NORADRENERGIC RECEPTORS IN PAIN AND ANALGESIA

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Much attention has lately been drawn to the role of noradrenaline in both narcotic and non-narcotic induced analgesia and to the relative contribution of spinal and supraspinal structures in suppression of responses to painful stimulation. The present study describes a series of experiments carried out to evaluate whether α-adrenoceptors modulate nociception in rats. The analgesia test employed was the electrical stimulation method originally developed by Carroll and Lim (1960) and later modified and extensively described (Paalzow, 1976). This method allows to study the effect of drugs on pain reactions relayed through different levels of the CNS. The approach here was to record a noxious response not elicited in spinally transected rats and thus mediated from structures above the level of the spinal cord. The parameter registered was a vocalisation only during pain stimulation response, which is abolished after transections below the medulla oblongata.

The antinociceptive effect of the α-adrenoceptor agonist clonidine was recorded after subcutaneous administration of the drug in doses from 50 μg/kg up to 2000 μg/kg. Both low and high doses of clonidine produced antinociception on the pain threshold studied. A careful analysis of the dose-response curve showed, however, that the net effect recorded involved the sum of responses from at least two functional systems or receptor sites. When the dose-response relationship of clonidine induced antinociception was studied after α_1-receptor blockade by means of phenoxybenzamine, it was found that this effect comprised contributions from opposite noradrenergic systems. It is suggested that separate adrenergic receptors mediate clonidine antinociception at different levels in the pain transmission. The determinant of the population of receptors being activated after systemic administration of clonidine is the dose given.

Low doses of the α_2-receptor blocking agent yohimbine (0.25-2 mg/kg s.c.) were found to dose-dependently increase the sensitivity to pain whereas doses over 4 mg/kg instead induced slight but significant antinociception. Naloxone (5 mg/kg) initially potentiated yohimbine induced increased pain sensitivity but hereafter rapidly reversed the decreased pain thresholds to normal suggesting the involvement of endogenous opoid pathways in the regulation of pain transmission on the threshold studied.

The results show, that adrenergic mechanisms are capable of modulating pain signals, however, the α-adrenoceptors seem to play different functional roles at different levels of response integration. Moreover in the control of pain adrenergic and opoid pathways may be associated.

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INTERACTIONS OF NALOXONE WITH KETAMINE OR ALTHESIN AT HIGH PRESSURE

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This paper reports results of some studies concerned with the mechanism of pharmacological protection against the high pressure neurological syndrome (HPNS), which is characterized in animals by tremors and convulsions. We have previously shown (Green et al, 1977) that Althesin and ketamine are anaesthetics which are effective in preventing the HPNS.

Naloxone is known to antagonize ketamine analgesia, but not that produced by Althesin (Lawrence & Livingston, 1980). We have studied naloxone alone in rats to determine its effect on the HPNS, and subsequently administered it to rats which were protected against the HPNS with ketamine or Althesin.

Sprague-Dawley rats (♂250-400g) with cannulated tail veins were used in all experiments. After preparation the rat was placed in a small cage, which was mounted over a strain gauge. The amplified signal from the strain gauge was used to determine tremor onset pressure and percentage of time with tremor.

In the experiments using naloxone alone, it was administered during compression with He-O_2 as a continuous i.v. infusion: mean total dose 44.3 mg/kg, (s.e.mean 6.4), mean rate 1.24 mg/kg/min (s.e.m. 0.16). This reduced the onset pressure for tremor to 44.7 (s.e.m. 0.6) ATA compared with controls (59.4 (s.e.m. 1.8) ATA). In subsequent experiments, ketamine or Althesin was infused at anaesthetic doses throughout compression. At a pressure at which tremor would be severe in untreated rats (88-92 ATA), compression was halted and the dose of anaesthetic reduced to be just sufficient to prevent tremor. Naloxone was then infused for 3 minute period (mean total dose ketamine rats 27.7 (s.e.m. 8.3) mg/kg, Althesin rats 33.3 (s.e.m. 8.3) mg/kg), and any tremor was recorded continuously for subsequent analysis. Results are shown in the table below:

Table 1 Percentage time with tremor

	Pre Naloxone (3 min)	During Naloxone (3 min)	After Naloxone (1 min)
Ketamine (n = 7)	4.3	3.5	4.4
s.e.mean	1.5	0.9	1.3
p		n.s.	n.s.
Althesin (n = 6)	10.3	6.0	64.9
s.e.mean	1.2	0.8	7.6
p		< 0.1	< 0.005

There was no significant change in the incidence of tremor in the minute immediately after naloxone in the ketamine treated rats, but there was a sudden increase in tremor after naloxone in the Althesin treated rats.

We have currently no explanation for the reversal by this dose of naloxone of the anti-HPNS action of Althesin. However, these data indicate that there may be more than one mechanism whereby drugs which will prevent the same aspect of HPNS (i.e. tremor) exert their effect.

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EFFECTS OF MORPHINE, NALTREXONE AND (+)-AMPHETAMINE ON EXPLORATION AND GENERAL ACTIVITY IN THE YOUNG RAT

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Measuring exploratory behaviour requires careful consideration, and it is not sufficient solely to rely on the measurement of locomotor activity in a novel environment (File, 1981). We chose to measure investigatory responses directed towards novel objects, which were placed in an alcove reached from a larger open field. Using this method, Robbins & Iversen (1973) showed that (+)-amphetamine increased locomotor activity, at the expense of exploratory activity. There are reports which suggest that pure opiate antagonists (naloxone, naltrexone) reduce exploration (e.g. Katz, 1979; Rodgers & Deacon, 1979). Our aims were to re-examine the effects of naltrexone, and to investigate any possible effect of morphine on exploration and/or general activity.

Subjects were 128 male hooded rats (General strain, 33-41 days old) from our colony. They were housed with free access to food (Diet 41B) and water, and were kept on a 12h light: 12h dark cycle, at a room temp 22-24°C. The apparatus was a modified Berlyne box, consisting of an open-field section (40x30cm), with a short alley off it (15x8cm), leading to an alcove (30x6cm). The box was equipped with 4 parallel photobeams crossing the open-field, one of which marked the entrance to the alley. Interruptions of the photobeams were automatically recorded. Each rat was run in the box for 5 consecutive days (a 5 min trial per day), as habituation experience. Drug conditions were tested on day 6. The rats were randomly allocated to 8 equal treatment groups: morphine sulphate (0.3, 1,3 and 10 mg/kg, s.c.); naltrexone hydrochloride (0.3 and 1 mg/kg, s.c.); (+)-amphetamine sulphate (1.5 mg/kg, i.p); saline vehicle control. Each group was divided into two; half were run on trial 6 as on habituation trials, and half were run with a number of small novel objects placed in the alcove. A trial lasted 5 min. An observer recorded episodes of locomotion, rearing, time in alcove, and contact with novel objects by depressing keys. All data were logged-on-line (recording frequencies and durations) by microcomputer.

Confirming a previous report, (+)-amphetamine, given 40 min before the test, significantly increased the duration of locomotion ($p<0.05$) and the frequency of photobeam interruptions ($p<0.05$), whilst it reduced mean duration of contact with novel objects ($p<0.01$). It also reduced the mean duration of rearing ($p<0.01$). On the other hand, morphine (0.3 - 3 mg/kg) given 30 min before the test, exerted no significant effect on any measure taken; at 10 mg/kg, all behaviour was non-specifically depressed. Naltrexone, particularly at 0.3 mg/kg, given 15 min before the test, did show clear behavioural effects. It increased the duration of locomotion in the open field ($p<0.05$), and the frequency of photobeam interruptions ($p<0.01$), whilst it reduced the total time in contact with novel objects ($p<0.01$). Naltrexone did not affect rearing.

Morphine, a μ opiate receptor agonist, displayed no effect on either general activity or exploration, at doses below 10 mg/kg. It seems unlikely therefore that exploratory activity in the rat is dependent upon an action at μ receptors. Naltrexone affected both behaviour categories, in ways which were similar to those of (+)-amphetamine. In short, naltrexone seemed to have a general activating effect, which led to a reduction in exploratory activity.

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INVOLVEMENT OF D_2, BUT NOT D_1 RECEPTORS IN STRIATAL DOPAMINERGIC TRANSMISSION

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Evidence suggests the existence of at least two categories of dopamine (DA) receptors in the CNS, the type D_1 (coupled to adenylate cyclase) and the type D_2 (Spano et al, 1978). It is however not yet firmly established whether these two DA receptor subtypes are involved in DA neurotransmission. Striatal cholinergic neurons which are target cells for the nigro-striatal DA system represent a convenient system to test the relative involvement of DA receptor subtypes in DA neurotransmission. We have recently studied the influence of various DA receptor agonists and antagonists, relatively selective for DA receptor subtypes, on striatal ACh transmission and obtained evidence that a single (probably the D_2) type of DA receptor is involved in the regulation of striatal ACh neuron activity (Scatton, 1982). In order to get a further insight into this problem we have presently investigated the effect of SKF 38393 and LY 141865, two recently developed DA agonists acting specifically on D_1 and D_2 receptor, respectively (see Stoof and Kebabian, 1981), on striatal ACh and DA turnover in the rat.

Experiments were performed on male Sprague Dawley rats (140-160g). Drugs were administered i.p. 1 h before sacrifice. ACh concentrations were measured by a radioenzymatic technique (Scatton and Worms, 1979) and DA metabolite levels by high pressure liquid chromatography with electrochemical detection (Semerdjian-Rouquier et al, 1981).

Administration of LY 141865 (0.01-10 mg/kg) produced a dose-dependent and monophasic increase in ACh concentrations and a concomitant reduction of homovanillic acid and dihydroxyphenylacetic acid levels (ED_{50} 0.1 mg/kg) in the rat striatum. In contrast, SKF 38393 (1-30 mg/kg) failed to alter the concentrations of striatal ACh and DA metabolites.

The effects of these two DA agonists on the release of striatal ACh were also compared. In these experiments, rat striatal slices preloaded with ^{3}H-choline (Spec. activity 80 Ci/mmol, final concentration 0.05 µM) were superfused continuously in a chamber at a constant rate (0.5 ml/min). Tritiated ACh release was evoked by a 2 min exposure to 20 mM potassium. Addition of LY 141865 into the incubation medium reduced in a concentration dependent and monophasic manner the release of striatal ^{3}H-ACh evoked by potassium (IC_{50} 0.13 µM). In contrast, even in high concentrations (100 µM), SKF 38393 failed to affect the potassium-evoked release of striatal ^{3}H-ACh. Moreover, when given concomitantly with LY 141865 (0.01-1 µM), SKF 38393 (10 µM) failed to modify the ability of the former compound to diminish striatal ^{3}H-ACh release (IC_{50} 0.12 µM).

In conclusion, the present results provide further evidence for the involvement of D_2 but not D_1 DA receptors in striatal dopaminergic neurotransmission. In addition, D_1 receptors do not appear to exert any modulatory influence on D_2-receptor mediated responses.

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DIFFERENTIAL EFFECTS OF NEUROLEPTICS ON 3-PPP AND AMPHETAMINE INDUCED CIRCLING BEHAVIOUR IN 6-HYDROXY-DOPAMINE LESIONED RATS

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The selective presynaptic dopamine (DA) agonist 3-PPP (N-n-propyl-3-(3-hydroxyphenyl)-piperidine; Hjorth et al.,1981) has recently been shown to induce contralateral circling behaviour in 6-hydroxy-DA (6-OHDA) lesioned rats (Martin et al.,1981) presumably by stimulation of supersensitive DA receptors. In this study, the inhibitory effect of neuroleptics on 3-PPP has been compared to the inhibitory effect on amphetamine induced ipsilateral circling behaviour which is mediated by normal DA receptors.

Wistar rats were unilaterally lesioned with 6-OHDA (8 µg/4 µl) injected into the median forebrain bundle at the level of rostral substantia nigra. 3-PPP (5 mg/kg s.c.) or amphetamine (2.5 mg/kg s.c.) was injected with intervals of one week until stable circling behaviour was observed.

Table 1

Drug (pretreatment time, h)	ED50 ((µmol/kg s.c.), 95 per cent confidence limits) 3-PPP 5 mg/kg	amphetamine 2.5 mg/kg
Cis (Z)-flupentixol (2 h)	0.12 (0.08-0.2)	0.039 (0.02-0.06)
Chlorprothixene (2 h)	1.5 (0.6 -4.2)	0.64 (0.2 -1.7)
Fluphenazine (2 h)	0.13 (0.06-0.3)	0.028 (0.02-0.04)
Clozapine (1 h)	8.4 (3.3 -22)	21 (8 -60)
Thioridazine (2 h)	29 (15 -58)	31 (16 -64)
Haloperidol (2 h)	1.7 (0.8 -4.4)	0.10 (0.05-0.2)
Pimozide (2 h)	5.6 (2 -14)	0.23 (0.1 -0.4)
Sulpiride (4 h)	>117	100 (57 -183)
Prazosin (1 h)	1.9 (0.7 -5)	4.4 (0.7 -24)

3-PPP and amphetamine induced dose-dependent contralateral (ED50 0.5 mg/kg s.c.) and ipsilateral (ED50 0.7 mg/kg s.c.) circling behaviour, respectively. The inhibitory effects of neuroleptics and the α_1-antagonist prazosin are shown in table 1. Inhibition of amphetamine induced circling was observed after low doses and correlated closely to other neuroleptic in vivo models, e.g. inhibition of conditioned avoidance response (correlation coefficient r=0.99). 3-PPP-induced circling was inhibited by cis (Z)-flupentixol, chlorprothixene, clozapine, thioridazine and prazosin in approximately the same dose ranges, whereas fluphenazine was five times weaker. In contrast, haloperidol and pimozide showed 17 and 25 times lower potency, respectively (table 1). No inhibition of 3-PPP induced circling was found after sulpiride.

Since haloperidol and pimozide preferentially bind to D-2 DA receptors in vitro, whereas other neuroleptics non-selectively bind to D-1 and D-2 DA receptors (Hyttel 1981), the D-1 receptor blocking component may be important for potent inhibition of 3-PPP induced circling. However, more recent experiments have shown that the preferential D_2-blockers spiroperidol and clebopride have high 3-PPP-antagonistic activity. In conclusion, some neuroleptics show atypical effect in the 3-PPP circling model, but the activity profile of the drugs studied does not correlate to DA receptor types classified by binding experiments.

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CHOLINERGIC MANIPULATION OF PERI-ORAL MOVEMENTS INDUCED BY THE CHRONIC ADMINISTRATION OF NEUROLEPTIC DRUGS TO RATS

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The chronic administration of neuroleptic drugs to rats results in an enhanced incidence of purposeless jaw movements (Sahakian et al,1976; Clow et al,1979). Such behavioural change might be related to the production of tardive dyskinesia following chronic neuroleptic therapy in man. We have examined therefore the ability of a range of neuroleptics to induce peri-oral movements and the effect of manipulation of cholinergic function.

The continuous administration in drinking water for 4 months of haloperidol (1.4-1.6 mg/kg/day), trifluoperazine dihydrochloride (4.5-5.1 mg/kg/day) or sulpiride (102-110 mg/kg/day), but not clozapine (23-26 mg/kg/day), increased jaw movements compared to age-matched control animals.

Enhancement of cholinergic function by the administration of pilocarpine (4 mg/kg ip) or physostigmine (0.2 mg/kg ip) 20 min previously increased jaw movements in control animals (controls: 22 $\pm$ 3; pilocarpine: 145 $\pm$ 12; physostigmine: 65 $\pm$ 11; movements/5 min; $p < 0.05$). Both drugs also enhanced the movements produced by chronic haloperidol (1.4-1.6 mg/kg/day) treatment (haloperidol: 51 $\pm$ 4; plus pilocarpine: 171 $\pm$ 12; plus physostigmine: 88 $\pm$ 8; movements/5 min; $p < 0.05$). Administration of cholinergic agents induced gaping in both control and haloperidol treated animals but the number of gaping movements induced by physostigmine (0.2 mg/kg ip) was greater in haloperidol treated animals than in control rats.

In contrast, administration of the anticholinergic agents scopolamine (0.5 mg/kg ip 50 min previously) or atropine (25 mg/kg ip 20 min previously) reduced jaw movements in control animals (controls: 21 $\pm$ 2; scopolamine: 13 $\pm$ 1; atropine: 4 $\pm$ 1; movements/5 min; $p < 0.05$). These drugs also reduced mouthing movements in haloperidol treated rats (haloperidol: 45 $\pm$ 4; plus scopolamine: 14 $\pm$ 2; plus atropine: 14 $\pm$ 3; movements/5 min; $p < 0.05$). Anticholinergic drugs produced a relatively greater decrease in jaw movements in haloperidol treated animals than in control rats.

Manipulation of peripheral cholinergic function by administration of neostigmine (0.2 mg/kg ip 20 min previously) or scopolamine methylbromide (0.5 mg/kg ip 50 min previously) did not alter jaw movements either in control animals or in animals receiving chronic haloperidol intake.

Since tardive dyskinesias are exacerbated by anticholinergic drugs and relieved by cholinergic agents, the data suggests the nature of the increased incidence of jaw movements induced in rats by chronic neuroleptic intake is not identical to that occurring in man.

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THE DOSE-RELATED EFFECT OF ATROPINE ON CORTICAL EVENT-RELATED SLOW POTENTIALS IN THE CONSCIOUS RAT

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The contingent negative variation (CNV) (Walter et al, 1964) is a slow and sustained negative shift of the vertex EEG recorded from the scalp of conscious human subjects during the interval between a warning stimulus (S_1) and a response stimulus (S_2), especially when a motor response follows S_2. The CNV is the archetype of a group of brain potentials known as Event Related Slow Potentials (ERSPs). Psychoactive drugs, such as nicotine (Ashton et al, 1980) and atropine (Thompson et al, 1978), have been shown to alter the magnitude of the CNV. Thompson et al (1978) have argued that the use of drugs in the study of the CNV may provide useful information about the processes involved in its electrogenesis.

Pirch (1977, 1978) has described CNV-like ERSPs in rats using both passive and operant reward-conditioning paradigms. In these experiments rats were trained in a passive reward paradigm. Rats were chronically implanted with silver/silver chloride electrodes in contact with the dura via agar-saline pools. The active electrode was located over frontal cortex and the reference electrode over parietal cortex. EEGs were recorded by means of a.c. amplifiers (time constant 15s).

Presentation of a 100msec 1400 Hz tone alone evoked a slight negative shift in the EEG which quickly habituated with repeated presentation of the tone. However, when the tone (S_1) was paired 3 seconds later with automatic food reward (S_2) a negative shift, similar to the human CNV, developed during the S_1-S_2 interval and persisted with overtraining. Twenty-five trials were presented to a rat in a single session and only one session was conducted per day. Artefact-free trials were averaged on a PDP8 computer and the area of the ERSPs calculated.

Atropine (0.1, 0.2 and 0.4mg/Kg) was administered subcutaneously to 4 male Wistar rats who had developed steady ERSP responses. Three saline control sessions were conducted on the 3 days prior to the drug sessions. The area of the ERSPs measured 20-40 min after administration of atropine was compared with the area of the saline control value taken on the day preceding the drug sessions.

Atropine produced a decrease in the mean ERSP area of the rats in a dose-dependent manner. 0.1mg/Kg atropine caused a slight decrease in the ERSP area of 3 rats but an increase in the area of one rat (9.5%). The higher doses were depressant in all rats. 0.2 and 0.4mg/Kg atropine produced a significant decrease in the mean ERSP area compared with the mean saline control value ($P < 0.05$ and $P < 0.01$, respectively).

Thompson et al (1978) have shown that a single dose of atropine (0.4-0.5mg i.m.) produced a decrease in the CNV area in human subjects. The results of this study show that atropine also has a general depressant effect on the ERSPs of rats which is dose-related. The results support the suggestion (Thompson et al, 1978) that a cholinergic muscarinic mechanism may be involved in the genesis of ERSPs.

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IN VIVO DETERMINATION OF BRAIN 5-HYDROXYTRYPTAMINE AND DOPAMINE TURNOVER IN THE CONSCIOUS RAT USING REPEATED CSF SAMPLING

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Most methods for studying transmitter metabolism in the rat brain do not permit repeated determinations or subsequent behavioural studies in the same animals. Non-destructive methods involving CSF sampling have been used in man (e.g. Garelis et al, 1974) and to a lesser extent in primates (Baker & Ridley, 1979). Using HPLC, similar methods are applicable to rat CSF. We report a procedure for repetitive sampling of CSF from conscious freely moving rats and its use to determine central 5HT and DA turnover by the probenecid method.

Under pentabarbitone anaesthesia, Sprague-Dawley rats (230-270 g) were implanted via a cranial approach with polyethylene catheters in the cisterna magna. X-ray studies confirmed that the catheters avoided the cerebellum and were located in the cisterna magna. 2/3 days after implantation, rats were given either isotonic saline (1.0 ml/kg) or the 5HT synthesis inhibitor p-chlorophenylalanine (PCPA) (150 mg/kg i.p.). 24 hr later they were injected with probenecid (200 mg/kg i.p.). 25 µl CSF samples were collected, animals killed and brains removed at intervals (Table 1). Tryptophan, tyrosine, 5HIAA, 5HT (and in some instances, HVA and DOPAC) were determined by HPLC (Anderson et al, 1979). Main results are shown in Table 1.

TABLE 1. 5HT turnover by probenecid method: comparison of CSF and brain values.

Pretreatment	CSF ($nmol.ml^{-1}$. 60 min^{-1})	Brain ($nmol.g^{-1}$. 60 min^{-1})
Group 1		
Saline (n = 14)	1.57 ± 0.15 (r = 0.95)	1.12 ± 0.20 (r = 0.86)
PCPA (n = 14)	0.52 ± 0.07 (r = 0.91)	0.29 ± 0.06 (r = 0.83)
% change	-67 ($p < 0.001$)	-74 ($p < 0.001$)
Group 2		
Saline (n = 9)	1.50 ± 0.34	
PCPA (n = 6)	0.50 ± 0.15	
% change	-67 ($p < 0.001$)	

Group 1. CSF taken at 0, 30, 60 min and rats killed at these times; Group 2. CSF taken at 0, 30, 60 min from each rat; Turnover values obtained from the rate of increase of 5HIAA after probenecid; r values are correlation coefficient (Δ 5HIAA vs time). Results as means ± one SD).

CSF 5HIAA, HVA and DOPAC rose linearily over the first 60 min after injection. Turnover values based on CSF and brain determinations cannot be directly compared as they are expressed in different units. However, after PCPA treatment (when brain 5HT was depleted by 43%) 5HT turnover values obtained by both methods were comparably reduced (CSF, -67%; brain -74%). These findings indicate that differences of rat brain 5HT turnover are comparably reflected by CSF measurements. Some CSF samples were also used to estimate DA turnover. Neither this nor CSF concentrations of tryptophan or tyrosine were significantly affected by PCPA. The method described should be applicable in a wide range of pharmacological and physiological situations.

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PHOTOAFFINITY LABELLING OF THE BENZODIAZEPINE RECEPTOR DOES NOT OCCLUDE THE βCCE BINDING SITE

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β-Carboline-3-carboxylic acid ethyl ester (βCCE) has been shown to displace benzodiazepines potently and completely from their brain specific binding sites in CNS membrane preparations (Nielsen & Braestrup, 1980). These binding sites, as characterised with ^{3}H-diazepam or ^{3}H-flunitrazepam, can be effectively occluded by covalent attachment of flunitrazepam to the membrane matrix by photo activation (Möhler et al, 1980). We report here that such occlusion is not apparent if the binding sites are characterised using ^{3}H-βCCE as the ligand.

Well-washed, frozen rat hippocampal membranes, 1:320 in 0.1M Tris Citrate pH 7.1, were incubated on ice for 1 h in the presence or absence of 5nM flunitrazepam. 10ml aliquots were irradiated in petri-dishes (5cm diameter) at 10cm from a U.V. light (366nm nominal 8W) for 10 min. The membranes were washed 3 times with buffer 1:960 to remove free and reversibly bound flunitrazepam, and resuspended 1:80 for subsequent binding experiments.

The specific binding to photoaffinity labelled (PAL) tissue of 0.5nM ^{3}H-flunitrazepam was 27% of that in control tissue (irradiated in the absence of flunitrazepam and washed) whereas that of 0.5nM ^{3}H-βCCE was 94% of control, when non-specific binding was defined by 1μM βCCE.

Scatchard analysis of ^{3}H-flunitrazepam binding (0.2-20nM) confirmed (Möhler et al, 1980) that the reduction in ^{3}H-flunitrazepam binding was due to a decrease in Bmax (control 401.3±26.5, PAL 106.3±31.0 fmol/mg) with no change in Kd (control 2.46±0.86, PAL 2.78±0.86nM) whereas Scatchard analysis of ^{3}H-βCCE binding (0.2-6nM) revealed no change in Bmax (control 559.7±78.4, PAL 620.4±67.4 fmol/mg) and only a small increase in Kd (control 0.49±0.07, PAL 0.64±0.06nM) for this ligand (values mean ± SEM, n=3).

That ^{3}H-βCCE binding is not reduced implies that the photoaffinity label does not bind to the occupation site. However, occupation of the benzodiazepine receptor by flunitrazepam is necessary before the irreversible linkage can form since 1μM βCCE, 10μM diazepam or 10μM Ro15-1788 present in the initial incubation mixture can prevent subsequent photoaffinity labelling as determined by the decrease in the specific binding of 0.5nM ^{3}H-flunitrazepam.

A variety of compounds capable of potently displacing benzodiazepines from their CNS binding sites, were tested for their ability to distinguish sites specifically labelled by 0.5nM ^{3}H-βCCE in PAL from those in control hippocampal membranes. The apparent IC_{50}s for 'agonist' benzodiazepines were decreased in PAL tissue:- Diazepam: control 83.3±4.9nM, PAL 2312.8±292.4nM. Flunitrazepam: control 34.1±10.6nM, PAL 308.4±36.8nM (mean ± SEM, n=3). However, the IC_{50}s for the benzodiazepine antagonists Ro15-1788 (Hunkeler et al, 1981) and CGS 8216 (Czernik et al, 1981) and for the methyl, ethyl and n-propyl esters of β-carboline-3-carboxylic acid were similar in control and PAL tissue.

This data suggests that either the recognition properties for Ro15-1788, CGS 8216 and the β-carboline analogues are different from those of the classical 5-phenyl 1,4-benzodiazepines or that the disposition of the binding site for these compounds in the membrane structure is such that steric factors limit the access of only the 5-phenyl-1,4-benzodiazepines in the PAL membrane preparations.

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CAFFEINE AND FLURAZEPAM AFFECT GABA DEPOLARIZATIONS OF PRIMARY AFFERENT FIBRES IN THE IN VITRO SPINAL CORD

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Caffeine has complex effects on animal behaviour: in low doses it reduces motor activity whereas in high doses it has a stimulant action leading eventually to convulsions (Snyder, 1981). It is still unclear how the methylxanthine acts on central neurones, particularly in view of the fact that brain concentrations of caffeine attained in vivo are rather low. We considered the possibility that caffeine could affect motor activity by altering presynaptic inhibition which in the spinal cord is mediated by GABA (Nicoll & Alger, 1979; Nistri & Constanti, 1979). We therefore tested the effects of caffeine on the depolarization of primary afferent fibres either induced by trans-synaptic stimulation (PAD) or by exogenously-applied GABA.

The preparation used was the parasagittal spinal cord slice of the frog (R. temporaria) kept in vitro at 4°C to block GABA uptake (Nistri, 1981). Responses were recorded from lumbar dorsal roots with Ag/AgCl electrodes during bath application of compounds to the spinal cord tissue. PAD was evoked by stimulating an adjacent dorsal root or the opposite ventral root (Constanti & Nistri, 1976).

High concentrations of caffeine (0.5 - 1 mM) elicited intense electrical discharges (spikes and slow-depolarizing waves) recorded from afferent fibres and attenuated PAD. This excitatory action was associated with a reduction in the GABA-induced dorsal root depolarization: the GABA dose-response curve was shifted to the right in a nonparallel fashion and the maximal response was diminished. Lower concentrations of caffeine (10-100 μM) had relatively little stimulatory effect on the spinal cord, did not significantly change baseline potential levels, tended to prolong PAD and clearly enhanced GABA depolarizations: the GABA ED_{50} value (1 mM) was decreased by 40% with no change in GABA maximal response. This effect of caffeine was very slowly reversible (full recovery was usually observed one day later) and was unaffected by 5 μM bicuculline, suggesting that was not generated at GABA receptor sites. Flurazepam (5 μM) also enhanced GABA depolarizations but mixtures of caffeine (50 μM) and flurazepam were ineffective on GABA responses or PAD. The GABA enhancing action of caffeine was antagonized by Ca^{2+} blockers such as 2.5 mM Mn^{2+} or 0.5 mM Cd^{2+} in a manner distinct from their concurrent depression of synaptic neurotransmission.

We suggest that caffeine (and probably flurazepam) had a modulatory action on receptor systems linked to presynaptic inhibition. In low doses caffeine increased the effectiveness of GABA apparently through a Ca^{2+} -dependent postsynaptic mechanism while, in high doses, it antagonized the effect of GABA. These data may offer some electrophysiological evidence to account for the action of caffeine on motor activity.

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EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE IN THE STRIATUM AND CINGULATE CORTEX

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Vasoactive intestinal polypeptide (VIP) is widely considered to be a peptide neurotransmitter in the CNS. However, no consistent pattern has yet emerged from investigation of the various processes which are indicative of neurotransmission to elucidate the regions in which this peptide may play its principal functional roles. In rats, very high concentrations of VIP are present in anterior cingulate cortex whereas the striatum is almost devoid of VIP-nerve fibres (Loren et al. 1979); the striatum possesses the greatest density of VIP-binding sites (Taylor and Pert, 1979) and is more sensitive to VIP-mediated activation of adenyl cyclase than the cerebral cortex (Quik et al. 1978). We have examined using the quantitative autoradiographic ^{14}C-2-deoxyglucose technique (Sokoloff et al. 1977) both the local and distance functional consequences of injecting VIP directly into one caudate nucleus or anterior cingulate cortex of conscious rats.

The rate of glucose utilisation (which reflect energy generation and hence integrated functional events) was measured in over 50 regions of the CNS of conscious lightly restrained rats (Sokoloff et al. 1977). Measurements were initiated 10 minutes after the injection of VIP (20pmoles) or vehicle via stereotactically placed guide cannulae into one caudate nucleus or anterior cingulate cortex.

Intrastriatal injection. The administration of VIP marked increases in glucose use in the injected caudate nucleus from the levels observed after injection of the vehicle alone (105 ± 9 v 83 ± 5umoles/100g/min, p 0.02, mean $\pm$ SEM). The increased glucose use occurred in small punctate areas (100-500um wide in coronal sections) scattered throughout the striatum. In addition more diffuse reductions of glucose use were present in areas adjacent to the regions of functional activation such that autoradiograms from all VIP-injected striata displayed heteregenour mosaic of glucose use. Glucose use in a number of regions with major primary or secondary anatomical connections such as the contralateral caudate nucleus and habenular nuclear complex was altered by VIP injection whereas in contrast glucose use in the regions to which the major striatal projections are directed (pallidum and subs. nigra) was minimally altered by the peptide injection.

Intracortical injection. The injection of VIP directly into anterior cingulate cortex effect a local increase in glucose utilisation in this region (from 68 ± 3 to 94 ± 9umoles/100g/min); the magnitude of the increases being proportionately similar to that observed in the striatum with the same concentration of VIP. As with striatal injections, alteration in glucose use in a number of regions connected anatomically to the injected region, the most notable being the contralateral cingulate cortex.

The local functional consequence of administing VIP, at least in respect of the index of local integrated functional activity by which we have employed does not appear to be correlated directly with the known topography of VIP neuronal systems. Moreover, the technical approach described can identify in which efferent pathway, of the many possible, is activity modified by local VIP injection.

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REGIONAL BRAIN 2-DEOXYGLUCOSE UPTAKE DURING MOTOR HYPERACTIVITY INDUCED BY ADTN IN RAT NUCLEUS ACCUMBENS

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Dopamine agonists in nucleus accumbens (NA) cause locomotor stimulation. NA sends efferents to several limbic and basal ganglia-related subcortical structures (Williams et al, 1977; Nauta et al, 1978) but the areas mediating the hyperactivity are unknown. We report a preliminary study in which the regional uptake of (^{3}H)-2-deoxyglucose (2-DG) was measured by semi-quantitative autoradiography in the brains of rats made hyperactive by injection in NA of the dopamine agonist ADTN (Elkhawad & Woodruff, 1975).

Female Sprague Dawley rats (170-180 g) were anaesthetized, a jugular vein was cannulated, and ADTN (10 µg) or saline was injected bilaterally in NA. Each rat,when fully conscious (1 h), was placed in an activity cage. ADTN in NA caused intense hyperactivity which peaked after 4 h (mean score: ADTN-treated 2986/1h; saline-treated 4/1h). 4h after ADTN/saline in NA each rat received 500 µCi of 2-deoxy-D-2,6-^{3}H glucose (50 Ci/mmol,Amersham) i.v. The rats were killed 45 min after 2-DG injection, the brains rapidly frozen in iso-pentane at -35°C, sectioned (8 µm) at -25°C, and exposed to tritium-sensitive film, for 14 days. Grain density measurements were taken using a microdensitometer. Regional 2-DG uptake was expressed as the ratio of control: hyperactive brain sections (Table 1).

Table 1 Regional 2-DG in brains of control and hyperactive rats

Brain area	Autoradiographic density ratio (control:ADTN) n=5
Ventral pallidum/substantia innominata†	1:0.3 - 1:1.7
Globus pallidus	1:0.6
Substantia nigra - pars reticulata	1:1
Entopeduncular nucleus	1:1.3
Subthalamic nucleus †	1:2.7 - 1:4.0
Lateral habenula	1:1.3
Dorsolateral mesencephalic reticular formation	1:2.3

†Range of measurements made in different parts of the structure

The data indicate that NA-induced hyperactivity had no consistent effect on 2-DG uptake in the region of the ventral pallidum (Heimer & Wilson,1975) or substantia innominata which receive NA efferents (Williams et al,1977; Nauta et al,1978). 2-DG uptake was greatly increased bilaterally in the subthalamic nuclei of hyperactive rats. A region of high 2-DG uptake was found bilaterally in the dorsolateral aspect of the mesencephalic reticular formation of hyperactive rats. This region is apparently close to the deep mesencephalic nucleus described by Veazey & Severin (1980). Lesioning experiments are in progress to determine the role of these areas in mediating NA-induced hyperactivity.

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COMPARISON OF THE CARDIOVASCULAR AND VENTILATORY EFFECTS OF URAPIDIL AND CLONIDINE

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The mode of action of the new antihypertensive compound, urapidil, appears to involve both blockade of the postsynaptic and stimulation of the presynaptic alpha-receptors (Eltze, 1979). The presynaptic alpha-adrenergic effect of urapidil suggests that a part of its hypotensive effect might be due to the similar central mechanism which is considered to mediate the antihypertensive effect of clonidine. The possible central site of action of urapidil is also supported by the finding that it decreased sympathetic nervous discharges (Schoetensack et al., 1977). The aim of this work was to demonstrate the possible similarities in the cardiovascular or ventilatory effects of the compounds urapidil and clonidine.

Wistar rats anaesthetized with urethane were used. The drugs were injected either i.v. or i.c.v. at 20-30 min intervals and cumulative dose response curves were constructed. Mean arterial pressure (MAP) and heart rate were recorded from the femoral artery. Ventilation rate was recorded with a hot wire flow meter.

At the dosage of 3-24 nmol/kg about 2 times more clonidine was needed i.v. than i.c.v. to decrease MAP by 15%. Larger doses of 30-240 nmol/kg induced a maximal hypotensive effect of about 32%. In this dose range the hypotensive responses were equal upon both routes of administration. Urapidil i.v. reduced MAP at the doses of 0.1-10 µmol/kg (maximal effect -54%). The i.c.v. dose of 4 µmol was lethal to 3 out of 10 rats. To reduce MAP by 35% about twice as much urapidil was needed i.c.v. than i.v. The results suggest that urapidil predominantly decreased blood pressure via a peripheral mechanism.

At the doses of 30-240 µmol/kg clonidine i.v. or i.c.v. induced a dose related decrease in heart rate (maximally about -23%). The doses of 0.1-1 µmol/kg of urapidil i.v. induced a slight tachycardia of about 10%. The largest dose of 10 µmol/kg i.v. reduced heart rate by 10%. Urapidil i.c.v. had only minor effects on heart rate.

Clonidine 30-240 nmol/kg i.v. or i.c.v. decreased ventilation rate dose-dependently (maximal effects -27% i.v. and -15% i.c.v.). Urapidil, 0.01-10 µmol/kg, i.v. increased ventilation rate maximally by 10% whereas the doses of 0.004-4 µmol/kg i.c.v. had no consistant effects on ventilation. It is concluded that urapidil, in contrast to clonidine, exerts its hypotensive effect mainly via a peripheral mechanism. Furthermore, the dissimilarity between these two drugs is emphasized by the inability of urapidil to produce any reduction in heart or ventilation rate.

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EFFECTS OF β-ADRENOCEPTOR ANTAGONISTS ON CENTRALLY-EVOKED PRESSOR RESPONSES IN ANAESTHETIZED AND CONSCIOUS RATS

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We have previously described a perihypothalamic area of the rat brain, electrical stimulation of which causes a rise in arterial blood pressure and other cardiovascular changes characteristic of a defence reaction (Marshall, 1981). Microinjection of β-adrenoreceptor antagonists into this area stereospecifically inhibits these pressor responses (Allott, Greenwood & Marshall, 1982).

To avoid possible artefacts associated with stimulation and injection into the same brain area, we have been studying the effects of β-antagonists given intravenously on pressor responses to perihypothalamic stimulation (PHS). Male rats of the Alderley Park strain (180-220g) were anaesthetised with alphaxalone/alphadolone ("Saffan", Glaxo, 18mg kg^{-1} i.v.) and cannulae implanted in the jugular vein and carotid artery. A monopolar, cathodal electrode was implanted at A3.0, L0.5, H+4.0mm from ear-bar zero, with an indifferent electrode attached to the skull surface. Whilst still under anaesthesia, a control "anaesthetised" frequency-pressor response curve was constructed (250μA, 2msec pulse width, 5 secs duration). The animals were then allowed to recover for 4 hours and, in a restraining tube, a "conscious" frequency/pressor response curve constructed (100μA, 1msec pulse width, 2secs duration). Drugs were then given intravenously (0.1ml/100gms) and 5 minutes later the frequency/pressor response curve repeated. Immediately thereafter each animal was re-anaesthetised and, using the higher stimulus parameters, the initial anaesthetised frequency/pressor response curve repeated.

In the conscious state, pressor responses to PHS were markedly attenuated by L-propranolol but not by D-propranolol (1mg/kg). The same dose of the β_2-selective antagonist ICI 118,551 (Bilski et al 1980) also inhibited the responses, whereas the β_1-selective antagonist atenolol was ineffective. When the rats were re-anaesthetised, however, none of the drugs studied exerted any inhibition.

The results of the present experiments are thus consistent with our previous observations that pressor responses elicited by PHS are selectively antagonised by β_2-adrenoceptor antagonists and, furthermore, show that this action is also present in the conscious rat after intravenous drug administration. The failure to detect any significant drug action in the anaesthetised animals following systemic administration may reflect a relative lack of drug sensitivity at the higher stimulus parameters used or a masking effect of the anaesthetic by an as yet unidentified mechanism.

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PRE- AND POSTSYNAPTIC α-ADRENOCEPTOR EFFECTS OF SOME NEW HYPOTENSIVE IMIDAZOLE DERIVATIVES IN PITHED RATS

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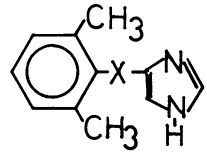
4(5)-(2,6-dimethylbenzyl)imidazole (MPV-207) and some of its alkyl bridge analogues have shown hypotensive and bradycardic activity in anaesthetised rats in pharmacological screening experiments. The hypotensive effect of MPV-207 may be due to stimulation of the central α-adrenoceptors (Kaipiainen et al, 1982). The pre- and postsynaptic α-adrenoceptor activities of MPV-207 and some of its alkyl bridge analogues were thus determined here by investigating the vasopressor effect and the inhibition of electrically stimulated tachycardia respectively in pithed rats (Gillespie et al, 1970).

The compounds were injected intravenously in a cumulative manner and the doses (μg/kg) inducing an increase of 50 mmHg in mean arterial pressure (ED50) and a 50 % inhibition of electrically stimulated (20 impulses, 0.4 Hz, 0.3 ms, 50 V) tachycardia (ID50) were determined. The ID50/ED50 ratio describing the α-adrenoceptor selectivity was calculated and compared with that of clonidine (Table 1).

MPV-207, which has the highest hypotensive and bradycardic potency of the MPV compounds , was approximately as potent as clonidine in stimulating pre- and postsynaptic α-adrenoceptors, but had a greater selectivity for presynaptic α-adrenoceptors. MPV-295, in which the methano bridge of MPV-207 is replaced by an ethano group, was less potent, but showed an equal selectivity towards the presynaptic receptors. The extension of the alkyl bridge to the prophano group of MPV-304 reduced not only the agonistic activity, but also the presynaptic selectivity. Finally, MPV-390, having a buthano bridge, proved inactive. The agonistic activity of these MPV compounds at the α-adrenoceptors correlated well with their hypotensive and bradycardic potency.

Table 1 Activity of clonidine and the MPV compounds at pre- and postsynaptic α-adrenoceptors in pithed rats

Compound	X	ED50	ID50	$\frac{ID50}{ED50}$	$\frac{PRE}{POST}$
Clonidine		3.8	7.4	1.95	1
MPV-207	CH_2	6.7	9.0	1.34	0.69
MPV-295	$(CH_2)_2$	55.6	72.0	1.29	0.66
MPV-304	$(CH_2)_3$	75.0	125.0	1.67	0.86
MPV-390	$(CH_2)_4$	-	-	-	-



In summary, the hypotensive compounds MPV-207, MPV-295 and MPV-304 showed an agonistic action at the pre- and postsynaptic α-adrenoceptors in pithed rats, an effect which, along with their hypotensive activity, was reduced by the extension of the alkyl bridge between the phenyl and imidazole moieties. Furthermore, these MPV compounds had a greater selectivity for the presynaptic α-adrenoceptors than did clonidine.

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DIFFERENCES BETWEEN NORADRENALINE AND CLONIDINE ON THE α_2-ADRENOCEPTOR MEDIATED INHIBITION OF THE RESPONSE OF THE RAT VAS DEFERENS

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Stimulation of presynaptic α_2-adrenoceptors inhibits the twitch response of the rat vas deferens elicited by field stimulation. The possibility that differences exist between the effects of clonidine and noradrenaline (NA) on presynaptic α_2-adrenoceptors was recently suggested (Pelayo et al., 1980 ; Langer and Dubocovich, 1981 ; Moret and Langer, 1982).

Under the experimental conditions described by Shepperson et al. (1981) we have examined the presynaptic inhibitory effects of NA and clonidine on the twitch response of the rat vas deferens at two frequencies of stimulation. Clonidine (3 - 100 nM) inhibited the twitch response of the rat vas deferens in a concentration-dependent manner. At 1 Hz the IC_{50} for clonidine was 13.0 $\pm$ 2.0 nM while at the lower frequency of 0.01 Hz this IC_{50} was significantly reduced to 4.3 $\pm$ 0.1 nM ($p<0.05$, Student's t-test). In contrast, the frequency-dependency of the inhibition by exogenous NA (30 nM - 100 µM) was reversed as compared to clonidine. At the low frequency (0.01 Hz) the IC_{50} for NA was 7.5 $\pm$ 0.9 µM while at 1 Hz, the IC_{50} was decreased significantly to 2.0 $\pm$ 0.5 µM ($p<0.05$, Student's t-test). These experiments were repeated without cocaine. This procedure slightly reduced the IC_{50} for clonidine : 3.3 $\pm$ 0.3 nM at 0.01 Hz and 8.6 $\pm$ 3.0 nM at 1.0 Hz and noradrenaline : 19.6 $\pm$ 2.0 µM at 0.01 Hz and 11.3 $\pm$ 3.0 µM at 1.0 Hz.

In view of the difference in the frequency-dependence between the inhibitory effects of NA and clonidine we determined pA_2 values with these α_2-adrenoceptor agonists against a novel, selective α_2-adrenoceptor antagonist : RX 781094 (Berridge et al., 1982). The pA_2 values were determined at a frequency of 0.1 Hz and in the presence of 1 µM cocaine, 1 µM propranolol and 0.3 µM prazosin. In order to assess the influence of endogenously released NA on the determination of pA_2 values we carried out these experiments in vasa deferentia from normal rats and from rats pretreated with reserpine (2.5 mg/kg s.c., 24 hr before the experiment). In the controls the pA_2 value for clonidine was 8.34 $\pm$ 0.03 from a Schild plot with a slope of -1.03 $\pm$ 0.03 (n=4). After pretreatment with reserpine the pA_2 value for clonidine was 7.87 $\pm$ 0.10 and the slope -1.03 $\pm$ 0.03 (n=3). The pA_2 value for NA in the controls was 7.47 $\pm$ 0.02 and the slope -0.72 $\pm$ 0.21 (n=8). After pretreatment with reserpine the pA_2 value for NA was 7.45 $\pm$ 0.20 and the slope -1.11 $\pm$ 0.20 (n=4). The difference in pA_2 values between clonidine and NA was significant ($p<0.05$) in the control groups where the endogenous NA was not depleted by reserpine.

It is concluded that differences exist between the effects of NA and clonidine on presynaptic α_2-adrenoceptors. Two important factors that contribute to these differences are the neuronal uptake of NA and the concentration of endogenously released NA in the synaptic cleft.

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MECHANISMS OF BRADYCARDIA

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Adrenergically-stimulated increases in the frequency of the sino-atrial node are associated with steepening of the slope of the slow diastolic depolarisation; conversely cholinergic stimuli flatten the slope (Hutter & Trautwein, 1956). In theory bradycardia could be achieved without any change in the slope of the slow diastolic depolarisation if (a) repolarisation were to be delayed; (b) the "take-off" potential, at which the slow diastolic depolarisation merges into the action potential upstroke, became more positive; (c) the upstroke itself were to be slowed. We have observed all three phenomena. The drug melperone, for example, slows the heart rate by mechanism (a). In panel A of the figure intracellular potentials recorded from a rabbit sinus node before and after exposure to 4.34 μM melperone have been superimposed. The bar indicates that repolarisation from the action potential peak to the point of maximum repolarisation was delayed by 98 ms, wholly accounting for the bradycardia because the bar above the 2nd peaks is also of 98 ms. Similar records obtained before and after exposure to 2.63 μM cibenzoline (B) show that bradycardia was caused partly by a slower action potential upstroke and more positive take-off, and partly by slower repolarisation, but there was again no change in the slope of the slow diastolic depolarisation. Finally (C) alinidine 6.25 μM produced a comparable bradycardia almost entirely by reducing the slope of the diastolic depolarisation, with no change in action potential upstroke and only slight delay of repolarisation. The slow diastolic depolarisation has been attributed to an inward current, i_f, which begins to be activated only at potentials negative to -50 mV (Brown & Difrancesco, 1980). Many rabbit sinus nodal cells, however, exhibit slow diastolic depolarisations from maximum diastolic potentials of -40 mV or less, and the node continues to beat regularly in solutions containing 14 mM potassium, in which E_K itself would be only -60 mV. In such solutions alinidine still induces bradycardia by slowing the diastolic depolarisation (Millar & Vaughan Williams, (1981).

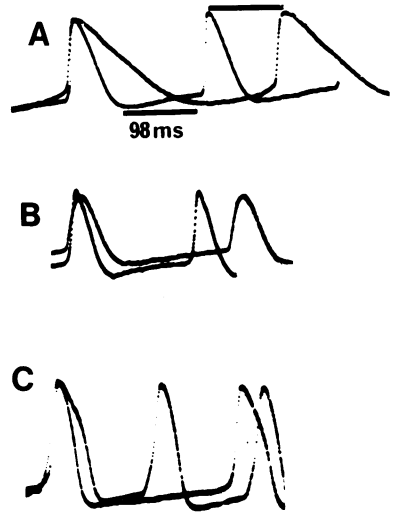

Figure. Intracellularly recorded potentials from single cells of the rabbit sinus node. Control records have superimposed upon them records taken subsequently in the presence of agents producing bradycardia, the Vmax points of the initial potentials coinciding. The oscilloscope traces were displayed from the memories of digital storage devices.

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INTERACTION BETWEEN INOTROPIC RESPONSE TO α-ADRENOCEPTOR STIMULATION AND TO OUABAIN

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Little is known about the mechanisms involved in the inotropic effect of α-adrenoceptor stimulation in myocardium. This effect is independent on a change if cellular cyclic AMP contents. α-Stimulation induced a small increase of the slow inward current, indicating increased Ca^{++} influx (Brückner & Scholz, 1980; Scholz, 1981). Another cyclic AMP independent inotropic effect is that caused by ouabain, which is thought to increase the amount of Ca^{++} reaching the myofilaments, presumably by inhibiting the sarcolemmal Na/K-ATPase. Studying interaction between the inotropic effects of ouabain and of α-adrenergic stimulation, might give some further insight into mechanisms coupled to α-receptors. In the present experiments were used isolated rat heart papillary muscles, electrically driven at 1 Hz. Isometrically developed tension was recorded. α-Adrenoceptor stimulation was obtained by adding phenylephrine in the presence of $5x10^{-6}$ mol/l propranolol.

Effects of α-stimulation on the response to ouabain. EC_{50} for ouabain, $8.9x10^{-7}$ mol/l, was not changed by α-stimulation. The presence of $4x10^{-7}$ mol/l phenylephrine did not change the maximum after ouabain. The maximum in the presence of 10^{-5} mol/l phenylephrine, however, was increased. The dose-additive effect thus obtained when ouabain was administered after the lower dose of phenylephrine, indicated at least partly similar mechanisms of action for ouabain and α-stimulation. The effect-additive response occurring when ouabain was given after the higher dose of phenylephrine, indicated different mechanisms of action for the two agents.

Effects of ouabain on the response to α-stimulation. EC_{50}, $1.4x10^{-6}$ mol/l, for phenylephrine was not significantly changed by ouabain; neither was the maximum altered. The dose-response curve for phenylephrine in the presence of 10^{-7} mol/l ouabain exhibited a dose-additive course. But the curve obtained in the presence of $4x10^{-6}$ mol/l ouabain revealed some peculiarities: Below about $4x10^{-7}$ mol/l phenylephrine did not evoke any positive inotropic effect, but instead induced a small but significant reduction in developed tension. Only at concentrations above $5x10^{-7}$ mol/l phenylephrine exerted a positive inotropic effect. When phenylephrine was added after the lower dose of ouabain, the combined effect thus indicated a common mechanism of action. When phenylephrine was given in the presence of the higher dose of ouabain, the lower part of the dose-response curve was clearly influenced by a negative inotropic component of α-stimulation. Thus it seems that ouabain in this case amplified the negative inotropic component of α-stimulation without correspondingly increasing the positive inotropic effect. This indicates an intricate interaction of the mechanisms involved.

The interactions between high doses of ouabain and phenylephrine were dependent upon the order of drug administration. When phenylephrine was added first, the effects were additive, but when ouabain was given first, the combined effect was not larger than that of phenylephrine alone. Thus a preceding inhibition of the Na/K-ATPase may hamper the positive inotropic effect of α-stimulation or increase the negative inotropic component.

In conclusion it seems that α-adrenoceptor stimulation and ouabain have some common mechanisms of action in rat myocardium but that also separate mechanisms are involved.

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THE EFFECT OF AGE ON PERIPHERAL α-ADRENOCEPTORS IN VIVO AND IN VITRO IN THE RABBIT

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Age related alterations in α adrenoceptor mediated responses have been reported by some workers (Cohen and Berkowitz, 1976; Elliott et al, 1982) while others have failed to find any change (Hayashi and Toda, 1978; Wyse et al, 1977). We examined 3 groups of male New Zealand white rabbits aged 2-3 months, 6-8 months and 24-36 months. In vivo pressor responses to phenylephrine, 10-50 μg/kg (α_1 adrenoceptor agonist), noradrenaline 1-25μg/kg (α_1/α_2 adrenoceptor agonist) and guanabenz 12.5-200 μg/kg (α_2 adrenoceptor selective agonist) were examined. The cardiovascular effects of the α_1 adrenoceptor antagonist prazosin (0.05-0.5 mg/kg) were also studied. Basal mean arterial pressure, heart rate and plasma catecholamines were measured. In vitro isometric contractile responses to phenylephrine and noradrenaline (10^{-9}-10^{-3}M) in isolated helical strips of aorta and renal artery were examined and specific binding of ^{3}H prazosin to spleen and heart membranes and ^{3}H clonidine to spleen membranes was studied. Mean arterial pressure rose from 80± 8 mm Hg in the youngest to 90± 11 mm Hg in the oldest group and plasma noradrenaline from 3.5 ± 1.2 to 6.9 ± 3.0 nM. No significant changes in in vivo responses to any of the α adrenoceptor agonists or to the antagonist prazosin were observed, nor was the specific binding of ^{3}H clonidine to spleen membranes altered. However the maximum number of specific prazosin binding sites was significantly reduced in spleen and heart membranes in the 6-8 month and 24-36 month animals.

		2-3 month	6-8 month	24-36 month
^{3}H clonidine binding in spleen	Bmax (f moles/mg protein)	174 ± 14	160 ± 8	171 ± 34
	K_D(nM)	29 ± 4	18 ± 2	19 ± 8
^{3}H Prazosin binding in spleen	Bmax (f moles/mg protein)	153 ± 25	91 ± 28*	87 ± 30*
	K_D(nM)	13 ± 4	12 ± 6	5 ± 4
^{3}H Prazosin binding in heart	Bmax (f moles/mg protein)	152 ± 15	72 ± 20*	81 ± 14*
	K_D(nM)	13 ± 4	16 ± 7	14 ± 4

(* $p < 0.05$)

In vitro, contractile responses to phenylephrine and noradrenaline were similar in 2-3 and 6-8 month old animals. There was a slight shift to the right in the dose response curve to both agonists in the 24-36 month animals, however the weight/unit length which was greater in the older animals, may have contributed to this apparent shift.

In previous studies we have shown that α_1 adrenoceptor mediated responses in young animals are not affected unless the number of prazosin binding sites is reduced by 40% or more (Hamilton et al, 1981); this we believe is due to the presence of 'spare' α_1 adrenoceptors. The lack of change in in vivo responses may reflect spare receptors, protecting the animals from the age-related decrease.

The effects of aging on ^{3}H clonidine and ^{3}H prazosin binding sites are a further indication of differences in the regulation of the two types of alpha receptor.

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WHY DO α_1-ADRENOCEPTOR ANTAGONISTS FAIL TO CAUSE REFLEX TACHYCARDIA?

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The absence of reflex tachycardia in response to the fall in blood pressure produced by the α_1-adrenoceptor antagonists prazosin and indoramin has been explained on the bases that they do not interfere with the negative feedback system which controls the release of noradrenaline (Cambridge et al, 1977). Furthermore, indoramin is said to produce 'cardiac stabilization' (Archibald, 1980). Large doses of indoramin have also been shown to reduce cardiac nerve activity (Baum & Shropshire, 1975) which may account for the absence of reflex tachycardia. Hence, the following experiments were done to study the effects of prazosin and indoramin on thoracic preganglionic sympathetic nerve activity.

Cats were anaesthetised with α-chloralose (70mg/kg i.v.) and pentobarbitone sodium (12mg). A pneumothorax was performed and the animals were artificially ventilated after paralysis with gallamine. Blood gases and pH were maintained within the physiological range. Brachial arterial pressure, heart rate, femoral arterial flow (from which conductance was derived) and preganglionic sympathetic nerve activity were recorded simultaneously. Confirmation that the sympathetic nerve activity, recorded from the third or fourth white ramus communicans, was preganglionic and under baroreceptor control was provided by its responses to noradrenaline and trimetaphan. Drugs were injected into the jugular vein or infused slowly into the brachial vein.

In all the experiments an initial infusion for 20 minutes of the vehicle alone (0.04M lactic acid) was given before the administration of the test solutions. Subsequent infusion of vehicle alone (3ml for 1h followed by 6ml for 1h and then 9ml for 1h), in 3 experiments, caused increases in blood pressure (14%), heart rate (38%) and preganglionic sympathetic nerve activity (311%) and a decrease in femoral arterial conductance (50%), all changes occurring gradually after the first hour. In another 3 experiments, infusion of indoramin (1mg/kg for 1h followed by 2mg/kg for 1h and then 3mg/kg for 1h) gradually reduced blood pressure (29%), heart rate (29%) and preganglionic sympathetic nerve activity (81%) and increased femoral arterial conductance (19%). When prazosin (3 experiments) was infused at the same rate and dose, it produced similar falls in blood pressure (18%), heart rate (13%) and preganglionic sympathetic nerve activity (68%) but a larger increase in femoral arterial conductance (65%). Its effects differed from those of indoramin in that they were rapid in onset, reaching a near maximum in the first 20 min. They remained at this maximal level for about 60 min after which, they all tended to return to baseline values.

The results suggest that the absence of reflex tachycardia, following the fall in blood pressure produced by prazosin and indoramin, is due to the reduction in preganglionic sympathetic nerve activity which is also an important component of their hypotensive action.

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CATECHOLAMINE HANDLING BY THE HUMAN FOREARM AT REST AND DURING ISOMETRIC EXERCISE AND LOWER BODY NEGATIVE PRESSURE

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Plasma catecholamine concentrations are often used to monitor sympatho-adrenal activity in humans. For practical reasons blood is usually sampled from the antecubital vein. Both noradrenaline (NA) and adrenaline (A) concentrations in this venous plasma will, however, be influenced by removal and/or release in the forearm. Stimuli causing changes in blood flow and sympathetic activity in the forearm may alter the relationship between arterial and forearm venous plasma catecholamines. This problem has been examined in healthy subjects at rest and in connection with contralateral isometric hand grip exercise (CIH) and lower body negative pressure (LBNP).

Eleven subjects participated in the study, which was approved by the Ethical Committee at the Karolinska Hospital. Nine experiments were performed with each provocation. Forearm blood flow was measured by venous occlusion plethysmography. Arterial and deep forearm venous blood was simultaneously sampled both at rest and during CIH and LBNP. Plasma catecholamines were analyzed by HPLC with electrochemical detection (Hjemdahl et al. 1979). CIH was performed at maximum voluntary effort during 10 min. LBNP was adjusted so as not to produce major changes in blood pressure and was maintained during 10 min.

At rest there were significant correlations between venous and arterial plasma concentrations of both NA and A ($r = 0.83$ and 0.78, respectively, based on 32 paired samples). Resting levels were (mean ± S.D.): arterial NA 1.19 ± 0.39 nM, venous NA 1.41 ± 0.68 nM, arterial A 0.38 ± 0.27 nM, venous A 0.11 ± 0.09 nM. There was an insignificant tendency towards release of NA (mainly in individuals with high NA levels) and a significant and pronounced removal of A by the forearm at rest. CIH increased blood pressure, heart rate, forearm blood flow and arterial NA and A and reduced forearm vascular resistance significantly. During CIH NA outflow from the forearm increased, especially in individuals with high noradrenaline levels. The removal of A in the forearm was reduced from 75 % to 39 %, on the average, during CIH. LBNP reduced mean arterial pressure by about 7 mm Hg and reduced forearm blood flow by 1/3. LBNP doubled the V-A difference for NA, which resulted in a significant release of NA. Removal of A by the forearm increased from 75 % to 88 % during LBNP. LBNP caused a significant increase in arterial but not venous plasma A. However, the significant correlations between arterial and venous plasma NA and A concentrations persisted during CIH and LBNP, even though the magnitudes of the V-A differences were altered.

The present results show a good correlation between arterial and venous plasma concentrations of both NA and A, in agreement with the results of Halter et al. (1980). However, there was a variable release of NA and a variable removal of A by the human forearm. Inter- and intraindividual variation in catecholamine handling in the arm may be related to changes in blood flow and sympathetic activity. Catecholamine analyses on forearm venous plasma will tend to overestimate changes in circulating NA and underestimate changes in circulating A induced by provocations.

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THE EFFECT OF ATENOLOL ON AORTIC NERVE DISCHARGE IN THE ANAESTHETIZED CAT

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In the anaesthetized cat, the administration of the β-adrenoreceptor blocking drug, atenolol, has been shown to reduce both the level of spontaneous sympathetic efferent discharge and to attenuate the responses of the sympathetic efferent nerves to pharmacologically induced falls in blood pressure (Scott, 1981). The present series of experiments was carried out to determine whether these changes were produced by changes in the baroreceptor input to the brain.

Seven cats were anaesthetized with α-chloralose (80 mg kg^{-1} i.p.) and artificially ventilated. Body temperature and the pH, pCO_2 and pO_2 of the arterial blood were monitored and maintained within normal limits. Extracellular recordings were made of aortic nerve discharge using conventional techniques. Blood pressure was raised or lowered by the injection of phenylephrine (1-15 μg kg^{-1}) and glyceryl-trinitrate (5-60 μg kg^{-1}) or sodium nitroprusside (2-8 μg kg^{-1}). The aortic nerve discharge was recorded over a range of blood pressures both during a control period of at least one hour and for one hour after the administration of atenolol (3 mg kg^{-1}).

In the control period immediately before giving atenolol the mean blood pressure was 108.7 ± 6.0 mmHg (mean ± s.e. mean) and the aortic nerve discharge was 59.7 ± 3.8 impulses s^{-1}. These values were not significantly different from the control values recorded one hour earlier. Immediately after atenolol there was a significant reduction in the blood pressure to 78.8 ± 11.9 mmHg ($P < 0.005$) and in aortic nerve discharge to 30.9 ± 9.4 impulses s^{-1} ($P < 0.005$). For the next 60 min the mean blood pressure remained significantly lower than during the control period before giving atenolol. However, 30 min after giving atenolol the aortic nerve discharge was 65.1 ± 2.8 impulses s^{-1} and 60 min after was 72.6 ± 2.1 impulses s^{-1}. The aortic nerve discharge 30 min and 60 min after atenolol was significantly higher than in the control period before giving the drug (P always < 0.025), even though the mean blood pressure was significantly lower ($P < 0.05$) during this period, compared to the control period.

There was however, no evidence that the responses of the aortic nerves to changes in blood pressure were attenuated after the administration of atenolol. Before atenolol the mean change in aortic nerve discharge was 0.91 ± 0.16 impulses s^{-1} per mmHg change in the blood pressure and one hour after giving atenolol the mean change in aortic nerve discharge was 0.93 ± 0.11 impulses s^{-1} $mmHg^{-1}$.

It is concluded that the decreases in the level of spontaneous sympathetic efferent discharge observed in previous studies (Scott, 1981) one hour after giving atenolol may be a result of an increased aortic nerve discharge which occurs in spite of the fall in blood pressure. However these experiments do not support the hypothesis that the attenuation of the sympathetic efferent nervous responses to falls in blood pressure is due to changes in the responsiveness of the baroreceptors themselves to changes in the blood pressure. This attenuation of the baroreceptor reflex is more likely to be due to a modulation of the reflex at some other site, possibly within the central nervous system.

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ALTERATIONS IN THE RESPONSE TO CONTRACTILE AND RELAXANT STIMULI IN NITROGLYCERIN TOLERANT RAT AORTA

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Tolerance towards the pharmacological effects of nitroglycerin (NG) may occur both in clinical practice and among workers in the explosive industry. In some cases, these workers develop angina pectoris symptoms during the withdrawal period (i.e. 1-2 days after the last exposure to NG) and cases of sudden death have been reported (Hogstedt, 1980). The cause of these symptoms is largely unknown as was also, until recently, the mechanism of action of NG on vascular smooth muscle. However, we have in recent works found evidence for cyclic guanosine-3´:5´-monophosphate (cGMP) as a mediator of NG induced vascular smooth muscle relaxation (Axelsson et al, 1979). We were also able to induce tolerance towards the relaxant effect of NG by incubating pieces of vascular smooth muscle in vitro at elevated pH in the presence of high NG concentrations. This tolerance also resulted in a substantial reduction in the cGMP elevating effect of a challenging dose of NG (Axelsson et al, 1982). In this study, these observations were further substantiated and extended in in vivo experiments on rats. Sprague-Dawley rats were treated with NG (50 mg/kg) 3 times daily for 3 consecutive days. 14-18 h after last treatment the rats were sacrificed and the aortas cut out. Rats treated with ethanol were used as controls. Pieces of aorta, weighing 5-7 mg, were used for tension studies in vitro and determination of cyclic nucleotides. The remaining aortic tissue was used for metabolic determinations. After precontraction of the aortic pieces with noradrenaline (2.5 μM), NG (44 μM) was found to relax the control vessels by 46 ± 3.5 %, while only causing 11 ± 1 % relaxation in NG-pretreated vessels. This reduced relaxant response towards NG was accompanied with a reduction in the cGMP level in the NG-pretreated vessels as compared to the control vessels (66 ± 9 pmol/g w.w. and 138 ± 15 pmol/g w.w., respectively) when determined 2 min after NG addition. The cAMP levels were not changed. The cGMP-dependent protein kinase activity was not found to be affected by NG-pretreatment. However, kinetic studies on cGMP phosphodiesterase showed an increase in V_{max} from 0.77 nmol/min x mg prot (control) to 1.34 nmol/min x mg prot for the enzyme isolated from NG-pretreated aortas. The K_m-value was also slightly elevated for the enzyme from this latter source. When the effect of the contracting agents phenylephrine and serotonin was investigated, no change in sensitivity towards serotonin could be established between control and NG-pretreated vessels. However, the sensitivity towards phenylephrine was about 4 times higher in aortic tissue from the NG-treated animals.

The results of this study indicate that several different factors might be involved in redeeming withdrawal symptoms in individuals previously exposed to NG. If cGMP is accepted as a mediator of vascular smooth muscle relaxation, the alterations in cGMP turnover together with the increased sensitivity towards certain contractile agents in vascular tissue from NG-treated animals might very well explain these symptoms.

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DIFFERENTIATION BETWEEN β_1 AND β_2 ADRENOCEPTORS MEDIATED INHIBITION OF SPONTANEOUS CONTRACTILITY IN THE RAT ISOLATED COLON STRIP

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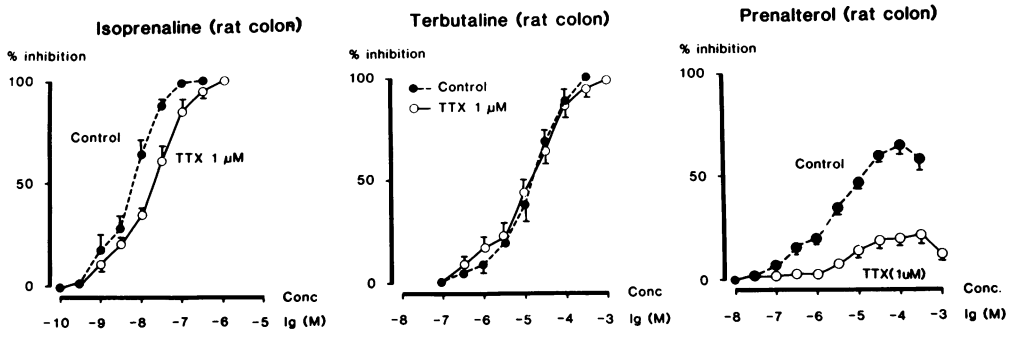
In the present investigation the inhibitory effect of isoprenaline (IPR), terbutaline (TRB) and prenalterol (PRE) was studied in the isolated rat colon strip. Furthermore, tetrodotoxin (TTX) was used to evaluate the sites of action of the three agonists. All three agonists inhibit concentration dependently, the spontaneous contractile activity in the circular rat colon strip, IPR and TRB by 100% and PRE by 70%. Metoprolol (1 uM) produced a significant righthand displacement of the concentration response curve for both IPR and PRE by approximately one log unit whereas the terbutaline curve was not significantly shifted. IPS 339 (1 uM), on the other hand, produced a shift of the same magnitude of the curves for IPR and TRB whereas the curve for PRE was not shifted significantly. This is in agreement with the findings in the cat colon strip (Ek & Lundgren, 1982).

In preparations treated with TTX (3 uM) the spontaneous activity was retained. However, the PRE induced response on colon activity is almost completely abolished after TTX while the TRB response is left unaffected, see Fig. 1. Furthermore, the concentration effect curve for IPR was significantly shifted to the right by TTX treatment, see Fig. 1.

These results indicate that the inhibitory effect of prenalterol, β_1-mediated, is produced at a level which is separated by at least one neuron from the effector organ, i.e. the circular smooth muscle cell. The lack of effect of TTX on the terbutaline, β_2-mediated, concentration effect curve may indicate that the β_2-adrenoceptor population is situated at the smooth muscle cells per se. The right-hand shift of the IPR, non-selective, concentration effect curve may be due to the disappearance of the β_1-mediated effect of IPR, the resulting effect is then entirely due to β_2-adrenoceptor stimulation. This is well in agreement with the concept of the β_1-adrenoceptor being the neuronal receptor and the β_2-adrenoceptor being the hormonal receptor, which was suggested by Carlsson & Hedberg (1976).

Figure 1



Log concentration-effect curves for inhibition of spontaneous contractions in the colon strip by isoprenaline, terbutaline and prenalterol with and without tetrodotoxin (3 uM). The curves are means of 6 experiments.

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DOSE-DEPENDENT METABOLISM OF THE FOOD FLAVOURING AGENT P-PROPYLANISOLE

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Allylbenzenes, both naturally occurring (anethole and estragole) and synthetic (p-propylanisole) are widely used as food flavouring agents. Concern has been expressed regarding their safety in view of hepatocarcinogenicity and hepatotoxicity in rodents and the meaningfullness of procedures which involve massive doses to laboratory species unless issues of species differences and dose-dependence of metabolism are taken into account. We have previously shown that the metabolism of estragole is dose-dependent in both the rat and mouse, and have now extended such studies to an evaluation of the possible dose-dependent metabolism of its saturated analogue, p-propylanisole (PPA).

[methoxy-^{14}C]-PPA was given to female Wistar albino rats p.o. and male CD-1 mice i.p. at doses ranging from 0.05-1500mg/kg and 0.2-20μCi/animal. The animals were housed in Metabowls, and urine, faeces and $^{14}CO_2$ in the expired air were collected. The urinary metabolites were separated by solvent extraction, TLC and HPLC, and characterized by MS, NMR and comparison with authentic samples. Three major ^{14}C-labelled urinary metabolites were excreted, 1'- and 2'-hydroxy-PPA (1'- OHPPA and 2'-OHPPA) and p-methoxyhippuric acid (PMHA). $^{14}CO_2$ was eliminated in the expired air, arising from the oxidative O-demethylation of PPA. The relative quantities of these metabolites varied markedly with dose, as shown in the Table.

Variation with dose of the excretion routes and metabolic pathways of ^{14}C-PPA in the Wistar rat.

	% dose in 72h						
Dose (mg/kg)	Urine	Faeces	$^{14}CO_2$	Total recovery	1'OH-PPA	2'OH-PPA	PMHA
0.05	8.0	n.d.*	81.6	89.6	0.7	1.8	4.0
0.5	13.7	3.6	74.6	91.6	1.0	3.3	5.3
5	22.2	1.5	65.4	89.1	2.7	5.3	7.6
50	26.0	2.0	56.2	84.2	5.6	6.6	7.8
500	28.7	2.1	50.7	81.5	6.8	8.0	8.5
1500	37.1	1.3	47.2	85.6	7.5	9.8	12.8

* not detected

Similar results were obtained in the male CD-1 mouse dosed i.p.

The various routes of PPA metabolism all exhibit dose-dependency, with the % dose undergoing O-demethylation falling with dose and the urinary recovery rising. The relative proportions of 1'-OH- and 2'-OH-PPA and PMHA in the urine all change markedly with dose. Thus, the metabolism and excretion of PPA is influenced by the dose given, and the pattern of dose-dependence observed is similar to that seen with estragole.

In view of the great discrepancy between the human exposure to PPA in foods (about 15μg/day) and the doses used for its toxicological evaluation in animals, these results emphasize the importance of considering dose-dependent metabolism when interpreting the significance for man of animal data obtained at very high doses.

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EFFECT OF RETINYLACETATE (VITAMIN A ANALOGUE) ON THE KINETICS OF GUANYLATECYCLASE

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In recent years, the potent anticancer activity of different vitamin A analogues (retinoids) has attracted much attention. Since cyclic guanosine 3':5'-monophosphate (cGMP) has been implied as a mediator of cellgrowth and transformation, we considered it of interest to investigate the effects of retinoids on the cGMP-forming enzyme, guanylatecyclase (GC). In an earlier paper, we reported that retinylacetate (RA) was a potent inhibitor of this enzyme (Rydell et al, 1981). In the present study, we have further investigated the mechanism of action of RA on GC. In these experiments crude soluble GC from rat liver was used.

GC, stimulated with 1 mM nitroprusside (NP), seemed to adhere to Michaelis-Menten kinetics, with an apparent K_m for MnGTP of 0.1 mM and with a V_{max} of 474 pmoles cGMP/min x mg prot. RA inhibited the enzyme in a non-competitive manner. In the presence of 1 mM RA, the maximal activity was about 10% of the control activity. However, with RA present in the assay, the Lineweaver-Burk plots were found to be non-linear. The concentration-activity curve for NP (with 1 mM GTP and 4 mM Mn^{2+}) was found to be bell shaped with an optimum at 0.5 mM NP. RA (0.1-1 mM) depressed the maximal enzyme activity and this inhibition could not be overcome by increasing the NP concentration. On the contrary, at low concentrations of NP (< 50 µM; i.e. at submaximal activation), RA somewhat enhanced the enzyme activity. For optimal activity GC seems to require free divalent cation (eg. Mn^{2+}). We therefore tested if the inhibitory effect of RA could be reversed by increasing the concentration of free Mn^{2+}. When increasing the Mn^{2+} concentration, in the presence of fixed conc. of GTP and NP (1 mM respectively), a bell shaped activity curve with an optimum at 2 mM Mn^{2+} was obtained. RA (0.1-1 mM) depressed the enzyme activity in a non-competitive fashion. GC is thought to contain essential sulfhydryl groups which may undergo redox-reactions with concomitant changes in the enzyme activity. With an assay mixture containing 1 mM GTP, 4 mM Mn^{2+} and 1 mM of the sulfhydryl reductant dithiothreitol (DTT), the NP concentration needed for optimal enzyme activation was about 100-fold less than in the absence of DTT. The maximal obtainable enzyme activity was also increased by about 50%. Furthermore, the inhibitory effect of RA was much less pronounced. RA (1 mM) caused only about 30% inhibition in the presence of DTT as compared to 80% in the absence of DTT.

These results seem to indicate critical sulfhydryl groups on GC as the major site of interaction with RA. Since RA is able to participate in redox-reactions, complex effects on the redox status of GC might be expected. Moreover, it can not be excluded that RA might bind to the enzyme and thus alter the response of GC towards different stimulatory agents. RA seems to function as a non-competitive antagonist against the natural substrate for GC (i.e. GTP) and also against other factors known to be of importance for the regulation of the enzyme activity (i.e. Mn^{2+}, NP). The importance of these findings is still unclear but could indicate that the interaction of RA with some site on the enzyme which is separated from other regulatory sites on the enzyme. However, a non-specific effect of RA on various sulfhydryl groups on GC can not be excluded. Further experiments are therefore needed to clarify the exact nature of the inhibitory action of RA on GC.

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CONJUGATION OF p-NITROPHENOL IN PERFUSED LIVERS ISOLATED FROM STREPTOZOTOCIN-INDUCED DIABETIC RATS

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It has recently been shown that 'native' microsomes from streptozotocin(stz)-induced diabetic male rats have a reduced capacity to form p-nitrophenol glucuronide. Triton X-100 activation abolished this defect which is thought to be due to altered microsomal membrane structure in stz-induced diabetes (Morrison and Hawksworth, 1982). The present study demonstrates alterations in p-nitrophenol conjugation by perfused livers isolated from male stz-treated rats.

Stz was administered i.v. (60 mg kg^{-1}). On day 5, when the blood glucose concentrations of treated rats were 382 ± 14 mg 100 ml^{-1} compared with 102 ± 3 mg 100 ml^{-1} in control rats, the livers were isolated and perfused with Krebs-Henseleit buffer, pH 7.4, containing 2.5% (w/v) bovine serum albumin and 12.5% (v/v) washed human erythrocytes. Perfusion flow rates ranged from 0.8-1.7 ml min^{-1} g^{-1} liver. p-nitrophenol was added as a bolus to the reservoir (110 ml, re-circulating mode) to obtain a perfusate concentration of 0.5 mM. Concentrations of p-nitrophenol and the sulphate and glucuronide conjugates were measured directly by reversed-phase ion-pair hplc (Diamond and Quebbemann, 1979).

At high substrate concentrations the elimination of p-nitrophenol showed saturation kinetics, but after 30 min there was a significant difference between the hepatic elimination in control rat livers and diabetic rat livers. The elimination half-life in control rat livers was 9.54 ± 0.93 min compared with 35.69 ± 12.25 min in diabetic rat livers (0.05< P<0.1). In addition perfusate concentrations of the sulphate and glucuronide were significantly different in control and diabetic rats after 15 min as shown in Figure 1.

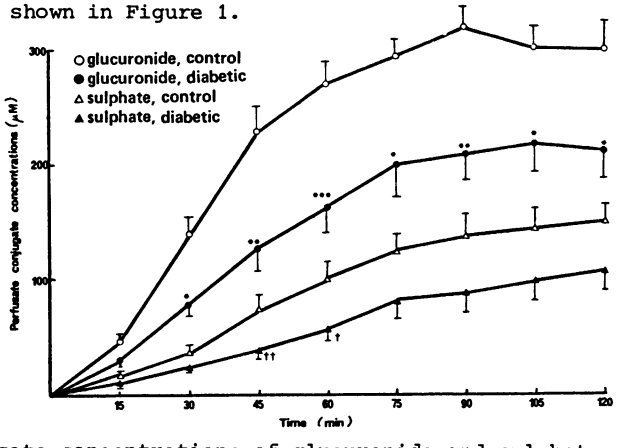

Figure 1 Perfusate concentrations of glucuronide and sulphate conjugates *P<0.05; ** P <0.01; ***P <0.005 for glucuronide: †P <0.05; ††P <0.02 for sulphate.

In control rat livers 10.5 ± 2.8% of the p-nitrophenol was excreted in the bile as the glucuronide and this was not significantly different in diabetic rat livers. The similarity of results obtained using 'native' microsomal fractions and the intact liver suggest that in vivo the glucuronyl transferase is in the 'native' form. The decreased sulphation in diabetic rat livers is thought to be a kinetic effect due to the persistence of high substrate concentrations.

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THE EFFECT OF ALDOSE REDUCTASE INHIBITION ON AXONAL TRANSPORT AND NERVE CONDUCTION VELOCITY IN STREPTOZOTOCIN-DIABETIC RATS

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Aldose reductase converts glucose to sorbitol in nervous tissue during hyperglycaemia. Thus, nerves of diabetic animals accumulate sorbitol which, because of its low membrane permeability, causes oedema in nerve trunks. Concomitantly nerve conduction velocity slows (Gabbay, 1973). Experimental diabetes also impairs axonal transport of choline acetyltransferase (ChAT) in the sciatic nerve (Schmidt et al, 1975). We have studied interrelations between these three phenomena by measuring the effects of aldose reductase inhibition in experimental diabetes. Three groups of rats (male Wistar, 260-310 g), diabetic, diabetic treated with aldose reductase inhibitor (ARI) and controls, were studied. Conduction velocity of left sciatic motoneurones (MNCV) was measured (method of Sharma & Thomas, 1974) weekly, twice before and 3 times after induction of diabetes with streptozotocin (75 mg/kg i.p.), and weekly in the control group. One diabetic group was given (50 mg/kg/day p.o.) an ARI (ICI 105552; 1-(3,4-dichlorobenzyl)-3-methyl-1,2-dihydro-2-oxoquinol-4-ylacetic acid), starting on the day of streptozotocin treatment. 3-4 weeks after streptozotocin the animals were anaesthetised with ether and a tight prolene ligature applied to the left sciatic nerve at the mid-femur level. 24 hr later the rats were killed, both sciatic nerves removed and cut into 3 mm segments, which were homogenised and assayed for ChAT activity (method of Fonnum, 1974). Aliquots of the homogenates of the unconstricted nerves were assayed for sorbitol by gas-liquid chromatography (Sweeley et al, 1963). Blood glucose was measured at death and concomitantly with each MNCV measurement. Untreated diabetic rats showed a progressive decrease in MNCV, significant (Table) after 3 weeks diabetes. MNCV did not change in control and ARI-treated groups. ChAT accumulation proximal to the constriction was reduced in diabetic rats. This impairment of axonal transport was prevented by ARI, although there was no difference in severity of diabetes between the two groups. The findings indicate that nerve sorbitol accumulation may promote impairment of both MNCV and orthograde axonal transport in experimental diabetes.

Mean values (±SEM) after 3-4 weeks' diabetes. Number of rats in brackets.

	MNCV (m/sec)	ChAT accumulation (nmol ACh/hr/nerve)	Sorbitol (nmol/mg/nerve)	Blood glucose (mmol/L)
Controls (9)	43.4 ± 1.5	9.6 ± 0.5	<0.1	4.2 ± 0.3
Diabetic (9)	38.2 ± 1.7*	5.9 ± 0.6 **	1.4 ± 0.1	17.2 ± 1.2
Diabetic-ARI (7)	45.0 ± 1.7	11.4 ± 1.0	<0.1	15.8 ± 1.3

*P<0.01 by comparison with pre-diabetic values (paired t); **p<0.001 (unpaired t).

J.H.M. was supported by the British Diabetic Association. We thank Dr. D.C.N. Earl of ICI Pharmaceuticals Division for gifts of drugs and for his support.

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DELIVERY OF CYTOTOXIC LDL-INCORPORATED ANTHRACYCLINES TO HUMAN GLIOMA CELLS WITH HIGH LDL-RECEPTOR ACTIVITY

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Drug selectivity in cancer chemotherapy can be enhanced if the drugs are linked to carriers, which are preferentially taken up by malignant cells. Low-density lipoprotein (LDL), the major cholesterol-carrying lipoprotein in human plasma, is taken up by human cells through receptor-mediated endocytosis (Goldstein & Brown, 1977). Rapidly dividing cells have high LDL receptor activity, whereas non-dividing cells have few if any LDL receptors (Kruth et al, 1979).

We have found that a human glioma cell line (U-251 MG) expresses LDL receptors and that the receptor activity is maximal when the cells are rapidly proliferating. By incubating LDL isolated from normal serum with daunorubicin at a high concentration at 40°C, a daunorubicin-LDL complex was obtained. After separation of free and LDL-incorporated daunorubicin on a Sephadex G-25 column, the complex containing 10-30 molecules of daunorubicin per LDL-particle, was stable on dialysis at 37°C for 40 h. Furthermore, when the density of the daunorubicin-LDL solution was increased to 1.21 g/ml by the addition of sodium bromide and submitted to ultracentrifugation, all the daunorubicin-LDL flotated to the top fraction. When free daunorubicin was treated in the same way a uniform drug distribution was obtained. The cellular accumulation of daunorubicin by U-251 MG-cells was higher after incubation of the cells with LDL-incorporated drug (1 μM) than after incubation of the cells with the same concentration of the free drug (figure 1). The addition of native LDL in 7-fold excess markedly reduced the cellular accumulation of LDL--incorporated but not of free daunorubicin. Furthermore, daunorubicin-LDL inhibited the growth of U-251 MG cells which could be counteracted by native LDL in excess.

In order to increase the drug incorporation into the LDL-particles we have used the reconstitution technique of Krieger et al (1980) to incorporate the lipophilic anthracycline derivative, N-trifluoroacetyladriamycin-14-valerate (AD 32) after extraction of the lipid core of the lipoprotein. The reconstituted LDL contained about 700 molecules of AD 32 per particle and exhibited β-mobility on agarose gel electrophoresis as does native LDL. Its toxic effect on the glioma cells was about 50 times higher than that of the daunorubicin-LDL complex and could be counteracted by the addition of LDL in excess, indicating that the AD 32-LDL complex exerts its toxicity after entering the cells via the LDL-receptor pathway.

Our results suggest that LDL can be used as a carrier for anthracycline cancer chemotherapeutics to preferentially kill cells with high LDL-receptor activities.

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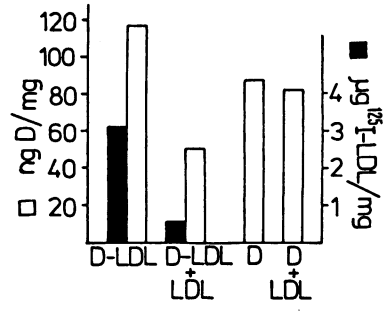

Figure 1 Uptake of daunorubicin and ^{125}I-LDL by U-251 MG cells. Cells maintained in lipoprotein--deficient medium were incubated for 4 hours with daunorubicin-^{125}I-LDL (D-LDL) and daunorubicin (D), with and without an excess of unlabeled LDL, respectively. Final concentrations: D-LDL = 1.0 μM daunorubicin, 23 μg ^{125}I-LDL/ml. D = 1.0 μM. Unlabeled LDL = 133 μg/ml. Cells were detached with a rubber policeman and aliquots were assayed for protein, daunorubicin and radioactivity.

ENDORPHINS AND CENTRAL CARDIOVASCULAR REGULATION

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β-Endorphin (β-LPH 61-91) induces a dose-related (1-1000 pg) fall in blood pressure and heart rate when administered directly into the nucleus tractus solitarii (NTS) of the urethane anaesthetised rat (Petty et al., 1981). The depressor response which is anatomically specific and restricted to the NTS, is naloxone reversible (Petty et al., 1982). These effects of β-endorphin are blocked by microinjection of β-endorphin antiserum, which causes a rise in pressure and tachycardia when administered alone (Petty et al., 1982). The possibility that these actions of β-endorphin are dependent on the entire molecule has been examined by means of fragments of the β-endorphin molecule, and the enkephalins applied locally to the NTS.

Microinjection of Des-Tyr-β-endorphin (β-LPH 62-91) induced a rise in mean arterial pressure (MAP) when compared to saline treated control ($p < 0.005$). This is the reverse response to that seen after the local application of β-endorphin, and is further confirmation of the activation of specific opiate receptors by the peptide to induce hypotension and bradycardia (Fredrickson, 1977; Guillemin et al. 1977).

α-Endorphin (β-LPH 61-76, 2 ng), like β-endorphin, caused a fall in both MAP and heart rate (HR), although the maximum change in MAP occurred 20 min after administration compared to 30-60 min after microinjection of β-endorphin. γ-Endorphin (β-LPH 61-77) however, induced a dose related (0.3-2000 pg) pressor effect and no apparent change in HR. Both Des-Tyr-α-endorphin and Des-Tyr-γ-endorphin (β-LPH 62-76, and 62-77 respectively) were without effect on MAP, indicating opiate receptor involvement.

Met-enkephalin and the stable analogue D-Ala^2 met-enkephalin produced dose related pressor responses upon local application. The maximum increase of 20 ± 6 and 18 ± 2 mm Hg respectively, occurred with a dose of 1 ng at about 40 min. after injection. In the case of D-Ala^2 met-enkephalin the rise in MAP was accompanied by tachycardia. Microinjection of antiserum to met-enkephalin (0.4 µl of a 1 : 50 dilution) induced a fall in MAP and reversed the pressor effect and tachycardia caused by D-Ala^2 met-enkephalin administration. The antiserum had no influence on the depressor response or the bradycardia induced by β-endorphin.

In conclusion, there are at least two endorphin systems involved in cardiovascular regulation at the level of the NTS, one which is depressor in nature (β-endorphin-like) and the other pressor (met-enkephalin-like).

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MODULATION OF DOPAMINE RECEPTOR ACTIVATION BY THE NEUROPEPTIDES VIP AND CCK

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The coexistence of specific intestinal, peptide hormones and conventional neurotransmitters is widespread in the central nervous system (Hökfelt et al, 1980). Since these neuropeptides have been shown to influence monoamine-linked behaviour (Kovacs et al, 1981), the present study has focussed on the biochemical interactions between the peptides, cholecystokinin (CCK) and vasoactive intestinal peptide (VIP), and central adenylate cyclase-linked dopamine receptors (D-1) and non adenylate cyclase-linked dopamine receptors (D-2).

VIP produced no direct activation of D-1 receptors, as indicated by the additive effect of VIP (0.2-10 μM) and DA (5-40 μM) on hypothalamic membrane adenylate cyclase (AC). Bromocriptine and lisuride (up to 50 μM) had no effect on the VIP-sensitive hypothalamic AC. Furthermore, dose-dependent activation by VIP of AC in hypothalamic slices was unaffected by preincubation with 1 μM bromocriptine. Finally VIP (2×10^{-7}M) did not alter ^{3}H-spiroperidol binding to D-2 receptors.

Sulphated cholecystokinin (CCK_8S, 10^{-6}M) significantly increased the affinity ($p < 0.01$) and decreased the number of ^{3}H-spiroperidol binding sites ($p < 0.05$) in rat striatal membranes. In contrast, in the nucleus accumbens, the opposite effect was observed; a decrease in affinity and an increase in the number of ^{3}H-spiroperidol binding sites. The activation of D-1 receptors in membrane preparations from amygdala, nucleus accumbens, olfactory tubercle, striatum and frontal cortex was not affected by CCK_8S (10^{-6}M).

Our results suggest a postsynaptic interaction between CCK and DA at the D-2 receptor but not the D-1 receptor. Although inactivation of D-2 receptors is considered to inhibit the VIP-sensitive AC in the pituitary gland (Onali et al, 1981) we failed to demonstrate a similar antagonistic relationship in striatal and hypothalamic membranes.

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PANCREATIC POLYPEPTIDES AND SYMPATHETIC VASOCONSTRICTION RESISTANT TO α-ADRENOCEPTOR ANTAGONISTS

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By immunohistochemistry it was found that sympathetic periarterial nerves in cat and human peripheral organs, such as the submandibular salivary gland contained, in addition to noradrenaline (NA), a pancreatic polypeptide (PP)-immunoreactive substance. The NA nerves around exocrine acini were however not PP-immunoreactive, suggesting that separate populations of NA neurons innervate blood vessels and exocrine elements. The NA neurons in the cat nictitating membrane were of the non--PP type. The PP immunoreactivity was further characterized in absorption studies with 36 amino acid peptides of the PP family: avian PP (APP), bovine PP (BPP), neuropeptide Y (NPY) and the peptide YY (PYY). It was found that APP and NPY abolished both the APP and BPP immunostaining. Since only NPY has been isolated from nervous tissue, this peptide may be present in vascular NA nerves. Electrical stimulation (0.2 ms, 10 V, 10 Hz) of the cervical sympathetic trunc caused a marked vasoconstriction in the cat submandibular gland simultaneously with a considerable salivation (10 drops/min) and a contraction of the nictitating membrane. After cessation of the stimulation there was a rapid vascular escape which was followed by a prolonged vasoconstrictory response with a gradual decline. Phentolamine or phenoxybenzamine, 3 mg/kg i.v. and/or 0.5 mg/kg i.a., abolished the salivation and contraction of the nictitating membrane. After α-adrenoceptor blockade the vascular response changed into an initial vasodilation followed by a small vasoconstriction. Following administration of propranolol, 1 mg/kg i.v. and/or 0.1 mg i.a., sympathetic nerve stimulation caused a significant slowly developing prolonged vasoconstriction without any rapid vascular escape. This suggests that part of the vasoconstrictory response to sympathetic nerve stimulation is resistant to α-adrenoceptor blockade on the contrary to the secretory effect and the contraction of the nictitating membrane. Local intra-arterial infusion of PYY and NPY (30 and 100 pmol x min^{-1}) caused a slowly developing submandibular vasoconstriction, which had a long duration with a successive decline. NA infusions, on the other hand, caused salivary secretion, nictitating membrane contractions and a strong short-lasting vasoconstriction. The rapid vascular escape after NA infusions was, however, not followed by any prolonged vasoconstriction as after sympathetic nerve stimulation. The relative molar vasoconstrictory potencies were about: PYY (1), NPY (3), NA (30), APP and BPP (100). The vasoconstrictory effects of the PP family were also seen after phentolamine or phenoxybenzamine administration as well as in chronically sympathectomized cats. This suggests that PP peptides have direct effects on vascular smooth muscle. Combined infusions of NA and NPY caused a vascular effect rather similar to the response to sympathetic nerve stimulation. NPY (and PYY) seem to have vasoconstrictory actions in many vascular beds, since bolus injections i.v. (with doses over 100 and 30 pmol/kg, respectively) caused a prolonged increase in systemic arterial blood pressure which was associated with a transient bradycardia. Following α-adrenoceptor blockade, NPY and PYY still increased systemic arterial blood pressure. After α- and β-adrenoceptorblockade combined with atropine, NPY and PYY caused very little effect on heart rate suggesting that the cardiac effects of the PP peptides were indirect via cardiovascular reflexes.

In conclusion, vascular NA nerves may also contain a vasoconstrictory peptide of the pancreatic polypeptide family such as NPY. This may account for the differences in the vascular response after NA and sympathetic nerve stimulation as well as the differential sensitivity to α-adrenoceptor antagonists between the vascular nerves and the nerves around exocrine acini or in the nictitating membrane

NEURONAL INVOLVEMENT IN THE RELEASE OF NEUROTENSIN-LIKE IMMUNOREACTIVITY (NTLI) FROM THE SMALL INTESTINE OF THE RAT

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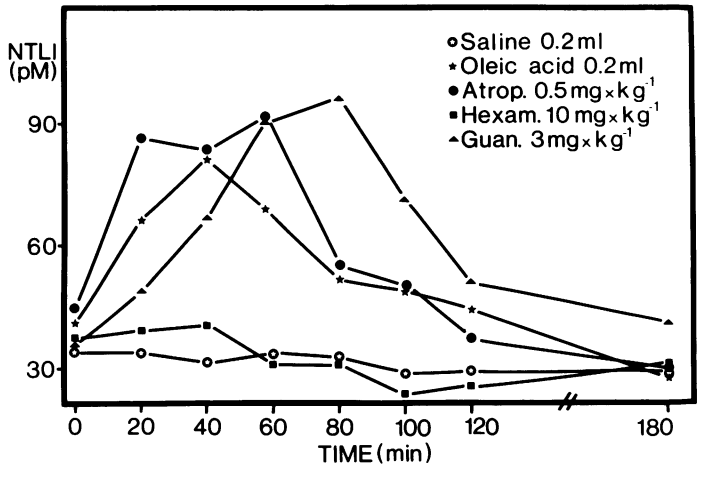
Ingestion of food, especially fat, induces a significant increase in plasma NTLI (Rosell & Rökaeus 1979). In the present study we have investigated the possible role of a neuronal mechanism for the release of NTLI. Intraduodenal instillation of oleic acid (0.2 ml) induced a significant increase of plasma NTLI at 20 min. The response lasted for about 100 min. Pretreatment with hexamethonium (10 mg x kg^{-1}) abolished the elevation of plasma NTLI induced by oleic acid. On the other hand, pretreatment with atropine (0.5 mg x kg^{-1}) or guanethidine (3 mg x kg^{-1}) did not change the plasma level of NTLI in response to oleic acid (Figure).

The results suggest that oleic acid evoked a release of NTLI via a nervous pathway in the small intestine which involves a preganglionic release of acetylcholine and a postganglionic nervous pathway of non-cholinergic, non-adrenergic type.



Concentration of plasma NTLI (median values) after administration of oleic acid at 0 min. Atropine, guanethidine and hexamethonium were infused intravenously between -15 min and +10 min.

EFFECTS OF (D-Pro^2, D-Trp^{7,9})-SUBSTANCE P ON THE DILATATION OF CEREBRAL ARTERIES PRODUCED BY SUBSTANCE P IN VITRO AND IN SITU

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Cerebral blood vessels have recently been found to receive a delicate network of substance P (SP) containing nerve fibres (Edvinsson et al, 1981; Uddman et al, 1981). Since SP may induce dilatation of isolated pial arteries both in vitro and in situ (Edvinsson et al, 1981) we have presently analysed this response by using a SP analogue with little agonistic activity but having strong antagonistic properties in other preparations (Leander et al, 1981; Rosell et al, 1981).

The effects of (D-Pro^2, D-Trp^{7,9})-SP, a structural analogue of SP, were examined on cerebrovascular responses to SP on cats in vitro using segments of the middle cerebral artery and in situ by microapplication of the peptides close to pial arterioles of α-chloraloze anaesthetized animals.

(D-Pro^2, D-Trp^{7,9})-SP in concentrations up to 6.6×10^{-6} M was without significant effect upon isolated middle cerebral arteries under normal conditions and in arteries contracted with prostaglandin $F_{2\alpha}$. SP effected concentration-dependent relaxations of middle cerebral arteries contracted by prostaglandin $F_{2\alpha}$ (mean ±s.e.m.; EC_{50}:2.0±1.6x10^{-9}M). The presence of (D-Pro^2, D-Trp^{7,9})-SP shifted the concentration-response curve of SP towards higher concentrations without significantly affecting the maximum response of the arteries to SP.

Table 1. Antagonism by (D-Pro^2, D-Trp^{7,9})-SP of substance P induced responses of pial arteries in situ

	n	Mean calibre (μm)±s.e.	(range)	Calibre alteration (%) ± s.e.
Mock CSF	25	145±12	(48-316)	0.9±1.5
Substance P (10^{-7}M)	27	131±9	(34-217)	14.5±2.0*
(D-Pro^2, D-Trp^{7,9})-SP (6.6×10^{-6}M)	13	155±14	(92-271)	6.3±2.2 n.s.
Substance P + (D-Pro^2,D-Trp^{7,9})-SP	13	170±17	(72-286)	1.5±2.1**

n = number of vessel segments examined
*P < 0.001 compared to mock CSF (Analysis of variance)
**P < 0.001 compared to substance P (10^{-7} M) (Analysis of variance)
n.s. Not signifantly different from mock CSF (Analysis of variance)

Perivascular microapplication of SP around individual pial arterioles in situ effected dose-dependent increases in vascular calibre (mean response 14.5±2.0 % with SP 10^{-7}M). The concomitant perivascular administration of (D-Pro^2, D-Trp^{7,9})-SP, which alone did not alter the arteriolar calibre, attenuated significantly the cerebrovascular response to SP (Table 1).

On the basis of the agonist-antagonist relation found, these observations point to the possibility of a specific SP receptor site in cerebral arteries and arterioles which mediate their dilatation.

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INDOMETHACIN INHIBITS THE INCREASE IN OUTFLOW OF AQUEOUS HUMOUR FROM THE RABBIT EYE INDUCED BY ADRENALINE OR CYCLIC AMP

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Adrenaline lowers intraocular pressure (IOP) in man, monkey and rabbit. This effect is due partly to an increase in the drainage (outflow) of aqueous humour from the anterior chamber. This action of adrenaline is mediated by β-adrenoceptors and is associated with an increase in cyclic AMP level in the aqueous humour. Cyclic AMP injected into the anterior chamber mimics the effect of adrenaline on outflow of aqueous humour.

In the rabbit, the fall in IOP caused by adrenaline is antagonised by topical pretreatment with indomethacin (Bhattacherjee & Hammond, 1977). The present work studied the effect of indomethacin on the increases in outflow of aqueous humour induced by adrenaline or cyclic AMP.

Male Dutch rabbits (1.6-2.2 kg) were anaesthetised with urethane. A needle (26-gauge) was inserted into the anterior chamber of each eye and connected to a pressure transducer to permit continuous measurement of IOP. The needle was also connected to a reservoir containing an aqueous humour substitute (AHS) of the following composition (mM): NaCl, 110; KCl, 3; $CaCl_2$, 1.4; $MgCl_2$, 0.5; $NaHCO_3$, 30; K_2HPO_4, 0.9; glucose 6; ascorbic acid, 1.0, at pH 7.4, after brief aeration with O_2-CO_2 (95:5, by volume).

The facility of outflow (C) of aqueous humour from the anterior chamber was estimated by measuring the flow of AHS from the reservoir into the eye when the reservoir was raised to exert a pressure of 4 mm Hg greater than the resting IOP. The AHS was allowed to flow into the eye for a period of 4 min in every 20-30 min.

Shortly after cannulation, the eyes were pretreated topically with either control solution (50 µl of Na_2CO_3 solution, 0.2 mg.ml^{-1}) or indomethacin (0.125 mg in Na_2CO_3 solution). Five determinations of C were carried out in both eyes. Both eyes usually showed a slow increase in C value with time, but indomethacin alone had no effect on outflow. Adrenaline (0.6 mg, as "Eppy" eyedrops, Smith & Nephew Pharmaceuticals) was then applied topically to both eyes and C was measured another six times. Adrenaline produced a significant ($P<0.001$) increase in C from 0.33± 0.03 to 0.60±0.05 µl.min^{-1}.mm Hg^{-1} (mean ± s.e. mean, n=6) within 50 min in the control eye. In the eye pretreated with indomethacin, the gradual increase in C seen during the pre-adrenaline determinations continued unaltered after adrenaline administration.

The experiment was repeated, substituting, for topical adrenaline, an intraocular injection of dibutyryl cyclic AMP (5 µl of 10 mM). The control eye now showed an increase in C from 0.43±0.08 to 0.87±0.10 µl.min^{-1}.mm Hg^{-1} (n=6) within 15 min following dibutyryl cyclic AMP injection. In the eye pretreated with indomethacin, dibutyryl cyclic AMP produced no significant change in outflow.

Indomethacin, therefore, antagonised the increase in outflow of aqueous humour induced in the rabbit eye by either adrenaline or dibutyryl cyclic AMP. Experiments are continuing to investigate the possible involvement of prostaglandins in these phenomena.

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INFLUENCE OF SELENIUM ON THE CARDIOVASCULAR EFFECTS OF ARACHIDONIC ACID IN SPONTANEOUSLY HYPERTENSIVE RATS

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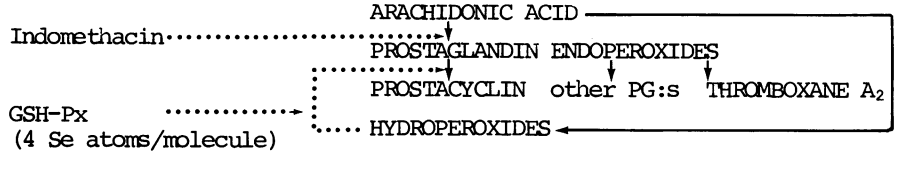
Selenium (Se) functions as a part of glutathione peroxidase (GSH-Px) in the avoidance of lipid peroxides (Flohé et al, 1973). Lipid peroxides inhibit the synthesis of prostacyclin, the main vasodilatatory and antiaggregatory metabolite of arachidonic acid (AA) in the vascular tissue (Gryglewski and Moncada, 1979). Recently a concomitant stimulation of GSH-Px activity and prostaglandin (PG) synthesis by dietary Se has been reported in the rat erythrocytes and platelets (Doni et al, 1981). The aim of the present study was to investigate the influence of Se on the cardiovascular effects of AA in spontaneously hypertensive rats (SHR).

Female SHR, 200 g, 20 weeks of age, were administered for a period of 27-34 days 1 mg/l Na_2SeO_3 dissolved in tap water, corresponding to a Se concentration of 0.3 ppm. Age-matched control rats, 180-210 g, received tap water, and both groups were fed standard rat pellets. Arterial and venous catheters were inserted into femoral vessels under chloral hydrate anaesthesia (300 mg/kg i.p.). The catheters were exteriosed at the nape of the neck and sealed until use. Mean arterial blood pressure and heart rate were recorded while the rats were awake and unrestrained in a plastic box.

The intravenous administration of AA at the doses of 500-2000 µg/rat dose-dependently decreased the blood pressure and induced a biphasic change in the heart rate with an initial brief bradycardia followed by a transient tachycardic effect. The decrease in the heart rate become apparent 30-60 s after each AA injection, while the maximum hypotensive and tachycardic responses were achieved 60-120 s after each injection. The AA-induced cardiovascular changes were due to its metabolites rather than AA itself, since the PG synthesis inhibitor, indomethacin (Flower, 1974) attenuated all the recorded effects of AA, when it was administered i.v. at a dose of 1 mg/kg 5 min before each AA injection. The hypotensive effect of AA was greater in magnitude in the Se-treated than in the control rats and the dose-response curve for AA was shifted to the left. The AA-induced bradycardic effect was also slightly more pronounced in the Se-treated group. However, Se had no significant effects of its own on the resting blood pressure or heart rate level. Since Se as a part of the enzyme GSH-Px is likely to reduce the level of fatty acid hydroperoxides, the potentiation of the AA-induced hypotension by Se-treatment might be due to an enhanced vascular formation of prostacyclin from AA (see Figure 1).

Figure 1. Interference of GSH-Px with the metabolism of arachidonic acid



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