Use-dependent effects of ganglion blocking drugs

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Studies on the kinetic properties of some ganglion blocking drugs have implicated two distinct mechanisms of action (Ascher, Large & Rang, 1979). Hexamethonium, decamethonium and tubocurarine appear to act by combining preferentially with the open state of an ion channel-receptor complex, and are characterized by sensitivity of the block to postsynaptic membrane potential. The actions of trimetaphan and mecamylamine, in contrast, are not voltage-dependent, and these drugs may well act as competitive antagonists.

In this study, the mode of action of both types of ganglion blocking drug on rat submandibular ganglion cells at 30°C, was further investigated. Synaptic currents (e.s.c.s), elicited by stimulation of the *chorda tympani*, were recorded by means of a two-microelectrode, voltage clamp technique (Ascher *et al.*, 1979). The aim was to test whether cumulative open-channel block occurs during a train of stimuli (10 pulses at 5 or 10 Hz). Such trains were repeated at 1 min intervals, and responses recorded at two different membrane potentials (−40 mV and −80 mV). Under control conditions the e.s.c. amplitude declined, reaching a plateau at around 50% reduction after the fifth response. Trimetaphan (5 μM) and mecamylamine (50 nM) reduced the e.s.c. amplitude by about 40% but had little effect on the rundown during repetitive stimulation. Hexamethonium (10 μM) and decamethonium (10 μM) had little effect at −40 mV but markedly increased the rundown at −80 mV, consistent with a cumulative channel-blocking action. A similar action was evident with tubocurarine (10 μM), but its effect at −40 mV was more pronounced. This may be due to some presynaptic effect, as proposed recently to explain a similar action at the neuromuscular junction (Magleby, Pallotta & Terrar, 1981) and is currently under further investigation.

Recovery from the additional block produced by repetitive stimulation in the presence of decamethonium or tubocurarine, was complete within 1–2 s after cessation of stimulation. However, with hexamethonium recovery from use-dependent block was much slower (time constant about 16 s). This recovery could be accelerated by stimulation during the recovery period with the cell depolarized to −40 mV. This suggests that hexamethonium molecules may be small enough to permit the channel to close, thus becoming trapped, their escape being facilitated by opening the channel with the cell depolarized. The presence of tubocurarine or decamethonium in the channel, in contrast, may prevent it from closing, so that these drugs dissociate more quickly, as at the neuromuscular junction (see, for example, Colquhoun, Dreyer & Sheridan, 1979).

These results confirm predictions of the two proposed mechanisms of action of ganglion blocking drugs and in addition suggest that hexamethonium may have a more prolonged blocking effect due to its ability to become trapped within the closed ion channel.

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Gallamine regulates muscarinic receptors in the heart and cerebral cortex

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Gallamine inhibits the chronotropic and negative inotropic actions of muscarinic agonists in the heart (Riker & Wescoe, 1951), yet it does not appear to be a general antagonist at muscarinic sites. This problem has been investigated by examining the effects of gallamine on the binding of the antagonist [^{3}H]-N-methylscopolamine ([^{3}H]-NMS) to muscarinic receptors in the rat heart and cerebral cortex.

Tissues were homogenized in 9 volumes of (mM), NaCl 100, $MgCl_2$ 10 and Hepes 20, pH 7.0 and the homogenates diluted further to give an appropriate concentration of receptor sites. The binding studies were carried out in the same buffer using procedures described previously (Hulme *et al.*, 1978).

Gallamine decreases the apparent affinity of [^{3}H]-NMS without affecting the binding capacity. However, the character of the gallamine effect differs from that shown by competitive inhibitors in that the inhibition is not linearly related to the concentration: a maximum effect is reached at about 10^{-3}–10^{-4} M. This maximum effect is such as to reduce the affinity of [^{3}H]-NMS fourteen-fold in the heart and ten-fold in the cortex.

This behaviour is consistent with the presence of negative heterotropic cooperativity in which gallamine and NMS can bind *simultaneously* to the receptor to form a ternary complex. It would be expected that such an interaction would involve alteration in the kinetics of association and dissociation of NMS with the receptor. In fact, both association and dissociation are markedly slowed but the greater effect is on association, so that NMS kinetics become extremely slow in the presence of gallamine.

The effects of gallamine are half maximal at a concentration of 1×10^{-6} M in the heart and 1×10^{-5} M in the cerebral cortex, and preliminary studies indicate comparable effects of gallamine on agonist binding. Thus a tissue specificity is present which is consistent with the known pharmacology. In contrast to the behaviour of gallamine, *competitive* muscarinic antagonists are in general more potent in the cerebral cortex than in the heart (Birdsall *et al.*, 1981).

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Functional evidence for super- and subsensitivity of acetylcholine autoreceptors after chronic drug treatment

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It was recently demonstrated that long-term treatment with paraoxon reduced muscarinic binding sites in the CNS (Smit, Ehlert, Yamamura, Roeske & Yamamura, 1980) whereas the opposite occurred when rats were chronically treated with scopolamine (Ben-Barak & Dudai, 1980; Majocha & Baldessarini, 1980), indicating that central cholinoceptors may adapt to prolonged stimulation by or deprivation of endogenous agonist. Cholinergic nerve terminals, in the CNS, possess presynaptic autoreceptors which control acetylcholine (ACh) release and seem to differ from postsynaptic muscarinic receptors (Szerb, 1980; Marchi, Paudice & Raiteri, 1981). It is conceivable that the above modifications in the number of muscarinic binding sites reflect adaptive changes both at pre- and postsynaptic receptor level. Our investigation was performed in order to establish whether long term blockade or stimulation of the presynaptic cholinoceptors would lead to development of super- or subsensitivity phenomena, as reflected by a functional increase or decrease of the responsiveness of autoreceptors towards the inhibitory effect of exogenous ACh.

Adult male Sprague-Dawley rats were treated daily, for 11 days, with scopolamine (10 mg/kg; s.c.) or paraoxon (0.15 mg/kg on day 1 and 2, followed by 0.07 mg/kg from day 3 to 11; s.c.). Control animals were injected with saline. The animals were sacrificed 48 h after the last injection. The effect of chronic treatment was compared with that of a single administration: animals were treated subcutaneously with scopolamine (10 mg/kg), paraoxon (0.15 mg/kg) or saline and killed 24 h later. Rat hippocampal synaptosomes were prelabelled with 0.1 μM [^{3}H]-choline for 4 min and aliquots of the suspension were superfused as previously described (Raiteri, Angelini & Levi, 1974). The inhibitory potency of exogenous ACh (5 μM) on the release of [^{3}H]-ACh elicited by 15 mM KCl differed depending on the drug treatment received by the animals: the percent inhibition of release in synaptosomes from rats chronically treated with paraoxon was significantly lower (21.1 ± 2.3; $P < 0.001$) than in controls (43.9 ± 2.0); the opposite was true in the case of nerve endings prepared from rats chronically treated with scopolamine (54.2 ± 0.5; $P < 0.05$). Exogenous ACh (5 μM) was equally effective in reducing [^{3}H]-ACh release in synaptosomes from rats pretreated with a single injection of paraoxon (0.15 mg/kg), scopolamine (10 mg/kg) or saline.

In conclusion, the results of the present investigation represent the first demonstration, obtained directly with experiments of release, that the negative feedback mechanism controlling ACh release can undergo adaptive changes after prolonged blockade or activation of ACh autoreceptors.

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5-Hydroxytryptamine-induced relaxation of porcine vena cava – a possible involvement of cyclic AMP

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Relaxation of vascular smooth muscle by some agents is thought to be mediated by cyclic AMP although this is controversial (see review by Diamond, 1978). Whilst 5-hydroxytryptamine (5-HT) is generally considered as a vasoconstrictor, a growing body of evidence suggests that it can also cause relaxation via interaction with specific 5-HT receptors (e.g. Eyre, 1975; Chand, 1981; Feniuk, Humphrey & Watts, 1981). In many instances, however, the receptor has not been fully characterized and the possible involvement of cyclic AMP has not been explored. We now wish to report on our analysis of the vasodilator action of 5-HT in the porcine vena cava.

Rings of abdominal vena cava were prepared for measurement of isometric contractions using a method similar to that of Edvinsson, Nielsen & Owman (1974) or measurement of cyclic AMP production in the presence of isobutylmethyl xanthine (1.0×10^{-4} mol/l) using a technique similar to that of Brown, Ekins & Albano (1972) which involved the use of an assay kit from the Radiochemical Centre, Amersham.

5-Hydroxytryptamine had no effect on the resting tension applied to the vessel (0.5 g) but relaxed preparations contracted with histamine, $PGF_{2\alpha}$ or α-methyl 5-HT. Relaxation was most pronounced with the latter spasmogen and 5-HT produced a half maximal relaxation (maximal relaxation was about 90% of total relaxable tone) at $7.0 \pm 1.1 \times 10^{-8}$ mol/l (mean ± s.e.mean, $n = 5$). The 5-HT mediated relaxation was not antagonized by propranolol (1×10^{-6} mol/l), indomethacin (3×10^{-5} mol/l) or atropine (1×10^{-6} mol/l). The effects of the 5-HT receptor blocking drugs, methysergide and cyproheptadine, could not be quantitated because the antagonists inhibited the α-methyl 5-HT induced tone.

5-Hydroxytryptamine (1.0×10^{-5} mol/l) raised cyclic AMP levels within 30 s and reached a peak within 1–2 min (approx 350% increase over basal values of 5.7 ± 0.2 pmol cyclic AMP/mg protein; mean ± s.e.mean, $n = 22$). This effect of 5-HT (1.0×10^{-7}–1.0×10^{-4} mol/l) was concentration-dependent and was antagonized by methysergide (1.0×10^{-6} mol/l) but not by cyproheptadine (1.0×10^{-7} mol/l). Methysergide produced a 12.5 fold shift in the 5-HT concentration-effect curve with an apparent mean pA_2 (95% confidence limits) of 7.19 (6.47–7.91), $n = 4$. In contrast α-methyl 5-HT (1.0×10^{-5} mol/l) had no effect on cyclic AMP levels.

The antagonism of the 5-HT mediated rise in cyclic AMP levels by methysergide yielded an apparent pA_2 value which was very similar to those obtained in the cat saphenous vein and guinea-pig ileum for 5-HT mediated relaxation (Feniuk, Humphrey & Watts, 1981). These findings suggest that in these preparations the receptors mediating elevation of cyclic AMP are similar to those mediating smooth muscle relaxation.

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Studies on the release of [^{3}H]-adrenaline in an isolated perfused adrenal gland-splanchnic nerve preparation from the rabbit

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Catecholamine secretion from the adrenal medulla in response to splanchnic nerve stimulation and to nicotinic cholinoreceptor agonists have been studied with the gland *in situ* (Bülbring, Burn & De Elio, 1948; Kirpekar & Cervoni, 1963). Various isolated perfused or sliced preparations have been used and secretion from these has been induced with high-potassium solutions and nicotinic agonists (Boonyaviroj & Gutman, 1979; Starke, Görlitz, Montel & Schümann, 1974). The present communication describes an isolated perfused preparation of the rabbit adrenal gland and splanchnic nerve. The preparation has been used to explore the possibility that stimulation-induced adrenal catecholamine secretion is subject to modulation through a mechanism involving α-adrenoceptors.

The rabbits were anaesthetized with urethane and the arterial supply to the left adrenal gland was cannulated *in situ*. The gland was removed, together with a distal portion of the splanchnic nerve, and was perfused at 0.5 ml/min with Krebs-Henseleit solution equilibrated with 5% CO_2 in O_2 at 37°C. Adrenal catecholamine stores were radiolabelled by perfusion with Krebs-Henseleit solution containing [^{3}H]-(±)-adrenaline (0.2 μM, 74 KBq/ml) for 20 min followed by 60 min of perfusion with adrenaline-free Krebs-Henseleit solution. The amounts of radioactivity released during resting periods and in response to 1 min periods of splanchnic nerve stimulation (5 Hz, 1 ms) or 2 min periods of perfusion with 1,1 dimethyl-4-phenyl-piperazinium (DMPP, 100 μM) or nicotine (100 μM) or acetylcholine (500 μM) were determined by measuring the amounts of radioactivity in 2 min fractions of the perfusate collected before, during and after stimulation or drug contact.

The radioactivity released during resting periods consisted of 48% (s.e.mean = 12, $n = 4$) [^{3}H]-adrenaline, the remainder being accounted for by non-catechol metabolites and oxidation products. The increased release of radioactivity in response to nerve stimulation consisted almost entirely of [^{3}H]-adrenaline and was abolished by tetrodotoxin (0.1 μM) and calcium-free Krebs-Henseleit solution and markedly reduced by hexamethonium (0.1 μM).

The release of radioactivity in response to nerve stimulation was unaltered in the presence of the α-adrenoreceptor agonists clonidine (1 μM), noradrenaline (0.1 μM) and oxymetazoline (10 μM) and the antagonists phentolamine (3 μM) and yohimbine (1 μM). These drugs in such concentrations alter transmitter outflow from noradrenergic nerves through a mechanism involving presynaptic receptors. The antagonist phenoxybenzamine (10 μM) enhanced release from the adrenal preparation.

DMPP-induced release of radioactivity was reduced by oxymetazoline (10 μM) and also by phentolamine (3 μM) and phenoxybenzamine (10 μM).

The findings do not support the possibility that catecholamine secretion from the rabbit adrenal gland in response to splanchnic nerve activity is subject to modulation through an α-adrenoreceptor mechanism. The mechanisms through which α-adrenoreceptor agonists and antagonists decrease DMPP-induced release have not been clarified.

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Binding of [^{3}H]-yohimbine to α-adrenoceptors on intact human platelets

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Stimulation of α-adrenoceptors on human platelets induces platelet aggregation (O'Brien, 1963) and inhibits adenylate cyclase (Salzman & Neri, 1969). These receptors have been characterized by radioligand binding techniques using [^{3}H]-dihydroergocryptine (Boullin & Elliott, 1979), [^{3}H]-clonidine (Shattil *et al.*, 1981) and [^{3}H]-adrenaline (Hoffman *et al.*, 1980). This communication describes the direct binding of [^{3}H]-yohimbine to intact human platelets and compares the results with previous binding studies.

Platelet rich plasma was prepared by centrifuging blood, anticoagulated with 1% EDTA, taken from healthy male volunteers (age 22–58). This was centrifuged at 1200 g for 7.5 min at 10°C to produce a platelet pellet which was gently resuspended in incubation buffer (0.1% EDTA, 150 mM NaCl, pH 7.5) to give a final cell density of approximately 0.8×10^8 platelet/ml. One ml aliquots of this suspension were incubated with [^{3}H]-yohimbine (1.4–14 nmol/l) for 20 min at 37°C. The incubations were terminated by centrifugation at 6500 g for 1 min in an Eppendorf 5412 Centrifuge producing platelet pellets which were washed and then sonicated in 500 μl of distilled water; 400 μl aliquots were counted for total radioactivity in a liquid scintillation spectrometer. Specific binding was calculated as the total radioactivity bound at a given free concentration minus the non-specific binding which occurred at the same free concentration in the presence of phentolamine (5 μM).

Specific binding of [^{3}H]-yohimbine is rapid ($T_{\frac{1}{2}}$ assoc = 1.93 min) and reversible ($T_{\frac{1}{2}}$ dissoc = 3.87 min). The rate constant for association (k_1) calculated from the second order rate curve is 0.055 nM min^{-1} and the rate constant for dissociation (k_2) calculated from the first order rate curve is 0.179 nM min^{-1}. Specific binding is saturable and Scatchard analysis shows a single receptor population with an affinity constant (Kd) of 3.37 ± 0.73 nM and a binding capacity of 39 ± 10 fmol/10^8 platelets (mean ± s.d., $n = 20$). The binding estimate of Kd agrees closely with the value derived from the kinetic rate constants ($Kd = {}^{k_2}/_{k_1} = 3.25$ nM). The rank order of potency for inhibition of specific [^{3}H]-yohimbine binding followed the expected pattern for an α_2-adrenoceptor (yohimbine > phentolamine > prazosin > methysergide > propranolol; clonidine > methoxamine) and showed stereospecificity ((−) adrenaline > (−) noradrenaline ≫ (+) adrenaline > (+) noradrenaline). Binding studies performed on a single subject examined four times in one week showed little variability (Kd = 3.4 nM ± 13%; capacity = 41 fmol/10^8 platelet ± 10%, mean ± coefficient of variation).

The number of [^{3}H]-yohimbine binding sites in intact platelets is substantially less than the number of [^{3}H]-dihydroergocryptine sites (72 fmol/10^8 platelets) previously identified in this laboratory (Boullin & Elliott, 1979). Paired studies on the same sample of blood show that in every case specific [^{3}H]-dihydroergocryptine binding is equal to or greater than specific [^{3}H]-yohimbine binding ($n = 8$). The reasons for this discrepancy are under investigation.

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Postsynaptic α-adrenoceptor subtypes mediating nerve-evoked contractions in rabbit blood vessels *in vitro*

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In a recent investigation, examining the effects of selective α-antagonists on concentration-response curves to selective α-agonists, we were unable to demonstrate postsynaptic α_2-adrenoceptors in rabbit aorta and portal vein *in vitro* (Docherty & Starke, 1981). However, since receptors close to the noradrenaline-secreting varicosities may contribute little to the overall response to an exogenous agonist, we have carried out further experiments to investigate the α-adrenoceptors which mediate nerve-evoked contractions in these tissues.

Tissues were obtained from rabbits of either sex (2–2.5 kg); the descending aorta was cut spirally into strips approximately 3 × 30 mm and the portal vein was cut longitudinally. Tissues were preincubated with [^{3}H]-noradrenaline, and were then suspended between parallel platinum electrodes in an organ bath under 4 g tension, and superfused with Krebs-Henseleit solution containing cocaine, corticosterone and propranolol (see Docherty & Starke, 1981). After 2 h equilibration, tissues were stimulated electrically (2 Hz for 3 min, supramaximal pulses, 0.3 ms) every 21 min. The effects of the antagonists rauwolscine (α_2-selective; Weitzell, Tanaka & Starke, 1979) and prazosin (α_1-selective; Cambridge, Davey & Massingham, 1977) were examined on the stimulation-evoked tritium overflow and contraction. In some experiments, tissues were exposed to the irreversible α-antagonist phenoxybenzamine (3×10^{-8} M) (α_1-selective; Constantine & Lebel, 1980) for 10 min before beginning superfusion.

In the aorta, rauwolscine (10^{-8}–10^{-6} M) increased the stimulation-evoked overflow of tritium and rauwolscine (10^{-7}–10^{-6} M) potentiated the stimulation-evoked contraction; prazosin (10^{-7} M) had no effect on the stimulation-evoked overflow of tritium, yet prazosin (10^{-9} M) reduced and (10^{-7} M) abolished the stimulation-evoked contraction. In the portal vein, rauwolscine (10^{-8}–10^{-6} M) potentiated the stimulation-evoked overflow but failed to increase the stimulation-evoked contraction; prazosin (10^{-7} M) had no effect on the stimulation-evoked overflow of tritium, prazosin (10^{-9}–10^{-6} M) reduced and only prazosin (10^{-5} M) almost abolished the stimulation-evoked contraction. These results suggest that, in the aorta, the postsynaptic receptors mediating stimulation-evoked contraction are α_1, since prazosin (10^{-7} M) abolished, and rauwolscine was able to potentiate contractions. However, in the portal vein, prazosin showed less potency, and rauwolscine failed to potentiate stimulation-evoked contractions.

Further experiments were carried out employing the portal vein, in which tissues were pre-exposed to phenoxybenzamine (3×10^{-8} M) to block irreversibly some of the α_1-adrenoceptors (contractile response to stimulation reduced to 38 ± 8% of that in control tissues). Under these conditions the presynaptic effects of antagonists were unaltered, but rauwolscine, in the concentration range which increased the stimulation-evoked overflow, decreased the evoked contraction, and became approximately equipotent with prazosin.

The results suggest that, in the portal vein but not the aorta, a proportion of the α-adrenoceptors mediating nerve-evoked contractions are of the α_2-type. Hence, although vascular α_2-receptors may be predominantly extrasynaptic in the dog hind limb (Langer, Massingham & Shepperson, 1980), this does not appear to be universally true.

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α_1- and α_2-adrenoceptor agonism is dependent on respiratory acid-base[2] balance

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The subclassification of post-junctional α-adrenoceptors has recently been reported using various 'selective' agonists and antagonists (Drew & Whiting, 1979; Docherty & McGrath, 1980).

We have now found further differences between the two receptor subtypes which may clarify their pharmacological nature and physiological role.

Male Wistar rats (250–275 g) were pithed and respired with O_2. Carotid arterial pressure was monitored. Drugs were injected via a jugular vein. Respiration rate was fixed at 60 strokes/min in all rats; in each rat stroke volume was set to give a constant pH and pCO_2 within the range 7.2–7.7 and 80–15 mmHg respectively. pO_2 remained greater than 300 mmHg. Carotid arterial blood samples were used for blood gas analysis. The diastolic pressor response to three α-adrenoceptor agonists, adrenaline (α_1 and α_2), phenylephrine ('selective' for α_1) and xylazine ('selective' for α_2) and their susceptibility to the 'selective' antagonists prazosin (α_1) and rauwolscine (α_2) were studied at different values of pH and pCO_2. Throughout this abstract respiratory-induced changes in acid-base balance will be referred to as changes in pH. Propranolol (1 mg/kg) was administered in each experiment.

The pressor response to adrenaline (1 μg/kg) was greater at high pH than at low pH but a more dramatic effect was seen in the effectiveness of antagonists. Adrenaline's response was more sensitive to prazosin (0.1 mg/kg) at high pH compared with low pH and was less sensitive to rauwolscine (0.2 mg/kg) at high pH compared with low pH. This suggested that adrenaline's α_1-agonism was relatively dominant at high pH and α_2-agonism at low pH.

Dose response curves to phenylephrine and xylazine were, therefore, constructed over the same pH range. Phenylephrine (α_1) was more potent at high pH than at low pH. Xylazine (α_2) was more potent at low pH than at high pH. This confirms that α_1- and α_2-agonists are more potent at high and low pH respectively.

As a corollary, it could be shown that the minor effects of the agonists changed with pH. (a) The response to high doses of xylazine were more susceptible to prazosin (1 mg/kg) at high than at low pH indicating α_1-agonism by xylazine at high pH. (b) The responses to high doses of phenylephrine were more susceptible to rauwolscine (1 mg/kg) at low pH than at high pH indicating an increase in phenylephrine's α_2-agonism (Flavahan & McGrath, 1981) at low pH.

In conclusion, high pH (hypocapnia) provides optimum conditions for α_1-agonism and low pH (hypercapnia) for α_2-agonism. These results cannot distinguish between the influence of hydrogen ions and of carbon dioxide. However the mechanism behind this effect may clarify the nature of the agonist/receptor interactions and the difference between α_1- and α_2-adrenoceptors. Furthermore, local conditions may determine the relative role of each receptor subtype in different physiological responses and this may indicate the physiological basis for these different α-adrenoceptors.

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Altered breakdown of ADP in isolated lungs of rats with streptozotocin-induced diabetes

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Adenosine diphosphate (ADP) is extensively metabolized on a single passage through the pulmonary circulation of rat isolated lungs (Crutchley, Eling & Anderson, 1978). This ADP-ase activity is associated with endothelial cells and may be an important anti-thrombotic mechanism *in vivo* (Lieberman, Lewis & Peters, 1977) because ADP is a potent aggregator of platelets. In diabetes mellitus, platelet

aggregation is enhanced (Bern, 1978) and we have therefore studied the fate of [^{14}C]-ADP perfused through the pulmonary circulation of isolated lungs from male rats made diabetic by a single injection of streptozotocin (85 mg/kg). Blood glucose levels reached a steady value (>420 mg/100 ml) 7 days after treatment and animals were used thereafter. Control animals were injected on the same day with saline.

Lungs were perfused via the pulmonary artery with Krebs solution at 8 ml/min and [^{14}C]-ADP infused (0.8 ml/min; 1×10^6 dpm) for 10 s into the Krebs flow. Lung effluent was collected for 60 s and the radioactivity analysed by t.l.c. on silica gel (Norman, Follet & Hector, 1974). Two concentrations of ADP, 10 μM (low) and 1 mM (high) were used. At the low concentration, in effluent from either control or diabetic lungs, less than 1% of the ADP infusion (total 13 nmoles) survived passage through the lungs. Most of the effluent label was present as AMP but there was more in effluent from diabetic lungs than in that from control lungs (9.4 ± 0.5 vs. 8.1 ± 0.3 nmoles, $n = 6$; $P < 0.05$). The other major products were adenosine and hypoxanthine (which were not separated in this solvent system) and here there was less in diabetic lung effluent (0.6 ± 0.03 vs. 0.8 ± 0.04 nmoles, $n = 6$).

At the high concentrations, survival of ADP was greater than at 10 μM, 5–10% of the total infused. However, effluent AMP was the same in diabetic and control lungs (870 ± 40 vs. 957 ± 54 nmoles; $n = 6$). Differences between diabetic and control lung effluent content were observed for inosine (12.0 ± 0.8 vs. $20.g \pm 3.6$ nmoles) and for adenosine + hypoxanthine (27.1 ± 1.1 vs. 40.0 ± 30 nmoles; $n = 6$).

We suggest that lungs of diabetic rats exhibit less AMP-ase activity at both ADP concentrations thus decreasing the formation of adenosine, a potent anti-aggregatory substance. This defect and indications of decreased ADP-ase activity at high ADP concentrations suggest that in diabetic lungs the anti-aggregatory potential of the vascular endothelium is less than normal, thus contributing to increased platelet aggregation *in vivo*.

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Enzymes acting on a glandular kallikrein substrate appear in inflamed tissue from adjuvant arthritic rats

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Increased free kinin levels in arthritic synovial effusions have been frequently observed, and have been thought to be due to activation of plasma kallikrein (Keele & Eisen, 1972). We now describe hydrolases, active on a commercial glandular kallikrein substrate, and present in inflamed tissue from adjuvant arthritic rats.

Adjuvant disease was induced in male Wistar rats (250–300 g) by a single subcutaneous injection of Freund's adjuvant into the right hind paw (Newbould, 1963). Control animals received an injection of vehicle. Paw oedema was monitored daily by measuring hind paw diameters. On the 16th day after adjuvant injection the mean diameter of injected paws had increased to 371% ($P < 0.001$, $n = 6$) of the initial value, and the contra-lateral paw to 221% ($P < 0.001$, $n = 6$). The animals were then killed, the hind paws perfused free of blood, severed at the tibiotarsal joint and the skin removed. The whole paws were homogenized in distilled water (10 ml/g tissue wet wt.) and incubated under toluene (24 h, 22°C) to activate kallikreins. Enzyme assays were carried out on the aqueous supernatants using H-D-Val. Leu. Arg.-pNA. 2 HCl (S-2266, Kabi Vitrum Ltd.) a chromogenic substrate selective for glandular

kallikrein (Claeson *et al.*, 1979). Mean enzyme activity in vehicle-injected control paws was 5.0 (s.d. = 1.2) nMoles pNA released $min^{-1} g^{-1}$ tissue wet wt., while that in inflamed paws was significantly greater at 19.7 (s.d. = 5.1) ($P < 0.001$) ($n = 6$).

Following ammonium sulphate fractionation of the pooled supernatants, enzymic activity precipitated between 40 and 60% ammonium sulphate saturation (fraction C). Fraction C was further characterized by determining the activity of a single concentration of the enzyme on the glandular kallikrein substrate S-2266, a plasma kallikrein substrate H-D-Pro. Phe. Arg-*p*NA.2Hcl (S-2302, Kabi Vitrum Ltd.), and a plasmin substrate H-D-Val. Leu. Lys-*p*NA.2Hcl (S-2251, Kabi Vitrum Ltd.). The ratios of activity of fraction C on the substrates was 1:0.7:0.4. This was similar to a glandular kallikrein (1:0.8:0.2) (Claeson *et al.*, 1979) and differed from plasma kallikrein (1:32:1.2) and from plasmin (1:20:20). Fraction C also released kinin-like spasmogens when incubated with human kininogen as described by Frankish & Zeitlan (1980).

Fraction C was dissolved in 0.2 M tris buffer pH 8.5, dialyzed against distilled water (24 h) and chromatographed on Sephacryl S-200 (Pharmacia). Two peaks of activity were obtained, the mol. wt. of one was less than 190,000 daltons, the other lay between 534,000 and 690,000 daltons. The lower molecular weight activity was largely inhibited (93.6%) by aprotinin (Trasylol, Bayer Ltd.) (10^3 units/ml) and was inhibited by soya bean trypsin inhibitor (SBTI) (100 μg/ml) by 56.2%. The high molecular weight activity was less susceptible to aprotinin (77.0% maximum inhibition) and was 41.0% inhibited by SBTI.

The possibility that the two activity peaks are related to a free kallikrein-like enzyme and its macroglobulin complex is now being investigated.

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Radioimmunoassay of glandular kallikreins in human biological fluids

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A radioimmunoassay (RIA) method has been developed for quantitative measurement of glandular kallikreins in human biological fluids both in normal and pathological states. Although glandular kallikreins are physiological components of urine and exocrine secretions, there is increasing evidence that they may also be present in the circulation (Fink, Geiger, Witte, Biedermann, Seifert & Fritz, 1980).

Purified human urinary kallikrein (HUK) was iodinated with Na[^{125}I] by the Chloramine-T method of Hunter & Greenwood (1962). Antiserum to purified HUK was raised in rabbits. The antiserum was further purified by removal of antialbumin antibodies on a column of Sepharose-4B coupled to human serum albumin. The antigen ([^{125}I] HUK) and antibody (dilution 1:60,000) were reacted for 24 h at 4°C. Separation of the free and bound label was obtained by centrifugation after incubation of the reaction mixture with Donkey anti-rabbit serum (dilution 1/20) for 24 h at 4°C. From the standard curves generated, values for glandular kallikrein could be quantitatively measured within the range of 2 to 30 ng protein ml.

Measurable glandular kallikrein activity was detected in both human serum and plasma. Since HUK cross-reacts with glandular kallikrein from other tissue sources (saliva and pancreatic juice) of the same species no distinction as to the origin of the glandular kallikrein in blood samples can be made with this assay. Interestingly, the values in serum were consistently higher than those obtained for plasma. We considered that the difference may be due to the presence of circulating endogenous protease inhibitors (α_2 macroglobulin, α_1 anti-trypsin, c_1 esterase inhibitor and anti-thrombin III) known to react with glandular kallikreins and thereby lowering the true values for plasma. We consequently examined the effect of exogenously added protease inhibitors on the measurement of glandular kallikrein by RIA (Rabito, Scicli & Carretero (1980). Both Aprotonin (Trasylol m.w. 6500) and benzamidine (M.W. 157)

displaced [^{125}I] HUK from its antibody; in contrast other protease inhibitors, ovomucoid, soya bean and lima bean trypsin inhibitors, showed no such action.

When fully characterized we anticipate that the RIA will provide further information on the relative importance of endogenous protease inhibitors on basal circulatory values of glandular kallikreins. The major aim in developing this RIA is to measure human glandular kallikreins in biological fluids in a variety of clinical states.

We are particularly grateful to Professor H. Moriya for the purified human urinary kallikrein. K.D.B. thanks the MRC for a project grant.

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Relation of morphine dependence to naloxone contractions in guinea-pig isolated ileum treated with morphine

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In morphine-withdrawn ilea isolated from guinea-pigs implanted for 3 days with 2 morphine pellets, the inhibitory effects of the presynaptic α_2-adrenoceptor agonist clonidine on electrically-induced twitches were much reduced and the dose-response curve was flat. Addition of morphine to the bath restored the inhibitory effect (Gillan, Kosterlitz, Robson & Waterfield, 1979). We used naloxone to produce the morphine-withdrawn state and studied the relation of flattening of the clonidine dose-response curve to the ability of naloxone to produce contractions in ilea treated with morphine (Ehrenpreis, Light & Schonbuch, 1972; Hammond, Schneider & Collier, 1976).

Ilea exposed to one of three different conditions were used. Procedures for setting up and testing (0.5 ms, 0.1 Hz, maximal voltage) were described by Gillan *et al.* (1979). The first kind of tissue was removed from guinea-pigs implanted with two 75 mg morphine pellets for 12, 24 or 48 h. Ilea were set up in Krebs solution containing 1600 mM morphine. The second was tissue removed from naive guinea-pigs and preincubated in Krebs containing morphine (10 μM) at room temperature for 24 h. These ilea were then set up in Krebs containing morphine (1600 nM). The third kind of tissue was removed from naive guinea-pigs and set up in normal Krebs. After stabilization, morphine (1600 nM) was added to the Krebs and the tissue was incubated for 3 h.

Segments of whole gut from each of the treatment conditions underwent contractions on addition of naloxone (400 nM) to the bath fluid. In the tissue treated with pellet implants the mean peak increase in baseline tension produced by naloxone was not greater than that seen in tissue from the other two treatment conditions. Yet, in the tissue treated with pellet implants, the clonidine dose-response curve measured in the presence of naloxone was flat, whereas in tissue preincubated for 24 h at room temperature in morphine (10 μM) the curve was less flat; in tissue incubated for 3 h in the tissue bath in morphine (1600 nM), the clonidine curve was almost unaltered. Longitudinal muscle strips with the myenteric plexus were also studied. Naloxone contractions were only observed in ilea treated with pellet implants, but at the durations of pellet implantation studied (12 and 24 h), clonidine sensitivity was similar in the presence and absence of naloxone.

The naloxone contraction has generally been considered a manifestation of tolerance produced by morphine treatment (Schulz & Herz, 1976). However, it is clear that naloxone contractions are not related in a simple way to tolerance to clonidine seen in the morphine-withdrawn gut.

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Methionine-enkephalin[Arg^6,Phe^7] immunoreactivity in bovine chromaffin granules compared with caudate

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The opioid heptapeptide metenkephalin[Arg^6,Phe^7] has recently been described in bovine adrenal medulla and the striata of various species using chromatography followed by radioreceptor assay or nonspecific radioimmunoassay (Stern, Lewis, Kimura, Rossier, Gerber, Brink, Stein & Udenfriend, 1979; Rossier, Audiger, Ling, Cros & Udenfriend, 1980). We have developed a sensitive and specific radioimmunoassay by oxidation of met-enkephalin[Arg^6,Phe^7] to the sulphoxide form with H_2O_2, conjugation to thyroglobulin with glutaraldehyde and injection into rabbits, raising an antiserum which bound [I^{125}]-methionine-sulphoxide-enkephalin[Arg^6,Phe^7] at a titer of 1:600 (20% of trace bound). The assay has a sensitivity of 0.033 pmol and requires a free C-terminus of the met-sulphoxide-enkephalin[Arg^6,Phe^7].

It has recently been suggested that the pro-enkephalin molecule in bovine adrenal medulla has met-enkephalin[Arg^6,Phe^7] as its C-terminl sequence (Stern, Jones, Shively, Stein & Udenfriend, 1981). We have compared met-enkephalin[Arg^6,Phe^7] immunoreactivity in bovine caudate and bovine chromaffin granule preparations after Sephadex G75 chromatography in 50% acetic acid, and have also assayed these fractions with an assay specific for the C-terminus of the met-enkephalin pentapeptide. Chromatography was of 50% acetic acid extracts. A strikingly different pattern of activity with both immunoassays was seen when the caudate and chromaffin granules were compared. With the met-enkephalin[Arg^6,Phe^7] assay only a small part of the immunoreactivity in the chromaffin granules corresponded in size with the heptapeptide; the major peak had an apparent molecular weight of 3,000, with a small excluded peak. The met-enkephalin assay applied to these same chromaffin granule extracts showed one peak corresponding to the pentapeptide; the major peak, however, had an apparent molecular weight of 10,000. When assays for met-enkephalin[Arg^6,Phe^7] and for met-enkephalin were applied to the G75 chromatography fractions of the bovine caudate extract, the majority of the immunoreactivity was found in the fraction corresponding to heptapeptide and pentapeptide respectively.

These data lend support to the suggestion that the chromaffin granules contain large molecular weight species with met-enkephalin[Arg^6,Phe^7] at the N-terminus. However, the results also show a major difference exists in enkephalin processing between the adrenal medulla and the caudate. Large molecular weight forms predominate in the chromaffin granules, which are present in very small quantities, if at all, in the caudate.

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Specific antibodies to choline acetyltransferase induced by small total amounts of purified antigen

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Choline acetyltransferase (ChAT) catalyzes the synthesis of acetylcholine (ACh) from choline and AcetylCoA. This enzyme is closely associated with ACh in nervous tissues and is therefore believed to be a specific marker for cholinergic pathways. The biochemical properties of ChAT and its localization in the central nervous system can be only ambiguously established after purification and subsequent preparation of 'monospecific' antibodies to the enzyme. Due to the very low amount of enzyme in the nervous tissues of vertebrates, extensive purification is required. AntiChAT antibodies have so far been induced only after a long period immunization using large amounts of purified antigen. We report here a method which allowed us to produce reproducibly a strong immune response to ChAT within 2 months immunization of mice which received in total fewer than 20 μg of active enzyme.

ChAT was purified 18,000 fold from bovine caudate nuclei to a final specific activity of 21 μmol min^{-1} mg^{-1} protein. Several reported purification methods (Rossier, 1976a; Roskoski, Lim & Roskoski, 1975; Ryan & McClure, 1979) were combined. This included extraction of ChAT activity by high salt concentration, CM-Sephadex chromatography, ammonium sulfate fractionation, affinity chromatography on Blue-Sepharose gel and absorption onto hydroxylapatite. This last preparation, stable up to 4 months, was emulsified in complete Freunds adjuvant for the first injection (day 0) and injected without adjuvant for the subsequent boosts (days 7, 14, 35). The antisera to ChAT were characterized before (inhibitory antibodies) and after (total antibodies) precipitation of all immune complexes by protein A-Sepharose. All of 3 series of 4 mice immunized with 3 different preparations of ChAT absorbed on hydroxylapatite produced a transient inhibitory response which appeared 2 weeks after the first injection and lasted about 3 to 4 weeks; no more than 50% of the ChAT activity could be inhibited. Six weeks after the first injection the inhibitory response has nearly disappeared but strong non-inhibitory antibodies could be revealed after precipitation by protein A. These antisera required 0.013 to 0.064 μl (12 mice) to bind 50% of 250 pmol/min of ChAT activity present in controls. Our results compared well to those of other authors (Cozzari & Hartman, 1980; Malthe-Sørenssen, Lea, Fonnum & Eskeland, 1978; Rossier, 1976b; Singh & McGeer, 1974). Bovine preparations of different specific activities were similarly recognized by the antibodies. These antisera cross-reacted to different degrees with ChAT from different species: bovine caudate nucleus > pig brain > human cortex > rat brain > chicken brain > Torpedo electric organ. These antisera were assumed to be specific but not 'monospecific'.

The relatively simple purification and immunization procedures described here should prove invaluable for the subsequent production of monoclonal antibodies to ChAT.

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Investigation of triglycyl-oxytocin as a hormonogen in the conscious dog

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The addition of a tripeptide extension to the amino-terminus of neurohypophysial peptides leads to a prolongation of biological action in a variety of systems (Beránková-Ksandrová, Bisset, Jošt, Krejčí, Pliška, Rudinger, Rychlík & Šorm, 1966; Kynčl & Rudinger, 1970) releasing the active principle by slow enzymic cleavage. These analogues have been termed 'hormonogens'. The present study investigates the activation and clearance of triglycyl-oxytocin (TGOT) following intravenous (i.v.) injection and infusion, and subcutaneous (s.c.) injection.

Male beagle dogs (10–18 kg) were prepared with indwelling venous cannulae whose tips lay in the superior vena cava (Stevenson, Parsons & Alberti, 1978), the infusion cannula opening approximately 3 cm nearer the atrium than the sampling cannula. The concentrations of OT and TGOT were measured by simultaneous bioassay (Robinson & Walker, 1974) and radioimmunoassay (Robinson, 1980) of plasma samples obtained from conscious dogs following i.v. and s.c. administration of each peptide. Metabolic clearance rates and apparent distribution spaces of OT and TGOT were calculated using a single-pool model (Sönksen, Tompkins, Srivastava & Nabarro, 1973).

During constant i.v. infusion at 23 pmol kg^{-1} min^{-1} for 60 min the mean plateau levels (± s.e.mean) were 1.08 ± 0.08 pmol/ml ($n = 5$) and 1.58 ± 0.21 pmol/ml ($n = 6$), for OT and TGOT respectively.

After stopping the infusion the peptide concentrations fell rapidly over the first 8 min followed by a slower rate of disappearance over the next 30–60 min. Metabolic clearance rates (OT, 21.5 ± 1.4 ml kg^{-1} min^{-1}, $n = 6$; TGOT, 15.4 ± 1.2 ml kg^{-1} min^{-1}, $n = 8$) and the initial half-lives (OT, 4.2 ± 0.4 min; TGOT, 6.6 ± 0.4 min) were significantly different ($P < 0.002$). There was no difference in the apparent distribution space for the two peptides (129 ± 14 vs 147 ± 15 ml/kg).

In two experiments a single i.v. injection of OT or TGOT (1.5 nmol/kg) was given, and the disappearance of each peptide from the plasma followed for 10 min after injection. The apparent half-lives of OT and TGOT were 3.3 and 6.5 min respectively, i.e. very similar to the values found after i.v. infusion.

In cross-over experiments in four dogs, s.c. injection of TGOT (3.45 nmol/kg) produced a mean immunoassayable plasma level of 2.28 ± 0.15 pmol/ml between 10 and 30 min, over 3-fold higher than the level of OT following the injection of the hormone itself (0.63 ± 0.10 pmol/ml). Bioassayed levels of OT were very similar to the radio-immunoassayed levels, whether this peptide was administered by constant i.v. infusion or s.c. injection. Intravenous infusions of TGOT produced very little bioactivity (0.2% conversion to OT), whereas after s.c. injection, the amount of bioactive OT generated from TGOT rose steadily from 3% at 20 min to over 13% at 2 h.

These results indicate that TGOT is reasonably effective as a hormonogen after s.c. administration, but much less so when given intravenously.

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The effect of thyroidectomy or propylthiouracil on the sensitivity of the rat saphenous artery to phenylephrine

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Thyroidectomy increases the maximum responses of the rat anococcygeus muscle (Gibson & Pollock, 1975) and of the vasculature of rat hindquarters to agonists (Brown & Pollock, 1980). During thyroidectomy, the parathyroid glands were also removed. It was, therefore, difficult to know if the increased responses were due to a lack of thyroxine. In the present study plasma thyroxine levels were reduced by thyroidectomy or by treatment with propylthiouracil. In addition, the effect of replacement of thyroxine in thyroidectomized rats was examined.

Male Wistar rats received propylthiouracil in their drinking water (20 mg kg^{-1} day^{-1}), which also contained saccharin (20 mg/l). A control solution containing saccharin (20 mg/l) was administered to a second group of rats. Thyroidectomized male Wistar rats were obtained commercially. Two to three weeks after thyroidectomy rats received thyroxine (5 mg kg^{-1} day^{-1}, i.p.) or isotonic saline (0.9% w/v, 0.2 ml/100 g body wt., i.p.). Two to four weeks after commencement of treatment rats were killed by halothane. The isolated saphenous artery was perfused at 2 ml/min with Krebs solution (35°C). Log-dose response curves for phenylephrine were obtained and doses which produced 50% of the maximum response were determined. Plasma thyroxine levels were measured by competitive binding analysis. Means were compared with Student's *t*-test.

The results are shown in Table 1. Thyroidectomy increased the maximum response to phenylephrine but had no effect on the ED_{50} value. Propylthiouracil also increased the maximum response to phenylephrine but had no effect on the ED_{50} value. Administration of thyroxine to thyroidectomized rats did not reverse the effect of thyroidectomy. Therefore, it appears that a reduction in the thyroxine levels, whether by surgical thyroidectomy or by inhibition of synthesis, causes an increase in the maximum response of the rat saphenous artery but that this effect is not easily reversed by replacement of thyroxine.

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Table 1 Effect of thyroidectomy or propylthiouracil on the sensitivity of the rat saphenous artery

	Maximum Pressor Response (mm Hg)	*ED_{50}* (nmoles)	*Thyroxine Level* (μg T4/100 ml)
Control	136 ± 14 (13)	2.8 ± 0.6 (14)	9.3 ± 2.3 (5)
Thyroidectomized	224 ± 28** (10)	4.1 ± 1.5 (13)	2.3 ± 0.5* (5)
Saccharin Control	158 ± 14 (10)	1.3 ± 0.2 (8)	10.8 ± 2.3 (8)
Propylthiouracil	210 ± 19* (11)	2.0 ± 0.5 (9)	1.8 ± 0.7** (7)
Thyroidectomized + Saline	212 ± 25 (8)	2.4 ± 0.9 (5)	4.4 ± 0.9 (4)
Thyroidectomized + Thyroxine	213 ± 35 (7)	1.5 ± 0.5 (4)	19.4 ± 4.9* (3)

Values are means ± s.e.mean $0.05 < P < 0.01$*, $0.01 < P < 0.001$**

Turnover of 5-hydroxytryptamine (5-HT) and analgesic effect of morphine in the chicken

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Fowls have been found to have a low sensitivity to the analgesic effect of morphine, doses of 200 mg/kg having been reported to be necessary for suppressing the pain of pinching the toes (Schneider, 1961).

The fact that, in the mammal, the dose-response curve to morphine is shifted to the right by inhibiting the synthesis of 5-HT in the brain with *p*-

chlorophenylalanine (Tenen, 1968), suggested the investigation of 5-HT turnover in the chicken brain and of its relation to morphine analgesia. Three different breeds of chicks were used, and to our surprise the results varied from breed to breed. The breeds will be called A, B and C, A standing for White Leghorns from Houghton Poultry Research Station, B for White Leghorns from Orchards Farm, and C for Rhode Island × Sussex Cross from Orchards Farm. Turnover was measured, as previously done in the rat (Snelgar & Vogt, 1981), by injecting probenecid (200 mg/kg s.c.) at time zero, morphine HCl s.c. 30 min later, and killing the chicks by decapitation at 120 min. Breed A showed an increased turnover of 5-HT in several parts of the brain, as indicated by increased accumulation of 5-HIAA after morphine, but no such effect was detectable in breeds B and C. Meanwhile we had devised a method for testing analgesia in the chick which proved more sensitive than the toe pinch. It consisted of shining a light on the chick's head from which the feathers had been cut off in an area of about 1 cm^2. The time of exposure required till the bird moved its head away (usually a few seconds) was a measure of the pain threshold. In breed C morphine (100 mg/kg), only achieved analgesia in 57% of the chicks, in breed B, morphine (20 mg/kg) produced an analgesic effect in 80%, whereas in breed A morphine (7.5 mg/kg) caused analgesia in all animals. Some chicks responded even to 5 mg/kg. These results demonstrate the importance of an activation of 5-HT neurones for morphine analgesia in the chicken, and the large variation of that activation in different strains.

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Actions of benzodiazepines and other anticonvulsants on 5HT turnover in mouse brain

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Many anticonvulsant drugs elevate cerebral 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA) concentrations in rodents (Bonnycastle, Giarman & Paasonen, 1957; Jenner, Chadwick, Reynolds & Marsden, 1975). We have demonstrated that clonazepam reduces 5HT utilization without altering 5HT synthesis (Pratt, Jenner, Reynolds & Marsden, 1979). We now investigate whether this is a general effect of other anticonvulsant compounds.

Acute administration of clonazepam (0.5–8 mg/kg i.p. 3 h previously), diazepam (2–32 mg/kg i.p. 3 h previously), chlordiazepoxide (1–40 mg/kg i.p. 2 h previously) or diphenylhydantoin (5–320 mg/kg i.p. 1.5 h previously) increased mouse whole brain 5HT, 5HIAA and tryptophan concentrations. Carbamazepine (5–100 mg/kg i.p. 1 h previously) and phenobarbitone (10–80 mg/kg i.p. 2 h previously) elevated whole brain 5HT and 5HIAA, but not tryptophan levels, whereas flurazepam (5–80 mg/kg i.p. 2 h previously) only elevated the concentration of 5HIAA. In subsequent experiments each compound was administered in a single dose that caused a substantial rise in either brain 5HT or 5HIAA concentrations.

Pretreatment of mice with p-chlorophenylalanine methyl ester hydrochloride (PCPA, 200 mg/kg i.p.) for 3 days caused a 45–65% reduction in whole brain 5HT concentration. Clonazepam (4 mg/kg), diazepam (32 mg/kg), chlordiazepoxide (40 mg/kg), and carbamazepine (50 mg/kg), but not flurazepam (40 mg/kg), diphenylhydantoin (40 mg/kg), and phenobarbitone (80 mg/kg), partially reversed the PCPA-induced depletion of 5HT suggesting a reduction in 5HT utilization.

Clonazepam (4 mg/kg), diazepam (32 mg/kg) or chlordiazepoxide (40 mg/kg), but not flurazepam (40 mg/kg), carbamazepine (50 mg/kg), diphenylhydantoin (40 mg/kg), or phenobarbitone (80 mg/kg), increased the brain content and specific activity of [^{3}H]-tryptophan produced by pulse labelling with L-[G-^{3}H]-tryptophan (25 uCi sc) 0.5 h before death. Clonazepam (4 mg/kg), diazepam (32 mg/kg), chlordiazepoxide (40 mg/kg), and diphenylhydantoin (40 mg/kg), but not flurazepam (40 mg/kg) and phenobarbitone (80 mg/kg), in combination with L-

[G-^{3}H]-tryptophan (25 uCi sc) 2.0 h prior to death elevated brain [^{3}H]-tryptophan levels, but not specific activity, compared to control animals. In contrast, carbamazepine (50 mg/kg) reduced the brain content of [^{3}H]-tryptophan. This would suggest that some anticonvulsant drugs increase the uptake or availability of plasma tryptophan into brain.

Measurement of whole brain [^{3}H]-5HT levels 0.5 h following L-[G-^{3}H]-tryptophan, when incorporation into the 5HT pool was occurring, was unaffected by all drugs, indicating no effect on 5HT synthesis. However, clonazepam (4 mg/kg), diazepam (32 mg/kg), chlordiazepoxide (40 mg/kg) and diphenylhydantoin (40 mg/kg), but not flurazepam (40 mg/kg), carbamazepine (50 mg/kg) or phenobarbitone (80 mg/kg), elevated both brain content and specific activity of [^{3}H]-5HT at 2 h following administration of L-[G-^{3}H]-tryptophan, when [^{3}H]-5HT levels were falling. This suggests that some anticonvulsants reduce cerebral 5HT utilization.

We suggest that the effect of anticonvulsant drugs on cerebral 5HT parameters is due, at least in part, to decreased 5HT utilization with no action on 5HT synthesis. Anticonvulsants appear to have no common mechanism of action on the 5HT system, and the effects observed occur at dosage levels higher than those required for anticonvulsant activity (Krall, Penry, White, Kupferberg & Swinyard, 1978).

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Autoradiographical localization of [^{3}H]-Ro 15-1788, a selective benzodiazepine antagonist, in rat brain *in vitro*

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Benzodiazepines elicit their main pharmcological and therapeutical effects, as anxiolytics, anticonvulsants, hypnotics and muscle relaxants, by interacting with a specific target structure in the CNS, the benzodiazepine receptor. This is a membrane protein localized in synapses some of which are GABAergic (Möhler, Richards & Wu, 1981). The affinity of the benzodiazepines for this receptor is an important factor determining the potency of their action in the CNS. Electro-physiological and pharmacological studies have shown that the primary mechanism of action of this class of drugs is the enhancement of GABAergic inhibition (Haefely, Pieri, Polc & Schaffner, 1981). Recently, the imidazodiazepine, Ro 15-1788 has been shown to selectively antagonize all known CNS effects of benzodiazepines although *per se* it lacks major pharmacological activity (Hunkeler, Möhler, Pieri, Polc, Bonetti, Cumin, Schaffner & Haefely, 1981). In order to determine its site of action in the CNS we have used a recently developed technique for the autoradiographical localization of drug and neurotransmitter receptors (Kuhar, 1981).

Slide-mounted cryostat sections were incubated with [^{3}H]-Ro 15-1788 (s.a. 26.8 Ci/mmol; 2 nmol/l) or [^{3}H]-flunitrazepam (87.9 Ci/mmol; 1 nmol/l) in Tris HCl (170 mmol/l) for 40 min at 4°C. Alternating sections were incubated with the radiolabel in the presence of a nonradioactive benzodiazepine (1 μmol/l). The sections were then processed as described by Kuhar (1981).

In sections of rat brain, cervical spinal cord and retina, the distribution and density of [^{3}H]-Ro 15-1788 binding sites were almost indistinguishable from that found with [^{3}H]-flunitrazepam as radioligand. The intensity of labelling in the molecular layer of the cerebellum was higher than in the granule cell layer, while in myelin only background amounts of radioactivity were present. When adjacent sections were incubated in the presence of an excess of nonradioactive flunitrazepam, the intensity of labelling corresponded to background levels in

grey as well as in white matter. Other CNS regions of intense labelling included: molecular layers of hippocampus and dentate gyrus, cerebral cortex (most intense in layer IV), medial amygdaloid nucleus, dorsal horn of spinal cord (layer II and III) and inner plexiform layer of retina.

Binding sites for the antagonist, similar to flunitrazepam, were also found in the brain stem of rat embryo (day 15) and in a distinct cortical localization (intense in layers I and III–IV) already on day 20. No binding sites were observed earlier than day 14. However, unlike flunitrazepam, the antagonist did not show a high affinity for 'peripheral' binding sites, e.g. in ependyma, choroid plexus, kidney cortex and adrenal cortex.

These findings are in line with the selective antagonism of central benzodiazepine effects by Ro 15-1788.

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Rat brain benzodiazepine receptor number and GABA concentration following a seizure

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It has been shown that following a single electroconvulsive shock or a bicuculline-induced seizure rats exhibit a rapid increase in seizure threshold to infusion of GABA antagonist drugs lasting approximately 3 h (Nutt, Cowen & Green, 1981). In view of the report that there is an increase in the specific binding of diazepam to cerebral cortical membranes from rats during the 60 min following an electroconvulsive shock or injection of pentylenetetrazol (Paul & Skolnick, 1978), it seemed possible that the rise in threshold was associated with an increase in benzodiazepine receptor number. An investigation has now been made into this possible relationship.

A single electroconvulsive shock (ECS) 125 v, 1 s, sinusoidal was given to rats through earclip electrodes. They were killed 30 min later and the brains were rapidly dissected into six areas. A well-washed membrane preparation was used for the determination of diazepam binding (modified from Martin & Candy, 1980). No changes were found in the specific binding to membrane fragments from the pons/medulla, hypothalamus, corpus striatum and cerebellum and Scatchard analysis of binding to membrane fragments from the cortex and hippocampus showed no change in either Kd or Bmax produced by ECS when compared to handled controls. Nor was there any change in the specific binding to cortical or hippocampal membranes at 15, 60 or 120 min following an ECS.

The preparation used by Paul & Skolnick (1978) was a crude synaptosomal fraction as opposed to the well-washed membranes used in the present study and it seemed possible, therefore, that the changes previously reported might have resulted from changes in an endogenous factor. We have therefore determined the concentration of GABA at various times following an ECS or a bicuculline-induced seizure (0.32 mg/kg i.v.). The rats were killed by a focused microwave beam, the brain dissected into four regions and GABA measured by a fluorimetric, enzymatic assay (Baxter, 1972). Cortex, hippocampus, corpus striatum and hypothalamus all showed an increase in GABA concentration following the convulsion, a statistically significant change occurring at 30 min in all regions except corpus striatum. However, only in the hippocampus did the GABA concentration increase markedly by 5 min after the convulsion (control: 4.70 ± 0.16 μmol/g (20); ECS: 5.73 ± 0.34 (8) $P < 0.01$), returning to control values by 120 min. The time course of the change in this region, therefore, closely resembled the previously observed rise in seizure threshold. Furthermore a similar change was produced following a bicuculline-induced seizure. The seizure and GABA concentration change were prevented by pretreatment with (+)-propranolol (30 mg/kg) 5 min before ECS. This

drug has previously been shown to prevent also the rise in seizure threshold when given before ECS (Nutt *et al.*, 1981).

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Differences in the response of rat superior colliculus to muscimol and THIP

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Differences in the pharmacological profiles of the GABA agonists, muscimol and 4,5,6,7-tetrahydroisoxazolo-(5,4,-C)-pyridin-3-ol (THIP) can be reliably demonstrated using a variety of neurochemical approaches. Muscimol inhibits GABA uptake and has an affinity for benzodiazepine receptors, whereas THIP has little effect on these two systems (Krogsgaard-Larsen *et al.*, 1979). However, in the intact animal it has proven extremely difficult to differentiate between the central effects of the two drugs when administered systemically or by local injection (Arnt & Scheel-Krüger, 1980; Meldrum & Horton, 1980; Waszczak *et al.*, 1980). The rate of glucose phosphorylation in any region of the CNS appears to reflect function-related energy utilization. In the present study we have compared the functional consequences of muscimol and THIP, administered i.v. using the 2-deoxyglucose (2-DG) technique. Thus a simultaneous assessment of glucose use in anatomically discrete regions of the CNS was possible.

Local cerebral glucose utilization in conscious, lightly restrained, male Sprague-Dawley rats (300–350 g) was measured using the quantitative autoradiographic 2-DG method in the manner described in detail previously (Sokoloff *et al.*, 1977). The experiments were initiated by the injection of a pulse of [^{14}C]-2-DG (125 μCi/kg) delivered 20 min after the administration of either muscimol (0.15 to 1.5 mg/kg i.v.) or THIP (1 to 10 mg/kg i.v.). 45 min later the animals were decapitated and autoradiograms prepared from sections of the frozen brains. The autoradiograms were analysed densitometrically and glucose use derived using the operational equation for the method (Sokoloff *et al.*, 1977).

The administration of muscimol or THIP resulted in dose-dependent reductions in glucose utilization in primary visual cortex, lateral geniculate body and the deeper layers of the superior colliculus (Table 1) and

Table 1 Glucose utilization in primary visual areas following the administration of muscimol and THIP

Region	*Saline*	*Muscimol* (mg/kg) *0.5*	*1.5*	*THIP* (mg/kg) *1.0*	*10.0*
Visual Cortex, layer IV	97 ± 4	74 ± 7	55 ± 1*	96 ± 2	52 ± 3*
Lateral geniculate body	78 ± 3	60 ± 4	49 ± 3*	68 ± 3	58 ± 2*
Superior Colliculus, stratum griseum superficiale	84 ± 3	64 ± 6*	49 ± 2*	84 ± 9	80 ± 4
Superior Colliculus, stratum griseum profundum	82 ± 2	72 ± 6	67 ± 3*	77 ± 5	64 ± 3*

Data are derived from 22 animals and are presented as mean glucose utilization (μmoles $100\,g^{-1}\,min^{-1}$) ± s.e.mean. Statistical analysis was performed by means of an analysis of variance (Scheffe).
*$P < 0.05$.

a similar pattern of response to both agents could be demonstrated in 28 of the 61 regions examined. In 32 regions neither muscimol nor THIP altered the local rate of glucose utilization. In only one region of the CNS, i.e. the superficial layer of the superior colliculus, could a qualitative difference be demonstrated between the two putative GABA agonists. The different pattern of response in the superior colliculus was visually discernible on the autoradiograms.

Whether the unique differential response of glucose use in the superior colliculus represents differences in the action of muscimol and THIP within this region or a modification of activity in the retinotectal projection remains to be determined.

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Depressor effects of isoguvacine propyl ester and isoarecaidine propyl ester in the cat due to stimulation of central GABA receptors

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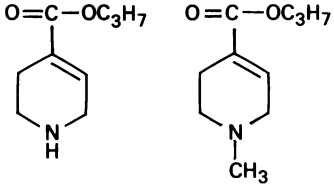

isoguvacine propyl ester — isoarecaidine propyl ester

GABA and muscimol, a GABA receptor agonist, lower blood pressure after intracerebroventricular injection into the cat (Williford *et al.*, 1980). Even in high doses, these drugs are practically ineffective after i.v. injection (Antonaccio & Taylor, 1977) mainly because of poor penetration into the CNS. Also, the GABA mimetic compounds synthesized so far (Krogsgaard-Larsen & Arnt, 1979) possess hydrophylic properties. The latter authors showed that isoguvacine excites GABA receptors and that guvacine inhibits GABA uptake.

The present study reports on the depressor action of both isoguvacine propyl ester (IGPE) and isoarecaidine propyl ester (IAPE). Cats were anaesthetized with α-glucochloralose and received gallamine hydrochloride (5 mg/kg) prior to drug administration. Drug solutions (140 μl) were infused for 1 min either intravenously or simultaneously via the left and right vertebral artery (v.a.) according to a method described previously (Porsius, 1980). IGPE and IAPE (as hydrochlorides) reduced blood pressure and heart rate upon i.v. infusion and bilateral administration into the v.a. in a dose dependent manner. The maximal effects are expressed as percentage ($\bar{x} \pm$ s.e.mean) of the initial mean arterial pressure (100%). IGPE (2×30 μg via the v.a.) reduced pressure to $45 \pm 3\%$ ($n = 3$). 30 min after dosing this value was $52 \pm 4\%$. IAPE (2×30 μg via the v.a.) reduced pressure to $41 \pm 4\%$ ($n = 5$). This value amounted to $48 \pm 5\%$, 30 min after infusion. Various doses of the GABA antagonists bicuculline and picrotoxin were infused during the reduced arterial pressure after bilateral application of the depressor agents via the v.a. during the first 30 min

after dosing. Both bicuculline (2×2, 2×5 and $2 \times 10\,\mu g$) and picrotoxin (2×10 and $2 \times 20\,\mu g$) counteracted the hypotension and bradycardia induced by the esters in a dose dependent manner. In untreated cats, bicuculline ($2 \times 10\,\mu g$) and picrotoxin ($2 \times 40\,\mu g$) induced transient and mild biphasic effects on pressure. Also, intravenous infusion of IAPE (4 mg/kg) reduced pressure to $54 \pm 4\%$ ($n = 5$). Pretreatment with $2 \times 40\,\mu g$ picrotoxin via the v.a. blocked this effect completely. In separate experiments the distribution of IAPE in brain tissue of the cat was established various minutes after the infusion of [^{14}C]-IAPE into the v.a. or a femoral vein. Moreover, it was demonstrated that the esters are rapidly hydrolyzed by brain tissue, both *in vivo* and *in vitro*. The results suggest that the esters lower blood pressure by stimulation of the GABAergic system within the pontomedullary region. It seems likely that IGPE and IAPE are pro-drugs and that the corresponding carboxylic acids (isoguvacine and isoarecaidine) are the active GABAergic drugs. Consequently, drugs are now available which can easily penetrate the CNS and stimulate central GABA mechanism.

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Allosteric interaction between the ^{3}H-imipramine binding site and the serotonin uptake mechanism

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[^{3}H]-Imipramine ([^{3}H]-IMI) has been shown to bind with high-affinity to specific sites in the brain and blood platelets of a variety of species including man (Langer & Briley, 1981).

Recently, evidence from a variety of different experimental approaches has suggested that the [^{3}H]-IMI binding site is probably associated with the uptake mechanism for serotonin (Briley, Langer, Raisman & Sette, 1981). To clarify the relationship between neuronal uptake of serotonin and the [^{3}H]-IMI binding site we have investigated in detail the inhibition by serotonin of [^{3}H]-IMI binding.

Serotonin, the only neurotransmitter to inhibit [^{3}H]-IMI binding ($IC_{50} = 1.0\,\mu M$), does so in a non-competitive manner, exhibiting a curvilinear Hofstee plot and a Hill coefficient of between 0.40 and 0.50. In contrast, tricyclic antidepressant drugs inhibit competitively the binding of [^{3}H]-IMI with high-affinity giving linear Hofstee plots and Hill coefficients of close to 1.00. This effect of serotonin may be interpreted as indicating the presence of either multiple high-affinity binding sites or allosteric interactions.

The existence of an allosteric interaction is suggested by the fact that the presence of serotonin increased the Ki for the inhibition of [^{3}H]-IMI binding by unlabelled imipramine. With serotonin ($1\,\mu M$), the Ki for imipramine was increased from 9.5 nM to 19.0 nM, while with serotonin ($5\,\mu M$), the Ki was increased nearly thirteen fold from 9.5 to 122.0 nM. This effect of serotonin, does not appear to be mediated through the serotonin receptor since the serotonin receptor antagonist, methysergide, could not reverse the effect of serotonin on the Ki for imipramine. Furthermore, the serotonin receptor agonist, LSD, unlike serotonin, was incapable of changing the Ki for imipramine. The modulation of the affinity of imipramine for the [^{3}H]-IMI binding site may, however, be mediated through the uptake mechanism for serotonin. Fluoxetine, a specific serotonin uptake blocker, at concentrations as low as 25 nM, gave similar results to micromolar concentrations of serotonin (the Ki of imipramine was increased from 15.0 to 34.0 nM).

These results suggest that the affinity of imipramine for the [^{3}H]-IMI binding site may be allos-

terically modulated by serotonin possibly acting through the neuronal serotonin uptake mechanism. This would imply that the [^{3}H]-IMI binding site and the recognition site for serotonin uptake are not indentical. Furthermore it is possible that the allosteric association demonstrated here between these sites is reciprocal and that serotonin uptake could be modulated by the [^{3}H]-IMI binding site. Such reciprocal allosteric interactions have already been demonstrated for the GABA receptor/benzodiazepine receptor complex (Briley & Langer, 1978; Guidotti, Toffano & Costa, 1978).

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Temperature and [^{3}H]-mepyramine binding to histamine H_1-receptors

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[^{3}H]-Mepyramine has been widely used in ligand-binding studies of histamine H_1-receptors, particularly in the central nervous system (Hill, Emson & Young, 1978; Tran, Chang & Snyder, 1978). Most of these studies have been carried out at 25–30°C, but there are suggestions from functional studies on intact smooth muscle that the property of mepyramine binding to H_1-receptors may vary with temperature (Cook, Kenakin & Krueger, 1977). This proposition is strengthened by the observation that [^{3}H]-mepyramine bound to sections of brain tissue at 4°C is not readily removed by washing in cold buffer (Palacios, Young & Kuhar, 1979). In view of these observations we have undertaken a study of the temperature-dependence of the kinetics of [^{3}H]-mepyramine binding to guinea-pig cerebellar homogenates.

Washed homogenates of guinea-pig cerebellum were prepared as described previously (Hill *et al.*, 1978) and the binding of [^{3}H]-mepyramine was measured either by a microcentrifugation method or by rapid filtration through Whatman GF/B glass-fibre filters. Receptor-specific binding of [^{3}H]-mepyramine was defined as the binding sensitive to inhibition by promethazine (2 μM). The rate of dissociation was measured by incubation of cerebellar homogenate with [^{3}H]-mepyramine (5 nM) in 50 mM Na-K phosphate buffer, pH 7.5, until equilibrium was attained and then diluting 100-fold into buffer, usually at the same temperature.

The rate of dissociation of [^{3}H]-mepyramine was highly temperature dependent. At 37°C dissociation was rapid ($T_{\frac{1}{2}} = 57$s), but at 4°C no measurable dissociation occurred up to 2 h after dilution. Addition of promethazine (2 μM) to the diluting buffer had no effect on the rate of dissociation. The kinetics of dissociation were solely a function of the temperature of the diluting buffer and were independent of the temperature of the incubation. An Arrhenius plot of $\log k_{-1}$ (dissociation rate constant) against 1/T was linear over the temperature range measured (15–37°C) and indicated an activation energy of 160 kJ mol^{-1} (38 kcal/mol).

Preliminary measurements of the association rate constant, k_1, by measuring the variation of k_{on} with the concentration of [^{3}H]-mepyramine ($k_{on} = k_1.[^3\text{H-mepyramine}] + k_{-1}$), indicate that k_1 also decreases markedly between 30°C and 4°C so that, in spite of a large change in both rate constants, there may be only a small change in affinity.

The marked temperature dependence of the dissociation of bound [^{3}H]-mepyramine has several implications for the interpretation of binding studies carried out at low temperature. The most important of these is that it is difficult to achieve true equilibration between [^{3}H]-mepyramine and 'competing' ligands, which can yield misleading values for affinity constants. Similarly the dependence of k_{on} on [^{3}H-mepyramine] at low temperature can lead to binding curves which are artifacts of inadequate equilibration at low concentrations of [^{3}H]-mepyramine.

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Iontophoresis of peptide neurotransmitter candidates in the caudal trigeminal nucleus of the rat: lack of correlation of effect with sensory responses of neurones

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The caudal trigeminal nucleus (ntV) is a site of termination of sensory nerve fibres from the face, and is a rostral analogue of the dorsal horn of the spinal cord. The peptides substance P and somatostatin are present in primary sensory neurones, and may also be found in spinal interneurones, as is neurotensin (Hökfelt, Johansson, Ljungdahl, Lundberg & Schultzberg, 1980). It is possible that these peptides have neurotransmitter roles in ntV, and it was thus of interest to investigate their actions on single neurones. In particular, it was of interest to see if there was any relationship between the effect of a peptide on a neurone and the sensory response of that neurone, as it has been reported that substance P only excites cat ntV neurones responding to noxious stimuli (Henry, Sessle, Lucier & Hu, 1980).

Extracellular single neurone recordings were made from ntV in urethane-anaesthetized rats using the centre barrel of 6 or 7 barrelled micropipettes. One outer barrel contained 1 M NaCl and was used for automatic balancing of the iontophoresis current. The remaining barrels contained combinations of one of the following: Na L-glutamate (0.5 M, pH 8.5), acetylcholine chloride (0.5 M, pH 3.5), substance P (2.5 mM in 150 mM NaCl), somatostatin (1 mM in 150 mM NaCl), neurotensin (1 mM in 150 mM NaCl), and 150 mM NaCl. Effects of the peptides on the spontaneous activity of neurones were observed or, in the absence of such activity, on glutamate-evoked activity, and wherever possible, the responses of neurones to noxious and non-noxious sensory stimuli were noted.

Both substance P and somatostatin were found to excite the majority (58% and 75% respectively) of ntV neurones tested (31 and 16, respectively), and there appeared to be no difference between those neurones that were spontaneously active and those that were glutamate-driven. However, although neurotensin excited 8 of 25 spontaneous neurones, it inhibited 9 of 27 glutamate-driven neurones. All three peptides had effects on neurones irrespective of whether the neurone responded to noxious stimuli.

The excitatory effects of substance P and somatostatin are consistent with a neurotransmitter role for these peptides. However, it does not appear possible to infer, from our data, that they serve any particular sensory modality. The difference in effect of neurotensin on spontaneous and glutamate-driven neurones suggests that neurotensin interneurones may affect the operation of excitatory amino acid-mediated transmission, which has been shown to occur in ntV (Salt & Hill, 1981).

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Experiments to investigate the neural origin of the high pressure neurological syndrome

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A high pressure neurological syndrome (HPNS) has been described in a variety of animals (Brauer *et al.*, 1974). Following compression with helium, which has been shown to act only as a pressure-transmitting agent (Miller *et al.*, 1967), the syndrome in mice is expressed progressively with increasing pressure as tremors, convulsions and death. General anaesthetics postpone the onset of these signs to higher pressures (Lever *et al.*, 1971). Reserpine reduces onset pressures, and this effect is lessened by treatment with L-tryptophan or tranylcypromine (Brauer *et al.*, 1978). In a study of whole brain amines, the signs of HPNS could not be associated with changes in any single amine (Koblin *et al.*, 1980). Current studies with Mr A. Dobbie suggest that while pretreatment with 6-OH dopamine reduces onset pressures, pretreatment with L-dopa is ineffective. Agents that facilitate GABA transmission significantly increase onset pressure (Bichard *et al.*, 1981). Classical antiepileptics have thus far proved unrewarding (Halsey & Wardley-Smith, 1980).

Pharmacological study, therefore, while it has discovered means for facilitating or antagonizing HPNS, has so far provided no clear lead as to the site or nature of the neural structures in which the tremors and convulsions are initiated. Experiments have therefore been made with classical ablation methods, using chronically operated animals in which sufficient time was allowed after operation for the elimination of the anaesthetics used.

Male CD1 mice, weight 30–40 g, were anaesthetized for aseptic decerebration or decortication with pentobarbitone sodium (75 mg/kg i.p., Sagatal). Series A were controls, series B were decerebrated at the mid-collicular level, and series C were decorticated using fine controlled suction and maintaining as far as was possible the integrity of the subcortical structures. From 24 h to 7 days was allowed for recovery before pressure was applied with helium at a rate of 1 atm/min in a 1.5 litre pressure vessel. Body temperatures at pressure were monitored by rectal probe and maintained at 36–36.5°C. Table 1 summarizes the threshold pressures at which fine tremor, coarse tremor and convulsions occurred.

One interpretation of these results is as follows: (a) There is a site for initiation of convulsions below the level of decerebration, which is normally held under some descending inhibitory influence. (b) Since the sensitization to convulsion is greater in the decerebrate than in the decorticate animal, some degree of inhibitory control resides within the subcortical structures. (c) There is a site of initiation of fine tremor above the level of decerebration and below that of decortication, which is under descending cortical inhibition. But other possibilities exist, and subsequent analysis will depend on detailed histological examination of the lesions made, together with more specific ablation now being employed.

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Table 1 Thresholds of HPNS (atm ± s.e.mean) of normal, decerebate and decorticate mice

	Fine tremors	*Coarse tremors*	*Convulsions*	*n*
A. Controls	46 ± 2	71 ± 4	100 ± 5	8
B. Decerebrate	nil	nil	68 ± 1[(c)]	6
C. Decorticate	30 ± 2[(a)]	67 ± 2[(b)]	76 ± 3[(d)]	5

(a) $P < 0.001$ vs control; (b) $P \simeq 0.6$ vs control; (c) $P < 0.001$ vs control, < 0.05 vs decorticate; (d) $P < 0.01$ vs control.

Cortical lesions differently affect neuroleptic- and non-neuroleptic induced catalepsy in rats

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There is now reasonable evidence that the excitatory (frontal) cortico-striatal and cortico-nigral pathways are at least partially glutamatergic in nature (cf. Fonnum, Soreide, Kvale, Walker & Walaas, 1981). We have recently reported that bilateral lesions of these cortical efferents result in a decreased cataleptogenic activity of haloperidol and an increased ability of apomorphine to induce stereotypies, in rats (Bartholini, Lloyd, Scatton & Worms, 1981). In the present study, we have investigated the effect of bilateral lesions of the frontal cortex on the cataleptic state induced by cisflupenthixol, reserpine, tetrabenazine (TBNZ) and morphine in the rat.

Male Sprague-Dawley rats (Charles River, France) weighing 200 to 250 g were used. The frontal cortex was ablated bilaterally by gentle aspiration through a glass needle. Catalepsy was estimated (time remaining on four corks, maximum 120 s; Worms & Lloyd, 1979) every 30 min for 3 h, starting either immediately, or 2 h (reserpine), after intraperitoneal drug injection.

Three weeks after bilateral ablations of the frontal cortex, the catalepsy induced by cis-flupenthixol (0.8 mg/kg, ip) was reduced (as compared to sham-operated rats) by 52% (cumulated catalepsy time in s $= 378 \pm 33$ and 182 ± 36 in sham and lesioned rats, respectively; $P < 0.01$). Reserpine (2 mg/kg, ip) induced catalepsy was diminished by 31% as compared to sham-operated rats (cumulated catalepsy time in s $= 276 \pm 35$ and 191 ± 29 in sham and lesioned rats, respectively; not significant). Catalepsy times after TBNZ (3 mg/kg, ip) were 150 ± 36 and 205 ± 41 s in sham and lesioned rats respectively. Finally, morphine (25 mg/kg, ip) induced catalepsy was not modified by this lesion (2 h cumulated catalepsy times: 174 ± 27 and 180 ± 41 s in sham and lesioned rats, respectively).

These data indicate that: (1) the catalepsy due to postsynaptic blockade of dopamine (DA) receptors (cis-flupenthixol; haloperidol, see Bartholini *et al.*, 1981) is more sensitive to the impairment of cortical projections than is the catalepsy induced by presynaptic depletion of DA (reserpine; TBNZ); (2) morphine-induced catalepsy is not dependent on the integrity of the frontal cortex, thus confirming that this effect of morphine is not localized in the striatum, but more probably in other brain regions not innervated by frontal-cortical efferents, e.g. the reticular formation (Dunstan, Broekkamp & Lloyd, 1981).

Thus, these results suggest that the cortical influences on DA-mediated events are preferentially exerted distally to DA nerve terminals, quite possibly in the striatum; in fact, cortical lesions do not alter DA turnover (Bartholini *et al.*, 1981) but diminish the turnover of acetylcholine (Wood, Moroni, Cheney & Costa, 1979) in the striatum.

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Buprenorphine-induced hyperthermia in rats: selective development of sensitization and tolerance

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We studied the effects of single and multiple doses of buprenorphine on the body temperature of male Sprague-Dawley rats (250–300 g) at an ambient temperature of 20°C. Rectal temperatures were taken with a thermistor probe at 0, 30, 60, 90, 120, 180 and 240 min after s.c. injection of either saline or buprenorphine (0.001, 0.01, 0.10, 0.30, 1, 3, 10 and 30 mg/kg; $n=4$). Buprenorphine raised body temperature at all doses tested (but had no marked effect at any dose if the animals were restrained). The magnitude of the hyperthermia increased with dose up to 10 mg/kg. The maximum mean rises obtained with 10 and 30 mg/kg levels were 1.83 ± 0.24°C (s.e.) at (90 min) and 1.73 ± 0.20°C (at 120 min), respectively. When the rats were rechallenged with the same doses of buprenorphine 24 h later, the lower doses (0.001 to 0.10 mg/kg) gave increased hyperthermias whereas acute tolerance developed to the higher doses (1 to 30 mg/kg).

Additional rats ($n=8$) were injected at 16.00 h daily with saline, or representative low (0.10 mg/kg) and high (3 mg/kg) doses of buprenorphine. Interestingly, complete tolerance to the hyperthermia occurred over 7 injections of the 3 mg/kg dose whereas sensitization developed at the 0.10 mg/kg level over 9 injections.

We conclude that (i) contrary to our previous experience with morphine where both hyperthermia (2.5–10 mg/kg) and hypothermia (20–40 mg/kg) were obtained in unrestrained Sprague-Dawley rats, buprenorphine induces only hyperthermia over a 30,000-fold dose-range; (ii) on the basis of tolerance studies, the hyperthermic effects of low and high doses of buprenorphine are mediated differently; however, there is a common link since pretreating rats with naloxone (3 mg/kg, s.c. at −15 min and −1 min) can attenuate both types of hyperthermia.

Effects of caffeine and chlor-desmethyldiazepam and their interaction on rota-rod performance and Y-maze in mice

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Interactions between acutely administered stimulants of the caffeine type and central nervous system depressants of the benzodiazepine type have been studied in a variety of behavioural situations (Traversa, de Angelis, Bertolissi, Nardini & Vertua, 1981). In light of our previous results, the present study was undertaken to better characterize the changes in the motor activity and in the exploratory behaviour that occur as result of an acute i.p. treatment of caffeine and a new benzodiazepine, chlor-desmethyldiazepam (Cl-DMDZ) administered separately or in combination. The first series of experiments was performed by using a rota-rod test (Dunham & Miya, 1957). The second series of experiments utilized the Y-maze (Soubrie, Thiebot, Simon & Boissier, 1977).

With respect to the rota-rod test, mice were selected as described below and tested 60 min after drug administration. Animals falling from the rod (5 rpm) 3 times within 40 s were considered as negative. Mice staying for 180 s on the rolling rod were considered as positive. Caffeine, as the free base (2.5, 5.0, 1.0, 1.25, 0.63 mg/kg) increased ($P<0.001$) rota-rod performance in negative mice, especially at intermediate doses. Chlor-desmethyldiazepam (2.50, 1.25, 0.63, 0.32 mg/kg) decreased ($P<0.01$) rota-rod performance in positive mice. Combining a fixed dose of caffeine with various doses of Cl-DMDZ, the benzodiazepine-induced decrease of the performance was antagonized by caffeine.

The exploratory behaviour in naive mice was measured in a Y-maze ($27 \times 12 \times 4.5$ cm) under two experimental conditions: aggregated and isolated (7 days) animals. Exploration (maze arm entries), habituation and spontaneous alternation were scored for 5 min at each trial. In controls no statistical difference in the above mentioned parameters was seen between aggregated and isolated mice. In aggregated mice, caffeine (40, 20, 10, 5.0 mg/kg) produced no significant variation in the activity scores. In isolated mice, the stimulant induced an increase ($P<0.01$) in

exploration only at 20 and 40 mg/kg. After Cl-DMDZ administration (1.25, 0.63, 0.32 mg/kg), exploration was higher ($P < 0.001$) in aggregated than in isolated mice. An increase ($P < 0.001$) in the habituation index and no variation in the alternation index was found in both experimental conditions. Both in aggregated and isolated animals, the association of caffeine with chlor-desmethyldiazepam showed that the stimulant may decrease, increase or not change the response obtained with Cl-DMDZ alone according to the particular dose combinations.

These data support other studies (Traversa, de Angelis & Vertua, 1980) that interactions between psychostimulants and benzodiazepines are complex and not predictable on the basis of the effects of the drugs administered singly.

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Changes in plasma noradrenaline concentration following intravenous isoprenaline in man and dogs

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Isoprenaline (IPN) was infused for periods of 5 min to test cardiovascular responsiveness in anaesthetized dogs (maximum dose 11.5 μg/min) and man (3 μg/min). A novel HPLC assay (Davies, Molyneux & Newman, 1981) was used to determine plasma levels in the last minute of infusion in two dogs and two men. Plasma noradrenaline (NA) rose with increasing isoprenaline infusion in both species (Table 1). No consistent changes in plasma adrenaline were observed. In one dog, it was found that the highest noradrenaline levels seen could be matched by exogenous noradrenaline infusion, sufficient to raise the mean arterial pressure by about 10 mm Hg.

There was no correlation between the noradrenaline increase and any fall in blood pressure due to isoprenaline. We do not think the increased noradrenaline resulted from sympathetic activity following baroreflex activation, although nitroprusside induced hypotension did produce similar noradrenaline levels.

Table 1

IPN infusion (μg/min)	*Plasma levels* (pmole/ml) *dog 1* NA	IPN	*dog 2* NA	IPN
0	4.6	*	4.6	*
1.0	8.5	1.3	8.6	1.5
2.25	12.8	3.5	7.3	3.4
4.75	15.0	5.0	9.1	10.2
11.50	18.6	20.5	12.8	20.8
	man 1		*man 2*	
0	1.0	*	0.7	*
1.0	1.0	*	1.5	*
2.0	1.2	*	1.8	1.5
3.0	3.1	2.8	2.8	2.8

*denotes non-detectable levels

In one dog, other possible mechanisms were investigated. To determine whether exogenous isoprenaline might have been blocking noradrenaline uptake processes, the neuronal and extraneuronal uptake of noradrenaline was blocked with a single intravenous injection of desmethylimipramine (DMI, 10 mg/kg) and normetanephrine (NMN, 50 mg/kg) (Benedict, Fillenz & Stanford, 1978). Following this, plasma noradrenaline rose to 92 pmole/ml, thereafter falling exponentially with a

half-life of 20 min. This fall was unaffected by subsequent isoprenaline infusion which produced the same plasma levels as before. Exogenous noradrenaline infusion resulted in very high plasma levels but no rise in blood pressure after DMI and NMN. Thus it appears that isoprenaline is not affected by the blocked noradrenaline uptake processes.

Finally, a single intravenous injection of salbutamol (5.6 μg/kg) was followed by a brief rise in plasma noradrenaline to a level of 12.6 pmole/ml, suggesting that in the dog presynaptic β-receptor mediated noradrenaline release (Stjarne & Brundin, 1975) could have produced the plasma levels seen following isoprenaline infusion.

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Effects of indomethacin on blood pressure, catecholamine release and adrenal blood flow in the anaesthetized, laparotomized dog

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A problem in studying adreno-medullary reflexes is the massive fall in blood pressure following laparotomy and handling of the viscera. Terragno *et al.* (1977) reported that laparotomy causes release of prostaglandins and that indomethacin, given after laparotomy, restores the blood pressure. We gave indomethacin (5 mg/kg) in divided doses to dogs before and after laparotomy. This enabled us to maintain a mean blood pressure around 100 mmHg for up to 3 h, whereas without indomethacin it was difficult to maintain a blood pressure of 70 mmHg.

In contrast to Feuerstein *et al.*'s (1979) results in the cat, indomethacin did not affect the amounts of adrenaline or noradrenaline released at rest or in response to chemoreceptor or barareceptor stimulation. Indomethacin did however reduce both the resting adrenal blood flow and the increase in blood flow during the reflex release of catecholamines.

The depression of the resting pressure flow curve (see Figure 1a) suggests the involvement of prostaglandins in maintaining adrenal blood flow. Figure 1b was derived using these curves to correct the flow changes accompanying the reflex rise in blood pressure. The results in Figure 1b after indomethacin imply a marked vasoconstriction associated with the

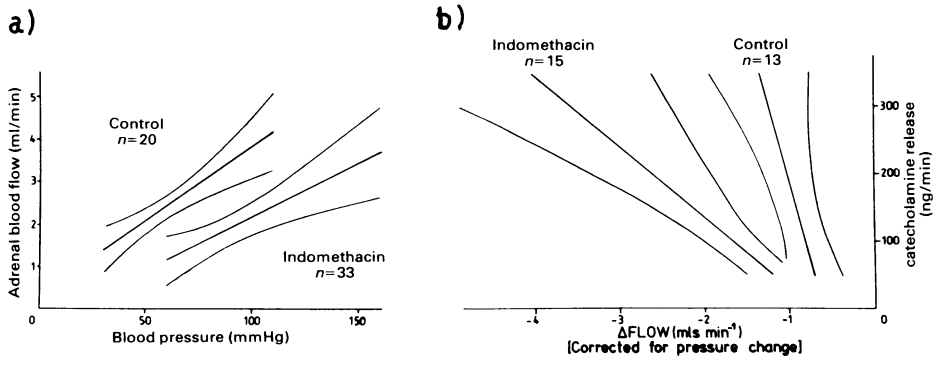

Figure 1 a) Resting pressure flow curves for left adrenal gland with and without indomethacin. b) Regression of change in adrenal flow (corrected for pressure from Figure 1a) on catecholamine release. All lines show 95% confidence limits.

release of catecholamines which is not seen without indomethacin. This suggests that prostaglandins are released with catecholamines and maintain adrenal blood flow by opposing their constricting action. This may also explain why platelet aggregation due to high concentrations of catecholamines does not occur in adrenal veins.

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A possible mechanism for the action of atenolol on the baroreceptor reflex in the anaesthetized cat

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Acute administration of the β-adrenoceptor blocking drug, atenolol (3 mg/kg), attenuates the reflex responses of the sympathetic nerves to changes in blood pressure (Scott, 1981). β-Adrenoceptor antagonists are known to influence the release of renin from the kidney (e.g. Loeffler, Stockigt & Ganong, 1972) and it is thought that changes in the plasma renin activity may modulate the baroreceptor reflex (e.g. Clough, Conway, Hatton & Scott, 1979). To determine whether the attenuation of the baroreceptor reflex produced by atenolol is mimicked by changes in the release of renin, the plasma renin activity was reduced by tying the renal arteries and veins.

Ten cats were anaesthetized with α-chloralose (80 mg/kg i.p.) and artificially ventilated. Body temperature and the pH, pCO_2 and pO_2 of the arterial blood were monitored and maintained within normal limits. In seven cats, loose ties were placed around both renal arteries and veins and, in a further three cats, around the mesenteric artery and vein. Recordings were made of the sympathetic efferent discharge (SED) from few-fibre preparations of the lumbar, splanchnic or renal nerves. Blood pressure was raised or lowered by the administration of phenylephrine (2–10 μg/kg) or glyceryltrinitrate (2–20 μg/kg) respectively. The sympathetic nervous response to changes in blood pressure was investigated both before and for 2 h after tightening the ligatures.

Before tying off the renal arteries and veins, the mean increase in the SED as a result of the injection of glyceryltrinitrate was 0.33 ± 0.057 impulses s^{-1} $mmHg^{-1}$ fall in the blood pressure (mean ± s.e.mean range 0.165 to 0.609 impulses/s; $n = 7$). Thirty min after tying off the kidneys, there was no significant change in the responses of the sympathetic nerves to falls in blood pressure. The mean change in the SED was 0.25 ± 0.081 impulses s^{-1} $mmHg^{-1}$ (range 0.049 to 0.46 impulses s^{-1} $mmHg^{-1}$). However, 60 min after tying off the kidneys there was a significant reduction ($P < 0.025$) in the responses of the sympathetic nerves to a fall in blood pressure. The mean change in the SED was 0.083 ± 0.059 impulses s^{-1} $mmHg^{-1}$ (range −0.082 to 0.389 impulses s^{-1} $mmHg^{-1}$).

In control experiments in three cats, tying off the mesenteric vessels did not result in any attenuation of the sympathetic nervous response to a fall in blood pressure.

Inhibition of SED occurred in all cats in response to raising blood pressure and this was unaffected by tying off the kidneys.

It is concluded that a substance, possible renin, released from the kidney is able to modify the sympathetic nervous response to a fall in blood pressure. It is therefore suggested that the β-adrenoceptor antagonist, atenolol, may exert at least some of its effects on the sympathetic nerves by reducing plasma renin levels.

I would like to thank I.C.I. Pharmaceuticals Division and the British Heart Foundation for their generous support and Mrs Sheila Millward for her invaluable technical assistance.

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Urethane attenuates pressor responses produced by α-adrenoceptor agonists and some other vasoconstrictor agents in pithed rats.

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We have previously suggested that in urethane-anaesthetized rats oxymetazoline and clonidine were less effective in producing bradycardia than in pentobarbitone-anaesthetized rats because urethane inhibited the neuronal α_2-adrenoceptor-mediated mechanism responsible for this response (Armstrong, Cavero & Lefèvre-Borg, 1980). There is evidence that in rats α_2-adrenoceptors are also located in blood vessels the stimulation of which evokes an increase in blood pressure (Drew & Whiting, 1979). Therefore, our previous study has now been extended to the effect of urethane on the pressor responses produced by several vasoconstrictor agents.

Male rats (Sprague Dawley, Charles River France) weighing 230–250 g were pithed during a brief period of ether anaesthesia and artificially ventilated with room air. Recordings were made of the mean carotid arterial blood pressure. (Armstrong *et al.*, 1980.) After a period of stabilization, the animals were given either urethane (1.2 g/kg) or, in parallel preparations, distilled water (0.1 ml/kg) by intraperitoneal injection. Fifteen min later both preparations received a series of progressively increasing cumulative intravenous doses of one of the following agents: the preferential α_2-adrenoceptor agonist M-7 (Drew, 1980); the α_1- and α_2-adrenoceptor agonists oxymetazoline (OXY), clonidine (CL) and noradrenaline (NE) (Docherty & McGrath, 1980); the selective α_1-adrenoceptor agonists phenylephrine (PHE) and cirazoline (CIR) (Roach, Lefèvre-Borg & Cavero, 1978); and also angiotensin II (ANG) and 5-hydroxytryptamine (5-HT). Linear regression analysis was used to calculate the slope and the dose needed to produce a pressor response of 60 mmHg (ED_{60}). The maximum response produced by the highest agonist dose injected was measured (E_{max}). The results shown in the following table are mean values (± s.e.mean) of the parameters characterising the dose-response curves.

The pressor-response curves for the adrenoceptor agonists and angiotensin II were shifted to the right in rats given urethane. In the case of M-7 the maximum response was also markedly depressed. Pressor responses elicited by 5-hydroxytryptamine were not significantly affected.

These results indicate that urethane, in the dose often used to anaesthetize rats, depresses pressor responses produced by a variety of agents. The responses most inhibited were those produced by α_2-adrenoceptor agonists. Thus the use of urethane as an anaesthetic agent should be avoided for studies of agents acting at both pre- and postsynaptically located α_2-adrenoceptors.

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Table 1

	Control (n = 4–6)			*Urethane* (n = 5–6)			*Ratio*
	Slope	ED_{60} (μg/kg, i.v.)	E_{max} (mmHg)	*Slope*	ED_{60} (μg/kg, i.v.)	E_{max} (mmHg)	ED_{60U}/ED_{60C}
M-7	47±4	6.5±1.0	107±2	14±2*	–	62.0±2.0*	–
OXY	60±6	0.6±0.1	125±5	59±6	3.0±0.5*	112±5	5.1±1.0
Cl	51±3	2.4±0.2	107±3	51±1	11.7±1.0*	91±3*	4.9±0.3
NE	46±3	0.3±0.1	127±2	42±1	1.1±0.1*	127±4	4.1±0.4
ANG	60±7	0.3±0.06	119±5	62±5	0.9±0.2*	112±8	2.8±0.6
PHE	59±4	3.6±0.6	116±5	66±9	9.7±2.0*	115±4	2.7±0.7
CIR	107±7	0.9±0.1	142±2	116±7	1.5±0.1*	138±3	1.8±0.1
5-HT	71±7	46.0±7.0	96±5	75±4	60.0±5.0	90±4	1.3±0.1

*significantly different from control value ($P < 0,05$, unpaired t-test).

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Pharmacological mapping in the rabbit heart

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A method for studying the relative effects of drugs in various parts of the rabbit heart reveals that different regions exhibit markedly different responses to the same drug. Contractions of atrial and papillary muscles were measured, and intracellular potentials were recorded from the sinoatrial node (SAN) and atrial muscle, and from His bundle, terminal Purkinje and ventricular cells, before and after exposure to two drugs, alinidine and melperone. Both these compounds produce bradycardia in isolated hearts, but our SAN records have indicated that they do so by entirely different mechanisms. The increase in spontaneous cycle length induced by melperone (2.7 μM) of 66 ms was almost accounted for by an increase in SAN action potential duration (APD) of 58 ms, without any change of maximum diastolic depolarization or 'take-off' potential. The slope of the slow diastolic depolarization was only reduced significantly by much higher concentrations of melperone. In contrast, a lower concentration of alinidine (1.0 μM) induced a comparable increase in cycle length (56 ms), which was attributable mainly to a reduction (−34%) in the rate of slow diastolic depolarization, and only a small increase (18 ms) in APD (Millar & Vaughan Williams, 1981).

Even within the ventricular conducting system melperone had quantitatively different effects at proximal and distal sites. Melperone 10.7 μM prolonged action potential duration, measured at 90% of repolarization (APD_{90}) by 54% in the bundle of His and by 40% in ventricular muscle. In contrast, in the terminal Purkinje cells, such as are commonly used for electrophysiological studies under voltage clamped conditions, APD_{90} was increased by only 16%. The same concentration of melperone depressed the maximum rate of depolarization by 13% in ventricular muscle, but had an almost threefold greater (36%) effect in the bundle of His. Since the bundle of His appeared to be more sensitive than other tissues to the two classes of antiarrhythmic action possessed by melperone (depression of MRD and prolongation of APD) it would be of interest to study the effect of melperone in human arrhythmias involving the bundle, such as supraventricular tachycardias. Both melperone and alinidine have been shown to be antiarrhythmic (Petersen, 1978; Allen *et al.*, 1981), and both cause bradycardia, but differ in their electrophysiological effects in similar cells, and each drug has quantitatively different effects in different parts of the heart.

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In vitro and *Ex vivo* examination of irreversible antagonism of cardiac β-adrenoceptors

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The β-adrenoceptor antagonist Ro 03-7894 has been shown to behave in an apparently irreversible manner both *in vitro* (Nicholson & Broadley, 1978) and *in vivo* (Nicholson, Broadley, Burden & Natoff, 1979). We have further examined the *in vitro* antagonism by Ro 03-7894 of the responses of guinea-pig left atrial tension, right atrial rate and left ventricular papillary muscle tension.

The tissues were suspended in Krebs-bicarbonate solution gassed with 5% O_2 in oxygen at 38°C and incubated with metanephrine (10^{-5} M) throughout to inhibit extraneuronal uptake. Left atria and papillary muscles were paced at 2 Hz (threshold voltage + 50% and 5 ms pulse width) and isometric tension recorded. Cumulative dose-response curves to orciprenaline were constructed. The tissues were then incubated with Ro 03-7894 (7.6 or 3.8×10^{-4} M) for 30 min and after washout over 3 h, the orciprenaline was repeated. Corrections were made from control experiments performed identically but without antagonist. The orciprenaline maxima were significantly ($P<0.05$) reduced to $66.6 \pm 5.2\%$, $42.3 \pm 8.3\%$ and $66 \pm 3.9\%$ respectively in right and left atria and papillary muscles. Thus Ro 03-7894 exerts apparent irreversible β-adrenoceptor antagonism by its resistance to washout and depression of the maxima. We next examined Ro 03-7894 by an *ex vivo* method whereby animals were pretreated and cardiac β-adrenoceptors were subsequently analysed pharmacologically and by radioligand binding. Guinea-pigs were pretreated with Ro 03-7894 (10 mg/kg s.c.) at 24 h before sacrifice. The three cardiac preparations were removed and dose-response curves for isoprenaline, orciprenaline and salbutamol constructed (in that order). Control tissues were removed from untreated animals. Responses were expressed as a percentage of the isoprenaline maximum. Dose-response curves to all three agonists in tissues from Ro 03-7894-pretreated animals were displaced to the right of those in control tissues.

The EC_{50} values for isoprenaline in left atria (10.2 nM, $P<0.05$), right atria (7.8 nM, $P<0.001$) and papillary muscles (9.6 nM, $P<0.01$) after pretreatment were significantly greater than in control tissues (5.1, 2.6 and 3.5 nM respectively). The maximum responses of orciprenaline and salbutamol were consistently less than isoprenaline in left (87.7 ± 3.8 and $12.3 \pm 1.9\%$) and right atria (95.5 ± 1.8 and $47.3 \pm 3.2\%$) and papillary muscles (96.3 ± 1.9 and $31.7 \pm 2.9\%$). In preparations from Ro 03-7894-pretreated guinea-pigs, these maxima were further reduced to 64.6 ± 7.8 and $3.7 \pm 1.1\%$, 92 ± 3.1 and $33 \pm 3.1\%$ and 84 ± 4.7 and $12.7 \pm 3.4\%$, only orciprenaline on right atria being non-significant.

For comparison with the pharmacological data, β-adrenoceptor binding was assessed by a modified method of Nahorski (Rugg, Barnett & Nahorski, 1978) using ventricular membrane fractions obtained simultaneously from the same hearts. Scatchard analysis of the saturation curves for [^{3}H]-dihydroalprenolol binding indicated a significant ($P<0.05$) decrease in binding sites in the pretreated hearts (B_{max}, 101 ± 4.3 fmol/mg protein; $n=4$) compared with untreated hearts (B_{max}, 124 ± 6.7 fmol/mg protein; $n=6$). No change in dissociation constant was observed (K_D, 6.3 ± 0.7 and 6.7 ± 1.3 nM respectively). These pharmacological and radioligand binding data suggest that β-adrenoceptor antagonism by Ro 03-7894 persists at least 24 h after administration and are consistent with an irreversible or slowly dissociable blockade of cardiac β-adrenoceptors.

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Pharmacological studies with bopindolol: a new long acting β-adrenoceptor antagonist

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In 1974 Clark *et al.* described the pharmacological properties of LL 21-945 a long acting non-selective β-adrenoceptor antagonist bearing a pivaloyl ester on the β-carbon atom of the propanolamine side chain. This report describes the pharmacological properties of bopindolol (4-[benzoyloxy-3-tert-butylaminopropoxy]-2-methylindole hydrogen malonate) which is an indole β-adrenoceptor antagonist bearing a benzoyl ester residue on the β-carbon atom of the propanolamine side chain.

In guinea-pig isolated atria (Saameli, 1972) bopindolol at concentrations between 3.2×10^{-9} and 2×10^{-6} M produced an inhibition of the positive chronotropic and inotropic effects of adrenaline and was found to be 17 times more potent than propranolol as a β-adrenoceptor antagonist.

In normal anaesthetized cats ($n = 3$/dose) which had been vagotomized and spinalized by ligation of the cord at the level of the second cervical vertebra, bopindolol in doses between 4 and 2500 μg/kg i.v. produced modest increases in heart rate and myocardial contractile force (maximum effects being 24 ± 3 beats/min and 34 ± 7% respectively). In similar experiments pindolol also produced positive chronotropic and inotropic effects but propranolol did not show sympathomimetic activity.

In pentobarbitone anaesthetized dogs ($n = 5$/dose) bopindolol in doses of 8, 16 and 32 μg/kg i.v. produced a dose-dependent inhibition of isoprenaline-induced (0.5 μg/kg i.v.) tachycardia and was approximately four times more potent than propranolol. The inhibition of the depressor effects of isoprenaline parallelled that of the cardiac effects indicating that the β-adrenoceptor blockade is non-selective. The β-adrenoceptor blockade was slow in onset, maximum effects being attained 75–155 min after injection, and persisted without evidence of decline for the remainder of the 5 h experimental period.

The duration of action of bopindolol was further investigated following intravenous administration of doses between 3 and 300 μg/kg in the conscious beagle dog ($n = 3$/dose). The maximum inhibition of isoprenaline-induced tachycardia occurred 1–4 h after drug administration and persisted for more than 24 h. In similar experiments in which pindolol and propranolol were tested in equipotent doses β-adrenoceptor blockade was no longer detectable 24 h after drug administration.

Since the drug exerts a long duration of action the possibility of a cumulation of the pharmacological effect was investigated in experiments in which doses of 10 or 30 μg/kg/day i.v. were administered to groups of 2 conscious beagle dogs for a period of 10 days. With neither of the doses was there any evidence of a cumulation of the effect during the treatment period and the response to isoprenaline had returned to normal 3 days after cessation of treatment.

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Structure-activity relations for negative chronotropic action of adenosine in rat atria

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Adenosine has negative chronotropic and inotropic actions on the heart. The aim of the present study was to determine the structure-activity relations for the negative chronotropic action of adenosine in rat atria as such data is required for the classification of the adenosine receptor mediating this effect.

Rat isolated atria were suspended in medium at 30°C and their spontaneous rate of beating recorded. The concentration inhibiting this rate by 30% (IC_{30}) was determined and expressed as the logarithm of

this concentration. The IC_{30} for adenosine was −4.54.

Absence of the 2′ hydroxyl group (2′-deoxyadenosine) and substitution at the 2′ and 3′ positions (2′,3′-diacetyladenosine; 2′,3′-isopropylideneadenosine) resulted in a loss of activity. Substitution at the 5′ position was compatible with a high level of activity (adenosine 5′-cyclopropylcarboxamide, $IC_{30} = -7.57$; adenosine 5′-acetate, $IC_{30} = -5.15$).

Halide substitution at C^2 of the purine moiety resulted in potent analogues (2-chloradenosine, $IC_{30} = -6.42$). Absence of an amino group at C^6 of the purine moiety resulted in loss of activity (inosine; 6-mercaptopurine riboside; 6-methoxypurine riboside). Benzyl, phenyl or phenylisopropyl substituents at N^6 resulted in analogues with increased potency (N^6-benzyladenosine, $IC_{30} = -5.23$; N^6-phenyladenosine, $IC_{30} = -7.34$). The L isomer of N^6-phenylisopropyladenosine (L-PIA) was considerably more potent than the D-isomer (D-PIA) ($IC_{30} = -8.24$ and -6.45 respectively). Bromide substitution at C^8 resulted in loss of activity.

The present study has shown that the negative chronotropic action of adenosine in rat atria requires hydroxyl groups at the 2′ and 3′ positions and an amino group at C^6. Substitutions at the 5′, C^2 and N^6 positions are compatible with activity. These findings indicate that adenosine acts at an R site (Londos & Wolff, 1977) to produce this effect. The rank order of potency with L-PIA > adenosine 5′-cyclopropylcarboxamide > 2′-chloroadenosine = D-PIA ⩾ adenosine and the marked difference in potencies of L-PIA and D-PIA suggests that adenosine act at an adenosine receptor of the A_1 sub-type (van Calker, Müller & Hambrecht, 1978; Daly, Bruns & Snyder, 1981) in rat atria.

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Marked suppression by nifedipine, niludipine and nisoldipine of ventricular fibrillation resulting from acute myocardial ischaemia

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In a previous communication to the Society (Kane, McDonald & Parratt, 1981) we described the protective effect of low-dose nifedipine against the early, life-threatening ventricular arrhythmias that result from acute coronary artery ligation in anaesthetized rats. A comparision has now been made between this antiarrhythmic effect of nifedipine and that of two structurally similar 'calcium antagonists', niludipine and nisoldipine (Kazda, Garthoff, Meyer, Schlossmann, Stoepel, Towart, Vater & Wehinger, 1980).

The model was that previously described (Kane, McDonald & Parratt, 1979; Clark, Foreman, Kane, McDonald & Parratt, 1980). In this model ventricular ectopic activity is pronounced between 5 and 25 min post-ligation and there are periods of both ventricular tachycardia (VT) and fibrillation (VF); this is often spontaneously reversible. After 25 min there are few ventricular ectopic beats (VEB's) until about 60–90 min post-ligation (Clark *et al.*, 1980). The drugs were given intravenously 15 min prior to ligation and the results of this treatment on the incidence and severity of early ventricular arrhythmias are given in Table 1. Although the number of VEB's was not significantly reduced (except by niludipine) there was a marked reduction in the duration of VF (Table 1). All three drugs were also highly effective if given orally, in a dose of 3 mg/kg, 1–1.25 h before ligation (30–45 min before anaesthesia). In this more severe model the duration of VF was reduced by all three drugs by 74–92%; none of the treated animals died compared with a mortality of 40% in the control group.

These findings may have important clinical reper-

Table 1 The effect of calcium slow-channel blocking drugs on early post-infarction ventricular arrhythmias in anaesthetized rats (mean ± s.e., $n = 10$ for each group)

	Total no. of VEB's	*Duration(s) of: VT*	*VF*	*Incidence of VF (and mortality) %*
Solvent controls	1494 ± 546	66 ± 8	48 ± 9	50*(10)
Nifedipine (5 μg/kg)	1129 ± 214	67 ± 4	0*	0* (0)
Nifedipine (10 μg/kg)	558 ± 79	27 ± 5	0*	0* (0)
Niludipine (10 μg/kg)	267 ± 45*	5 ± 2*	8 ± 1*	20 (0)
Niludipine (50 μg/kg)	188 ± 64*	5 ± 2*	(2.5)	10* (0)
Nisoldipine (5 μg/kg)	892 ± 212	45 ± 7	21 ± 4	0* (0)
Nisoldipine (50 μg/kg)	456 ± 118	17 ± 4*	0*	0* (0)

*$P < 0.05$

cussions. If given to patients after a first myocardial infarction these drugs might reduce both the likelihood of sudden cardiac death from fibrillation and the degree of myocardial ischaemic damage resulting from a subsequent infarction. Certainly both these beneficial actions can be demonstrated in animal models.

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Action of Ketanserin, a new 5-hydroxytryptamine antagonist, on human isolated blood vessels

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Ketanserin, 3-(2-(4-(4-fluorobenzoyl)-1-piperidinyl)ethyl)-2, 4(IH, 3H)-quinazolinedione, is a new 5-hydroxytryptamine (5-HT) antagonist with a spectrum of activity which does not reflect the 5-HT receptor classification of Gaddum & Picarelli (1957) but may be consistent with recent studies which indicate that vascular 5-HT receptors are more varied than was previously recognized (e.g. Apperley, Feniuk, Humphrey & Levy, 1980). We have therefore investigated the nature of 5-HT antagonism by ketanserin on human vasculature.

Gastric and colonic arteries and veins and saphenous veins, obtained from surgical operations, were cut spirally to produce muscle strips which were suspended in Krebs' solution (37°C, 5% CO_2 in O_2, 0.5 g load). Contractions to 5-HT were recorded through isotonic transducers connected to pen recorders as described previously (Grimmer & Leathard, 1981).

Ketanserin, caused rapid antagonism of 5-HT, its effects being maximal within 10 min. The antagonism appeared to be non-competitive and insurmountable in each type of vessel studied. Therefore, to assess the antagonism quantitatively IC_{50} values (concentrations producing 50% inhibition of 5-HT

Table 1 Molar concentrations of ketanserin which cause 50% inhibition of 5-HT in various human blood vessels. *P* calculated using Mann-Whitney *U*-test.

Vessel	*n*	IC_{50} (M)	*Interquartile range*	*P*
Gastric Artery	10	1.6×10^{-8}	$5.1 \times 10^{-9} - 3.7 \times 10^{-8}$	> 0.05
Colonic Artery	10	3.2×10^{-8}	$5.8 \times 10^{-9} - 5.3 \times 10^{-8}$	
Gastric Vein	11	5.6×10^{-9}	$2.0 \times 10^{-9} - 2.2 \times 10^{-8}$	< 0.01
Colonic Vein	13	2.5×10^{-8}	$6.6 \times 10^{-9} - 1.3 \times 10^{-7}$	
Saphenous Vein	11	7.6×10^{-8}	$2.5 \times 10^{-8} - 1.1 \times 10^{-6}$	< 0.01

responses) were determined by recording contractions to repeated submaximal doses of 5-HT in the absence and presence of increasing concentrations of ketanserin. Simultaneous control experiments showed that repeated doses of 5-HT produced reproducible contractions.

The values obtained (Table 1) indicate that ketanserin is equipotent in arteries from the two regions studied whereas the effectiveness on veins differs between regions ($P < 0.01$, Mann-Whitney *U*-test), possibly suggesting differences in receptor populations. A further difference was the observation of slight stimulant activity of ketanserin quite frequently on saphenous but rarely on mesenteric veins. On saphenous veins methysergide too caused contraction as well as non-competitive 5-HT antagonism ($IC_{50} = 5.6 \times 10^{-7}$ M, $9.8 \times 10^{-8} - 1.1 \times 10^{-6}$ M, $n = 6$), the apparent agonist activity being more marked than with ketanserin. The receptors here may resemble those described in dog saphenous vein (Apperley *et al.*, 1980), but our results have also shown differences in the mesenteric vessels which require further investigation.

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Comparative effects of bepridil and verapamil on isolated coronary and systemic arterial smooth muscle

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Previous investigations into the slow-channel blocking effects of bepridil in cardiac and intestinal smooth muscle of the guinea-pig have suggested that this agent differs from classical 'Ca^{2+} antagonists' in that the inhibition is only partial (Vogel *et al.*, 1979) and follows non-competitive kinetics (Labrid *et al.*, 1979). However, since the profile of 'Ca^{2+} antagonists' has been shown to depend crucially upon the actual tissue and physiological medium used (Spedding, 1981; Sanner & Prusa, 1981) it was of interest to compare the actions of bepridil and verapamil on isolated vascular smooth muscle from the rabbit (aortic strip) and pig (coronary artery strip) under different experimental conditions.

In rabbit aortic strips, bathed in Krebs-Henseleit solution (37°C), both bepridil (1.24 and 12.4 μM) and verapamil (0.11 and 1.1 μM) inhibited contractile responses to KCl (20 to 300 mM) and verapamil (1.1 μM) also caused a rightward shift of dose-response curves to noradrenaline. Neither drug depressed the maximum response in this case.

Cumulative Ca^{2+} dose-response curves were obtained in K^+-depolarized rabbit aortic strips (Broeckaert & Godfraind, 1979). Both drugs inhibited these responses in an apparently non-competitive manner as shown for bepridil in Figure

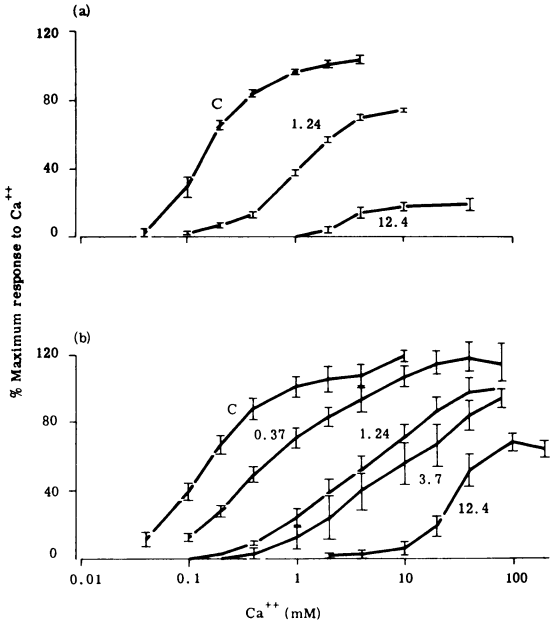

Figure 1 Log dose-response curves to calcium in K^+ depolarized rabbit aortic strips bathed (a) in bicarbonate-buffered or (b) Tris-buffered Krebs. Curves were obtained in the absence (c) and in the presence (μM) of bepridil. Each point is mean ± s.e.mean of 3–6 observations.

1a. When these experiments were repeated in a tris-buffered medium, the dose-response curves obtained in the presence of either drug indicated competitive antagonism (Figure 1b). Schild analysis yielded pA_2 values of 6.71 (95% confidence limits, 6.45 to 7.24) for bepridil and 7.76 (7.37 to 8.82) for verapamil.

Both bepridil (0.075 to 2.5 μM) and verapamil (0.0066 to 0.22 μM) relaxed K^+-depolarized pig coronary artery strips bathed in Krebs solution containing 1 mM Ca^{2+} (Fleckenstein, 1976). The responses to bepridil were slower to develop. The relaxant effects were reversed by increasing the Ca^{2+}-concentration in the bathing medium to 6 mM.

In pig coronary artery strips, both drugs inhibited the contractile response evoked by anoxia in the presence of submaximal concentrations of noradrenaline (Van Neuten & Vanhoutte, 1980).

These results imply that in contrast to the situation in cardiac and intestinal smooth muscle, bepridil and verapamil show similar profiles with regard to coronary and systemic vascular smooth muscle. In addition, the composition of the bathing medium is an important consideration in studies designed to investigate 'Ca^{2+}-antagonism'.

Inhibition of anoxia-induced contractions in coronary arterial muscle by bepridil and verapamil may have clinical relevance to the treatment of coronary vasospasm.

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The K^+/Ca^{2+} ratio and calcium slow channel antagonists on arrhythmias in the isolated perfused coronary ligated rat heart

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Ligation of the main left coronary artery of the isolated rat heart perfused with Krebs-Henseleit solution (K^+, 5.9 mM; Ca^{2+}, 2.5 mM) produced a low incidence of ventricular arrhythmias, despite the appearance of profound ischaemic damage (Kannengiesser, Lubbe & Opie, 1975). However, occlusion of this coronary artery during perfusion with a lowered K^+ concentration (K^+, 2.5 mM; Ca^{2+}, 2.5 mM) produced ventricular arrhythmias which had a distinctive time course. The first 3 min post-ligation had a very low incidence of premature ventricular contractions (PVC's). PVC's then increased in incidence with a peak of instability occurring at 10 min. Hearts then reverted to a more stable state for the remainder of the 30 min of recording.

The incidence of electrical instability produced by ligation during perfusion with a lowered potassium concentration was significantly reduced by the concomitant reduction of the calcium concentration (K^+, 2.5 mM; Ca^{2+}, 1.2 mM) (see Table 1). Two calcium slow channel antagonists, verapamil and nifedipine, were used to determine whether the protective effect of low calcium perfusion could be mimicked by these drugs (see Table 2).

The highest concentration of verapamil used (1×10^{-7} M) produced a significant ($P<0.01$) reduction in the PVC's during ligation. Verapamil also reduced the incidence of ventricular tachycardia and ventricular fibrillation although these reductions did not attain statistical significance using chi-squared analysis. Nifedipine (1×10^{-7} M) did not effect any index of electrical instability during ligation. The solvent for nifedipine, ethanol (0.0007% v/v), similarly did not influence arrhythmia production.

In summary, the electrical instability produced by ligation during perfusion with a low potassium concentration may be reduced by a lowered calcium concentration, as has been shown in non-ischaemic tissues (Eisner & Lederer, 1979). Although verapamil exerted a protective effect, this may be due to its local anaesthetic properties (Bayer, Kalusche, Kaufmann & Mannhold, 1975), since nifedipine did not influence the incidence of arrhythmias.

Table 1 The effect of the K^+/Ca^{2+} ratio on the incidence of premature ventricular contractions (PVC's; mean ± s.e.mean), ventricular tachycardia (VT; percentage incidence) and ventricular fibrillation (VF; percentage incidence) during 30 min of ligation

Ionic milieu (mM) (Ca^{2+})	(K^+)	*n*	*PVC's*	*% VT*	*% VT*
2.5	5.9	6	4 ± 3	17	0
2.5	2.5	15	797 ± 101	47	27
1.2	2.5	12	21 ± 8	8	0

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Table 2 The effect of verapamil, nifedipine and nifedipine solvent (ethanol) on ventricular arrhythmias produced by ligation during perfusion with 2.5 mM K^+, 2.5 mM Ca^{2+}. Statistical difference from control denoted* ($P<0.01$) using Wilcoxon Rank Sum test.

Drug Treatment	*n*	*PVC's*	*% VT*	*% VF*
Verapamil 1×10^{-8} M	5	559 ± 151	60	0
Verapamil 1×10^{-7} M	8	297 ± 74*	13	0
Nifedipine 1×10^{-7} M	7	791 ± 164	43	14
Nifedipine solvent (Ethanol 0.0007% v/v)	8	600 ± 130	38	13

Coronary vasodilator- and benzodiazepine-inhibition of site-specific binding of nitrobenzylthioinosine, an inhibitor of nucleoside transport, to human erythrocytes

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Diazepam has been shown to potentiate adenosine action in the CNS (Phillis, 1979) and in peripheral neuroeffector systems (Clanachan & Marshall, 1980a, b), possibly via inhibition of adenosine transport. We have evaluated the affinities of several coronary vasodilators and benzodiazepines (BDZs) for the nucleoside transport system of human erythrocytes through inhibition of the site-specific binding of the nucleoside transport inhibitor, nitrobenzylthioinosine (NBMPR). Nucleoside transporter activity ceases when specific membrane sites, evidently on the transporter elements, are occupied by NBMPR and certain congeners (Cass, Gaudette & Paterson, 1974).

Washed human erythrocytes ($1.5-3 \times 10^7$) were incubated for 20 min at 25°C in Dulbecco's phosphate buffered saline (1 ml final volume) containing [G-^{3}H]NBMPR (0.25–5 nM) to determine cellular binding parameters for NBMPR. The inhibitory potencies of the coronary vasodilators and BDZs were determined by incubating cells with [G-^{3}H]NBMPR (1 nM) and graded concentrations of the test compounds.

As has been reported previously (Cass *et al.*, 1974; Jarvis & Young, 1980), the site-specific binding of NBMPR to human erythrocytes was to a single class of sites with a dissociation constant for NBMPR of 0.97 ± 0.13 nM ($n = 6$). The maximum number of binding sites per erythrocyte was $12{,}000 \pm 1100$. The coronary vasodilators (Figure 1) competitively inhibited the binding of NBMPR with an order of potency (dilazep > hexobendine > dipyridamole > lidoflazine > papaverine) similar to that found for their inhibition of adenosine transport (Mustaffa, 1979). The BDZs (Figure 1), in a range of concentrations (1–300 μM) which potentiates adenosine action *in vitro* (Clanachan & Marshall, 1980b), also competitively inhibited NBMPR binding (flunitrazepam = nitrazepam = diazepam = desmethyldiazepam > clonazepam > oxazepam > chlordiazepoxide > lorazepam > flurazepam).

The low affinity of the BDZs for the nucleoside transport system indicates that significant inhibition of nucleoside transport in erythrocytes is unlikely to occur following anxiolytic doses of the BDZs, but may occur at the higher plasma concentrations associated with the induction of anaesthesia. Potentiation of the extracellular effects of adenosine following transport inhibition may explain the well-documented coronary vasodilation following diazepam administration in man (Ikram, Rubin & Jewkes, 1973).

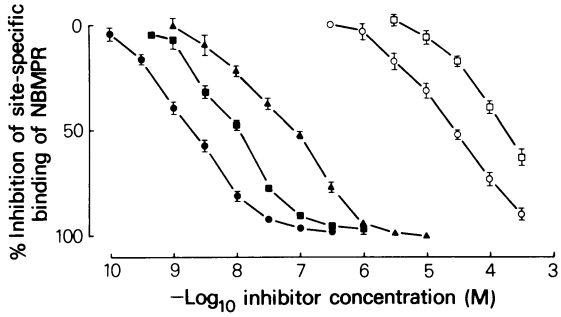

Figure 1 Percentage inhibition of site-specific binding (approximately 6000 molecules/cell) of NBMPR (1 nM) to human erythrocytes by different concentrations of dilazep (●). dipyridamole (■), lidoflazine (▲), diazepam (○), and lorazepam (□).

Assays, performed in 1.5 ml polypropylene centrifuge tubes, were initiated by addition of cells and terminated by centrifugation for 10 s in an Eppendorf 5412 microcentrifuge. Cells were washed once and digested prior to assay of [^{3}H] activity by liquid scintillation counting. Site-specific binding was defined as the difference between total binding and the binding which remained cell-associated when assays were conducted in the presence of a second nucleoside transport inhibitor, nitrobenzylthioguanosine (10 μM). Values shown are means ± s.e.mean, $n = 6$.

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