POSITIVE INOTROPIC AND CHRONOTROPIC EFFECTS OF NORADRENALINE WHICH CANNOT BE EXPLAINED BY α- OR β-ADRENOCEPTOR STIMULATION

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Selective α_1-adrenoceptor agonists such as methoxamine induce positive inotropic responses (Shibata et al. 1980). Whether the naturally occurring catecholamines adrenaline (AD) and noradrenaline (NA) also elicit α-mediated inotropy is questionable. Schümann (1983) claims that only AD stimulates α-adrenoceptors of rabbit papillary muscle. We have shown that AD exerts α-mediated positive inotropy of rat atria only in the presence of β-blockade by pindolol (Broadley et al. 1985). This study examines whether the same is true for NA.

Tension responses of paced left atria (2Hz, 5ms, threshold voltage + 50%) and rate responses of spontaneous right atria set up in Krebs-bicarbonate solution at 30°C gassed with 5% CO_2 in O_2 were recorded. One cumulative dose-response curve to NA was constructed in the absence or presence of pindolol (PIND) and/or prazosin (PRAZ). Metanephrine (10μM) and desipramine (1μM) were present throughout. Pindolol caused rightwards shifts of NA dose-response curves in left and right atria. The EC50 values in the presence of 1 and 3μM pindolol did not, however, differ significantly (P>0.05) in either atria (Table 1). This failure to further displace the curve with increase in antagonist concentration suggests that the

Table 1. Effects of pindolol and prazosin on noradrenaline EC50 values (μM)

Antagonist	n	Right Atrium	Left Atrium
None	6	0.0302(0.0199-0.0455)	0.0834(0.0312-0.222)
PIND 1μM	12	2.82(1.66-4.80)	4.47(2.15-9.29)
PIND 3μM	16	3.47(2.14-5.62)	2.88(1.91-4.36)
PIND 10μM	7	28.2(12.2-64.9)	26.3(10.6-65.2)
PIND 30μM	4	21.6(7.76-61.7)	24.3(5.13-112)
PRAZ 0.1μM	6	0.0258(0.0128-0.0518)	0.0448(0.0266-0.0752)
PIND 1μM + PRAZ 0.1μM	9	4.47(2.49-7.99)	5.37(2.51-11.5)
PIND 10μM + PRAZ 0.1μM	8	18.2(5.86-56.5)	25.7(9.12-72.4)

responses are no longer β-adrenoceptor-mediated. Since the same phenomenon occurred in left atria with AD, and the curve was displaced further by prazosin, it was considered that NA might also stimulate α-adrenoceptors. However, in the combined presence of pindolol (1μM) and prazosin (0.1μM) the EC50 values for NA were identical to those with pindolol alone. Furthermore, prazosin alone (0.1μM) did not antagonize NA.

To confirm this observation, an alternative experimental design was used, in which a curve to NA was followed after washout by one in the presence of antagonist. The mean ($n \geq 3$) dose-ratios at the EC50 for pindolol 1 and 3μM were not significantly different from each other in left (94.8±16.4 and 117.2±13.1) or right atria (44.7±5.9 and 42.7±9.6). Prazosin (0.1μM) and pindolol (1μM) produced no further antagonism (dose-ratios 111.6±30.8 and 43.5±3.4). Thus the responses to NA at pindolol 1μM were not antagonized further by either α- (prazosin) or β-blockade (pindolol (3μM).

On increasing the concentration of pindolol to 10μM there was further antagonism but at 30μM antagonism again halted. Here prazosin also failed to antagonize, indicating that unlike AD, NA does not exert cardiac effects via α-adrenoceptors even with substantial β-blockade. The reason for the secondary shift by pindolol remains obscure. It is possibly due to blockade by pindolol of receptor types other than typical α- or β-adrenoceptors.

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EVIDENCE FOR OPIATE AND DOPAMINE INTERACTION IN STRIATUM

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Recent studies using the selective D1 dopamine receptor antagonist, SCH23390, have shown that activation of adenylate cyclase in striatum involves contributions from both stimulatory (D1 receptor mediated) and inhibitory (D2 receptor mediated) influences (Onali et al, 1984). We have used SCH23390 to 'unmask' D2 receptor specific inhibition of adenylate cyclase in striatal membranes and to investigate possible interactions with the opiate receptor agonist, morphine.

Adenylate cyclase activity was determined with crude P2 preparations of rat striatal homogenates. Samples were assayed for cyclic AMP according to the method of Brown et al, 1971.

Morphine (0.1-1000μM) reduced basal adenylate cyclase activity in a dose dependent manner producing a maximum inhibition of 30-40%. The inhibition produced by $\geqslant$10μM morphine was statistically significant ($p \leqslant 0.05$; n=3-6) and was dose-dependently reversed by naloxone (0.1-100μM).

Dopamine (1-300μM) activated adenylate cyclase in a dose-dependent manner ($\geqslant$3μM, $p \leqslant 0.05$; n=6). In the presence of 0.1μM SCH23390, dopamine significantly inhibited enzyme activity ($\geqslant$10μM dopamine, $p \leqslant 0.05$; n=6). The maximum inhibition observed was of the order of 30%. 1μM (-)sulpiride, the D2 receptor antagonist, blocked this inhibition.

Morphine (10μM) did not further reduce adenylate cyclase activity when enzyme inhibition by dopamine was maximal. This was shown by the lack of effect of morphine on the overall enhancement of adenylate cyclase activity by high concentrations of dopamine, which activate both D1 and D2 receptors maximally. It was also shown by the inability of morphine to enhance the D2-mediated inhibition of adenylate cyclase in the presence of 100μM dopamine and 0.1μM SCH23390 (see Table).

DRUG	% BASAL ADENYLATE CYCLASE ACTIVITY
10μM morphine	75 ± 8
100μM dopamine	
alone	123 ± 3
plus 10μM morphine	127 ± 3
plus 0.1μM SCH23390	76 ± 6
plus 0.1μM SCH23390 & 10μM morphine	73 ± 4

These results demonstrate that in striatal membranes, dopamine and opiate receptors are coupled to the same adenylate cyclase domain. This suggests that changes in neuronal cyclic AMP formation may be involved in interactions between opiates and dopamine in the basal ganglia.

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THE EFFECT OF ISOPRENALINE ON THE SECRETORY RESPONSE TO SUBSTANCE P IN THE RAT PAROTID SALIVARY GLAND

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The physiological stimulants of amylase secretion from the rat parotid gland include acetylcholine, noradrenaline and the tachykinin, substance P. Recent studies from this laboratory have provided evidence that substance P may be a secreto-motor neurotransmitter in the parotid (Sharkey & Templeton 1984). It is generally accepted that these secretagogues activate the secretory mechanism by two alternative intracellular pathways. Whereas α-adrenoceptor agonists, muscarinic agonists and substance P use Ca^{++} as a second messenger, β-adrenoceptor agonists employ cAMP. The observation that in other systems substance P may influence cyclic AMP metabolism (Rougon et al. 1983) suggested the possibility of interaction between second messenger pathways activated by tachykinins and β-agonists. This possibility was investigated in the rat parotid gland.

The ventromedial portions of both parotid glands from a male Wistar rat, which had been killed by cervical dislocation, were trimmed, chopped and then places in a 1ml chamber maintained at 37 °C. The tissue was superfused with a Krebs-Henseleit bicarbonate buffered saline (KHB) and the amylase released was monitored continuously by an automated fluorimetric method as described by Matthews et al. (1974). Superfusion of the tissue with KHB containing either isoprenaline (IPR) (10^{-9}-10^{-5} M) or substance P methyl ester (10^{-8}-10^{-5} M) for 15 s. elicited a specific dose-dependent increase in amylase secretion (Table 1). Continuous superfusion of the gland with KHB containing low concentrations of IPR, which alone had no effect, resulted in a potentiation of the response to substance P (Table 1).

In separate experiments the role of cAMP in this response was examined. Isoprenaline stimulated cAMP accumulation in a dose dependent fashion with an EC_{50} of 7 μM. Substance P (10^{-6}-10^{-4} M) had no effect upon the ability of the parotid to accumulate cAMP nor did it alter the response to IPR.

The ability of IPR to potentiate the effect of substance P cannot be explained by changes in cyclic nucleotide production. Studies are continuing to investigate the role of Ca^{++} and phosphoinositides in this response.

Table 1 Effect of IPR on substance p evoked amylase secretion

Substance P (M)	α-amylase units/ g tissue	α-amylase units/ g tissue in presence of 2 x 10^{-9} M IPR	α-amylase units/ g tissue in presence of 10^{-8} M IPR
1 x 10^{-8}	43.10 ± 16.13 (6)	86.50 ± 21.35 (3)	125.54 ± 17.08 (4)
3 x 10^{-8}	54.37 ± 14.01 (6)	162.58 ± 25.98 (3)	326.64 ± 66.30 (6)
1 x 10^{-7}	96.73 ± 20.45 (7)	228.50 ± 24.87 (7)	332.58 ± 97.85 (7)
3 x 10^{-7}	145.25 ± 28.47 (7)	346.65 ± 68.70 (3)	621.79 ± 125.66 (6)
1 x 10^{-6}	274.19 ± 43.52 (7)	521.63 ± 81.62 (3)	807.55 ± 184.99 (5)
3 x 10^{-6}	392.14 ± 59.31 (3)	858.93 ± 150.84 (3)	897.61 ± 203.61 (4)
1 x 10^{-5}	543.39 ± 98.81 (3)	942.79 ± 108.64 (3)	1001.66 (2)

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IS THE EFFECT OF INTRACEREBROVENTRICULAR 5,7-DIHYDROXYTRYPTAMINE ON MORPHINE ANALGESIA TIME-DEPENDENT?

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In recent years several investigations have implicated central serotonin (5-HT) mechanisms in morphine-induced analgesia. Much evidence has been obtained using selective 5-HT neurotoxins (5,6-and 5,7-dihydroxytryptamine) to modify regional serotonergic transmission. However, intracerebroventricular (ICV) administration of these toxins (a procedure that markedly reduces 5-HT levels in all brain areas) has given conflicting findings as regards the effect on morphine analgesia. The present study investigated whether the effect of ICV 5,7-dihydroxytryptamine (5,7-DHT) on morphine analgesia was time-dependent: the effect of morphine was studied in the tail immersion test in rats given 5,7-DHT three or ten days earlier.

Male CD-COBS rats, 220-250 g, were injected with desipramine (25 mg/kg i.p.) followed, thirty minutes later, under chloral hydrate anaesthesia, by ICV 5,7-DHT, 150 µg, dissolved in 20 µl of 0.01% ascorbic acid solution. Controls received desipramine plus the vehicle for 5,7-DHT. Experimental and control animals were divided in two groups: antinociceptive testing was three days later for one group and ten days later for the other. Serotonin was determined in the brain and spinal cord at both times using HPLC.

Three days after 5,7-DHT administration 5-HT content in brain and spinal cord was reduced about 60%; in the tail immersion test the analgesic effect of morphine (3 mg/kg s.c.) was significantly antagonized. Sixty min after morphine the mean response latencies ± S.E. were: vehicle + morphine 28.4 ± 1.1, 5,7-DHT + morphine 8.7 ± 1.7, $p<0.01$ (Student's t test). Ten days after 5,7-DHT, serotonin was reduced to about 20% of controls in both areas, but the effect of 3 mg/kg morphine in the tail immersion test was similar in experimental and control animals (vehicle ± morphine 25.5 ± 2.0, 5,7-DHT + morphine 22.3 ± 2.8, $p>0.01$). To test the hypothesis that a functional recovery, possibly due to post-synaptic receptor supersensitivity, was responsible for the lack of effect on morphine analgesia ten days after 5,7-DHT, various doses of the 5-HT receptor antagonist metergoline were administered s.c. 3 h before morphine to rats treated ten days earlier with 5,7-DHT or its vehicle. In control animals 1 mg/kg metergoline had no effect but 3 and 5 mg/kg significantly reduced morphine's effect; in 5,7-DHT-treated rats metergoline still reduced morphine's effect and the inhibiting effect of the 3 mg/kg dose was actually potentiated.

In conclusion, this study found a reduction of morphine analgesia in the tail immersion test three but not ten days after ICV 5,7-DHT. Ten days after 5,7-DHT, metergoline's effect on morphine analgesia was still present, indicating that functional recovery of the 5-HT system could partly explain the different results.

EVALUATION OF μ AND κ OPIOID AGONISTS IN THE GUINEA-PIG PAW PRESSURE TEST

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Several rodent models detect the central antinociceptive activity of opioid agonists, but drugs selective for μ or κ receptors show different profiles of activity in these tests (Tyers, 1980). The guinea-pig brain contains a higher proportion of κ binding sites than the rat or mouse brain (Kosterlitz et al., 1981; Gillan and Kosterlitz, 1982; Neil, 1984) suggesting that an antinociceptive model in this species might be particularly sensitive to κ agonists. We have thus compared the potency of some μ and κ opioid receptor agonists in three antinociceptive tests, the guinea-pig and rat paw pressure tests and the mouse abdominal constriction test.

Antinociceptive tests in the mouse and rat were performed as previously described (Tyers, 1980). Guinea-pig nociceptive pressure thresholds were measured in weanling Dunkin-Hartley guinea-pigs (180-220g) in the same way as for the rat paw pressure test, but with a 75g instead of a 25g load on the Analgesymeter.

The selective κ agonists U50488 (U), ethylketocyclazocine (E), tifluadom (T), bremazocine (Br), proxorphan (Pr), moxazocine (Mx) and Mr 2034 (Mr) were consistently more potent in the guinea-pig than in the rat paw pressure test (Fig. 1). In fact, two κ-selective agonists, xorphanol and oxilorphan, were inactive in the rat model but were effective antinociceptive agents in the guinea-pig. The selective μ agonists morphine (M), D-propoxyphene (D), fentanyl (F), pentazocine (Pe), nalbuphine (N) and buprenorphine (Bu) were of approximately equal potency in the guinea-pig and rat.

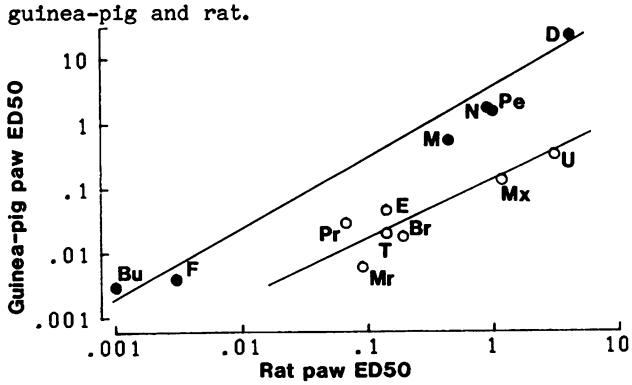

Fig. 1: Potency of opioids (mg/kg) in the guinea-pig and rat paw pressure tests. For μ-selective agonists (filled circles) r=0.99 and the slope =1.03. For κ-selective agonists (open circles) r=0.88 and the slope=0.79.

All of the compounds listed above were antinociceptive in the mouse abdominal constriction test. The potency of μ-selective agonists was similar in this test to their potency in the rat paw pressure test, while the potency of κ-selective agonists was similar to their potency in the guinea-pig paw pressure test. The mouse abdominal constriction test is thus highly sensitive to both μ and κ agonists, while a comparison of potencies in the rat and guinea-pig paw pressure tests may provide additional evidence for their receptor selectivity in vivo.

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BINDING AND PHARMACOLOGICAL PROFILE OF A HIGHLY SELECTIVE LIGAND FOR THE κ-OPIOID RECEPTOR - U-69,593

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The characterization of the κ-opioid binding site has been hampered by the lack of selective tritiated ligands. Some of the fragments of prodynorphin are highly selective for the κ-binding site but they are rapidly hydrolysed by tissue peptidases (Gillan et al, 1985). The non-peptide U-50,488H (trans-3,4-dichloro-N-methyl-N-(2-(1-pyrrolidinyl)cyclohexyl)benzeneacetamide: The Upjohn Company) is a selective κ-ligand (Gillan et al, 1983) which however is not available in tritiated form. Recently, the related compound U-69,593 (5α,7α,8β-(-)-N-methyl-N-(7-(1-pyrrolidinyl)-1-oxaspiro [4,5]dec-8-yl)benzeneacetamide; The Upjohn Company) has been tritiated and reported to be a selective ligand for the κ-binding site (Lahti et al, 1985). We have tested the unlabelled compound in both binding and pharmacological assays.

The potency of U-69,593 to displace the binding of selective tritiated ligands was measured in homogenates of guinea-pig brain at 25°C. The μ-binding site was selectively labelled with [^{3}H]-[D-Ala^2,MePhe^4,Gly-ol^5]enkephalin (1 nM), the δ-binding site with [^{3}H]-[D-Ala^2,D-Leu^5]enkephalin (0.7 nM) in the presence of 30 nM [D-Ala^2,MePhe^4,Gly-ol^5]enkephalin and the κ-binding site with [^{3}H]-(-)-bremazocine (0.1 nM) in the presence of 100 nM each of unlabelled μ- and δ-ligand (Corbett et al, 1984). The compound was tested for agonist and antagonist activity in the vasa deferentia of the rabbit which contains only κ-receptors, of the hamster which contains only δ-receptors and of the rat which contains mainly μ-receptors and in the myenteric plexus-longitudinal muscle preparation of the guinea-pig which contains both μ- and κ-receptors (Corbett et al, 1985).

U-69,593 is a highly selective ligand for the κ-binding site with a K_i of 5.58± 1.11 nM (n = 5). The corresponding values at the μ- and δ-sites are 2350±425 nM (n = 4) and 19670±1420 nM (n = 4), respectively. In bioassays, it is a potent agonist in the rabbit vas deferens (IC_{50} = 33.1±6.2 nM; n = 5) and the guinea-pig myenteric plexus (IC_{50} = 1.95±0.28 nM; n = 4). In contrast, it has no agonist or antagonist activity in either the hamster or the rat vasa deferentia at 10 μM. In the myenteric plexus, the K_e of naloxone against U-69,593 is 17.8±3.8 nM (n = 4).

Thus, in both binding and pharmacological assays, U-69,593 is a highly selective ligand for the κ-receptor. The availability of [^{3}H]-69,593 will facilitate the characterization of the κ-binding site.

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THE EFFECT OF PERTUSSIS TOXIN PRETREATMENT ON μ- AND κ- OPIOID MODULATION OF NEUROTRANSMITTER OUTPUT

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Selective κ-receptor opioid agonists such as ethylketazocine (EKC) share with μ-receptor agonists such as normorphine (NM) the ability to decrease neurotransmitter (ACh) output in the guinea-pig isolated ileum, which may be readily quantified in terms of reduction in twitch response to low frequency transmural electrical stimulation (Hutchinson et al., 1975). The mechanism of this modulatory action of NM appears to involve a reduction in cAMP levels in the neurone as pretreatment with a factor from Bordetella pertussis toxin, which ribosylates the N_i inhibitory subunit of adenylate cyclase, markedly attenuates its action in this preparation (Lujan et al., 1984; Tucker, 1984). However, little is known about the mechanism of κ-receptor mediated effects.

The present study was designed to compare dose-response curves for NM, EKC and adenosine within ileum preparations taken from sham injected (control) or pertussis toxin pretreated (test) animals. Guinea-pigs in the weight range 150-200 g were injected i.p. three days prior to experiment with vehicle control or 125 μg/kg toxin (supplied by Dr L Irons, Porton Down). Subsequently ileum preparations in Krebs fluid at 38°C were stimulated transmurally at 0.1 Hz with square wave pulses of 1 ms duration and supramaximal voltage to give isometrically recorded twitch responses. Cumulative inhibitory dose-response curves were obtained for each of the three agonists using 0.5 log dose intervals.

EKC has a high potency in this system with an IC_{50} of about 1 nM as compared to 100 nM for NM, but is less readily antagonised by naloxone. Adenosine served as a non-opioid control. In preparations (n=12) from pertussis treated animals the uncoupling of inhibitory modulation mediated by the three receptor types correlated quite closely and was characterised by a rightward shift in the log dose-response lines with a marked depression of the maximum inhibition obtainable even with high concentrations of agonists. However, comparison of EC_{50} values for carbachol in test and control preparations gave no evidence of any change in sensitivity of muscarinic receptors, so this action of the toxin may be ascribed to the neurone (see also, Hall & Morton, 1985).

In conclusion, it seems likely that the κ-receptor binding site is, in these neurones, coupled in the same way as the μ-receptor (and the adenosine receptor), all acting via the N_i regulatory subunit to reduce cAMP levels which in some way results in a decrease in evoked ACh release.

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IMIPRAMINE ENHANCES THE ANALGESIC EFFECT OF MORPHINE BUT NOT THE ELECTROCONVULSIVE SHOCK-INDUCED ANALGESIA IN RATS

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Several reports in the literature indicate that maximal electroconvulsive shock (ECS) in rats cause a naloxone-reversible opioid-like antinociception, catalepsy, respiratory depression and electroencephalographic hypersyncrony (Tortella F.C. et al., 1981; Tortella F.C. and Cowan A., 1982), by activating the endogenous opioid systems and releasing β-endorphin. Administration of morphine or opioid peptides induce a similar pattern of activity (Urca G. et al., 1977). Moreover, a cross-sensitivity between repeated ECS and morphine tolerance in the rat has also been demonstrated (Urca G. et al., 1981).

Tricyclic antidepressants have been shown to potentiate the analgesic effect of opioids (Botney and Fields, 1983; Malseed R.T. and Goldstein F.J., 1979; Liu .J. and Wang R.I.H., 1975), probably by blocking serotonin uptake and, therefore, by enhancing the action of serotonin in the spinal terminals of an opioid-mediated intrinsic analgesia system.

We have tested the effect of imipramine on the morphine and ECS-induced analgesia in rats.
The degree of analgesia was assessed by the tail-immersion method. Morphine was injected i.p. at the dose of 5 mg/kg. ECS-analgesia was elicited according to the method of Holaday and Belenky (Holaday J.W. and Belenky G.L., 1980). The antinociception produced 30 min after ECS was comparable to that seen 30 min after morphine, showing that the two methods gave the same degree of analgesia.

The administration of imipramine (p.o. at the dosages of 15, 30 and 45 mg/kg) given 30 min before the injection of morphine, induced a dose-dependent increase of the opioid analgesia. The increase in morphine analgesia was significant at the doses of imipramine of 30 and 45 mg/kg ($P < 0.05$ and $P < 0.01$ AxB factorial ANOVA).
On the other hand, no enhancement of ECS-induced analgesia occurred following oral administration of imipramine at the same doses previously used.

The results of these experiments seem to confirm that different opiate receptors mediate morphine or ECS-induced analgesia. The activation of descending epsilon and mu systems for β-endorphin and morphine, respectively, has been proposed (Tseng L.F. and Fusimoto J.M., 1985). Imipramine seems to increase only analgesia mediated by stimulation of mu receptors.

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EFFECTS OF FEEDING CONDITIONS ON THE CUEING PROPERTIES OF MORPHINE

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The feeding state is an important variable when assessing the reinforcing properties of narcotics (Carrol & Meish, 1984); in fact increased drug self-administration occurs if food intake is restricted. It is well known that opiates can function as discriminative as well as reinforcing stimuli and it is generally assumed (Colpaert, 1977) that the reinforcing action constitutes part of the stimulus complex composing the narcotic cue. Since there are no data relative to a food deprivation effect on the discriminative stimulus properties of narcotics, the present work was performed to investigate this problem.

Male Sprague Dawley rats were trained to discriminate morphine (10 mg/kg i.p.) from saline (2 cc/kg i.p.) in a two lever food reinforced operant task (tandem V.I. 60 F.R. 10). Food pellets were available only during a 90 min period, beginning 1 h after each daily experimental session; water was always freely available. After stable discriminative responding had been established, the rats were submitted to morphine generalization tests when food deprived (training conditions) or after 15 min supplemental feeding in the home cages. In both cases morphine lever selection increased with the dose (see Figure 1); however a Waud (1972) analysis of the data indicated that the ED_{50} value was significantly ($P < 0.05$) lower for food deprived (6.09 mg/kg) than for partially satiated (7.79 mg/kg) rats. On the other hand a similar dose-related decrease in response rate was obtained in both feeding conditions.

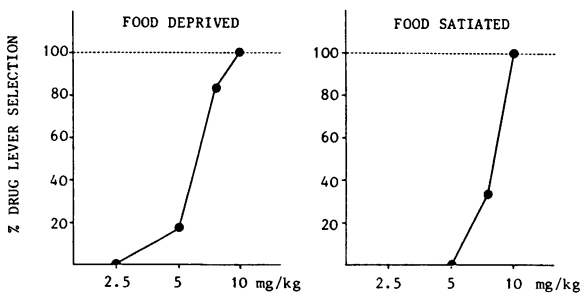

Figure 1 Morphine generalization gradients; each point is based on 5-6 determinations

The present results indicate that an augmented food intake lowers the sensitivity to the narcotic cue and thus suggest that food deprivation can increase not only the reinforcing but also the discriminative stimulus properties of opiates.

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708P

INFUSION OF MPTP INTO CELL BODY AND TERMINAL AREAS OF THE NIGROSTRIATAL PATHWAY IN PRIMATE BRAIN

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1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) given systemically to primates induces a syndrome resembling that of Parkinson's disease in man. However, from studies using this route of administration it has not been possible to determine whether the loss of nigrostriatal dopamine reflects a specific neurotoxic action in the substantia nigra (SN), or if direct effects in the striatum are contributory. To resolve this, in a model which avoids the complications of peripheral administrations of a neurotoxin to primates, we have slowly and persistently infused MPTP directly into the SN and caudate nucleus (CN) of primate brain.

Common marmosets (Callithrix jacchus, male, 350-450g) were anaesthetised with Saffan(R), 1ml/kg body weight i.m., and subject to standard stereotaxic surgery for the implantation of stainless steel guide cannulae (0.64mm diameter) to allow subsequent unilateral infusion of MPTP, 10 or 40µg/24h, into the area of the zona compacta of the SN (Ant. 5.0, Lat. ±2.3, Vert. 13.7mm below dura) or central area of the CN (Ant. 9.5, Lat. ±2.5, Vert. 6.9mm below dura) (atlas of Stephan and colleagues). After 14 days marmosets were re-anaesthetised for subcutaneous implantation in the scapula region of one Alzet(R) osmotic minipump delivering MPTP (0.46µl/h) into the right SN (10µg/24h, n = 4) or right CN (10µg/24h, n = 2, or 40µg/24h, n = 2). Animals were visually observed via remote video recording and were also placed for 1h per day in cages fitted with 4 computer-linked infrared units strategically placed to allow the detection of movement about the cage.

Within 6h of commencing infusion of MPTP, 10µg/24h, into the SN marmosets developed marked motor deficits seen most clearly as loss of movement in the contralateral front limb, but also apparent as loss of movement in the contralateral hind limb with restricted movement of the contralateral facial muscles. Thus, animals became 'unsteady' on the perches, fed preferentially by using their right front limb, and showed a very characteristic drooling of food from the left hand side of the mouth (movement reduced from 85-130 counts/60 min to 5-50 counts/60 min). By 24h marmosets also showed contralateral twisting of the neck and trunk region and all movements were in the contralateral direction. In two marmosets these contralateral movements were so intense as to restrict feeding behaviour, and infusion of MPTP was terminated at 48h. The contralateral deficits were maintained when infusion continued for 13 days. Challenge with l-dopa (50 and 100mg/kg i.p. after benserazide) exacerbated the contralateral movements during infusion (day 10) and induced marked hyperactivity. In those animals receiving 48h infusion spontaneous contralateral deficits were not apparent following withdrawal of MPTP, and were not then revealed by l-dopa. However, after 13-day MPTP infusions contralateral deficits, exacerbated by l-dopa, continued into the post-infusion period (at least 7 days). In contrast, 10µg/24h MPTP infused into the marmoset CN failed to cause any change in motor responding, although some ipsilateral movements became apparent during the infusion of 40µg/24h MPTP. Biochemical correlates are being determined.

It is concluded that MPTP infused into the marmoset SN can cause marked motor deficits which are not replicated from the CN. The discrete intracerebral infusion of MPTP in the marmoset is forwarded as a useful approach to developing primate models for analysis of MPTP neurotoxicity.

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ANTINOCICEPTION PRODUCED BY A STEREOISOMER OF SULPIRIDE COMBINED WITH A DOPAMINE D_1 AGONIST

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The involvement of dopamine receptors in the modulation of pain perception is generally accepted (Akil and Liebeskind 1975). It has been shown that stimulation of D-2 receptors facilitates antinociception whilst D-1 receptor stimulation evokes hyperalgesic responses (Bensreti et al. 1983). A recent investigation into the stereospecificity of the dopamine antagonist sulpiride reports that although the (-)-isomer shows a marked selectivity for D-2 receptors, the (+)-isomer exhibits considerable affinity for the D-1 receptor (Leff et al. 1984).

The present study set out to determine the effects of sulpiride's two isomeric forms on nociceptive sensitivity in the mouse in the presence of selective dopamine agonists. Nociceptive response reaction times were determined using the mouse tail immersion test at 48°C. Isomers of sulpiride ($10mg.kg^{-1}$, i.p.) were administered 30 min prior to the test dopamine agonist. All agonists were administered by intracerebroventricular (icv) injection, whereupon nociceptive sensitivity was monitored repeatedly for a 100 min period. Dopamine (50µg per animal) and the D-2 agonist quinpirole hydrochloride (LY 171555; 100µg per animal) produced a significant antinociceptive effect, manifested as an increased tail flick latency (TFL), ($P<0.05$ and $P<0.01$, respectively - Dunnett's 't' test). In contrast, the D-1 agonist SKF 38393 (2,3,4,5-tethydro-1-phenyl-1H-3 -benzazepine-7,8-diol hydrochloride; 20µg per animal) produced a small but significant hyperalgesic response, ($P<0.05$), manifested by a fall in the TFL. However, when animals were pretreated with (-)-sulpiride (but not with (+)-sulpiride), the hyperalgesic effect of SKF 38393 was converted into an antinociceptive effect ($P<0.05$). Administration of the sulpiride isomers alone produced a slight elevation of the TFL values. When tested against the antinociceptive action of the D-2 agonist quinpirole, both sulpiride isomers attenuated the antinociceptive response.

These observations indicate that, in the presence of the D-2 antagonist (-)-sulpiride, SKF 38393 no longer induces hyperalgesia but conversely produces mild antinociception.

The results suggest a possible shift in the balance of the two dopamine receptor systems with respect to nociception in the presence of (-)-sulpiride. This is in agreement with other studies which have shown a similar profile of nociceptive activity of dopamine agonists administered to naive and dopamine-supersensitive animals (Barasi et al. 1985).

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ALTERATIONS IN MOTOR BEHAVIOUR PRODUCED BY THE ISOMERS OF 3-PPP IN THE MPTP-TREATED MARMOSET

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(-)-3-(3-hydroxyphenyl)-N'n-propylpiperidine (3-PPP) is an agonist at dopamine autoreceptors but an antagonist at dopamine post-synaptic receptors (Hjorth et al 1983). (+)-3-PPP also is a dopamine agonist at autoreceptors but stimulates postsynaptic dopamine receptors. We have studied the effects of (-)- and (+)-3-PPP in marmosets treated with the nigro-striatal neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

Common marmosets (282-385 g) of either sex were treated intraperitoneally with MPTP (dissolved in a minimum quantity of 70% ethanol and diluted with sterile 0.9% saline) in a cumulative dose of 7-10 mg/kg. On test days animals were transferred to a novel cage environment. Following drug or vehicle administration the behaviour of animals was video-recorded over the following 120 min. Total numbers of movement of animals was assessed from the video recording.

Marmosets treated with MPTP 4-6 weeks previously exhibited fewer and less co-ordinated movements than control animals when examined in the home cage. Administration of (-)-3-PPP (1-8 mg/kg i.p.) to both control and MPTP-induced marmosets caused a trend towards a dose dependent decrease in movement counts (Table 1). The administration of a low dose of (+)-3-PPP (1 mg/kg i.p.) to control marmosets caused some suppression of activity. At 2 mg/kg (+)-3-PPP caused an initial suppression of activity followed by a brief burst of enhanced motor activity. Higher doses of (+)-3-PPP increased the movement counts compared to saline treated control. In contrast (+)-3-PPP (1-8 mg/kg i.p.) only caused dose-dependent behavioural activation in MPTP-treated marmosets. The increase in motor activity caused by (+)-3-PPP was of greater intensity and duration than that observed in normal control animals.

Table 1 Movement counts following (-)- and (+)-3-PPP administration

Drug	0 - 5 min		30 - 35 min	
(mg/kg ip)	MPTP	Control	MPTP	Control
Saline	9.0±4.9	13.0±5.4	0.3±0.3	2.8±2.8
(-)-3-PPP				
1	4.5±4.2	10.0±4.3	0.3±0.3	0.8±0.8
2	2.8±1.9	4.3±1.7	0.3±0.3	0.3±0.3
4	2.8±0.9	3.3±1.1	0	0
8	0	2.8±0.6	0	0
(+)-3-PPP				
1	10.3±9.9	7.4±3.4	3.3±2.4	1.5±1.5
2	17.8±11.6	3.8±1.7	29.0±10.5*	2.0±1.2
4	24.5±16.0	23.5±15.0	42.0±9.3*	37.8±14.7
8	22.5±9.3	52.5±17.8	94.5±6.8*+	60.0±10.0*

* $p < 0.05$ compared to saline treated-animals. + $p < 0.05$ compared to controls.

Loss of dopamine neurons following MPTP treatment causes no change in the actions of (-)-3-PPP. In contrast, (+)-3-PPP produces only motor activation in MPTP treated animals probably reflecting the loss of presnyaptic autoreceptors.

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BEHAVIOURAL AND ADRENOCORTICAL RESPONSES TO DIAZEPAM IN TWO DESIGNS OF ELEVATED X-MAZE APPARATUS

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Previous studies have shown that diazepam (Dz) increases the rate and degree to which rats exhibit adaptation of the adrenocortical response to an aversive stimulus whereas its withdrawal from stressed rats causes a marked increase in plasma corticosterone (Balfour & Copland, 1985). The aim of the present study was to correlate the effects of Dz on adrenocortical activity with changes in locomotor activity.

Male Sprague-Dawley rats, weighing approximately 250 g at the beginning of the study, were given daily intragastric injections of Dz (25 mg/kg: n = 12 per gp) or vehicle (40% propylene glycol (v/v) in water; n = 6 per gp) for 20 days. Thirty minutes after each injection the rats were placed at the centre of an x-maze raised 1 m from the laboratory floor and composed of 4 enclosed runways (43 cm x 9 cm with 15 cm sides) and the number of runway entries was recorded for 15 minutes. A second group of rats received the same treatment with drug or vehicle but were placed on an elevated x-maze composed of 4 open runways (43 cm x 9 cm with 3 cm sides). On days 21 to 25 half the rats previously treated with Dz were given vehicle prior to each trial in the maze, the remaining animals continuing to receive the treatment they had been given on days 1 to 20. Immediately after the last trial on day 25 the rats were killed and blood samples taken for the determination of plasma corticosterone (Mattingly, 1962). Plasma corticosterone was also measured in behaviourally naive unstressed rats (n = 6) which were given 25 daily injections of vehicle but were not exposed to either of the mazes.

Analysis of the data for days 1 to 20 showed that, for both mazes, repeated exposure to the apparatus was associated with a significant reduction ($F(4, 20) = 4.3$; $p < 0.05$) in total entries made per trial and that Dz also decreased ($F(5, 25) = 11.4$; $p < 0.01$) the number of entries. No interaction between the effect of Dz and the trial day was observed. Analysis of the data for days 21 to 25 revealed no effect of trial day on total entries but that the treatments had a significant effect ($F(5, 25) = 8.6$; $p < 0.01$). Subsequent analysis showed that vehicle-treated rats tested in the open maze were less active ($p < 0.05$) than those tested in the enclosed maze and that Dz reduced activity ($p < 0.01$) in both mazes. In the open maze Dz withdrawal resulted in complete recovery of locomotor activity to control levels whereas in the enclosed maze the Dz-withdrawn rats remained less active than controls ($p < 0.05$). Plasma corticosterone levels in both Dz- and vehicle-treated rats tested in the open maze (12 ± 2 µg/100 ml and 18 ± 5 µg/100 ml respectively) were not significantly different from unstressed controls (18 ± 3 µg/100 ml). Dz withdrawal increased ($p < 0.05$) the levels to 38 ± 2 µg/100 ml. Plasma corticosterone levels in Dz-treated rats tested in the enclosed maze (10 ± 4 µg/ml) were lower ($p < 0.05$ and $p < 0.01$ respectively) than those found in vehicle-treated rats (28 ± 4 µg/ml) and withdrawn rats (39 ± 4 µg/100 ml) tested in the same maze. No correlations between plasma corticosterone and activity in either of the mazes were apparent.

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ACTION OF OPIOID ANTAGONISTS ON MEPTAZINOL- AND LEVORPHANOL-INDUCED HYPERPHAGIA

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The novel analgesic meptazinol has been shown in both in vitro and in vivo tests to possess a pharmacological profile which differs from that of classical opioids such as morphine (Spiegel & Pasternak, 1984). Agents acting as agonists at μ, κ and δ-opioid receptors have been reported to increase food intake in a variety of animal species (Morley et al. 1983). Hence, in the present investigation it was of interest to examine the effects of the unique μ-ligand meptazinol on the food intake of non-deprived rats - both alone and in combination with the opioid antagonists Mr1452 and ICI154,129. We also report here, for comparison, corresponding experiments performed using the more traditional μ-agonist levorphanol.

Individually-housed male Wistar rats (300-350g) were allowed free access to powdered standard rat diet and tap water at all times. Meptazinol (m-(3-ethyl-1-methyl-hexahydro-1-H-azepin-3-yl)phenol hydrochloride), levorphanol, and Mr1452 [(-)-5,9-α-dimethyl-2-(3-furyl methyl)-2'-hydroxy-6,7-benzomorphan] were injected i.p. in a dose volume of 1ml/kg. ICI154,129 (N,N-bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH) was injected directly into the lateral ventricle through permanently indwelling cannulae in a dose volume of 10μl/rat. Experiments were carried out during the daylight period and animals were given 30 min pretreatment with the agonist. Feeding jars were weighed at the time of drug administration and after 1, 2 and 4h to enable calculation of mean group cumulative food intakes(g/kg rat weight±SEM). Statistical comparisons were made using the analysis of variance and Dunnett's t-test.

Rats injected with either meptazinol (2mg/kg i.p.) or levorphanol (1mg/kg i.p.) ate significantly greater amounts than saline-treated controls over the 4h test period. Mr1452 (1mg/kg i.p.) significantly reduced the hyperphagia induced by both meptazinol (2mg/kg i.p.) and levorphanol (1mg/kg i.p.); however, a dose of the selective δ-antagonist ICI154,129 (10μg/rat i.c.v.) which inhibited the feeding response to the δ-agonist D-Ala^2-D-Leu^5-enkephalin (Jackson & Sewell, 1984) suppressed meptazinol- but not levorphanol-induced food intake. The doses of antagonist used in this study did not have any appreciable effects on baseline feeding levels per se.

Thus, it appears that meptazinol closely resembles the μ-agonist levorphanol in producing an increase in food intake which appears to be mediated via opioid receptors. However, our findings indicate that unlike levorphanol, the appetitive effects of meptazinol involve either direct or indirect activity at δ-opioid receptors. This observation is interesting in relation to the reports that meptazinol acts selectively at the high affinity μ_1-site common to both the enkephalins and morphine (see Siegel & Pasternak, 1984). It is also of note that the putative μ_1-antagonist naloxonazine has been shown to possess activity at central δ-receptors in vivo (Dray & Nunan, 1984).

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TURN PREFERENCE DOES NOT INFLUENCE THE RESPONSE OF MICE IN PUTATIVE MODELS OF DEPRESSION AND ANXIETY

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Schizophrenia may be due to a dysfunction of the left hemisphere while depression may be associated with right hemisphere dysfunction (Gruzelier, 1981). Reynolds (1983) reported that the amygdala in particular exhibited an imbalance in schizophrenia since post mortem dopamine levels were higher in the left than in the right amygdala. The amygdala also may play a role in depression since a unilateral lesion of the rat amygdala greatly reduced the efficacy of imipramine in the Porsolt test (Gorka et al, 1979), a putative model of depression (Porsolt, 1981). Finally the unilateral infusion of dopamine into rat amygdala evoked a distinctive locomotor response dependent upon the direction in which the animal had consistently moved in a turn preference test (Costall et al, 1985). Using a similar turn preference protocol we have examined whether hemispheric dominance influences the response of mice in two non-drug behavioural models, namely the Porsolt model of depression and the chimney test for detecting anxiolytic activity (Boissier et al, 1960).

Male CD1 mice (18-22g at the start of the study; Charles River) were assessed for turn preference by either (a) removing them from their cage, placing them in an open field and noting their immediate turning movement, or (b) by suspending them vertically by the tail and observing their turn preference which proved to be a less immediate response. Mice were subjected to the same test procedure on 5 consecutive mornings and only those always turning right or left or making no turning movement were used in the following tests on day 5. Firstly the time taken (up to a maximum of 60 sec) to back up a vertical chimney (copper tube 300mm x 26mm) was measured. Later their mobility during a 4 min period in the Porsolt test was measured in arbitrary units using a Doppler recording system.

Although many mice exhibited a turn preference, the response was only poorly reproducible. Thus of mice initially identified as turning either right (R) or left (L) or neither (N) only 13%, 9% and 13% (method (a)) or 18%, 18% or 21% (method (b)) respectively maintained this preference for 5 days. The turning preference, selected by either method, did not alter either the time taken to back up the chimney or the mobility in the Porsolt test. Thus the mean (± SEM) climbing times (sec) were R: 20.3±2.0; L: 25.0±5.3 and N: 26.7±3.9 after selection by method (a) and R: 15.0±3.1; L: 10.9±1.6 and N: 15.9±2.0 after selection by method (b). Mean (± SEM) mobility in the Porsolt test was R: 191±20; L: 181±20; N: 182±28 (method (a)) and R: 192±28; L: 197±31 and N: 190±36 (method (b)). At least 15 mice per group were examined in each model; none of the differences were significant ($p>0.05$; Student's t-test).

If turn preference is an indication of hemisphere imbalance in rodents then it appears to have no effect on the response of mice in putative models of either depression or anxiety.

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ORGOTEIN EFFECT ON DOXORUBICIN-INDUCED CARDIAC LIPID PEROXIDATION IN MICE

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Doxorubicin (DXR), an anthraquinone antitumor antibiotic, is known to cause cardiac toxic effects (Bristow, 1982). One proposed mechanism of DXR cardiotoxicity is through the conversion of the anthraquinone nucleus to a free radical semiquinone with the consequent production of superoxide anions. These, in turn, initiate free radical mediated chain reactions resulting in the conversion of membrane unsaturated fatty acids to lipoperoxides (Handa & Sato, 1976; Bus & Gibson, 1979). On the other hand, the heart contains low levels of superoxide dismutase (SOD) which, in the presence of increased superoxide production from DXR, may increase the chance of cardiac muscle damage from reactive oxygen (Doroshow et al., 1980). Furthermore, it was shown (Myers et al., 1977) that after acute DXR intoxication malondialdheyde, an index of lipid peroxidation, increased in mouse cardiac tissue and its cytological lesions were similar to those observed in vitamin-E deficiency.

In this paper we have studied the effect of repeated administrations (5 and 50 mg/kg, s.c., for 6 days) of orgotein (Serono), a cuprozinc SOD, in counteracting the lipid peroxidation induced in mice heart by the i.v. injection of 7.5 mg/kg DXR. Vitamin E (85 U/mouse) was used as a reference drug. The thiobarbituric acid test procedure, recommended by Uchiyama and Mihara (1977), was used for the assay of lipoperoxides in cardiac tissue homogenates obtained 72 hrs following DXR injection.

DXR administration raised the cardiac lipoperoxide levels with a peak value (+ 32%) at 72 hrs. Orgotein treatment prevented this increase that was found to be of 23 and 8%, at the doses of 5 and 50 mg/kg, respectively. Similarly, in the vitamin-E treated group the lipid peroxidation was found to be increased by 4%.

These results suggest that orgotein may be of benefit in protecting from DXR cardiotoxicity. Although with the obvious caution in extrapolation of animal data to human, it would be of great interest to assay this drug in patients under antiblastic therapy.

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RESTORATION BY ORGOTEIN OF RAT CARDIAC CELL BEATING INHIBITED BY DOXORUBICIN

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Doxorubicin (DXR) is an effective anti-cancer agent which produces an irreversible cardiomyopathy in a significant number of cancer patients. One proposed mechanism of cardiotoxicity of DXR centers around lipid peroxidation initiated by free radical formation (Goodman & Hochstein, 1977; Bachur et al., 1979; Facchinetti et al., 1982). It was also shown that the addition of DXR to cultured heart cells reduces their spontaneous beating rate, possibly through an action on the cellular membrane ATP-ase sites (Necco et al., 1976).

In this study we have investigated the effects of orgotein, a cuprozinc superoxide dismutase, on the inhibition of beating rate provoked by DXR in cultured cardiac cells of newborn rats. The method used for culturing the cardiac cells was that proposed by Harary and Farley (1963), with substantial modifications.

DXR (0.1 μg/ml) inhibited the beating frequency by 36.6, 47.3 and 62.8% after 1, 2 and 3 days, respectively. The addition of orgotein (Serono) at 10 μg/ml to the culture medium partially prevented such an inhibition which was of 25, 36.6 and 44.7%. A lesser inhibition (20.6, 17.8 and 28.7%) of the beating frequency was achieved when a higher concentration (100 μg/ml) of orgotein was used. Ouabain (5×10^{-5} M) similarly prevented the inhibiting effects of DXR which were reduced to 10, 13.3 and 12.8% after 1, 2 and 3 days, respectively. The addition of DXR at 10 μg/ml for only 30 mins resulted in a stronger inhibition of the beating rate which was found to be 29.6, 74.6 and 94.2% after 1, 2 and 3 days, respectively. When 10 μg/ml orgotein were added to the cultures the inhibition was slightly reduced to 20.9, 49.2 and 93.5%. Conversely, a more marked protection was obtained with 100 μg/ml orgotein which gave beating rate inhibitions of 7.5, 39.6 and 77.3% after 1, 2 and 3 days respectively. Similarly to the above experiment, ouabain exerted a very high protection against DXR inhibiting effects which were found to be of 23.3, 26.2 and 32.9%.

The results of this study suggest that orgotein could counteract the harmful effects of the superoxide free radicals which, through a peroxidative process, may damage the ATP-ase sites of the cellular membrane thus resulting in an impaired cell function.

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716P

CARDIAC PROTECTIVE ACTIVITY WITH L-ARGININE METHYL ESTER AGAINST ISCHAEMIA AND REPERFUSION-INDUCED ARRHYTHMIAS IN THE RAT

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It has been reported that methyl esters(ME)of certain amino acids such as L-Leucine lead to lysosomal disfunction and cardiac toxicity (1), while other ME such as L-Arginine ME (LAM) exhibit a strong protective activity against ultrastructural and functional damage induced on the isolated perfused guinea pig in ischemic and reperfusion conditions (2).

In order to verify in a vivo model the function of LAM, the antiarrhythmic activity (AA) of amino acid arginine (LA) and two of its ME, LAM and benzoil arginine ME (BAM) was assessed against arrhythmias occurring during 30 min. of acute occlusion and upon 5 min. of rapid reperfusion of the left coronary artery in anaesthetized rats.

Lidocaine, a membrane stabilizing drug was also evaluated for AA in this model. The serum enzyme levels, GOT, GPT, CPK, LDH, β-glucoronidase, the fibrin and fibrinogen degradation products (FDP) were determined at the end of the ischemic and reperfusion period (35 min.).

Only lidocaine and LAM (3 mg/kg i.v.) significantly reduced both the number and the lenght of arrhythmias occurring during occlusion and the incidence of tachycardia or ventricular fibrillation (VT, VF) occurring after occlusion and reperfusion of coronary artery.

LA and BAM were inactive on both ischemic and reperfusion arrhythmias.

The serum cytoplasmic enzyme increase observed after coronary occlusion-reperfusion was generally unaffected after treatment except for GOT and LDH levels which were reduced slightly with lidocaine and LAM respectively. On the contrary, the increase of β-glucoronidase and FDP serum levels observed in this experimental model was almost totally counteracted with lidocaine and LAM.

These in vivo data confirm the antiarrhythmic activity of LAM, previously observed on an ischemic reperfused heart model.
The parallel and similar activity of LAM and LID on reduction of occlusion-reperfusion arrhythmias and on serum β-glucoronidase level decrease can suggest the relevance of safe keeping the myocardial lysosomal integrity which appears damaged in this model; the lack of activity of an other arginin ME such as BAM requires closer investigation for delineating the ME function contribution in this cardiac protective effect.

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A DILTIAZEM SENSITIVE COMPONENT OF PRESSOR RESPONSES OF PITHED RATS TO CIRAZOLINE

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Ruffolo et al (1984) used phenoxybenzamine (PBZ) to uncover a diltiazem sensitive component of pressor responses in pithed rats to cirazoline (CIR) which they attributed to a reduction in receptor reserve by PBZ. The aim of this study was to determine whether a high dose of the potent α_1-adrenoceptor antagonist prazosin (PZ) would have a similar effect to PBZ.

Male Wistar rats (180-220g) were anaesthetised with pentobarbitone sodium (60mg/kg i.p). The tracheae were cannulated, the animals pithed and artificially respired with room air. Blood pressure recordings were monitored from the right carotid artery. Drugs were administered via either the left jugular vein or the left femoral vein. Cumulative dose response curves to CIR (0.1-10µg/kg) were constructed (control). 15 minutes pretreatment with prazosin (PZ) (0.3mg/kg) caused a parallel shift of the control curve to the right by two log cycles. Phenoxybenzamine (PBZ) (0.2mg/kg) in addition to rightward displacement, also caused a reduction in both the slope and the maximum. The effect of DLZ (25µg/kg/min infusion) or the α_2-adrenoceptor antagonist yohimbine (YOH) (0.3mg/kg) was determined on dose response curves to CIR alone and CIR in the presence of PZ or PBZ (Table 1).

Table 1. Maximum and EC50 of pressor effects to CIR.

	saline	DLZ	YOH	PZ	PZ+DLZ
Max.pressor effect (mm Hg)	109±6.2	94±5.2	98±4.9	101±2.6	68±8.0*
EC50	1.08 (1.07-1.08)	1.63 (1.62-1.64)	1.20 (1.19-1.21)	106 (105.8-106.9)	504 (498.6-510.3)

	PZ+YOH	PBZ	PBZ+DLZ	PBZ+YOH
Max.pressor effect (mm Hg)	84±4.5*	82±3.3	36±7.8	80±2.2
EC50	252 (250.3-253.9)	22.7 (22.4-22.9)	-	52.9 (52.2-53.6)

*=significant difference from displaced control. Student's t-test ($p<0.05$). EC50=dose CIR (µg/kg used to increase diastolic blood pressure to 50% of undisplaced control maximum. Figures in brackets represent the 95% confidence limits for the EC50. n=4-7.

These results show that high doses of prazosin will uncover a DLZ sensitive pressor component of CIR; this may be attributed to stimulation of α_2-adrenoceptors. The inability of YOH to block DLZ sensitive component of the CIR pressor responses after PBZ pretreatment infers that PBZ, in the concentration used, is blocking α_2-adrenoceptors. This observation supports the view *vide supra* of Ruffolo et al (1984)

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BIPHASIC EFFECT OF BAYER K 8644 ON THE EFFICIENCY OF THE RAT WORKING HEART IN VITRO

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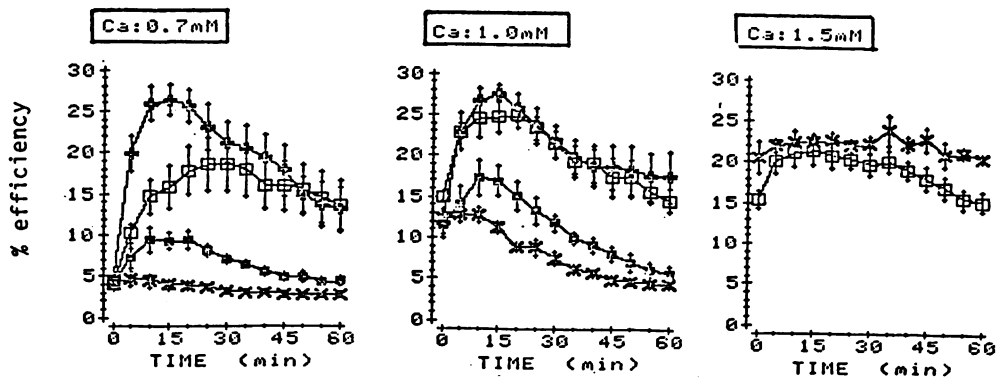
Bayer k 8644 has been shown to enhance myocardial contractility, an effect attributed to facilitation of calcium entry through membrane slow channels in myocytes (Thomas et al., 1984). We have now studied the kinetics of this compounds cardiac effects using rat isolated working hearts.

Hearts were perfused via the pulmonary vein with Kreb's solution (containing 0.7 mM, 1.0 mM or 1.5 mM Ca^{++}), gassed with 95% O_2 and Co_2 and heated to 37°C as described previously (Armstrong and Ferrandon, 1985). Perfusion was maintained at a preload of 9 mmHg and the afterload at 59 mmHg. Cardiac function was assessed from measurements of the left ventricular phasic pressure and its componants and from aortic and coronary outflows. Bayer k 8644 was dissolved in dimethylsulphoxide (DMSO) and subsequently diluted in Kreb's solution to achieve the final concentration used. Control hearts studied in parallel received the same concentrations of DMSO alone. Cardiac efficiency (%) was assessed using the formula : (cardiac output x left ventricular systolic pressure x 0.0000136 / oxygen extracted by the heart) x 100. The graphs shown below are of mean values (± sem) of efficiency occurring initially and then at intervals from the start of perfusion with Bayer (0 nM ✕ ,3 nM ■ ,10 nM □ or 100 nM ✱).



Bayer did not alter values of heart rate or coronary flow but did change left ventricular contractility (+dp/dt) and relaxation (-dp/dt) in a biphasic manner. In either case the maximum value achieved was proportional to the concentration of Bayer studied. Thereafter, values declined. For example when 0.7 mM Ca^{++} was used, the initial value of contractility was 1700 ± 100 mmHg/sec and that for relaxation was 1200 ± 110 mmHg/sec. After 15 min of perfusion with 100 nM Bayer k 8644 the corresponding values were 4600 ± 300 mmHg/sec and 3100 ± 200 mmHg/sec and after 60 min they were 2500 ± 130 mmHg/sec and 1800 ± 120 mmHg respectively.

Thus Bayer k 8644 enhanced mechanical performance of the rat heart, an effect that was more evident at low than at physiological concentrations of ionized Ca^{++}. Furthermore, at each concentration of Bayer tested, cardiac stimulation reached a maximum but this was not sustained subsequently decreasing towards or to below baseline values as reported by Thomas et al. (1984).

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CARDIOVASCULAR RESPONSES TO NIFEDIPINE IN COMBINATION WITH β-ADRENOCEPTOR ANTAGONISTS OR CLONIDINE IN CONSCIOUS SH RATS

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In conscious experimental animals and patients with essential hypertension, vasodilators with selectivity for vascular smooth muscle cause a lowering of blood pressure (BP) which is accompanied by reflex tachycardia and increased cardiac output. To minimize the reflex cardiac effects such pure vasodilators may be given in combination with beta-adrenoceptor blocking agents (Zacest et al., 1972 ; Andersson et al., 1984) and in some cases with clonidine (Pettinger et al., 1977 ; Valesco et al., 1981), a centrally acting antihypertensive drug. In the present study, cardiovascular responses to nifedipine have been studied in conscious spontaneously hypertensive (SH) rats in the presence and absence of clonidine. The results are compared to those obtained with nifedipine in combination with beta-adrenoceptor blocking agents.

SH rats (Iffa Credo, France) weighing 300-360 g, were cannulated (PE 50) in the femoral artery and vein under light ether anaesthesia. Mean arterial BP and heart rate (HR) were recorded by standard techniques. Effectiveness of beta-adrenoceptor blockade was assessed by antagonism of the chronotropic response to isoprenaline (0.3 µg/kg, i.v.).

Injections of nifedipine, 0.1-3.0 µmol/kg i.v., caused dose-related falls in BP which were accompanied by dose-related increases in HR. Clonidine, 0.038 µmol/kg i.v., resulted in a fall in both BP (19 ± 2mm Hg) and HR (38 ± 4 beats/min) and the hypotensive response was preceded by a transient pressor effect. Administration of nifedipine, 0.1-3.0 µmol/kg, 5 min after pretreatment with clonidine, resulted in falls in BP, at all doses greater in magnitude and duration than those obtained with nifedipine alone. Furthermore, the reflex increase in HR was transient and at the 0.1 and 0.3 µmol/kg dose did not exceed the HR measured immediately prior to clonidine. Injection of propranolol, 3.34 µmol/kg i.v., initially increased the BP (11 ± 1mm Hg) and caused an equivalent degree of bradycardia (34 ± 4 beats/min) to that seen with clonidine. Nifedipine, 0.1-1.0 µmol/kg i.v., injected 5 min following propranolol resulted in augmented falls in BP compared to the same doses of nifedipine given alone, with the single exception of the 0.1 µmol/kg dose. The reflex tachycardia was markedly reduced at all doses. Treatment with atenolol, 3.76-11.28 µmol/kg i.v., 5 min prior to injection of nidefipine, 0.3 µmol/kg i.v., also resulted in a reduction in the reflex tachycardia with a small augmentation of the effect on BP compared to nifedipine given alone.

The cardiovascular responses to nifedipine in conscious SH rats are those expected of a typical dihydropyridine vasodilator. Pretreatment with clonidine or beta-adrenoceptor blocking agents augmented the hypotensive effects of nifedipine and markedly attenuated the reflex tachycardia. At the dose used, clonidine was more effective than either propranolol or atenolol in this latter respect.

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HAS THE INHIBITION OF Na^+/Ca^{++} EXCHANGE BY AMRINONE RELEVANCE IN THE VASCULAR EFFECT OF THE DRUG?

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Amrinone is a drug proposed as cardiotonic agent in the therapy of congestive heart failure for its inotropic effect and vasodilator properties.These effects of amrinone on myocardium and vascular smooth muscle are ascribed to the inhibition of phosphodiesterase activity by the drug(Carpenedo,1984).Recently amrinone has been shown to inhibit also the Na^+/Ca^{++}exchange of cardiac sarcolemmal vesicles prepared from beef heart(Carpenedo,1984).The Na^+/Ca^{++}exchange mechanism normally acts as a system for active extrusion of $Ca^{++}$from the cells,promoting an inward Na^+movement coupled to an outward Ca^+movement(Sheu & Fozzard,1982, Blaustein,1977).The Na^+/Ca^{++} exchange may play a critical role in the regulation of the vascular smooth muscle tone(Blaustein,1977).An important inhibition of the exchange by amrinone is not consistent with the drug vasodilator action,which however may be the net result of the inhibition of phosphodiesterase activity on one hand and of the Na^+/Ca^{++}exchange mechanism on the other.The present study was designed to evaluate the relevance of amrinone Na^+/Ca^{++}exchange mechanism inhibition on the vascular effect of the drug.

Amrinone was tested on isolated strips of guinea-pig aorta in which contractures were elicited by changing the ionic conditions of the medium.High K^+induces membrane depolarization and consequent opening of the Ca^{++}channels.Extracellular Na^+or K^+ reduction(NaCl-free or KCl-free medium)is thought to activate Ca^{++}influx through the Na^+/Ca^{++}exchange mechanism.

Amrinone caused a dose-dependent inhibition of the high-KCl or KCl-free induced contractures.The effect was equivalent in both conditions.In the high-KCl the IC_{50} was 0.166mM and the maximum inhibition was 87.5%±7,in the KCl-free the $IC_{50}$was 0.173mM and the maximum inhibition 81.22%±5.Aortic strips contracted by KCL-free or by NaCl-free medium relaxed when the modified solutions were replaced with physiological salt solution.Amrinone did not delay the relaxation.

The inhibitory effect of amrinone on the high-KCl induced contracture is likely accounted for the phosphodiesterase inhibition by the drug.If amrinone,in addition to the action on phosphodiesterase,exerts a relevant inhibition also on Na^+/Ca^{++} exchange,we would observe a greater effect on KCL-free induced than on high-KCl induced contracture.Moreover the drug would delay the aortic strips relaxation produced by readmiting NaCl or KCL in the medium.According to our observations it seeems unlikely that the observed inhibition of Na^+/Ca^{++}exchange by amrinone has relevance in the vascular effect of the drug

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THE RESPONSE OF THE GUINEA-PIG HEART TO ISOPRENALINE AND ITS PROPOSED MODULATION BY ENDOGENOUS ADENOSINE

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It has been suggested that in the heart an increased level of endogenous adenosine formed in response to catecholamine stimulation is able to attenuate the response to the amine (Schrader et al. 1977). More recently Dobson (1983) was able to demonstrate this effect in rat atria where endogenous adenosine appeared to reduce both the size and the duration of the response to isoprenaline (ISO). Therefore it was of interest to see if these findings could be extended to the guinea-pig isolated heart.

Langendorff hearts were perfused at 7ml $minute^{-1}$ with Krebs-bicarbonate solution at 38°C gassed with 5% CO_2 in O_2. Contractile force, rate and perfusion pressure were recorded. The hearts were infused with ISO (3ng/ml) for a 6 min period and 15 min later adenosine deaminase (0.3U/ml) (DEAM) or 8-phenyltheophylline (3µg/ml) (PHEN) were infused, during which the exposure to ISO was repeated 15 min later. These experiments were then repeated using electrically paced whole ventricles (5Hz, 5ms, 1.5-2 x threshold voltage).

Measurements were made of peak contractile force developed in the presence of ISO and at 12, 24, 36 sec, 1, 1.5, 2, 4, 8 and 12 min thereafter. Pretreatment responses to isoprenaline were corrected using time matched controls. Measurements of rate were also made at these same times.

ISO increased the contractile force and rate (in unpaced hearts). The tension however rapidly subsided during the period of ISO infusion whilst the rate was sustained. If endogenous adenosine was released during the infusion with ISO, then its well known negative inotropic effects might attenuate the response and explain the rapid decline in tension. Enzymatic breakdown with DEAM or antagonism of adenosine receptors by PHEN, however, failed to modify the decay in response of spontaneous or paced hearts, or to significantly ($P>0.05$) affect peak tension responses (Table 1). Exogenous adenosine (10µg) responses were however abolished.

Table 1. Effects of DEAM and PHEN on ISO peak responses

Heart	Treatment	Peak of ISO response: control		treated	
		tension (g)	rate(bpm)	tension	rate(bpm)
Spontaneous	DEAM	8.3±0.6	393±13	8.2±0.6	*371±15
	PHEN	10.6±0.8	363± 7	9.8±1.3	363± 5
Paced	DEAM	9.6±0.8	-	10.7±0.9	-
	PHEN	7.6±0.7	-	7.7±0.8	-

The peak rate response to ISO occurred 4 min after the tension peak. PHEN did not significantly change this peak, but DEAM caused a small but significant ($P<0.05$) decrease in the rate peak.

On changing normally beating hearts to paced ventricles, the rate of decline of the tension response to ISO was significantly ($P<0.05$) less in the paced preparation. Thus spontaneous rate changes contribute in part to the declining tension, but endogenously produced adenosine does not appear to explain the residual fade. Its reputed modulating role does not therefore apply to the response of normoxic guinea-pig perfused hearts to ISO.

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PROTECTIVE EFFECT OF NICARDIPINE AGAINST MYOCARDIAL ISCHAEMIA AND REPERFUSION IN ISOLATED RABBIT HEARTS

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During ischaemia developed pressure declines, diastolic pressure increases, tissue ATP and CP are depleted; the ATP producing activity of the mitochondria is compromised whilst their avidity for calcium increases. On reperfusion after prolonged ischaemia there is no recovery of developed pressure, further increase of diastolic pressure as well as of mitochondrial calcium with a further reduction of mitochondrial oxidative phosphorylating capacities. Using these changes as indices of ischaemic and reperfusion damage, we have investigated whether nicardipine, a calcium channel inhibitor, protects the myocardium against ischaemic damage. Nicardipine was administered in two ways:
1) Group 1: profilactically to the rabbits before isolation of the heart; rabbits were injected i.p. twice daily with 2 mg/kg of nicardipine for 4-5 days.
2) Group 2: directly to the isolated heart after ischaemia dissolved in the perfusate (10^{-6}M) during post-ischaemic reperfusion.
The hearts were isolated and Langhendorff-perfused. Ischaemia was induced reducing coronary flow to 1 ml/min for 90 min followed by 30 min of reperfusion. Hearts were paced at 180 beats/min and maintained at 37°C irrespective of coronary flow. The data obtained are reported in the following table.

		Developed pressure mmHg	Diastolic pressure mmHg	Tissue ATP µmol/g d.w.	Tissue CP µmol/g d.w.	Mitochondrial Ca++ nmol/mg	Mitochondrial ATP produc. nmol/mg/min
CONTROL	Aerobia	55.5±4	0	18.2±1	31.9±1	14.1±1	362±17
	Isch.	0	34.3±11	2.8±1	6.7±1	28.4±3	321±13
	Reperf.	11.1±4	57.6±3	4.0±1	7.7±1	65.2±10	132±6
GROUP 1	Aerobia	53.5±6	0	18.7±1	31.4±1	17.5±1	368±11
	Isch.	0	8.7±2*	14.8±1**	24.3±2**	24.7±1	341±17
	Reperf.	26.5±11*	36.0±13*	15.9±1**	22.0±1**	34.8±4*	312±19**
GROUP 2	Reperf.	11.0±3	67.7±10	4±1	6.9±1	75.9±12	130±9

* $P < 0.05$; ** $P < 0.01$

These results indicate that nicardipine administered before ischaemia is able to protect the myocardium against ischaemic and reperfusion damage. Likewise, its mechanism of action depends on the ability to ensure sufficient ATP and CP to maintain homoestasis with respect to calcium. Under these conditions mitochondrial calcium overload does not occur and mitochondria function is preserved. This hypothesis is confirmed by the finding that, when added during reperfusion, with tissue ATP and CP contents already reduced, nicardipine did not protect the myocardium against reperfusion damage.

Ca^{++}-DEPENDENCE OF NORADRENALINE-INDUCED CONTRACTION IN RAT AORTIC SMOOTH MUSCLE

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Smooth muscle contraction to noradrenaline (NA) has two components which are dependent to different extents on external Ca^{++} levels (van Breemen, 1977). An initial transient contraction (ITC) is still present in very low $[Ca^{++}]_o$ but a secondary contraction requires a higher level of $[Ca^{++}]_o$. Addition of EGTA will selectively reduce the secondary response but produces an unknown $[Ca^{++}]_o$ making it impossible to estimate accurately the Ca^{++} dependence of the components. However, free $[Ca^{++}]_o$ ($[Ca^{++}]_{free}$) can be accurately calculated in a buffered system incorporating known levels of $[Ca^{++}]_{total}$, EGTA and nitrilotriacetic acid (NTA) (Miller & Smith, 1984). In the rat anococcygeal muscle the ITC to NA (3μM) could be isolated when $[Ca^{++}]_{free}$ was <1μM, but the secondary Ca^{++} dependent contraction had a Ca^{++} requirement equivalent to a pD_2 value of 4.2. BAY K 8644 (1μM) increased this to 4.9 and nifedipine (10μM) decreased it to 3.6 (McGrath et al, 1984; McGrath, 1985; and unpublished observations). Without buffering this exercise was not possible.

The object of the present study was to carry out a similar analysis on rat aorta, which has been used extensively to study Ca^{++}-dependence. Rings of aorta were set up as described by Carrier & White (1985) in either a modified Krebs Bicarbonate or Tris buffered saline. $[Ca^{++}]_{free}$ was modified by omitting Ca^{++} salts from the saline and/or by adding EGTA or by buffering Ca^{++} with EGTA and NTA (2.5mM each). Responses were obtained to NA (0.3-1μM).

Similar results were obtained with Bicarbonate or Tris buffered solutions. Omission of Ca^{++} caused only a 60% reduction in the secondary response to NA leaving a more prominent ITC. Addition of EGTA up to 5mM steadily reduced the maintained response but left an ITC. In Ca^{++} buffered media a maintained component was still present even at $[Ca^{++}]_{free}$ <1μM. Ca^{++} concentration/response curves were constructed in unbuffered or buffered media. In buffered saline the Ca^{++} pD_2 was 3.8. This value was decreased to 3.2 by nifedipine (1μM) but was not affected by BAY K 8644 (1μM). (In unbuffered saline the pD_2 was 4.0)

The results from the anococcygeus show that Ca^{++} pD_2 values between 3 and 5 can be estimated. In that tissue, inhibition of Ca^{++} entry by nifedipine and facilitation by BAY K 8644 could be shown. In rat aorta, the Ca^{++} sensitivity was slightly lower, was similarly sensitive to nifedipine yet was not affected by BAY K 8644. This indicates that although BAY K 8644 can facilitate NA-induced Ca^{++} entry in some smooth muscle preparations it does not do so in rat aorta.

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COMPARATIVE EFFECTS OF CALCIUM ANTAGONISTS ON INHIBITORY JUNCTION POTENTIALS OF CIRCULAR MUSCLE OF GUINEA-PIG ILEUM

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Since calcium ions play an essential role in neurotransmission, "calcium antagonists" would be expected to depress neuro-effector transmission. French and Scott (1981), however, showed that verapamil, but not nifedipine, potentiated the non-adrenergic contractions of the prostatic portion of the rat vas deferens to single pulse stimulation. Recently, Beattie et al. (1985) reported that manganese (Mn^{2+}) ions and verapamil, but not nifedipine, prevented action potential conduction into the terminal varicosities in the guinea-pig vas deferens.

We have examined the effects of verapamil, nifedipine and Mn^{2+} on the non-cholinergic, non-adrenergic inhibitory junction potential (i.j.p.) of circular muscle of guinea-pig ileum. Isolated preparations of ileum were constantly perfused at 28°C with a modified Krebs solution containing hyoscine (2 μM). Intracellular electrophysiological recordings were made with microelectrodes (30-60 MΩ) filled with 2M KCl solution. The mean resting membrane potential (r.m.p.) was -51.2±0.6 mV (± s.e. mean; n = 182); cells with r.m.p. of less than -45 mV were not studied. I.j.p.s, evoked by transmural stimulation, were recorded up to 8 mm from the transmural electrodes; within this limit, the amplitude of the i.j.p. was constant. The amplitude of the i.j.p. was corrected for non-linear summation to minimise the effect of variations in r.m.p.

Verapamil (3-50 μM) and nifedipine (0.3-3.0 μM) caused a marked potentiation (30-135% increase) of the amplitude of the i.j.p. in response to single pulse stimuli, without significantly affecting the time-course of the i.j.p. or the r.m.p. When, however, verapamil (100-300 μM) or nifedipine (10 μM) was used, the amplitude of the i.j.p. was reduced and, in some preparations, abolished. At a concentration of 300 μM, verapamil almost certainly caused a depolarization because few cells with a r.m.p. of -45 mV or more were impaled, whereas nifedipine (10 μM) did not significantly affect r.m.p. Mn^{2+} (0.3-0.5 mM) had no significant effect on r.m.p. or on the amplitude of the i.j.p. evoked by a single pulse but markedly prolonged its time to half-decay (1538 ± 66 ms, compared with 335 ± 23 ms in controls; mean ± s.e. mean). In the presence of Mn^{2+}, 3-6 consecutive pulses at 1 Hz caused a marked decrease in the amplitude of the second and subsequent i.j.p.s, an effect not seen with verapamil or nifedipine. Additionally, only in the presence of Mn^{2+} did post-tetanic stimulation after trains of 6 pulses at 6 Hz fail to elicit an i.j.p., for up to 5 min. Experiments are in progress to determine possible pre- and post-junctional modes of action of these agents on the i.j.p.

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EFFECTS OF CHANGES IN CALCIUM AND PHOSPHATE CONCENTRATIONS ON THE RESPONSES OF ISOLATED CARDIAC MUSCLE TO INOTROPIC DRUGS

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The failing myocardium is characterised by a deficit in the stimulus-contraction coupling mechanism which can be mimicked by placing isolated cardiac muscle in physiological salt solutions containing low concentrations of calcium ions (Bailey et al, 1968). However, failed hearts do not often contain reduced calcium ion concentrations and, very often, they show increased concentrations in the mitochondria (Bowman & Rand, 1980). This phenomenon can be mimicked by exposing cardiac mitochondria to high phosphate concentrations (Vaghy et al, 1981). We have therefore compared the effects of several positively inotropic compounds on these two models of myocardial failure.

Guinea-pig isolated left atria mounted in well oxygenated, modified McEwen (1956) solutions were driven by 0.5 ms pulses of supramaximal voltage (c5V) at 1.5 Hz and cumulative dose-response curves were obtained to ouabain, isoprenaline, aminophylline and M66 (1-(4-aminophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline).

McEwen (1956) solution contains 2.16 mM Ca^{2+} and in this solution isoprenaline (10^{-9} - 3.7 x 10^{-7}M) was most effective, whilst aminophylline (10^{-5} - 4.5 x 10^{-3}M) produced 70% of this response. Ouabain (10^{-7} - 5 x 10^{-6}M) and M66 (10^{-7} - 10^{-4}M) were equieffective at about 50% of the maximum isoprenaline response. In the presence of 1.08 mM Ca^{++} the responses to ouabain were increased, those to aminophylline were maintained, those to isoprenaline were reduced by 25% and those to M66 were reduced by 50%. In the presence of 0.54 mM Ca^{2+} the responses to all four inotropic agents were reduced and the responses to M66 were completely abolished.

In the presence of 4.32 mM Ca^{++} responses to all agents were approximately the same at about 75% of the control ouabain response.

Ringer Locke solution contains no phosphate and when mounted in this solution the basal contractions of atria were about 60% of those in control McEwen's solution. In Locke solution the responses to isoprenaline, ouabain, aminophylline and M66 were all obtainable although the maximum tension generated was less than that in McEwen's solution. In the presence of increasing concentrations of phosphate (4.5 mM, 9.0 mM, 18 mM) there were marginal increases in force of contraction but the responses to inotropic agents were unaffected.

It is concluded that M66 is very dependent on extracellular calcium for its positive inotropic response and that cardiac mitochondria would appear to be well protected against changes in extracellular phosphate concentration.

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CARDIAC EFFECTS OF 2-METHYL-1,4-NAPHTOQUINONE (MENADIONE)

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Several quinones employed as therapeutic agents, i.e. the antineoplastic anthracyclines doxorubicin and daunorubicin and the vitamin K congener menadione, modify heart contractility in different ways. "In vitro" anthracyclines produce biphasic inotropic responses (van Boxtel et al.,1978). Menadione exerts positive inotropic and chronotropic effects through an adrenergic mechanism (Tôth et al., 1966).

Anthracyclines and menadione have toxic effects: cardiotoxicity for doxorubicin and daunorubicin (Lefrak et al.,1973) and hepatotoxicity for menadione (Thor et al, 1982). The cytotoxic effects of quinones are probably mediated through their one-electron reduction to semiquinone radicals which subsequently enter redox cycles with molecular oxygen to produce active oxygen species (Powis et al., 1981).

We report evidence that menadione has complex effects upon myocardial contractility. After the addition of 2-45 μM menadione to electrically driven guinea-pig left atria three temporal phases can be observed:
i, an initial transient decrease in inotropism that appears not to be mediated by a release of acetylcholine;
ii, a subsequent marked increase in contractility, only partially dependent on an adrenergic mechanism, that is not completely reversed by β-blocking agents and persists in atria isolated from reserpinized (2 mg/kg i.p. daily for 2 days) guinea-pigs. The not catecholamine-mediated increase of contractility is not histamine-dependent and is antagonized by 50-100 μM xilocaine and by 0.1-0.5 μM verapamil. Menadione (30 μM) partially restores cardiac contractility depressed by 0.05 μM tetrodotoxin in preparations isolated from reserpinized animals;
iii, a final increase in resting tension that preceeds a not-reversible contracture. This systolic effect is not antagonized by chelating agents, sulfhydryl group donors and antioxidant compounds.

Among the enzymatic systems and exchangers involved in cardiac contractility, cAMP phosphodiesterase is inhibited by menadione in a concentration-dependent way. Consistent degrees of inhibition of sarcotubular Ca^{2+}ATPase and Na^{+}/Ca^{2+} exchange are evident after a long preincubation time. Na^{+}/K^{+} ATPase is not affected at all.

The presence of microsomal metabolizing systems does not alter the inhibitory effects of the drug.

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EFFECT OF L-CARNITINE, ACETYL-L-CARNITINE AND L-PROPIONYL-CARNITINE ON LIPID PEROXIDATION OF CARDIAC MITOCHONDRIA

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It has been suggested that L-Carnitine derivatives may exert protection on membrane damage induced by lipid peroxidation. To investigate this hypotesis we have chemically induced lipid peroxidation on myocardial mitochondria. Freshly isolated rabbit heart mitochondria were incubated for 30 minutes at 20° C with ferrous ions (0.2 mM). Mitochondrial function was measured in terms of RCR, QO_2, ADP/O (determined polarographically) and of Ca^{2+} transport (determined by means of a selective Ca^{2+} electrode). Lipid peroxidation was evaluated following malonaldehyde (MDA) production. The effect of L-Carnitine, Acetyl-L-Carnitine and L-Propionil-Carnitine were also determined. The results on mitochondrial function are summarized in the following table.

	RCR	QO_2(4) n a./O_2/mg prot.	ADP/O
CONTROL	14.8 ± 1.4	22.7 ± 0.5	2.98 ± 0.03
AFTER Fe2+ INC. (0.2 mM)	7.1 ± 0.9	46.0 ± 6.9	2.73 ± 0.06
AFTER Fe2+ INC. + L-CARNITINE (1mM)	8.2 ± 0.7	39.0 ± 4.1	2.71 ± 0.04
AFTER Fe2+ INC. + L-ACETYL-CARN. (1mM)	8.8 ± 1.2	34.5 ± 5.0	2.74 ± 0.03
AFTER Fe2+ INC. + L-PROPIONIL-CARN. (1mM)	10.2 ± 0.8*	28.3 ± 0.7**	2.91 ± 0.03*
AFTER Fe2+ INC. + PROPIONIC ACID (1mM)	7.9 ± 0.6	35.8 ± 1.6	2.70 ± 0.03

Incubation with Fe^{2+} induced an increase in MDA formation (from 0.56 ± 0.11 to 6.5 ± 0.7 n moles/mg prot) and a decrease of RCR, QO_2 and ADP/O. Accordingly there was a marked compromition of the mitochondrial capacity to accumulate and retain calcium. L-Carnitine and Acetyl-L-Carnitine (tested at concentration from 0.5 to 1.5 mM) were almost ineffective, whilst L-Propionil-Carnitine (tested at concentration from 0.5 to 2 mM) was able to reduce the decline in mitochondrial function, the increase in MDA and to maintain Ca^{2+} transport capacities. The effect of L-Propionil-Carnitine was more evident at 1 mM. Further investigation have excluded that the beneficial effect of L-Propionil-Carnitine was dependent on propionic acid (the data reported in the table) or on direct antioxidant action. It is likely, therefore, that L-Propionil-Carnitine interacts with mitochondria membrane leading to a stabilizing effect. These results indicate that lipid peroxidation causes severe impairement on mitochondrial function which is protected by L-Propionil-Carnitine.

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EFFECT OF PROPIONYL-L-CARNITINE-TAURINE AMIDE ON RECOVERY OF CARDIAC FUNCTIONS AS AFTER ISCHAEMIC HYPOXIA IN RAT WORKING HEART

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Previous studies have suggested that L-Carnitine can protect the ischemic myocardium in animals under experimental ischemia. More recent research has shown that two derivatives of L-Carnitine: Propionyl-L-Carnitine (ST 261) and Propionyl-L-Carnitine-Taurine Amide (ST 520), can also protect the ischemic rat heart.

In the present study we compared the effects of ST 520 ($4x10^{-9}$, $4x10^{-8}$, $4x10^{-7}$ M), ST 261 ($4x10^{-8}$, $4x10^{-7}$ M) and taurine ($4x10^{-8}$, $4x10^{-7}$ M) on the cardiac output, coronary flow, (CF) systolic and diastolic pressure (SP-DP) in hearts subjected to ischemic hypoxia (10 min) and reperfused (30 min) according to the working heart preparation. The data obtained in this experiment were indicative of the heart performance in both isometric and isotonic systole, e.g. the systolic and diastolic pressure X output values in normoxic and in the 30 min reperfusion after ischemia in controls, and in ST 520 $4x10^{-7}$ M reperfused hearts were as follows:

	SP x OUTPUT	DP x OUTPUT
CONTROLS		
BASAL	4433.7±131.16	1608.3±43.72
30' after ischemia	3354.0±138.26	1504.4±54.65
ST 520		
BASAL	4307.8±215.07	1803.5±68.45
30' after ischemia	4383.6±363.84	2361.5±221.43

Experimental results suggested that ST 520 improves the recovery of the ischemic myocardium. ST 261 and taurine evidenced a milder or no activity depending on the parameters considered.

EFFECTS OF REPEATED SUBCUTANEOUS ADMINISTRATION OF PARAQUAT

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Paraquat is a herbicide which has been responsible for a number of fatalities because of its toxicity to the lung. Smith et al, (1979) showed that paraquat is selectively accumulated by the lung which accounts in part for the specific toxic effect on that organ. Evidence suggests that both Type I and Type II pneumocytes are the sites of accumulation. In humans paraquat causes diffuse alveolar damage with oedema, haemorrhage, and increased fibroblastic activity leading to alveolar fibrosis (Whimster and de Poitiers, 1982). The picture in animals is similar and has been divided into a destructive phase in which Type I and Type II pneumocytes are destroyed or damaged, followed by a proliferative phase in which macrophages, profibroblasts and fibroblasts become more numerous with an associated accumulation of collagen (Smith et al, 1974).

Paraquat, dissolved in saline (g/l) was injected subcutaneously into male Porton Wistar-derived rats (200-250 g) at a dose volume of 1 ml/kg. Groups of at least 5 rats received 3 mg/kg or 4 mg/kg 5 days/week; 4 mg/kg or 6 mg/kg 3 times/week for up to 3 weeks. They were killed at 10, 21 or 28 days. An additional group dosed at 3 mg/kg/day was killed at 5 days. Control groups received solvent only and were killed at 28 days. The animals were observed and weighed daily: if an individual animal had lost greater than 20% of its initial body weight dosing was stopped. After sacrifice the lungs were inflated "in situ" with 10% neutral buffered formalin and samples for histological examination were taken from the upper and lower lobes of the right lung and from the left lung. 4 μm sections were stained with haematoxylin and eosin or with Masson's trichrome.

No deaths were observed in the 3 mg/kg 5 days/week study or in the 4 mg/kg 3 days/week exposure. In the 4 mg/kg 5 days/week study 5 animals died during dosing and 3 animals became sufficiently affected for the dosing to be suspended. In the 6 mg/kg 3 days/week study 3 deaths occurred and dosing was not curtailed in any of the remainder. Generally there was significant weight loss in all of the paraquat dosed groups. In the 4 mg/kg/day group there was a rise in respiration rate during the course of the experiment reflecting the decrease in body weight. Histological findings in the test animals could largely be divided into 2 types; destructive as exemplified by increases in alveolar macrophages, alveolitis and oedema, and reparative as shown by foci of Type II pneumocyte hyperplasia, alveolar thickening, interstitial fibrosis and sometimes intra-alveolar fibrosis. In order to reveal trends in pathology the findings were graded into mild, moderate or severe on the basis of their extent and density. It was found that macrophages became more prominent with time as did the prevalence of alveolitis. Type II pneumocyte hyperplasia and fibrosis followed a similar course becoming more extensive in the latter stages of dosing especially after 10 days. No distinct disruptive phase, followed by proliferation, as reported by (Smith et al, 1978) could be distinguished. This was due to the multiple insults of the dosing regime. A notable feature was that cessation of dosing at 19 days was followed by a decrease in the extent and severity of fibrosis.

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THE RELAXANT EFFECTS OF AN ATRIAL PEPTIDE AND NITROPRUSSIDE ON RAT VASCULAR MUSCLE DEPEND ON SPASMOGEN CONCENTRATION

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Atrial natriuretic factors (ANFs) are peptides which may be the effector hormones of a new endocrine system with a physiological role in extracellular fluid volume and electrolyte homeostasis (Needleman et al., 1984). In addition to their natriuretic properties and ability to inhibit aldosterone production, low concentrations of ANFs have been shown to relax precontracted vascular smooth muscle in vitro. The greater effect of an ANF in antagonising vascular responses not associated with marked cell membrane depolarisation led to the suggestion that ANFs cause vasodilatation in a similar way to sodium nitroprusside (SNP) (Winquist et al., 1984). The inhibitory effects of SNP on the responses of canine renal artery to low concentrations of potassium (K^+) and noradrenaline (NA) are much greater than those obtained with high concentrations (Karaki et al., 1980). The present study examines the dependence of ANF-induced relaxation on K^+ and NA concentration.

Isolated helical strips of rat aorta, bathed in Krebs' solution at 37° C under a resting tension of 1g, were pre-contracted with a range of concentrations of KCl or NA and subsequently relaxed by the cumulative addition of SNP or ANF(6-33). (ANF(6-33) = SerLeuArgArgSerSerCysPheGlyGlyArgIleAspArgIleGlyAlaGlnSerGlyLeuGlyCysAsnSerPheArgTyr)

Table 1	KCl concn. (mM)			NA concn. (nM)		
	20	40	60	10	100	1000
ANF(6-33)	2.55	8.55	9.76	0.494	5.29	23.7
	(1.38-5.00)	(4.97-24.5)	(5.8-20.0)	(0.15-2.10)	(1.67-17.3)	(5.60-63.0)
	100 ± 6.0	70 ± 6.2	36 ± 1.7	91 ± 3.6	82 ± 7.8	78 ± 6.6
SNP	2.29	4.12	8.88	0.253	0.332	1.72
	(0.52-4.92)	(2.40-7.11)	(5.50-24.3)	(0.19-0.43)	(0.18-0.52)	(0.77-2.9)
	90 ± 3.8	78 ± 4.1	65 ± 4.3	100 ± 0	100 ± 0	96 ± 2.3

Geometric mean IC_{50} values in nmol/l (range) and max. relaxant effect (%±SE) N > 7

Table 1 shows that both ANF(6-33) and SNP are less effective in relaxing contractions induced by higher concentrations of spasmogen. Increasing spasmogen concentration caused both a depression of maximum and a shift to the right of dose response curves for both ANF(6-33) and SNP. Karaki et al (1980) attributed the similar action of SNP in canine renal artery to the ability of high concentrations of spasmogens to increase uptake of the lanthanum-resistant calcium fraction, producing a strong stimulus to contraction which SNP was unable to overcome. The differential depolarising effects of the various spasmogen concentrations or the abilities of ANF and SNP to elevate intracellular cGMP levels may also contribute to the observed effect. Recently, Bergey and Kotler (1985) have also shown a similarity in the effects of SNP and ANF(10-33) on rabbit aortic strips.

These results support the hypothesis that ANFs and SNP share a common mechanism of action.

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IS INHIBITORY REGULATION OF ADENYLATE CYCLASE INVOLVED IN THE ACTION OF SPASMOGENS ON INTESTINAL SMOOTH MUSCLE?

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Pretreatment of animals with an optimal dose of Bordetella pertussis toxin is known to effectively uncouple the inhibitory influence on adenylate cyclase of a number of receptor types acting via the N_i regulatory subunit.

The availability of such pretreated guinea-pigs in the laboratory from a parallel study on presynaptic modulation of neurotransmitter release (Fosbraey et al., 1985) where the degree of poisoning of this control mechanism could readily be quantified, allowed a speculative investigation of possible involvement of the N_i subunit in postsynaptic excitation of smooth muscle of the ileum by exogenously applied neurotransmitters and peptides.

Our reasons for interest were twofold. First, we were investigating a suggestion by Holzer & Petsche (1983) that substance P depolarises intestinal smooth muscle cells by decreasing potassium permeability, as is the case for certain neurones, and the coupling mechanism here is unknown. Secondly, muscarinic receptors are able to couple to the N_i subunit in some tissues including cardiac muscle and some neurones and, though it is clear that the principal mode of depolarisation of intestinal smooth muscle is an increase in cation permeability not involving adenylate cyclase (Bolton, 1979), it seemed possible that there may also be some N_i coupling, conceivably through another muscarinic receptor subtype.

Longitudinal muscle strips were prepared from the ileum of guinea-pigs in a 150-220 g body weight range that had been injected 3 days previously with Pertussis toxin (125 µg/kg i.p.; toxin supplied by Dr L Irons, Porton Downs) or vehicle control. Isometric contractions on application of agonists were recorded using Krebs bathing solution at 31°C containing hexamethonium (100 µM), guanethidine (10 µM) and atropine (1 µM) to minimise the effects on the preparation of neurotransmitter overspill from disinhibited nerves of the myenteric plexus. Dose-response lines were obtained at three dose-levels with three or more replicates for histamine, substance P, eledoisin, bradykinin and carbachol, using histamine as an internal control in all strips and obtaining EC_{50} estimates in terms of its maximum. Another series was also run with TTX (300 nM) substituted for atropine in the bathing fluid in view of the elevated doses of carbachol necessary in its presence.

Comparison of EC_{50} estimates in test and control groups showed no strong evidence of a difference in sensitivity to any of the spasmogens ($p > 0.05$, $n=8$) due to Pertussis treatment. Thus it seems unlikely that the N_i regulatory subunit is involved to any important extent in the contractile actions of these five spasmogens.

J.M. Hall has an M.R.C. studentship.

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CARDIOVASCULAR AND BRONCHODILATING PROPERTIES OF TRAPIDIL

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Trapidil, 5-methyl-7-dietylamino-s-triazolo(1,5-a)pyrimidine, a compound previously proposed as a therapeutic agent for ischemic heart disease, is reported to have miscellaneous pharmacological-biochemical actions generally correlated to its anti-phosphodiesterase activity (coronary vasodilation; inhibition of platelet aggregation and thromboxane effects and/or synthesis; increase in erythrocyte deformability).

In the present study we investigated both cardiovascular and bronchodilating properties of trapidil in different animal species.

In anesthetized beagle dogs trapidil (3 and 10 umol kg^{-1} i.v.) produced a marked increase in coronary blood flow (+15.4 and +61.1%) and a considerable reduction of coronary (-24.2 and -45.9%) and peripheral (-27.0 and -46.1%) vascular resistance. These effects were accompanied by an appreciable increase in cardiac output and dP/dt max and by a moderate positive chronotropism.

The bronchodilating effects of trapidil were investigated in anesthetized guinea-pigs by the overflow method (Konzett-Roessler). The compound inhibited dose-dependently the bronchoconstriction induced by 22-33 nmol kg^{-1} i.v. histamine, 110-165 nmol kg^{-1} i.v. acetylcholine and 17-34 nmol kg^{-1} i.v. 5-HT; the ID_{50} against the three agonists resulted respectively 6, 48 and 10 umol kg^{-1} i.v.

In the anesthetized cat and dog the i.v. administration of trapidil (10-30 umol kg^{-1}) inhibited the rise in pulmonary artery blood pressure and airway resistance both induced either by i.v. infusion of histamine (9-18 nmol kg^{-1} min^{-1}) or 5-HT (50 nmol kg^{-1} min^{-1}).

This study, besides confirming the peripheral and coronary vasodilating properties of trapidil, suggests that the drug could be useful in the management of bronchoconstriction, especially in the presence of a concomitant increase in pulmonary vascular resistance.

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EFFECTS OF SELECTIVE 5-HYDROXYTRYPTAMINE AGONISTS ON CAROTID BODY CHEMORECEPTOR DISCHARGE IN ANAESTHETIZED CATS

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Intracarotid (i.c.) injection of 5-hydroxytryptamine (5-HT) in cats has the following effects on chemosensory discharge: dose-related chemodepression, selectively blocked by the 'neuronal' 5-HT antagonist MDL 72222 (MDL); delayed chemoexcitation, selectively blocked by the 5-HT_2 antagonist ketanserin, and intense, but transient chemoexcitation preceding 5-HT-evoked chemodepression, which is also selectively blocked by MDL (Kirby & McQueen, 1984). We have further studied the 5-HT receptors that might be responsible for these effects using the following selective agonists: 2-Methyl,5-hydroxytryptamine (2Me5-HT), active at MDL-sensitive sites (see Humphrey, 1984); 5-methoxytryptamine (5-MeT), which is inactive at MDL-sensitive sites (Fozard, 1984); 8-OH-DPAT, a 5-HT_{1A} agonist (Middlemiss & Fozard, 1983), and RU 24969, a selective 5-HT_{1B} agonist (Cortés et al, 1984).

Chemoreceptor discharge was recorded in pentobarbitone-anaesthetized cats, artificially ventilated with air and paralysed with gallamine (for details see McQueen, 1977), and responses evoked by i.c. injections of the 5-HT agonists were compared.

During experiments in which the typical effects of injection of 5-HT (1-25 µg) were obtained, 2Me5-HT (1-50 µg; n=4) caused chemodepression equal to, or greater than that seen with 5-HT; marked transient chemoexcitation was elicited by 5-HT in all four experiments, whereas 2Me5-HT had only weak effects. 2Me5-HT caused variable secondary chemoexcitation, and hypotension similar to that induced by 5-HT. In contrast, 5-MeT (1-100 µg; n=3) caused only slight changes in chemoreceptor discharge, although its vascular effects were similar to those evoked by 5-HT.

RU 24969 (0.5-50 µg; n=2) caused marked dose-dependent prolonged chemoexcitation, preceded in one experiment by slight chemodepression - less than that seen with 5-HT. There was no initial transient chemoexcitation, nor any obvious effect on blood pressure. 8-OH-DPAT (0.5-50 µg; n=2) caused prolonged dose-related hypotension; there was neither initial transient nor delayed chemoexcitation, but chemodepression, greater than that caused by 5-HT occurred, and persisted after 5-HT-evoked chemodepression had been blocked by MDL (100 µg kg^{-1}) in one of the experiments.

The rank order of potency of these agonists for the three phases of the '5-HT response' appears to be: transient excitation 5-HT >> 2Me5-HT; chemodepression 8-OH-DPAT > 2Me5-HT and 5-HT >> RU24969 and 5MeT; secondary excitation RU 24969 > 5-HT > 2Me5-HT >> 8-OH-DPAT and 5-MeT. These results provide further evidence that 'neuronal' 5-HT receptors are associated with the carotid chemoreceptors, and also emphasise the complexity of the 5-HT effects on chemosensory discharge, the significance of which remains to be determined.

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SELECTIVE BLOCKADE BY ICS 205-930 OF 5-HT DEPOLARISATIONS OF RABBIT VAGAL AFFERENT AND SYMPATHETIC GANGLION CELLS

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5-HT has a potent depolarizing action on vagal afferent cell bodies in the nodose ganglion (NG) and on sympathetic neurones in the superior cervical ganglion (SCG) (Wallis et al, 1982). ICS 205-930, (3α-tropanyl)-1H-indole-3-carboxylic acid ester, has been shown to antagonize the indirect stimulant action of 5-HT on the rabbit heart and the 5-HT-induced depression of the rabbit vagus nerve compound action potential (Donatsch et al,1984). We report here the effects of this agent using a method which allows the depolarizations induced by 5-HT to be recorded directly. The membrane sucrose-gap method was employed to record membrane potential change from a population of neurones in NG and SCG, prepared as described by Azami et al (1985). Ganglia were continuously superfused with Krebs solution at 19-21° C. Reproducible depolarizations were obtained by injecting into the superfusion stream to the ganglion amounts of 5-HT ranging from 10-80 nmol (0.05 to 0.4 ml of solutions in Krebs) for NG and 20-320 nmol for SCG. ED_{50} values for 5-HT on NG and SCG were approximately 17 and 80 nmol, respectively.

ICS 205-930 was a potent antagonist of 5-HT responses in both tissues, the threshold for blockade being around 10^{-11}M. Dose-response curves to 5-HT were constructed before exposure and after equilibration with the antagonist for at least 1 h. ICS 205-930 (10^{-11} and 10^{-10}M) caused a rightward, parallel shift of the DR curves and blockade was surmountable, whereas 10^{-9} and 10^{-8}M caused a rightward shift of the curves with a reduction of slope and of their maxima. Apparent pA_2 values were determined in experiments where a parallel shift occurred (a) from a Schild plot and (b) from $pA_2 = -\log[B]+\log[DR-1]$, where B is the concentration of antagonist. Results are shown below with, for comparison, values for the selective 5-HT antagonist (Azami et al, 1985) tropine 3,5-dichlorobenzoate (MDL 72222).

	ICS 205-930		MDL 72222	
	NG	SCG	NG	SCG
a)	9.75	10.55	7.81	7.94
b)	10.23±0.15	10.44±0.11	7.73±0.09	7.78±0.10

Lower line shows means ± s.e.mean.

Thus, ICS 205-930 showed a similar blocking potency on NG and SCG and was around 350 times more potent than MDL 72222.

The selectivity of blockade was assessed by using as control agonists DMPP and GABA on NG and DMPP on SCG. ICS 205-930 (10^{-11}-10^{-9}M) did not alter the amplitude of responses (50% maximal) to DMPP or GABA in either ganglion. We conclude that ICS 205-930 is a highly potent and selective antagonist of the depolarizing action of 5-HT in NG and SCG cells.

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5-HYDROXYTRYPTAMINE RECEPTORS IN THE HUMAN SAPHENOUS VEIN

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In human saphenous veins, nerve stimulation-evoked contractions are mediated predominantly by alpha$_2$-adrenoceptors (Docherty & Hyland, 1985) and both noradrenaline (NA) and 5-hydroxytryptamine (5-HT) are potent veniconstrictors (Müller-Schweinitzer, 1984). We have chosen to examine the receptors mediating the contractions produced by 5-HT in this tissue.

Human saphenous veins were obtained from coronary artery bypass grafts of patients (predominantly male) in the age range 37-70 years. Tissues were cut spirally into strips and were bathed in Krebs-Henseleit solution at 37°C under 1 g tension. Cumulative concentration-response curves were carried out to 5-HT alone or following 45 min exposure to an antagonist drug.

5-HT contracted the human saphenous vein with an EC50 (concentration producing 50% of maximum contraction) of 0.24 uM (95% confidence limits 0.16-0.39 uM), and there was no significant correlation between potency and age. Desipramine (1 uM) did not significantly alter the potency of 5-HT, so that the contractions to 5-HT did not appear to involve an indirect component by release of NA.

The neuronal 5-HT antagonist MDL 72222 (Fozard, 1984), in concentrations of up to 1 uM, did not significantly affect the contractions to 5-HT. The 5-HT$_2$ antagonist ketanserin (1 uM) did not significantly affect the contraction to low concentrations of 5-HT but shifted the contraction to high concentrations of 5-HT, and the 5-HT$_2$ antagonist cyproheptadine (1 uM) acted non-competitively, reducing the maximum contraction to 5-HT. Yohimbine competitively antagonised the contractions to 5-HT with a pA$_2$ value of 5.66. This potency of yohimbine is much lower than its potency at alpha$_2$-adrenoceptors in this tissue.

In conclusion, the contractions of the human saphenous vein produced by 5-HT appear to be mediated predominantly by 'atypical' postjunctional 5-HT receptors at which yohimbine is a competitive antagonist. Additionally, 5-HT$_2$ receptors may be present.

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EFFECT OF NEUROCHEMICAL LESIONS ON THE CARDIOVASCULAR RESPONSES TO 8-HYDROXY-2-(DI-N-PROPYLAMINO)-TETRALIN (8-OH-DPAT) IN RATS

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8-OH-DPAT is a central 5-hydroxytryptamine (5-HT) receptor agonist (Hjorth et al., 1982) which shows selectivity for the putative 5-HT_{1A} receptor (Middlemiss and Fozard, 1983). The compound has been suggested to lower blood pressure (BP) in rats via activation of central 5-HT receptors (Fozard and Mir, 1985). The hypotensive effects of 8-OH-DPAT appear to involve a catecholaminergic link (Fozard and McDermott, 1985), since α_2-adrenoceptor antagonists can block its response despite 8-OH-DPAT lacking direct agonist effects at α_2-adrenoceptors. The objective of the present study was to determine if the hypotensive effects of 8-OH-DPAT are pre- or post-synaptically mediated and to investigate further the catecholaminergic involvement in the response.

Cardiovascular responses to 8-OH-DPAT given i.v. in pentobarbitone anaesthetized rats (male, Sprague-Dawley, 280-350g) were determined after various treatments designed to reduce monoamine levels in the brain. BP and heart rate (HR) were measured using standard techniques. Neurotoxins were injected intracerebroventricularly (ICV) or directly into specific brain areas using stereotaxic procedures. An 8-10 days post-lesion recovery time was allowed prior to testing with 8-OH-DPAT. Immediately after testing, the rats were killed and the monoamine concentrations of selected brain regions assayed using HPLC with electrochemical detection (Wagner et al.,1982).

8-OH-DPAT, 2-128 µg/kg i.v., caused dose-related falls in BP and HR. ICV injection of 5,7-dihydroxytryptamine (5,7-DHT), 150 µg/kg (4 µl/rat), following pretreatment with desmethylimipramine, 10 mg/kg i.p., reduced the cardiovascular responses to all doses of 8-OH-DPAT compared to sham-operated controls. The hypotensive effect of 8-OH-DPAT was further inhibited by injecting 5,7-DHT lesioned rats with p-chlorophenylalanine (PCPA), 300 mg/kg i.p., 3 days before testing with 8-OH-DPAT. PCPA alone did not change the effects of 8-OH-DPAT. Lesioning with 5,7-DHT reduced brain 5-HT by 63%, PCPA alone reduced brain 5-HT by 74% and combining PCPA with 5.7-DHT reduced brain 5-HT by 88%. Catecholamine concentrations were little changed following these treatments.

The cardiovascular effects of 8-OH-DPAT, 32 µg/kg i.v., were unchanged following ICV injection of 6-hydroxydopamine, 250 µg/kg, (4 µl/rat) or by DSP4, 1 mg/kg i.p., injected 10 days before test. Both treatments resulted in a 77 - 99% depletion of noradrenaline (NA) in different brain regions except for the hypothalamus (33-44%) and pons-medulla (24-27%). In an attempt to reduce specifically hypothalamic NA levels, DSP4, 10 µg/side, was injected bilaterally into the ventral noradrenergic bundle (VNB) (co-ordinates : Ant.-0.48 ; Vert.-0.35; Lat.$\pm$ 0.12mm). This treatment did not alter the responses to 8-OH-DPAT, 32 µg/kg i.v., and there was no reduction of NA in any part of the brain suggesting that DSP4 administered into the VNB is not effective in producing noradrenergic lesions.

These results confirm that depletion of central 5-HT does not per se affect the responses to 8-OH-DPAT (Fozard and McDermott, 1985) although the integrity of the 5-HT nerve terminals appears to be important. A catecholaminergic involvement in the response to 8-OH-DPAT is not apparent from lesioning of noradrenergic neurones. It is possible that the remaining concentration of NA is sufficient for a normal response to 8-OH-DPAT or that areas like the pons-medulla or hypothalamus, which are little affected by the lesions, are involved in the cardiovascular response to 8-OH-DPAT.

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THE MECHANISM OF THE HYPOTENSIVE ACTION OF 5-CARBOXAMIDOTRYPTAMINE IN CONSCIOUS DOCA-SALT HYPERTENSIVE RATS

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5-Carboxamidotryptamine (5-CT) is a potent agonist at 5-HT receptors mediating relaxation of vascular smooth muscle in vitro (Feniuk et al., 1984) but also has other effects (Feniuk et al., 1981). The present study was undertaken to determine the mechanism of the hypotensive action of 5-CT in conscious DOCA-salt hypertensive rats.

Male rats, CD Charles River, were made hypertensive and blood pressure and heart rate measured as described elsewhere (Dalton et al., 1985). Drugs were administered either into the lateral cerebral ventricle (i.c.v.) or intravenously (i.v.) into a cannulated jugular vein. All values are shown as mean $\pm$ s.e.m.

5-Carboxamidotryptamine (0.1-10 µg i.v., n=7) produced immediate dose related decreases in diastolic blood pressure (maximum decrease 33 $\pm$ 6 to 77 $\pm$ 4 mmHg) and increases in heart rate (maximum increase 59 $\pm$ 16 to 115 $\pm$ 15 b/min). However, 5-CT administered centrally (0.1-10 µg i.c.v., n=7) was approximately 10-30 times weaker in producing decreases in blood pressure. These results suggest that the hypotensive action of 5-CT is predominantly mediated by a peripheral mechanism. Ganglion blockade with mecamylamine (5 mg/kg i.v.) reduced blood pressure and heart rate. After mecamylamine, 5-CT still produced vasodepression (see Fig. 1) but the tachycardia was abolished indicating that the latter was reflexly mediated. In these animals, the hypotensive action of 5-CT was not markedly affected by the 5-HT_2 antagonist, ketanserin (0.03-1 mg/kg i.v., n=6) or the M-receptor antagonist MDL 72222 (2 mg/kg i.v., n=4) (Fozard, 1984), but was dose-dependently antagonised by methiothepin (0.1-1 mg/kg i.v., n=6) and methysergide (0.1-1 mg/kg i.v., n=6) (see Fig. 1). These doses of antagonists did not modify the vasodepressor effects of isoprenaline, 0.03-0.1 µg/kg i.v.

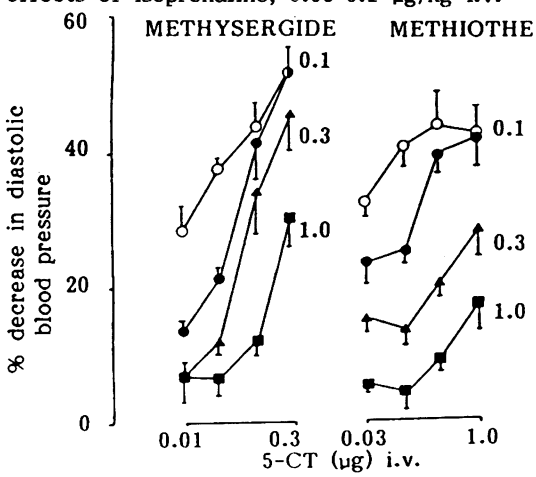

Figure 1: The effect of methysergide and methiothepin on the vasodepressor effect of 5-CT (µg) given intravenously to conscious DOCA-salt hypertensive rats pretreated with mecamylamine (5 mg/kg i.v.). Control responses are shown as open circles and responses after antagonist treatment (mg/kg i.v.) are shown as closed symbols. Values shown are mean $\pm$ s.e.m. Neither antagonist caused any significant change in diastolic blood pressure.

The results of this study suggest that the vasodepressor action of 5-CT is not mediated via classical M or 5-HT_2 receptors but appears predominantly mediated through a direct vasodilator action at a "5-HT_1-like" receptor (Feniuk et al., 1984; Trevethick et al., 1984).

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DRUG ACTION OF SEROTONIN MECHANISMS CAN ENHANCE FIELD STIMULATION-INDUCED CONTRACTIONS OF STOMACH SMOOTH MUSCLE

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The antagonism by serotonin of metoclopramide's action to facilitate field stimulation (FS)-induced contraction responses of stomach smooth muscle strips would indicate an action of metoclopramide via blockade of an 'inhibitory' serotonin receptor system. However, serotonin itself fails to reduce contractions to FS (Gunning & Naylor, 1985). In the present study we further investigate the role of serotonin to regulate contractions of stomach smooth muscle, and in the actions of metoclopramide.

Male Dunkin-Hartley guinea-pigs (450-550g) were killed by cervical dislocation and the stomachs removed. Gastric body longitudinal muscle strips (one from each animal, 20mm long and 5mm wide, with the mucosal layer removed) were placed in tissue baths containing oxygenated (95% O_2, 5% CO_2) Krebs-Henseleit solution at 37°C. 1g tension was applied to the tissues which were allowed to equilibrate for 45 min before being subject to electrical stimulation (platinum wire electrodes placed 5mm apart, supramaximal voltage, 0.1ms pulse width, 0.1-10Hz) or drug treatments. Levels of serotonin in treated and non-treated stomach strips were determined using HPLC with electrochemical detection. At least 6 tissues were used for each treatment and the significance of differences between treatments determined using the Mann-Whitney U test.

5-Hydroxytryptophan (5HTP, 3×10^{-7}-2.5×10^{-4}M) caused concentration-related enhancements (up to 200% of control values at the highest concentration, $P<0.001$) of the FS-induced contractions throughout the frequency range, without change in the resting tissue tension. The l-aromatic amino acid decarboxylase inhibitor monofluoromethyldopa (Fozard et al, 1980) (6×10^{-7}-10^{-4}M) and the tryptophan hydroxylase inhibitor parachlorophenylalanine (Koe & Weissman, 1966) (2.5×10^{-7}-2.5×10^{-5}M) similarly caused concentration-related enhancements of the FS-induced contractions to a maximum of 150-200% of control values ($P<0.001$). The ability of 5HTP (3×10^{-5}M) to enhance FS-induced contractions was abolished by a 30 min pretreatment with monofluoromethyldopa (2.5×10^{-5}M) which alone caused a modest increase in FS-induced contractions. Metoclopramide (10^{-7}-10^{-5}M) caused concentration-related increases (up to 250%, $P<0.001$) in FS-induced contractions. This action was exaggerated further (by 200% as compared to metoclopramide alone, $P<0.001$) when strips were taken from guinea-pigs pretreated for 5h with monofluoromethyldopa (40mg/kg i.p. which reduced serotonin levels by 50-70%, $P<0.001$). The neuronal 5HT receptor antagonist MDL 72222 (10^{-9}-10^{-8}M) caused concentration-related increases (approx. 200%, $P<0.01$-$P<0.001$) in FS-induced contractions, but increasing concentrations of MDL 72222 (10^{-7} and 10^{-6}M) were less effective and at 10^{-5}M the FS-induced contractions were abolished.

These findings are consistent with a hypothesis that within the body longitudinal muscle of the guinea-pig stomach there exists to modify FS-induced contractions (a) a dominant inhibitory 5HT system which is antagonised by metoclopramide and MDL 72222 to facilitate FS-induced contractions, and which is normally subject to a maximal endogenous serotonin stimulation, and (b) a second facilitatory 5HT system responsive to exogenous 5HTP/5HT and which may afford a site of action for higher concentrations of MDL 72222 and perhaps even metoclopramide to thus indirectly 'reduce' FS-induced contractions.

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