), 40mg/kg, or GR38032F (GR), 0.2mg/kg on their respective anti-anxiety effects. Drugs and vehicle controls (5% acacia - V) were given orally twice daily for 7 or 21 days and rats tested for possible tolerance under high light unfamiliar conditions and 24h after the last dose for withdrawal effects under low light familiar conditions. The effect of the benzodiazepine antagonist flumazenil (Fz) on both diazepam and GR38032F treated rats was examined and the ability of diazepam and GR38032F to influence Fz-induced withdrawal was also assessed. Statistical analysis was by analysis of variance and subsequent Dunnett's t-test.

Rats chronically doses with Dz became tolerant to the anxiolytic effects of a submaximally effective dose (1.5mg/kg) after both 7 and 21 days treatment as manifest by a failure of this dose to increase SI in the chronically treated rats. (7 days: V - 21.3±3.5s; V/Dz1.5 - 41.3±3.05s, $P<0.01$ vs V; Dz40/Dz1.5 - 21.1±4.3s, $P<0.01$ vs V/Dz1.5; 21 days: V - 11.8±1.0s; V/Dz1.5 - 25.4±2.3, $P<0.01$ vs V; Dz40/Dz1.5 - 12.3±1.9s, $P<0.01$ vs V/Dz1.5). No such tolerance was observed with a similar regimen of GR38032F treatment (e.g. 21 days: V - 11.8±1.0s; V/GR0.01 - 37.7±4.4s, $P<0.01$ vs V; GR0.2/GR0.01 - 31.7±4.1s, $P<0.01$ vs V). Withdrawal effects, as measured by a reduction in SI, could be induced spontaneously after 21 days treatment with Dz (V - 25.2±3.1s; Dz40 - 10.6±2.6s, $P<0.05$ vs V) but after 7 days treatment only by using Fz (10mg/kg p.o.) (V - 55.7±8.7s; Dz40 - 44.8±4.1s; Dz40/Fz10 - 12.0±2.2s, $P<0.01$ vs Dz40). The reduction in SI seen with Fz could be attenuated both by concomitant treatment with Dz, 2mg/kg (50.5±9.9s, $P<0.01$ vs Dz40/Fz10) and GR, 0.01mg/kg (37.8±7.2s, $P<0.05$ vs Dz40/Fz10). No withdrawal effects could be induced to GR38032F either spontaneously (V - 25.2±3.1s; GR0.2 - 22.1±0.2s) or with Fz treatment (v - 32.0±4.1s; GR0.2/Fz10 - 27.6±5.6s) after 21 and 7 days chronic treatment respectively.

These data show that tolerance can develop readily and rapidly to the anxiolytic effects of diazepam but not GR38032F on chronic treatment. Furthermore, the diazepam withdrawal effects can be attenuated by GR38032F. This suggests that GR38032F should be free of tolerance and withdrawal problems in the clinic and could be used to treat individuals both with and without prior benzodiazepine exposure.

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[3H]GBR 12935 BINDING TO DOPAMINE UPTAKE SITES IN SCHIZOPHRENIA

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The dopamine (DA) hypothesis of schizophrenia remains the most convincing biochemical explanation, although evidence to support a link between brain dopamine and the illness is mainly of a pharmacological nature. However, measurements of DA and its metabolites *in vivo*, and in post-mortem brain tissue, have provided only inconclusive evidence. Even the most consistent abnormality reported, the increase in DA D2 receptors in the striatum, may be explained by the effects of long term neuroleptic treatment. Recently, however, there has been a report that synaptosomes prepared from the accumbens and caudate nuclei taken post-mortem from schizophrenic patients show substantial and significant elevations in the active uptake of DA (Haberland and Hetey, 1987). Since the assessment of active uptake processes in post-mortem brain is very dependent on the treatment and storage of the tissue (Hardy et al, 1982) we decided to investigate these findings by employing a ligand-binding assay for the DA uptake site. Only recently has a suitably selective ligand been developed for identification of the presynaptic DA uptake site. We utilised this ligand, [3H]GBR 12935, as a means of measuring the density of these sites in post-mortem striatal tissue from a series of schizophrenic subjects and matched controls, in an attempt to detect any differences between the two groups.

Binding of [3H]GBR 12935 to caudate nucleus taken post-mortem from seven patients with a diagnosis of schizophrenia and from seven age, sex and post-mortem delay matched controls, was studied essentially as described by Janowsky et al. (1986). Tissue was homogenized in 10 vol. ice-cold sucrose (0.32M). Following centrifugation at 1000g for 10 mins at 4°C, the supernatant was centrifuged at 48,000g for 10 mins and the resulting pellet homogenized in 100 vol. 50mM phosphate-HCl (pH 7.7) containing 0.01% bovine serum albumin. Assay tubes containing 200µl of this final resuspension, varying quantities of [3H]GBR 12935 and buffer to a total volume of 1ml, were incubated for 45mins at 20°C. The final concentration of ligand varied from 0.2-4nM. Tubes were vacuum filtered over glass-fibre filters (previously soaked in 0.05% polyethyleneimine for at least one hour). Filters were rinsed with 4ml of ice-cold buffer and the radioactivity was then determined by liquid spectrometry. Non-specific binding was determined in the presence of 10µM amfonelic acid. Bmax and Kd values were obtained by linear regression analysis of Scatchard plots. Student t-tests were used for statistical analysis of resulting data.

Our results indicated no significant difference in Kd values between schizophrenics and controls and no change in maximal number of binding sites between the two groups (table 1). Bilateral assessment of ligand binding indicated a lack of asymmetry in both receptor density and affinity. There was also no correlation between Bmax values and previously assessed DA or homovanillic acid concentrations. Our results do not support the hypothesis that there is a disorder of nigrostriatal DAergic neurones in schizophrenia, providing no support for the proposal of increased caudate DA uptake in the disease.

Table1 Binding of [3H]GBR 12935 to post-mortem caudate nucleus (Bmax)

	Left	Right
Control	1954±564	2338±675
Schizophrenics	2098±713	2164±711

mean±s.d. in fmol/mg protein

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THE EFFECT OF INHIBITION OF DOPAMINE SYNTHESIS ON DIHYDROXYPHENYL-ACETIC ACID (DOPAC) FORMATION IN THE RAT BRAIN

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There is evidence that in conditions of overactive dopamine synthesis in the rat striatum, dopamine can be deaminated to DOPAC before its release has occurred (Soares-da-Silva, 1987). More recently, it has been suggested that even in basal conditions of dopamine synthesis in the rat brain a major portion of DOPAC is derived from an intraneuronal pool of newly synthesized dopamine (Zetterström et al., 1988). Although DOPAC has been considered for years as an index of dopamine release or utilization, there is increasing evidence that it might serve as an index of dopamine synthesis. The question we were interested in was that of studying the effects of dopamine synthesis inhibition on DOPAC formation in three areas of the rat brain (striatum, accumbens and prefrontal cortex) on which DOPAC/dopamine ratios are known to be different.

Male Wistar rats, 250-300 g, were divided in three groups of 5 animals each, treated i.p. with alpha-methyl-p-tyrosine (200 mg/kg) and sacrificed at different times. Sixty, 120 or 180 minutes after alpha-methyl-p-tyrosine treatment the rats were decapitated, brains removed and placed on an ice-cold porcelain plate and the striatum, accumbens and prefrontal cortex dissected, weighed and stored in 0.1 mM perchloric acid. The assay of dopamine, DOPAC and homovanillic acid (HVA) was effected by HPLC-ECD. Dopamine, DOPAC and HVA tissue content in the three brain areas were logarithmically transformed, plotted against duration of tyrosine hydroxylase inhibition and the rate constant of amine or amine metabolite decline calculated. The rate constant of dopamine decline k (min^{-1}) was significantly higher in the prefrontal cortex (0.0071±0.0015) than in the accumbens (0.0033±0.0003) or the striatum (0.0015±0.0002). In the striatm and accumbens the rate constant of DOPAC decline k (min^{-1}), 0.0038±0.0005 and 0.0066±0.0013 respectively, were significantly greater than the corresponding rate constant of dopamine decline. Only in the striatum was HVA consistently found after tyrosine hydroxylase inhibition and the rate constant of HVA decline k (min^{-1}); 0.0035±0.0005) was similar to that for DOPAC. In control conditions, tissue content DOPAC/dopamine ratios in the striatum, accumbens and prefrontal cortex were 0.15±0.02, 0.26±0.02 and 0.40±0.04, respectively).

These results show that the rate of dopamine synthesis is not the same in all brain areas endowed with dopaminergic innervation and suggest that the main source of amine for the formation of DOPAC resides in newly synthesized dopamine. For these reasons, DOPAC appears to be a better index of dopamine synthesis than of its release or utilization.

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IN VIVO VOLTAMMETRIC DETECTION OF 3-METHOXYTYRAMINE (3-MT) IN PARGYLINE-TREATED MICE

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Electrically-treated 12 μm carbon fibre electrodes (CFE) combined with differential pulse voltammetry (DPV) allow the in vivo detection of ascorbate (Peak 1, -50mV), DOPAC (Peak 2, +100mV) 5HIAA (Peak 3, +300mV) and HVA (Peak 4, +400mV) in striatum of anaesthetised rats (Crespi et al., 1984). However, in the striata of pargyline-treated mice we found that the size of Peak 4 unexpectedly increased, suggesting that Peak 4 may be due to oxidation of the dopamine (DA) metabolite 3-methoxytyramine (3-MT). We now present further evidence that Peak 4 represents oxidation of 3-MT in pargyline-treated mice.

CFEs were prepared as described as Crespi et al. (1984). Following in vitro characterisation they were implanted into the striata of Balb/CJ or CBA/J mice anaesthetised with chloral hydrate (500 mg/kg i.p.). DPVs were obtained every 6 min. Mice were injected 60-90 min later with saline (2 ml/kg) or pargyline (150 mg/kg) i.p. and DPVs were recorded for a further 180 min. Animals were killed, striata removed and stored at -80°C until analysis of HVA and 3-MT by HPLC with ECD (Heal et al., 1988).

The sizes of Peaks 1 and 3 in vivo were similar to those observed in rats, whereas Peak 2 was 50% smaller (Crespi et al., 1984). Pargyline abolished Peak 2 and decreased Peak 3 by 70%. In addition, Peak 4, previously a shoulder on Peak 3, became a distinct peak at +400mV and was 2-3 times larger in Balb/CJ than CBA/J mice. Furthermore, infusion of 0.1M KCl close to the electrode, which increases DA release (Crespi et al., 1988), resulted in a 150% or 60% increase in the size of Peak 4 in Balb/CJ or CBA/J mice respectively. The HVA and 3-MT concentrations in the striata of control and pargyline-treated mice are shown in Table 1.

	Balb/CJ mice		CBA/J mice	
	HVA	3-MT	HVA	3-MT
Control	676 ± 92	304 ± 48	676 ± 224	140 ± 32
Pargyline	44 ± 20	1856 ± 440	32 ± 8	1160 ± 108

Values are mean ± s.d.mean ng/g wet wt tissue (n=7).

When deamination of DA by MAO is inhibited by pargyline, DA can only be metabolised by extraneuronal COMT (Davies & Heal, 1986). Thus, DOPAC and HVA levels fall following pargyline and 3-MT levels rise as illustrated in Table 1. Since Peak 4 increased in size by about 100% following pargyline, our data support the view that, in pargyline-treated mice, Peak 4 represents mainly oxidation of 3-MT and that it may be a useful index of DA release in situations where DA itself cannot be detected (Crespi et al., 1988).

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ORAL N-ACETYLCYSTEINE (NAC) PREVENTS LUNG INJURY INDUCED BY α-NAPHTHYLTHIOUREA (ANTU) IN RATS

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Pulmonary oedema in rats induced by intraperitoneal injection of ANTU may be mediated by oxygen derived free radicals (Fox et al, 1983). One of the natural defences against free radicals is glutathione (GSH) and its depletion may be a mechanism underlying oxidant lung injury. Depleted GSH in lung is replenished by treatment with NAC (Berggren et al, 1984) and we have therefore studied the effects of NAC, given orally, on lung injury caused by ANTU.

Male rats (200-250g) were injected with ANTU and the lungs removed at various times up to 28h later. Two physical measures of lung injury were made; pleural exudate was weighed and the lung dry:wet weight ratio determined. In other similarly treated rats, the pharmacokinetics of exogenous prostaglandin E_2 (PGE_2) were measured in isolated lungs perfused via the pulmonary circulation, with Krebs solution at a flow rate of $8ml.min^{-1}$. These assays were also performed using lungs from rats pretreated with NAC in drinking water for at least 24h before, and after, ANTU treatment. Our initial experiments used $10mg.kg^{-1}$ ANTU and a range of NAC concentrations (1, 3 and 6 %, w/v). At 4h after ANTU, the lung dry:wet weight ratio was only slightly improved by 6% NAC (ANTU alone, 16.6±0.2%; ANTU + NAC, 17.2±0.2%; N=4-6, $p<0.05$). A lower dose of ANTU (3mg/kg) induced less severe lung injury (at 4h, lung dry:wet weight ratio, 16.7±0.2%; exudate, 2.0±0.3g, N=10). After treatment with 6% NAC for 24h before ANTU and for the subsequent 4h, pleural exudate was reduced to 0.2±0.1g and lung dry:wet weight ratio improved to 17.9±0.2% (N=14). The pharmacokinetics of exogenous ^{14}C-PGE_2 (bolus injection of 500ng, $2x10^4$ dpm) in isolated lungs from rats treated with the lower dose of ANTU ($3mg.kg^{-1}$) were also altered by NAC pretreatment. Thus the $T_{1/2}$ value (time for 50% of injected radioactivity to efflux from lung) in normal lungs was 36±1s and was raised by 4h after ANTU to 48±2s. NAC reduced this increased $T_{1/2}$ to 40±1s (N=9). Survival of unlabelled PGE_2 (500ng; assayed by RIA) after a single pass through the pulmonary circulation, was 10±3% in lungs from untreated rats and increased to 22±4% after ANTU. Treatment with NAC reduced this higher value to 6±1% (N=6).

Another pharmacokinetic consequence of ANTU-induced lung injury is a decrease in ^3H-adenosine uptake which leads to a shorter $T_{1/2}$ (Bakhle & Grantham, 1987). After a bolus injection of ^3H-adenosine (10nmole, 5μCi), the $T_{1/2}$ value in normal lungs was over 120 s. This was reduced to 31±3s, 4h after ANTU and, following pretreatment with 6% NAC, the $T_{1/2}$ was restored to > 120s (N=5). Treatment of normal rats with NAC alone did not affect any of the variables measured.

From these results we conclude that orally administered NAC does diminish the lung injury induced by ANTU as measured by the pleural exudate and pulmonary oedema. NAC also prevents the pharmacokinetic changes caused by ANTU. These findings and our previous results (Bakhle & Grantham, 1986), suggest that NAC may provide a useful model from which to develop treatments for acute lung injury.

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INHIBITION OF ANTIGEN-INDUCED BRONCHOPULMONARY EOSINOPHILIA BY SELECTIVE LEUKOTRIENE B_4 (LTB_4) ANTAGONISTS

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6-[6-(3-hydroxy-1E,5Z-undecadien-1-yl)-2-pyridinyl]-1,5-hexanediol(**U-75,302**) 6-[6-(3-hydroxy-5Z-undecen-1-yl)-2-pyridinyl]-1,5-hexanediol (**U-77,692**), and 6-[6-(3-hydroxy-1E-undecen-1-yl)-2-pyridinyl]-5Z-hexen-1-ol (**U-78,489**) are novel synthetic, structural analogs of LTB_4 which antagonize LTB_4 binding to neutrophils (ID_{50s} for U-75,302, U-77,692 and U-78,489 on human neutrophils were 0.38 μM, 10 μM and 0.3 μM respectively). On guinea pig lung parenchymal strips, U-75,302 has been shown to selectively antagonize the myotropic activity of LTB_4 but not other myotropic agonists including the thromboxane A_2 mimetic U-46,619, LTC_4, LTD_4, AGEPC, $PGF_{2\alpha}$ and histamine '(Lawson et. al. in press)'.

Previously we have shown that antigen inhalation in sensitized guinea pigs produces a marked eosinophil-rich accumulation of leukocytes in the airway lumen '(Dunn et. al., 1988)'. In the present study we compared ovalbumin antigen-induced increases in eosinophil populations in guinea pig bronchoalveolar lavage (BAL) fluid 24h after antigen challenge in groups of 6 vehicle-treated, sensitized guinea pigs or in animals treated with U-75,302, U-77,692 or U-78,489, 30 mg/kg p.o. given 1h before and 7h after challenge. U-75,302, U-77,692 and U-78,489 produced a statistically significant ($p<0.05$) 64.1, 57.9 and 66.6% inhibition respectively of the antigen-induced lung eosinophilia.

U-75,302 was selected for further evaluation. In a follow-up dose-ranging experiment U-75,302, 1.0, 10.0 and 30.0 mg/kg was shown to produce 12.2% ($p>0.05$), 43.2% ($p<0.05$) and 61.1% ($p<0.05$) inhibition respectively of the bronchopulmonary eosinophilia.

In a separate study, we compared the histopathological changes in guinea pig lungs at various time intervals after ovalbumin inhalation in U-75,302 or vehicle-treated guinea pigs. Between 6 and 18h after antigen provocation, eosinophils were seen in tracts between smooth muscle cell layers, accumulating in large numbers in the bronchial mucosal epithelium in vehicle-treated guinea pigs but not in U-75,302-treated animals.

These data suggest that LTB_4 may be an important mediator of antigen-induced bronchopulmonary eosinophilia in sensitized guinea pigs.

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EFFECT OF WEB 2086 ON EARLY AND LATE AIRWAYS RESPONSES TO OVALBUMIN CHALLENGE IN CONSCIOUS GUINEA-PIGS

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Platelet activating factor (PAF) has been suggested to be a mediator in late phase responses in asthma and to be intimately involved in the pathogenesis of increased bronchial hyperresponsiveness (Page, 1988). We report the effects of a PAF-antagonist, WEB 2086, on early and late phase responses in a guinea-pig model of asthma (Hutson *et al*, 1988).

Male Dunkin Hartley guinea-pigs (500-700 g) were sensitized to ovalbumin (1% inhaled for 3 min weekly for 2 weeks). Groups of 16 sensitized animals received 0.5 mg/kg p.o. of WEB 2086 or saline (control), 1 h before or 6 h after challenge with aerosolized ovalbumin (2% for 5 min) under cover of mepyramine (10 mg/kg i.p.). In control animals, specific airways conductance (sGaw), assessed in conscious animals by whole body plethysmography, showed three falls in control animals; an early response (EAR) peaking at 2 h, a late response (LAR) peaking at 17 h and a second late response (LLAR) at 72 h. Bronchoalveolar lavage (BAL) revealed significant increases in neutrophils at 17 h and eosinophils at 17 h and 72 h (Hutson *et al*, 1988).

WEB 2086 administered 1 h before challenge had no effect on the immediate fall in sGaw occurring within 5 min, but partially inhibited the EAR measured at 2 h by 43% ($p<0.025$). The LAR was not significantly affected at 17 h, but the fall in sGaw occurring at 24 h was inhibited ($p<0.005$). There was no significant effect on the LLAR. The increases in neutrophils and eosinophils in BAL at 17 h were inhibited by 88% ($p<0.01$) and 56% ($p<0.05$) respectively. No effect was observed on any cell type at 72 h. When administered 6 h after challenge, WEB 2086 completely inhibited the LAR and LLAR ($p<0.001$) and signficantly inhibited the rise in eosinophils at 72 h by 50% ($p<0.05$). No effect was observed on the rise in neutrophils or eosinophils at 17 h. Although lymphocytes were not increased after ovalbumin challenge, they were significantly reduced from baseline levels at 72 h ($p<0.01$) in the WEB 2086 group.

These results confirm observations that PAF antagonists are capable of inhibiting late phase responses in the rabbit (Page *et al*, 1988) and sheep (Stevenson *et al*, 1987). The observation that WEB 2086 administered 6 h after challenge has a more pronounced effect on the LAR and LLAR, suggests that PAF release after the EAR is more critical for the development of these late responses.

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THE RESPONSIVENESS OF LUNGS FROM OXYGEN TREATED RATS TO PAF-ACETHER

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PAF-acether has been detected in the bronchoalveolar lavage fluid from rats which had been exposed to oxygen (O_2) for 12h. Exposure to O_2 for 24h and 48h, however, elicited changes only in lyso PAF levels (Court & Kingston, 1987). We now report the pharmacological actions of PAF-acether on O_2 inflamed rat lungs.

Male Wistar rats (250-300g) were exposed to oxygen as described by Court & Kingston (1987) for periods of either 12h, 24h or 48h. The control group of rats (0h), were not exposed to 100% O_2. The lungs were excised and perfused either singly, through the airways, or double perfused through the pulmonary artery and the airways simultaneously. The perfusion methods are described elsewhere (Court & Kingston, 1988).

PAF-acether (13.8nmol) administered to lungs perfused solely by the airways gave increases in the perfusion pressure of 0.35±0.08, 0.51±0.06, 0.74±0.12 and 0.83±0.06 mmHg (mean ± s.e. mean, n=4) after 0h, 12h, 24h and 48h respectively of O_2 exposure. The increase in perfusion pressure was significant ($P<0.05$) between the responses at 0h and 48h and also between 12h and 48h.

In the double perfusion the increase in the vascular perfusion pressure was significantly different ($P<0.05$) from 0h rats that received the same dose of PAF-acether (Table 1). The increase in the airway perfusion pressure following oxygen exposure (Table 1), was in most cases not significantly different from 0h. The responses of the 24h and 48h preparations, to 0.08nmol PAF-acether were, however, significantly different ($P<0.05$) from 0h.

Table 1 The change in the Perfusion Pressure of the vasculature (vasc.) and airways (air.) in double perfused lungs following the vascular administration of PAF-acether. The results are expressed as the mean ± s.e. mean (n=4). One response to PAF-acether was examined per preparation.

	PAF acether (nmol)	Period of Oxygen exposure (h) 0	12	24	48
vasc.	0.05	1.33±0.17	2.78±0.13	3.48±0.14	4.39±0.24
	0.23	2.50±0.25	3.63±0.29	4.17±0.43	6.63±0.42
	0.91	3.30±0.44	5.38±0.24	6.63±0.42	7.68±0.75
air.	0.08	0.82±0.14	1.20±0.16	1.26±0.05	1.41±0.15
	0.34	1.21±0.13	1.35±0.12	1.51±0.10	1.69±0.15
	1.38	1.30±0.17	1.54±0.11	1.89±0.22	1.95±0.19

We have previously observed that the addition of one dose of PAF-acether to rat isolated lungs, either single or double perfused, will desensitize the tissue to a second dose of PAF-acether. We have found that oxygen treated rats release PAF-acether into the airways (Court & Kingston, 1987). In the present investigation the lungs from oxygen treated rats were more responsive to PAF-acether than the lungs from control animals. Therefore, it would appear that endogenously released PAF-acether does not desensitize the lungs to the effect of exogenous PAF-acether.

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PROTEOLYTIC DESTRUCTION OF LIPOCORTIN 1 BY ALVEOLAR PHAGOCYTES FROM BRONCHOALVEOLAR LAVAGE FLUID: PROTECTION BY α_1-ANTIPROTEASE

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Lipocortins can suppress acute inflammation and the release of lipid mediators from target cells through a mechanism which involves binding to the external cell surface (reviewed in Flower, 1984). Migrating phagocytic cells are abundant at sites of inflammation and membrane bound lipocortin could be vulnerable to destruction by secreted proteases. We have therefore investigated the effect of phagocyte-derived proteases on the integrity of lipocortin 1.

Human alveolar phagocytes were obtained from bronchoalveolar lavage fluid (BALF) of 8 sequential patients undergoing routine fibreoptic bronchoscopy from diagnostic purposes (Smith et al 1985). Cells were collected by centrifugation (300 xg, 15 min, 4°C) and cultured in low protein hybridoma medium at a density of 10^6/ml. After 1h the conditioned media (CM1) were removed and separated from any non adherent cells (mainly polymorphonuclear leukocytes). Fresh media were then added to the adherent cells (mainly macrophages) and harvested after a further 3h (CM2). Aliquots of CM1 and CM2 (equivalent to 10^5 cells) or unconditioned (control) medium were preincubated (30 min, 37°C) with α_1-protease inhibitor (α_1 PI; 10 ug/ml) or EDTA (100uM) to inhibit serine and metal ion dependent proteinases respectively. To assess proteolytic destruction of lipocortin, samples (2ug, 0.05 nmoles) of authentic highly purified (>99%) human recombinant lipocortin 1 (a gift from The Biogen Research Corp Inc) were incubated for 24-48h with CM1, CM2 or control medium with or without inhibitors. The reaction was terminated by boiling the sample in 5% mercaptoethanol: native and proteolyzed lipocortin was separated by SDS-polyacrylamide gel electrophoresis and the bands containing lipocortin 1 or its major degradation products detected by immunoblotting using a specific rabbit anti-human lipocortin 1 polyclonal antibody (Biogen) and a peroxidase-conjugated second antibody. After visualisation of the bands with diaminobenzidine the relative proportion of the native (37 kda) to the degraded (34kda or smaller) species was assessed by quantitative reflectance densitometry. The elastase activity of the samples was measured as described by Smith et al 1985.

Table 1 Degradation of lipocortin 1 by human phagocyte derived proteases.

	% lipocortin remaining in native from (median range))		
Medium	no addition	+ α1PI	+ EDTA
Control (n=8)	93(58-95)	92(89-95)	91(90-93)
CM1 (n=8)	45(0-94)*†	93(39-100)	70(0-100)*†
CM2 (n=7)	88(0-100)	98(42-100)	55(0-100)

†*Significantly different from *control or †α1PI value, $P<0.05$, using Wilcoxon Signed Rank test for paired data (two tailed).

Lipocortin was significantly degraded by CM1 but not CM2 or control medium. α1PI, but not EDTA protected lipocortin from degradation suggesting a serine protease. Elastase was present in all CM1 and most CM2 (CM1, 2.5 (0.5-9.2) and CM2 0.2 (0.0-9.7) neutrophil elastase equivalents, ng/10^5 elastase secreting cells (median, range)), but it seems unlikely that elastase alone was responsible for hydrolysis since there was no correlation between elastase activity and lipocortin proteolysis in individual samples. Subjects deficient in α1PI are susceptible to early onset pulmonary emphysema and uncontrolled destruction of endogenous anti-inflammatory proteins such as lipocortin could contribute to this disease state.

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BRADYKININ-INDUCED BRONCHOCONSTRICTION IN THE GUINEA-PIG VIA A β_2 RECEPTOR POPULATION

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Bradykinin has been characterised as a bronchoconstrictor agent in the guinea-pig for a number of years (Bhoola et al., 1962), but the receptor population responsible has not been characterised. The recent availability of a number of bradykinin analogues as selective antagonists for the B_1 or B_2 receptor populations has facilitated our understanding of the mechanisms of action of bradykinin in smooth muscle preparations (Regoli et al., 1986). In the present series of experiments we have investigaed the activity of the B_2 antagonist D-arg-[Hyp^3-Thi^{5,8}-D-Phe^7]-Bk and the B_1 antagonist des-Arg-Leu^8-Bk to influence bronchoconstriction induced by bradykinin or a number of other spasmogens in the anaesthetised guinea-pig. Male Dunkin-Hartley guinea-pigs were anaesthetised with urethane (7ml/kg) and the jugular vein cannulated for the injection of drugs and the carotid artery cannulated for the measurement of blood pressure. A tracheal cannula was inserted and connected to a small animal ventilator. The animals were ventilated with room air (1ml/100g body weight, at 70 strokes per minute). Air overflow as an index of intrathoracic pressure (ITP) was measured at the side arm of the tracheal cannula by means of a pressure transducer. I.v. administration of bradykinin (1ug/kg) induced bronchoconstriction, whereas the administration of the B_1 agonist des-Arg^9-Bk (1-100ug/kg) was ineffective. Bradykinin induced bronchoconstriction was abolished by pretreatment of the guinea-pigs with the B_2 antagonist D-Arg-[Hyp^3-Thi^{5,8}-D-Phe^7]-Bk (400ug/kg i.v.) (ITP before = 154 $\pm$ 28.4; ITP after = 0, n=6). Responses are expressed as mean $\pm$ s.e.m. % increase in ITP. The response to bradykinin recovered over a 5 minute period. No significant inhibition was observed following pretreatment with the B_1 antagonist des-Arg-Leu^8-Bk (ITP before = 103 $\pm$ 25.8; ITP after = 80.2 $\pm$ 13.2; NS Students t test, n=5). The B_2 antagonist was inactive against i.v. platelet activating factor (30ng/kg), histamine (10ug/kg), acetylcholine (10ug/kg), bombesin (240ng/kg) or vagal stimulation. These results suggest that bradykinin-induced bronchoconstriction in the guinea-pig is caused by activation of B_2 rather than B_1 receptors. It is hoped that the availability of B_2 receptor antagonists will allow the determination of the role of bradykinin in respiratory disease.

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MODULATION OF BRADYKININ-INDUCED RESPONSES BY INDOMETHACIN AND REMOVAL OF THE EPITHELIUM IN GUINEA-PIG TRACHEA IN VITRO

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There is considerable evidence that epithelium lining guinea-pig trachea (GPT) influences the responses to various agonists (Hay et al, 1986). Since bradykinin (Bk) is a potent bronchoconstrictor in asthmatics (Fuller et al, 1987) and epithelial damage occurs in asthma, we investigated the effect of epithelial removal on Bk-induced responses of GPT.

Tracheae from male guinea pigs (Dunkin-Hartley, 500-600g) were halved. One half was kept intact (control) while the other was stripped of epithelium by gentle rubbing with a cotton swab. Microscopic examination of preparations (HE stain), confirmed the presence and absence of epithelium in control and rubbed GPTs respectively. GPTs were cut longitudinally, opposite the smooth muscle and then in zig-zag strips. They were either placed in 10-ml organ baths, containing a modified Krebs-Henseleit solution, or were superfused with Tyrode's solution at 37°C. Where applicable, a combination of antagonists to histamine, acetylcholine, 5-hydroxytryptamine and α- and β-adrenoceptors (see Piper & Vane, 1969) and/or indomethacin (indo) were administered continuously throughout the experiment. In the superfusion system, BK was compared with salbutamol and U-44069, the β_2-adrenoceptor and thromboxane mimetic respectively, given as bolus injections (10-100µl) into the superfusing buffer. In the 10-ml baths, cumulative concentration-response curves to Bk were obtained. In all experiments, n = 4-7.

Bk induced dose-related responses on all preparations. In superfusion,Bk (3pmol-3nmol) caused a profound relaxation, preceded by an initial transitory peak, on control GPTs and was largely equiactive with salbutamol (30pmol-3nmol). In denuded GPTs, Bk produced contractions and was approximately 10 times more active than U-44069 (0.3-3nmol). Removal of epithelium had no effect on responses to salbutamol or to U-44069. Combined antagonists did not affect responses of GPTs to Bk. In contrast, indo (1.4µM) abolished spontaneous activity of rubbed GPTs and greatly reduced Bk-induced contractions (e.g. those due to 0.1 and 1 nmol were inhibited by 93±5 and 79±1% respectively). Moreover, response of Bk on intact GPTs was notably altered since dose-related contractions were obtained in the presence of indo.

In the 10-ml baths, Bk (10^{-9}-10^{-5}M) caused relaxations (preceded by a transitory peak) and contractions on intact and rubbed GPTs respectively, which agrees with findings described by Bewley et al, 1987. In the presence of indo (2.8µM), Bk had little effect on rubbed GPTs but contracted intact tracheae.

It is of interest that only intact GPTs developed intrinsic tone, suggesting that epithelium is required for maintenance of airway tone. Results indicate that both removal of the epithelium and treatment with indo lead to a reversal of the Bk-induced relaxation of GPT, suggesting involvement of a cyclo-oxygenase product of arachidonic acid metabolism.

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ICI 169,369 IS BOTH A COMPETITIVE ANTAGONIST AND ACTIVATOR OF THE 5-HT_2 RECEPTOR SYSTEM OF GUINEA-PIG TRACHEA

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5-Hydroxytryptamine (5-HT) contracts tracheal smooth muscle through 5-HT_2 receptors. Ketanserin antagonises the effects of 5-HT competitively but also depresses by 1/3 the maximum response to 5-HT. Methysergide antagonises non-competitively and markedly depresses the maximum response to 5-HT. Ketanserin can partially prevent the depression of the response to 5-HT caused by methysergide. These findings led Lemoine & Kaumann (1986) to conclude that tracheal 5-HT_2 receptors exist in 2 interconvertible states R⇌R' as proposed for arterial 5-HT_2 receptors (Kaumann & Frenken, 1985). Drugs that favour the highly active R-state and low active R'-state have been designated activators and deactivators respectively; in several blood vessels ketanserin is an activator, methysergide a deactivator (Kaumann, 1988). In guinea-pig trachea ketanserin is a partial deactivator, methysergide a marked deactivator (Lemoine & Kaumann, 1986). ICI 169,369 [2-(2-dimethylamino ethylthio)-3-phenylquinoline] is a new 5-HT_2 receptor antagonist (Blackburn et al, 1986) and also an activator in blood vessels (Frenken & Kaumann, 1987). We have investigated the blocking potency of ICI 169,369 and whether it is an activator of the 5-HT_2 receptor system of guinea-pig trachea. We used tracheal helicoids in Krebs solution with 6 μM cocaine and 0.2 mM ascorbate at 32.5°C. We determined 2 successive concentration-effect curves (CEC) on each tissue. ICI 169,369 (incubated for 2h) shifted the CEC in parallel and surmountable manner. The slope of the Schild-plot was 1.13 on 11 tissues from 7 guinea-pigs. A pK_B value of 9.05±0.08 was estimated for ICI 169,369 (10-1000 nM). The depressant effects of methysergide and ketanserin on the maximum response to 5-HT were completely or partially prevented by ICI 169,369.

Table 1. Data are x ± SEM.

	n	% of 1st maximum		log CR[a]
Second CEC	4	101±7		-0.03±0.02
ICI 169,369 100 nM[b]	4	96±5		2.02±0.06
Ketanserin 3nM	6	68±5)	P<0.03	0.93±0.03
ICI 169,369 100nM + ketanserin 3nM	7	86±5)		2.07±0.09
Methysergide 3nM	5	16±2)	P<0.001	c
ICI 169,369 100nM + methysergide 3nM	6	97±5)		2.19±0.05

a CR concentration-ratio
b The antagonists were incubated for 2h, except ICI 169,369 2½h when combined
c CR values not estimated because 5-HT CEC were biphasic (Lemoine & Kaumann, 1986)

We conclude that i) ICI169,369 is both a competitive antagonist and activator, ii) ICI 169,369 shifts the equilibrium of the 5-HT_2 receptor more towards the active R-state than ketanserin, iii) ICI 169,369 is a more effective activator against methysergide than against ketanserin because the affinity of ketanserin for the 5-HT_2 receptor system may be higher than the affinity of methysergide.

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EVIDENCE FOR $GABA_B$ RECEPTORS ON NON-ADRENERGIC NON-CHOLINERGIC NERVES IN GUINEA-PIG AIRWAYS

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Recent evidence suggests that GABA and its receptors are present in peripheral nerves (Giotti et al, 1983). In guinea-pig trachea GABA decreases the contractile properties of airway smooth muscle to cholinergic nerve stimulation (Tamaoki et al, 1987). Non-adrenergic non-cholinergic (NANC) bronchoconstriction evoked by vagal stimulation *in vivo* may be due to the release of neuropeptides such as substance P (SP) and neurokinin A from sensory nerves (Lundberg et al, 1983). We have now studied the effect of GABA on the NANC bronchoconstrictor response evoked by vagal stimulation to evaluate whether GABA or the specific agonists -4,5,6,7-tetrahydro-isoxazolo[5,4-c]pyridin-3-ol (THIP) ($GABA_A$-selective) and baclofen ($GABA_B$) have an inhibitory action on sensory neurones.

Male Dunkin-Hartley outbred guinea-pigs were anaesthetised with urethane (8ml kg^{-1} of a 25% solution i.p.). The left jugular vein was cannulated for the injection of drugs and the left carotid artery for monitoring blood pressure. The animal was ventilated (60 strokes min^{-1} of 1ml laboratory air) via a tracheal cannula and airway pressure recorded. Both cervical vagus nerves were dissected free and sectioned. The peripheral ends of the cut nerves were placed on platinum electrodes and electrically stimulated using pulses of 5ms and 5V for 30s. The frequency used was either 3Hz or 5Hz depending on the size of the NANC response obtained. Bilateral vagal stimulation 30min after atropine and propranolol (1mg kg^{-1}) gave a NANC bronchoconstrictor response with a mean tracheal pressure of 21.9 $\pm$1.04 cm H_2O (n=70) which was reproducible in any given animal. GABA (10ug kg^{-1} - 10mg kg^{-1}) did not alter basal tracheal pressure but reduced the NANC response in a dose-dependent manner (ED_{50}=158ug kg^{-1} with a maximum inhibition of 74 $\pm$ 3.4% at 10mg kg^{-1}). GABA-induced inhibition was not blocked by the $GABA_A$ antagonist bicuculline (2mg kg^{-1}) and the $GABA_A$ agonist THIP only inhibited the NANC response at a high dose. The $GABA_B$ agonist ($\pm$)baclofen (10ug kg^{-1} - 3mg kg^{-1}) gave a dose-dependent inhibition of the NANC response (ED_{50}=100ug kg^{-1} with a maximum inhibition of 35.5 $\pm$ 2.8% at 3mg kg^{-1}). Responses to exogenous SP (5ug kg^{-1} or 25ug kg^{-1}) were not inhibited by exogenous GABA (500ug kg^{-1}) or baclofen (2mg kg^{-1}).

We conclude that GABA may inhibit the release of transmitter from NANC nerves via $GABA_B$ receptors and that GABA may play a role in regulation of neurogenic responses within airways.

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MUSCARINIC RECEPTOR SUBTYPES IN GUINEA-PIG AND HUMAN LUNG

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Subtypes of muscarinic receptors have now been recognised in many tissues (Eglen & Whiting,1986). The aim of the present study was to evaluate the effects of a range of selective muscarinic antagonists on binding of [^{3}H]quinuclidinyl benzilate (QNB) to guinea pig and human lung membranes to define the muscarinic subtypes present in lung.

Receptor binding studies to lung membranes from guinea pig and human (obtained at surgery) were performed using [^{3}H]QNB (1.0nM). Experiments were carried out in an assay volume of 250µl of Tris-HCl buffer (25mM Tris, 154mM NaCl; pH 7.4) at 25°C. Incubations were for 60 mim. Bound ligand was separated from free by vacuum filtration. Nonspecific binding was defined using 1µM atropine. Specific binding was displaced by various selective muscarinic antagonists and the inhibitory dissociation constants (K_i) are shown below:

Compound	Guinea pig lung		Human lung	
	K_i(M)	n_H	K_i(M)	n_H
Atropine	2.63×10^{-9}	0.97	8.86×10^{-10}	0.95
4-DAMP	1.84×10^{-8}	0.95	4.01×10^{-9}	0.92
HHSiD	8.90×10^{-7}	0.93	1.08×10^{-7}	0.91
AF-DX 116	5.55×10^{-8}	0.93	3.70×10^{-7}	0.96
Methoctramine	2.75×10^{-7}	0.93	1.04×10^{-6}	0.96
Pirenzepine	3.40×10^{-7}	0.66*	3.29×10^{-8}	0.54*

Values are from 3-5 experiments. SEM was always less than 15%.
*Represents a n_H significantly different ($p<0.05$) to unity.

Our results show that both 4-DAMP and hexahydrosiladifenidol (HHSiD) displace specific binding with a high affinity indicating M_3 (smooth muscle and gland type) receptors in human and guinea pig lung which would be consistent with the presence of M_3 receptors on airway smooth muscle. AF-DX 116 and methoctramine by contrast displace specific binding with a low affinity suggesting that there may be no significant population of M_2 (cardiac type) receptors in the lung of either species. The results with pirenzepine are most difficult to explain. The low Hill number (n_H) suggests the presence of both high affinity (M_1) and low affinity sites. A computerised curve fitting programme indicated that the proportion of M_1 receptors in guinea pig lung was 51% and in human lung 64%. Using [^{3}H]pirenzepine (PZ), we comfirmed the presence of M_1 receptors in human lung corresponding to the high affinity PZ sites determined in competition studies. The localization and function of M_1-receptors in lung is uncertain but autoradiographic studies are now necessary to determine their cellular localization.

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MUSCARINIC RECEPTOR STIMULATED TURNOVER OF POLYPHOSPHOINOSITIDES AND INOSITOL POLYPHOSPHATES IN BOVINE TRACHEAL SMOOTH MUSCLE

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Inositol 1,4,5-trisphosphate releases calcium from internal stores in permeabilized canine tracheal smooth muscle cells (Hashimoto et al. 1985) and may have an important role in the initiation of agonist-induced contractions in airway smooth muscle. We have examined the effect of muscarinic receptor stimulation on inositol polyphosphate production and the turnover of membrane phosphoinositides (PI, PIP, PIP_2) in bovine tracheal smooth muscle (BTSM).

Muscle slices (300 x 300 µm) were preincubated for 60 min. in oxygenated Krebs-Henseleit buffer prior to labelling with [^{3}H]myo-inositol (2.5 µCi (0.5 µm)/50 µl packed slices for 60 min). 50 µl of packed slices were stimulated with carbachol for 30 min. in a total volume of 300 µl. Incubations were terminated with 300 µl 1 M TCA, neutralized and inositol phosphates and deacylated phosphoinositides separated on Dowex AG 1 x 8 columns (200-400 mesh; formate form) (Downes et al. 1986).

In the presence of 5 mM Li^+ carbachol produced a 47 ± 7 (mean ± SEM) fold increase in total InsPs (EC_{50} 2 µM) which was atropine sensitive. Carbachol also stimulated the incorporation of [^{3}H]inositol into the individual phosphoinositide pools (PI 2.1, PIP 2.9, PIP_2 3.7 fold increases over basal; PI representing 88%, PIP 4%, PIP_2 8% total phosphoinositide pool). This effect was independent of Li^+ and maximal with 10^{-6} M carbachol and could be abolished with labelling periods > 6 hr. or by labelling in the presence of 10^{-6} M carbachol. This latter method, followed by repeated washes to remove carbachol, was used to achieve steady-state labelling.

With either labelling protocols, higher concentrations of carbachol (10^{-4} M) resulted in a fall in the labelling of the polyphosphoinositides (PI 94 ± 2%, PIP 51 ± 2%, PIP_2 44 ± 3%, of 10^{-6} M carbachol levels) with an even greater reduction evident in the presence of 5 mM Li^+ (PI 63 ± 4%, PIP 45 ± 4%, PIP_2 35 ± 3%). This data indicates that the conversion of PI to PIP may be rate limiting when phospholipase C is maximally stimulated and that re-cycling of inositol derived from PIP_2 hydrolysis is important in maintaining PI pool size.

Table 1 Inositol polyphosphate production following carbachol 10^{-4} M stimulation

	$InsP_1$	$InsP_2$	$InsP_3$	$InsP_4$	
5 sec.	1.2 ± 0.1	2.4 ± 0.4	1.8 ± 0.3	1.0 ± 0.05	Steady-state
1 min.	5.2 ± 0.5	7.0 ± 1.9	2.3 ± 0.5	1.0 ± 0.1	labelling. Fold
15 min.	10 ± 1.4	11 ± 2.7	5.8 ± 1.3	2.5 ± 0.2	stimulations
30 min.	16 ± 2.7	17 ± 4.0	7.1 ± 1.3	3.2 ± 0.4	(Mean ± SEM, n = 3)

HPLC analysis of the '$InsP_3$' fraction following 30 min. carbachol (10^{-4} M) stimulation identified 90% as Ins(1,3,4)P_3 and 10% as Ins(1,4,5)P_3. These rapid and marked increases in the inositol polyphosphates following muscarinic receptor stimulation support a second messenger role for some of these products in pharmacomechanical-coupling in airway smooth muscle.

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PERIPHERAL ADMINISTRATION OF CHOLECYSTOKININ AND FENFLURAMINE CHANGES THE ACTIVITY OF NEURONES IN HYPOTHALAMUS

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There is good evidence to suggest that the ventromedial nucleus of the hypothalamus (VMH) is involved in the control of food intake and satiety (Oomura, 1986). In vivo experiments have demonstrated that glucose and satiety agents cause changes in neuronal activity in the VMH (Ono et al, 1987). Recently the peripheral cholecystokinin (CCK) receptor antagonist L364718 was shown to increase food intake by attenuating the action of endogenous CCK (Hewson et al, 1988) and CCK8S has been shown to excite VMH neurones (Boden and Hill, 1988). In this study, agents known to suppress food intake were injected intravenously or intraperitonealy into rats to determine whether they would consistently change neuronal activity in the VMH. The effects of vagotomy on the actions of these agents was examined to determine whether they had a predominantly peripheral or central site of action. 300 to 500 gm male Wistar rats were anaesthetised with 0.5 gm/kg urethane supplemented with 0-1% Halothane in 40%O_2 60%N_2O. Animals were mounted in a stereotaxic frame to allow access to the hypothalamus through the roof of the mouth (Dreifuss & Ruff, 1972). Animals with bilateral vagotomies were artificially respired. The cut vagus nerve was electrically stimulated with a suction electrode attached to its cut central end. Extracellular recordings of neuronal activity from glass micropipettes were made by conventional techniques. The position of the recording electrode within the VMH was marked with Pontamine Sky Blue dye at the end of the experiments. Peripheral administration of 1 to 100ug/kg CCK8S changed the firing rate of the majority of neurones in the VMH. Following IV injection of CCK8S 10 neurones were inhibited, 2 excited and 1 was not affected. Following IP injection of CCK8S 10 neurones were inhibited, 1 excited and 2 were not affected. 100ug/kg of the CCK receptor antagonist L364718 completely reversed the effects of CCK8S on 5 of 10 neurones, on a further 3 neurones the CCK8S response was attenuated by L364718, which did not change neuronal firing when administered alone on 4 neurones. In animals with bilateral vagotomies CCK8S was found to have a weak excitatory action on 7 of 10 neurones with 1 neurone weakly inhibited. Neither CCK8S nor L364718 had effects on blood pressure at the doses used in this study. 0.1mg/kg fenfluramine inhibited 5 and excited 2 of 10 neurones. On all three neurones tested 0.1mg/kg ketanserin reversed the action of fenfluramine. CCK8S responses on 3 neurones were not affected by ketanserin. Electrical stimulation of the cut central end of the vagus nerve with a 5s pulse train of 0.5ms, 100Hz pulses inhibited 11 of 13 neurones in the VMH. These observations suggest that peripheral administration of CCK and fenfluramine modify feeding behaviour by different mechanisms. The predominantly inhibitory effect of CCK8S actions was probably mediated via a peripheral site of CCK action. This is in contrast to its excitatory action on VMH neurones in vitro.

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EFFECTS OF α_2-AGONISTS AND ANTAGONISTS ON PUPIL DIAMETER ARE CORRELATED WITH [^{3}H]-IDAZOXAN BINDING IN VIVO IN MOUSE BRAIN

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α2-adrenergic receptors have been characterised in mouse brain in vivo using 3H-idazoxan binding (Assié and Briley 1986). Clonidine-induced mydriasis in mice has recently been shown to be mediated by central α2-adrenoceptors (Heal et al., 1988). We compare here the inhibition of 3H-idazoxan binding in vivo by α2-adrenoceptor agonists and antagonists with their effects on pupil diameter in the mouse to determine to what extent central 3H-idazoxan binding in vivo may be correlated with central α2-adrenoceptor function.

Male Swiss mice (20-24 g) were used. Pupil diameter was measured immediately before and 10 min after drug injection (i.p.) as described by Heal et al. (1988). When antagonists and agonists were used together, they were given simultaneously. Binding in vivo in the forebrain was measured 1 min after i.v. injection of 4 µCi/mouse 3H-idazoxan. Drugs were injected (i.p.) 30 min before 3H-idazoxan. Yohimbine 10 mg/kg i.p. was used to determine non-specific binding.

Doses of α2-agonists which inhibit 3H-idazoxan binding were similar to those that increased pupil diameter (Table 1).

Table 1 : Effects of α2-agonists on 3H-idazoxan binding and on pupil diameter.

Compound	Mydriasis 35% (mg/kg i.p.)	3H-idazoxan binding ID_{50} (mg/kg i.p.)
Clonidine	0.1	0.57
Guanabenz	3	4.3
Azepexole	30	73

α2-antagonists when given alone at doses which inhibited 50-70 % of 3H-idazoxan binding in vivo induced no change in pupil diameter. However, with exception of piperoxan, they inhibited the action of clonidine (Table 2).

Table 2 : Effects of α2-antagonists on 3H-idazoxan binding and on mydriasis induced by clonidine (0.3 mg/kg i.p.).

Compound	(mg/kg i.p.)	n	% inhibition binding	n	% inhibition mydriasis
Idazoxan	0.1	5	45.8	10	63.2
Yohimbine	0.5	4	53.3	10	63.2
Rauwolscine	1	4	45.1	10	52.6
Piperoxan	5	4	55.0	20	10.5

In conclusion, the effects of agonists and antagonists on the α2-adrenoceptor mediated mydriasis occur at doses that inhibit 3H-idazoxan binding in vivo which thus appears to be well correlated with physiological central α2-adrenoceptor function.

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CLEAR EVIDENCE THAT CLONIDINE HYPOACTIVITY AND MYDRIASIS ARE MEDIATED BY PRE- AND POSTSYNAPTIC α_2-ADRENOCEPTORS RESPECTIVELY

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Clonidine induces hypoactivity (a distinct form of sedation) and mydriasis (pupil dilatation) in rodents by activating central α_2-adrenoceptors (Drew et al, 1979; Heal et al, 1988a). Most CNS α_2-adrenoceptors are postsynaptic (Dausse et al, 1982). However the clonidine-induced decrease of brain MHPG reflects presynaptic α_2-adrenoceptor function (Heal et al, 1988b) and MHPG measurements tentatively suggested clonidine caused hypoactivity and mydriasis by a presynaptic mechanism (Heal et al, 1988c). We have now examined this further by using intracerebroventricular (i.c.v.) injections of methamphetamine to enhance noradrenergic function and selective lesioning techniques to reduce it.

Male C57/Bl/6Ola mice (Olac, Bicester) weighing 20-30g were used. Drugs were dissolved in saline or water and administered i.p. (1 ml/100g) or i.c.v. in 2µl using a stereotaxic injector (Heal, 1984). After pretreatment with zimeldine (5 mg/kg) to protect 5-HT neurones, DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine)(100 mg/kg) was injected twice 7 days apart. Hypoactivity was rated 0-3 on 5 behavioural parameters modified from Drew et al (1979). Pupil diameter was measured in conscious mice as described by Heal et al (1988a). Results are given ± s.e. mean. Pupil diameter 0.37 ± 0.01 mm (n=42) was dose-dependently increased by methamphetamine (0.1-5 mg/kg i.p.) with a 68% ($P<0.001$) enlargement at the highest dose. Identical effects were produced by i.c.v. injection of methamphetamine (0.5-10µg). Mydriasis induced by methamphetamine (0.5 mg/kg i.p.) was abolished by 2.5µg injections i.c.v. of idazoxan and yohimbine, but not prazosin or pindolol indicating this response was mediated by central α_2-adrenoceptors. Mydriasis induced by clonidine (0.05 mg/kg i.p.) was dose-dependently potentiated by simultaneous i.c.v. injection of methamphetamine (1-10µg) and these effects were significantly ($P<0.05$) greater than either treatment alone. Conversely, hypoactivity induced by clonidine (0.1 mg/kg i.p.) was dose-dependently and significantly ($P<0.05$) inhibited by i.c.v. methamphetamine (1-10µg). DSP-4 treatment decreased brain noradrenaline concentrations by 77% ($P<0.001$). Mydriasis induced by methamphetamine (0.5 mg/kg i.p.) was abolished ($P<0.001$) by lesioning, but the response to clonidine (0.1 mg/kg i.p.) was unaltered. However, DSP-4 lesioning inhibited the hypoactivity to clonidine (0.1 mg/kg i.p.) by 49% ($P<0.01$). Hence, clonidine mydriasis was potentiated by methamphetamine and was unaltered by noradrenergic lesioning. By contrast, hypoactivity was inhibited by methamphetamine or DSP-4 treatment. These results strongly argue that clonidine induces hypoactivity and mydriasis by stimulating pre- and postsynaptic α_2-adrenoceptors, respectively. In addition, the similarity between the ED_{50} values for these 2 responses reported by Heal et al (1988c) indicates clonidine doses <0.1 mg/kg (i.p.) do not selectively stimulate presynaptic α_2-adrenoceptors.

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MOTOR BEHAVIOURS FOLLOWING INTRATHECAL 2,5-DIMETHOXY α,4-DIMETHYL-BENZENE ETHAMINE HCl (DOM); A 5-HT_2 AGONIST

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Both systemic and intrathecal administration of the non-selective 5-hydroxytryptamine (5HT) agonist 5 methoxy-N,N^1-dimethyltryptamine evokes a well-defined behavioural syndrome in rats including wet-dog shakes (WDS). Intrathecal injection also induces brief back muscle contractions (BMC) which are attenuated by ritanserin pretreatment, suggesting the involvement of spinal $5HT_2$ receptors in this behaviour (Fone et al., 1988). The present study examined the behavioural response following intrathecal injection of 2,5-dimethoxy-α,4-dimethylbenzene ethamine hydrochloride (DOM) a selective $5HT_2$ receptor agonist (Glennon, 1987) given alone and after pretreatment with either ritanserin or the serotonergic neurotoxin 5,7-dihydroxytryptamine (57DHT) to fully characterise the involvement of spinally located $5HT_2$ receptors.

Male Wistar rats (280-310g) were cannulated intrathecally as described previously (Fone et al., 1987). Following a seven day recovery period one group of rats (n=7) received saline or DOM (2, 10 and 25 μg) intrathecally at four day intervals and four days later DOM (10 μg) was injected again 30 min after ritanserin (1 mg/kg i.p.). Another group of rats (n=6) were given DOM (10 μg) intrathecally two days before and ten days after 57DHT (2 x 150 μg, intrathecally, 11 and 13 days after surgery), 1 h after desipramine 25 mg/kg i.p.) and ventral spinal cord 5HT levels were determined by HPLC with electrochemical detection. The number of BMC were continuously counted (mean± s.e.mean) and other components of the 5HT syndrome were scored (on a scale of 0-3 for 20s every 2 min) for 30 min from the injection of saline or DOM.

Intrathecal DOM injection produced WDS and BMC which both increased in a dose-related manner (from 4±3 WDS and 31±6 BMC in 30 min with 2 μg up to 27±5 and 130±14, respectively, with 25 μg DOM) ($P<0.05$, ANOVA). No other components of the 5HT behavioural syndrome were observed after intrathecal DOM. Ritanserin pretreatment significantly attenuated both the WDS produced by DOM (10 μg) (from 13±5 to 1±1 WDS in 30 min, $P<0.05$) and BMC (101±11 to 1±1 BMC, $P<0.001$, Student's t-test). Intrathecal 57DHT pretreatment which reduced thoraco-lumbar ventral spinal cord 5HT levels 95% below control levels (7.2 ng/mg protein) as previously reported (Fone et al., 1987), significantly enhanced the number of BMC produced by DOM (from 82±14 to 157±16 in 30 min, $P<0.01$, Student's t-test) without altering the number of WDS (9±4 compared with 10±5).

Both the WDS and BMC produced by intrathecal injection of the selective $5HT_2$ agonist DOM were virtually abolished by ritanserin suggesting that these behaviours were mediated by $5HT_2$ receptor activation. However, since BMC, but not WDS, were potentiated by intrathecal 57DHT treatment the $5HT_2$ receptors mediating BMC may be post-synaptic to bulbospinal serotonergic neurones, whereas spinal serotonergic neurones do not appear to be involved in 5HT agonist-induced WDS behaviour.

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ARE THE HYPER- AND HYPOPHAGIC RESPONSES TO 8-OH-DPAT MEDIATED BY THE SAME NEURONAL MECHANISM?

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The administration of 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) to freely-feeding, satiated rats increases food intake (hyperphagia) (Dourish et al., 1986) but given to food-deprived rats or to rats fed only at specific times of the day a marked hypophagia is produced (Bendotti & Samanin, 1987). These experiments investigate the mechanism of action of 8-OHDPAT in these two experimental conditions.

Male Wistar rats (300-350 g) were given access to water and maintained on one of three feeding regimens: "freely feeding" (n=9) allowed free access to food; "fixed feeding" (n=6) only given food between 0900 and 1300 h; and "freely feeding on reversed light" (n=6), given free access to food but maintained on a reversed 12 hour light/dark cycle. The fixed feeding rats were trained for 14 days on their feeding regimen and the reversed light rats allowed 21 days to acclimatise. Food intake was measured in all groups between 0900 and 1300 h, 10 mins after the administration of 8-OHDPAT (0.32 mg/kg s.c.) or saline. In separate groups of rats, the behavioural syndrome (reciprocal forepaw treading, flat body posture and lateral head weaving) produced by 8-OHDPAT was scored every 10 min for 1 hr following either saline or 8-OHDPAT in fixed (n=6) and freely feeding rats (n=6) on a scale of 0-3, where 0=absent and 3=continuous. In a final group of rats 8-OHDPAT (500 ng) or saline was given through bilateral guide cannulae implanted two weeks earlier into the lateral ventricles of fixed feeding rats 10 min before food was presented at 9.00 h.

8-OHDPAT (0.32 mg/kg s.c.) produced a marked hyperphagia in freely feeding rats (food intake: saline treated rats=0.3±0.2 g/4 h; rats treated with 8-OHDPAT=2.4±0.3 g/4 h). In fixed feeding rats a significant hypophagia was produced (food intake: saline treated rats=16±1.5 g/4 h; rats treated with 8-OHDPAT=12.25±0.4 g/4 h). However there was no significant difference between the intensity of the behavioural syndrome observed in fixed or freely feeding rats. In freely feeding rats on a reversed 12 h light/dark cycle, 8-OHDPAT produced a hypophagic response. Bilateral i.c.v. injections of 8-OHDPAT (500 ng) had no effect on food intake in fixed feeding rats although Hutson et al. (1986) have shown that injections of 8-OHDPAT into the dorsal or medial raphe of freely feeding rats gave rise to hyperphagia.

These data suggest that food intake in rats can be affected by at least two 5HT systems, one existing in the periphery (hypophagia) and one existing in the brain (hyperphagia). The hypophagia seen in freely feeding rats given 8-OHDPAT in the dark phase of their light-dark cycle may be explained by the fact that rats normally eat in the dark phase and consequently the receptors involved in increasing food intake are fully stimulated so 8-OHDPAT can only act through the peripheral hypophagic 5HT system. The hypophagia seen in fixed feeding rats may have a similar explanation as the rats have been trained to accept their daily intake of food.

AJS is an SERC CASE student (Merrell-Dow). CAM is a Wellcome Senior Lecturer.

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INVOLVEMENT OF 5-HT_{1A} RECEPTORS IN THE ANTIDEPRESSANT-LIKE EFFECT OF 8-OH-DPAT IN A PUTATIVE MODEL OF DEPRESSION IN MICE

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The 5HT-1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT) has been reported to have an antidepressant-like effect in two rat models of depression (Cervo and Samanin, 1987; Kennett et al, 1987). We now report that in a model in mice capable of detecting potential antidepressants (Porsolt, 1981), 8-OHDPAT also exhibits antidepressant-like activity by a mechanism involving 5HT-1A receptors.

The mobility of male CD1 mice (20-26g; Charles River) in the test was measured by a Doppler recording system (Buckett et al, 1982) during a 4 min period 1h after drug administration. 8-OHDPAT or saline was injected subcutaneously immediately after oral administration of antagonists or vehicle.

After subcutaneous, but not oral, administration 8-OHDPAT (0.3-10 mg/kg) induced a dose-related increase in mobility and 3 mg/kg (s.c.) was selected as suitable for 8-OHDPAT-antagonism studies. Spiroxatrine (0.1-30 mg/kg p.o.), a putative 5HT-1A receptor antagonist (Nelson and Taylor, 1986), caused a dose-dependent inhibition of the 8-OHDPAT (3 mg/kg s.c.) response (Table 1). The 8-OHDPAT effect was also inhibited by (±)-pindolol but only at a high dose (30 mg/kg p.o.) normally indicative of 5HT-1A antagonist activity. In contrast, the 5HT-2 receptor antagonist ketanserin (0.1-3 mg/kg p.o.) and the α_2-adrenoceptor antagonist idazoxan (3-30 mg/kg p.o.) had no effect on the 8-OHDPAT (3 mg/kg s.c.)-induced increase in mobility (Table 1).

Table 1 Effect of oral administration of monoamine receptor antagonists on the 8-OHDPAT (3 mg/kg s.c.)-induced increase in mobility (Porsolt test)

Antagonist	Control‡ mobility	Antag. dose	Mobility	Antag. dose	Mobility	Antag. dose	Mobility	Antag. dose	Mobility
Spiroxatrine	315±24	0	554±33	10	319±23**	1	360±60*	0.1	465±36
(±)-Pindolol	275±32	0	507±33	30	330±33**	10	488±26	3	481±30
Ketanserin	244±37	0	526±39	3	454±62	1	466±46	0.3	479±35
Idazoxan	257±20	0	465±30	30	464±33	10	487±31	3	405±37

‡Control mice were administered vehicle only (p.o. and s.c.).
Mobility is expressed in arbitrary units as mean mobility ± s.e. mean.
**P<0.001, *P<0.01 vs 8-OHDPAT alone (antagonist dose = 0) (Student's t-test, 2-tailed). n = at least 10 mice/treatment.

The 8-OHDPAT-induced increase in mobility in this test confirms studies in rats showing that 8-OHDPAT exhibits antidepressant-like activity. In addition, our data demonstrate that this effect involves the 5HT-1A receptor agonist properties of 8-OHDPAT since it can be inhibited by putative 5HT-1A receptor antagonists but not by 5HT-2 or α_2-adrenoceptor antagonists.

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PREVENTION BY PURINES OF THE STRUCTURAL CHANGES CAUSED AT THE EXTRANEURONAL LEVEL BY SURGICAL AND CHEMICAL DENERVATION

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Surgical denervation of the lateral saphenous vein of the dog causes marked extraneuronal changes, both at the morphological and functional level. Constant i.v. infusions of noradrenaline did not prevent the changes induced by denervation (Branco et al.,1984). Adenosine, continuously infused under the same conditions,totally prevented the morphological consequences of denervation on the effector cells (Albino Teixeira et al.,1987). In an attempt to further investigate the factor(s) and mechanism(s) responsible for the trophic effects exerted by the sympathetic innervation on the dog saphenous vein, we decided to compare the effects of adenosine, inosine and N-ethylcarboxamidoadenosine (NECA) on denervation.

Dogs were anaesthetized (sodium pentobarbitone 30 $mg.kg^{-1}$ i.v.) and the right lateral saphenous vein exposed,dissected from the surrounding subcutaneous tissue and denervated by applying clamps for 5 min. An Alzet 2ML1 osmotic minipump was subcutaneously implanted and connected to the right saphenous vein. The pump delivered adenosine (10 $\mu g.kg^{-1}.h^{-1}$), inosine (10 $\mu g.kg^{-1}.h^{-1}$) or NECA (0.1 $\mu g.kg^{-1}.h^{-1}$).

In other series chemical denervation was induced by 6-hydroxydopamine (6-OHDA) (10 mg/kg on day 0 + 10 mg/kg on day 1 i.v.). Some of these dogs were also treated with adenosine (10$\mu g.kg^{-1}.h^{-1}$ administered through an Alzet minipump as described,from day 0 on).Five days after surgery or the first 6-OHDA injection dogs were re-operated, under pentobarbitone anaesthesia,segments of both infused and contralateral veins removed and used for determination of noradrenaline (NA) (by HPLC-ED) and for morphological study (light and ultrastructural microscopy).

6-OHDA results in morphological alterations similar to those described for surgical denervation: at the ultrastructural level both smooth muscle cells and fibroblasts exhibit exuberant nucleii,rich in euchromatin,nucleoli and indentations of the nuclear membrane,as well as a voluminous rough endoplasmic reticulum, all signs of increased synthetic activity. Light microscope morphometry showed that smooth muscle cell dimensions and connective tissue volume were significantly increased in denervated veins. Adenosine prevented the morphological changes induced by chemical denervation on smooth muscle cells, similarly to what happened in surgical denervation experiments (Albino Teixeira et al.,1987). Like adenosine,NECA infusions prevented the structural consequences of denervation in operated dogs. In contrast, inosine did not hinder the changes caused by surgical denervation.

The results are compatible with an involvement of purines in the trophic effects of sympathetic innervation. Moreover, the effects of adenosine do not appear to be mediated by inosine.

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PROBUCOL REDUCES THE EXTENT OF AORTIC ATHEROSCLEROSIS IN CHOLESTEROL-FED RABBITS

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Probucol reduces atherosclerosis in animals with elevated plasma concentrations of low density lipoproteins (Kita et al, 1987; Carew et al, 1987). To determine whether probucol conveyed similar protection during elevated plasma concentrations of the atherogenic lipoprotein, cholesterol ester-rich very low density lipoproteins (CER-VLDL), diet-induced hypercholesterolaemia was induced in rabbits (Daugherty et al, 1985). Five rabbits were fed cholesterol-enriched (2% v/v) supplemented with the drug for 60 days to yield plasma concentrations in the range of 50 - 100 μg/ml. A control group of five rabbits were fed a cholesterol-enriched diet alone.

Dietary consumption and body weight gains were comparable in the two groups. Plasma concentrations in control compared to probucol-treated animals of total cholesterol (45.1 vs 40.7 mmol/l), unesterified cholesterol (16.3 vs 14.9 mmol/l), triglycerides (1.6 vs 3.2 mmol/l), and phospholipids (8.7 vs 7.9 mmol/l) were not significantly altered by the administration of drug. Also, the mass of CER-VLDL in plasma and its physicochemical characteristics were also not changed by the drug. CER-VLDL from probucol-treated animals was only slightly less potent than the comparable fraction obtained from controls in augmenting the incorporation of [^{3}H]-oleate into cholesteryl-[^{3}H]-oleate in macrophages in culture; a criterion that is commonly considered to indicate the extent of atherogenicity of a specific lipoprotein fraction.

Despite the lack of effect of probucol on concentrations of plasma lipids, administration of the drug markedly decreased the extent of aortic atherosclerosis from 40.1% to 10.9% of surface area in the thoracic section, and from 25.8% to 4.5% in the abdominal section. Probucol treatment reduced the deposition of esterified and unesterified cholesterol by 51.2% and 28.2% respectively.

Accumulation and catabolism of CER-VLDL in tissues was determined using the residualizing label for protein, radioiodinated dilactitol tyramine (DLT; Daugherty et al, 1985). CER-VLDL from controls and probucol-treated animals was radiolabeled with ^{131}I and ^{125}I, respectively, and radiolabels were injected simultaneously into both groups. Metabolism of *I-DLT-CER-VLDL was much reduced in aortic tissue of probucol-treated animals compared to controls, but the drug did not influence metabolism in liver or adrenals. CER-VLDL was metabolized similarly in control and probucol-treated animals irrespective of the source of the lipoprotein. Thus, probucol reduced atherosclerosis in cholesterol-fed rabbits, possibly by reducing the metabolism of CER-VLDL in the arterial vessel wall.

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THE RELATIONSHIP OF PLASMA CHOLESTEROL LEVELS TO VASCULAR NORADRENALINE CONTENT IN NWZ RABBITS

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Relevance between membrane lipids and transport of noradrenaline (NA) across sympathetic nerve endings had been suggested by Paton[1]. Meanwhile, only recently, some authors[2,3,4] accepted hyperlipidaemic diets influence on neuronal storage and release of NA. The purpose of this work was to assess the effects of diets supplemented with cholesterol or cholesterol + L 44-0 (a new nicotinic acid derivative) on the content of NA in several vascular segments of rabbit.

47 male New Zealand White Rabbits were included in the study. 5 animals were sacrificed at the beginning (control group). The others were divided in 2 main groups: One of these was fed with normal diet plus cholesterol (0.1%) - diet A; The other was fed with normal diet plus cholesterol (0.1%) plus L 44-0 (0.1%) - diet B. At the beginning and after 4, 8 and 12 weeks, blood samples were obtained from the cannulating medium ear vein for the determination of plasma cholesterol and triglycerides levels. At the outset of experiment (control group) and after 4, 8 and 12 weeks, either from diet A group or from diet B group, 6 rabbits were sacrificed and femoral, renal, carotid and mesenteric arteries and femoral vein were cut off. All the vascular tissue samples were rapidly washed, blotted, weighed and stored inside tubes containing 1 ml of 0.1N perchloric acid at 4° C for further determination of catecholamines by HPLC-ECD.

In the series of rabbits fed with diet A the total, free and sterified plasma cholesterol levels increased about three times. In the series of rabbits fed with diet B the lipidic profile was already different: although at 4 weeks there was an evident increase of the total, free and sterified cholesterol, afterwards this increase was very small or null. The content of NA in the rabbit vascular segments were very different according to diet A or B and to blood vessel: In the group fed with diet A there was a great increase of NA in the femoral artery and vein and in the renal artery; In the group fed with diet B and in the same tissues there was a diminished or a null accumulation of NA (Table I). When in a group fed with diet A during 8 weeks this diet was changed for a normal diet (without cholesterol), 4 weeks later NA contents returned to control values.

Table I Content of NA in the rabbit vascular segments
(Values represent mean ± SEM, µg/g of tissue)

	Femoral a.	Femoral v.	Renal a.	Carotid a.	Mesent. a.
Before (control)	0.36±0.15	0.03±0.02	1.49±0.38	2.89±0.98	4.69±0.52
After 4 W. Diet A	1.42±0.32	1.06±0.36	2.34±0.43	2.85±0.52	5.88±1.16
8 W.	0.76±0.07	0.55±0.38	2.44±0.24	2.73±0.30	4.60±1.10
12 W.	0.61±0.09	0.53±0.17	2.36±0.14	2.82±0.22	4.39±1.16
After 4 W. Diet B	0.69±0.24	0.39±0.29	2.26±0.15	3.00±0.40	3.50±0.75
8 W.	0.32±0.08	0.16±0.08	1.91±0.10	2.59±0.12	3.34±0.86
12 W.	0.48±0.06	0.40±0.12	2.05±0.10	2.57±0.09	3.04±0.21

So, the content of noradrenaline in some vascular segments of the NWZ rabbits can be affected by plasma cholesterol levels. This fact could be due to alterations in the composition of membrane phospholipids with a consequent disturb of neuronal function of storage vesicules and neuronal terminals[2,3,4] and/or by an alteration in the prejunctional α_2 adrenoceptor number or sensitivity to NA activation[4].

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CHARACTERISATION OF THE ENDOTHELIAL-DEPENDENT RELAXATION OF NEONATAL PORCINE VENA CAVA EVOKED BY 5-HYDROXYTRYPTAMINE

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We have previously described an endothelial-independent relaxant response to 5-HT in isolated ring preparations of neonatal porcine vena cava (Sumner et al, 1987). We now describe some characteristics of an additional relaxant component in this preparation which is endothelium-dependent.

Endothelium-intact porcine vena cava rings were mounted in organ baths for isometric tension recording, and contracted to a constant tone with a concentration of spasmogen (see below) giving 50-75% of its own maximum response (~1g tension). Cumulative concentration-effect curves to test agonists were then constructed until either a maximum effect was produced or a concentration of 10μM was reached. Where the effects of antagonists were examined, these were added to the bath 30 minutes before the agonist. Experiments were controlled so as to monitor changes in tissue sensitivity to agonists and for effects of drug vehicle where appropriate. Agonist EC_{50} values (concentration required to produce 50% of the maximum relaxation) were determined for comparison with 5-HT and pEC_{50} values (negative $\log_{10}$ of the EC_{50} values) calculated. Results are expressed as the mean ± s.e.m. of at least three experiments.

In preparations contracted with α-methyl-5-HT, only endothelial-independent relaxation was seen to 5-HT (pEC_{50} 6.7 ± 0.3). 5-carboxamidotryptamine (5-CT) was a potent relaxant agonist under these conditions (pEC_{50} 8.3±0.1) and was antagonised by spiperone (pA_2 7.5±0.3) (Sumner et al, 1987). In marked contrast, when preparations were contracted with histamine or U46619, 5-HT also elicited an endothelial-dependent relaxation (pEC_{50} 8.4 ± 0.1). This response was mimicked by acetylcholine (pEC_{50} 8.2±0.1), α-methyl-5-HT (pEC_{50} 8.8 ± 0.2), 2-methyl-5-HT (pEC_{50} 7.2±0.1), 5-methoxy-tryptamine (pEC_{50} 7.9 ± 0.2), tryptamine (pEC_{50} 8.0±0.2) and RU24969 (pEC_{50} 7.4±0.3), but not by 5-CT, GR43175 or 8-hydroxy-DPAT at concentrations up to 10μM. In preparations contracted with U46619, relaxant responses to 5-HT and acetylcholine were abolished by endothelial removal (balloon catheter) or by methylene blue (10μM). The response to 5-HT (but not to acetylcholine) was also abolished by methiothepin (1μM), but not by spiperone, ketanserin, cyanopindolol or indomethacin (3μM).

The results suggest that endothelial cells in the porcine vena cava possess a novel 5-HT receptor, activation of which leads to EDRF release and subsequent vasorelaxation. This receptor appears to share some of the characteristics of the 5-HT receptor in the rabbit jugular vein (Martin et al, 1987), and is similar to that mediating contractile responses to 5-HT in the rat fundus (Cohen & Fludzinski, 1987).

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ERGOTAMINE-INDUCED REDUCTION IN ARTERIOVENOUS SHUNTING IS NOT MEDIATED BY 5-HT_1-LIKE OR 5-HT_2 RECEPTORS

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Ergotamine, a potent antimigraine drug, reduces carotid blood flow (CBF) due to a marked decrease in arteriovenous anastomotic blood flow (AVAF); only a small decrease in nutrient (arteriolar) blood flow (NF) is noticed. Phentolamine and/or pizotifen do not modify the effects of ergotamine thus excluding the involvement of α-adrenoceptors and 5-HT_2 receptors (Saxena et al., 1983). Since 5-HT_1-like receptors mediate AVAF reduction (Saxena et al., 1986), we investigated the involvement of such receptors in the effects of ergotamine.

Using the microsphere technique the partition of CBF into AVAF and NF was measured in 18 chloralose-anaesthetised cats (2.1-4.4 kg) and in 12 young pigs (19-27 kg) during pentobarbital anaesthesia. Increasing doses of ergotamine were given i.v. with intervals of 15 min. In the cat ergotamine decreased CBF, AVAF and NF (Table 1), whereas in the pig only CBF and AVAF were reduced (Table 2). Neither ketanserin (0.5 mg/kg) nor methiothepin (1 mg/kg) antagonized the effects of ergotamine on CBF and AVAF, but both drugs seem to reduce the effects on NF in the cat. Treatment with methiothepin (1 mg/kg) in the pig mildly reduced the ergotamine-induced changes in CBF and AVAF.

TABLE 1. Effect of Ergotamine (E) on CBF and Its Distribution in the Cat.

	Saline (S; n=6)			Ketanserin (K; n=6)			Methiothepin (M; n=6)		
	CBF	AVAF	NF	CBF	AVAF	NF	CBF	AVAF	NF
Baseline	17±1	8±1	10±1	21±3	10±2	11±2	22±3	10±3	10±1
S, K or M	17±1	8±1	9±1	14±2⁺	7±2⁺	7±1⁺	16±3⁺	7±2	8±1⁺
E 3 µg/kg	11±1*	5±1*	7±1*	11±2	4±1	7±1	14±2	5±2	8±1
E 10 µg/kg	8±1*	2±1*	6±1*	8±1*	2±1*	7±1	11±2*	3±1*	8±1
E 30 µg/kg	6±1*	1±1*	5±1*	6±1*	1±1*	6±1	9±2*	1±1*	7±1

Data: ml/min (Mean±sem); ⁺, $p<0.05$ vs baseline; *, $p<0.05$ vs S, K or M treatment

TABLE 2. Effect of Ergotamine (E) on CBF and Its Distribution in the Pig.

	No treatment (n=6)			Methiothepin (n=6)		
	CBF	AVAF	NF	CBF	AVAF	NF
Baseline	164±6	123±8	40±5	118±8	86±11	32±6
E 2.5 µg/kg	132±7⁺	80±11⁺	52±7	111±9	79±13	32±7
E 5.0 µg/kg	115±10⁺	63±12⁺	52±7	102±8⁺	69±11⁺	32±6
E 10.0 µg/kg	104±14⁺	48±10⁺	57±14	80±12⁺	48±11⁺	32±4
E 20.0 µg/kg	85±18⁺	30±10⁺	56±16	67±12⁺	34±11⁺	33±5

Data: ml/min (Mean±sem); ⁺, $p<0.05$ vs baseline

We conclude that the reduction of AVAF by ergotamine in both the cat and pig is not mediated by 5-HT_1-like or 5-HT_2 receptors. The reduction of NF in the cat may probably be mediated by 5-HT_2 receptors.

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ASPECTS OF THE PHYSIOPATHOLOGY OF MIGRAINE: [^{3}H]-PAROXETINE BINDING TO PLATELET MEMBRANES

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Most of the clinical manifestations of migraine could be produced by an abnormal reactivity of the cranial vessels (Skinhoj, 1973). Other explanations have also been advanced. Pearce (1984) considered that evidence favoured a primary neural mechanism and Hanington (1982) stated that migraine is a blood disorder due primarily to a platelet function alteration. This work aimed to test the hypothesis of an abnormality in the serotonin carrier of platelets.

The studied patients were females, aged between eighteen and forty years, with diagnosis of common migraine. The control subjects were people of our department without any known disease. All the studied subjects were out of medication for at least fourteen days. Platelets membranes were isolated by the method repported by Mellerup et al. (1983). The membranes isolated from the controls or patients have been used to draw a saturation curve with ^{3}H-paroxetine in order to calculate the maximal number of binding sites (B_{max}) and the affinity (K_D) of this ligand for the serotonin carrier. Specific binding was defined by 30 µM imipramine. We have also drawn competition curves between ^{3}H-paroxetine (0.3 nM) and flunarizine, amitriptyline, methysergide and propranolol. The platelet content in serotonin was determined by HPLC as described by Le Quan-Bui et al. (1983). On the other hand, migraine patients were prophylactically treated by propranolol (120 mg i.d.;n=19), flunarizine (10 mg i.d.; n = 11), amitriptyline (75 mg i.d.; n = 10) and pizotifen (1.5 mg i.d.; n = 9) and evaluated monthly for frequency, duration and severity of the migraine attacks as well as the consumption of analgesic medications.

Between control (n = 12) and migraine subjects (n = 22) there was not significant difference for the B_{max} (322.2 $\pm$ 32.1 and 289.3 $\pm$ 41.1 fmol/mg protein, respectively) or the K_D (0.43 $\pm$ 0.19 and 0.32 $\pm$ 0.09 nM, respectively) of specific ^{3}H--paroxetine binding. The platelet levels of serotonin did not significantly difer between the two groups (297.2 $\pm$ 51.5 and 325.8 $\pm$ 38.7 ng/ml) which suggests that there was no differences in the basal release or uptake of serotonin. Against ^{3}H-paroxetine binding amitriptyline was more potent (IC_{50} = 1.5 nM) than flunarizine (IC_{50} = 1.6 µM), propranolol (IC_{50} = 3.2 µM) or methysergide (20% of inhibition at 32 µM), whereas clinically the rank order of potency was flunarizine > > propranolol = pizotifen > amitriptyline.

These results lead to the conclusion that in the migraine patients there is not any abnormality in the platelet serotonin carrier which could explain the physiopathology of this disease or the efficacy of the preventive drugs.

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TOLERANCE TO THE CARDIOVASCULAR EFFECTS OF SK&F 101468-A, A DOPAMINE DA_2-RECEPTOR AGONIST, IN SPONTANEOUSLY HYPERTENSIVE RATS

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SK&F 101468-A is a potent, selective agonist for dopamine DA_2-receptors (Gallagher et al 1985). SK&F 101468-A, 0.5 $mg.kg^{-1}$, administered intravenously to anaesthetised spontaneously hypertensive rats (SHR; SK&F-Smirk strain), or orally in solution (20 or 40 $mg.kg^{-1}$) to conscious SHR produced a substantial reduction in mean blood pressure (Eden et al 1988). The present study was designed to investigate the effects of chronic administration of SK&F 101468-A. Rats were dosed with SK&F 101468-A, 10, 20 or 40 $mg.kg^{-1}$ p.o., b.i.d. for up to 14 days. The animals were anaesthetised with dial-urethane 12 hours after the last dose, and blood pressure and heart rate measured from a femoral artery. A femoral vein was cannulated for the infusion of SK&F 101468-A. Blood pressure and heart rate were recorded before, during and for 60 minutes after the start of an infusion of SK&F 101468-A 0.5 or 5 $mg.kg^{-1}$ over 5 min. Some animals were challenged with bromocriptine, 1 $mg.kg^{-1}$, instead of SK&F 101468-A to investigate possible cross-tolerance to another DA_2 receptor agonist.

Table 1. Changes in mean blood pressure (mmHg) 15 minutes after the infusion of SK&F 101468-A 0.5 $mg.kg^{-1}$ to SHR pretreated with saline, or SK&F 101468-A p.o. Values represent mean ± S.E.M. expressed in mmHg

	Pretreatment								
	Vehicle				SK&F 101468-A 40 $mg.kg^{-1}$				Saline
No. of doses	1	2	4	14	1	2	4	14	only
n	6	5	5	12	6	6	6	10	6
Resting	197±6	185±17	195±5	188±10	175±9	180±2	184±4	196±6	173±6
SK&F101468	-68±8	-79±5	-69±5	-77±6	-40±8*	-29±3*	-21±5*	-21±5*	-17±6

*Significantly different from vehicle control ($p<.05$);

The hypotensive response to the infusion of SK&F 101468-A was reduced in those animals pretreated with SK&F 101468-A, 20 or 40 $mg.kg^{-1}$ for 1 or more days compared to saline dosed rats. Resting blood pressure was not significantly different between any of the saline or drug treated groups. Similar effects were recorded when the acute SK&F 101468-A i.v. challenge was either increased by 10 fold or replaced by bromocriptine (1 $mg.kg^{-1}$). No tolerance was seen in the rats pretreated with 10 $mg.kg^{-1}$ b.i.d. This study has demonstrated: a) tolerance to the peripheral effects of SK&F 101468-A which cannot be overcome by increasing the dose and b) cross-tolerance with bromocriptine, another DA_2 agonist.

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