THE EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE AND p-CHLOROPHENYLALANINE ON THE EX VIVO RELEASE OF THYROTROPHIN RELEASING HORMONE

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In the ventral spinal cord and the nucleus accumbens of rat brain the serotoninergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) causes a reduction in levels of thyrotrophin releasing hormone (TRH) that has a positive correlation with the depletion of both 5HT and its metabolite 5HIAA. However, p-chlorophenylalanine (PCPA) which depletes 5HT by inhibiting tryptophan hydroxylase, causes a decrease in brain TRH levels only in the nucleus accumbens (Lighton et al, 1984). This may suggest that 5HT and TRH whilst co-existing in the ventral cord (Gilbert et al, 1982) has a different relationship in the nucleus accumbens. We have now studied the effects of 5,7-DHT and PCPA on the ex vivo release of TRH in regions of rat brain and spinal cord.

Male Wistar rats were given 5,7-DHT (200 µg i.c.v.) or PCPA (2x250 mg/kg i.p.). 14 days after 5,7-DHT and 24 h after the second injection of PCPA, rats were killed and the nucleus accumbens, septum, hypothalamus, dorsal and ventral lumbar spinal cord dissected out (Lighton et al, 1984), sliced and incubated as previously described (Bennett et al, 1983). After 5 min of a 20 min incubation period K^+ was added to a concentration of 56 mM. TRH was assayed in the supernatant and the incubated tissue by radioimmunoassay.

Initial experiments showed that K^+ (56 mM) stimulated basal TRH release from slices of the nucleus accumbens (+57%), septum (+129%), hypothalamus (+63%), ventral (+49%) and dorsal (+59%) spinal cord. 14 days following 5,7-DHT, K^+ stimulated TRH release from tissue slices was significantly reduced in the nucleus accumbens (-42%) and the ventral spinal cord (-67%) compared to vehicle injected controls. Similar percentage reductions were seen in the levels of TRH measured in the incubated tissue.

Table. Effect of PCPA on K^+ stimulated ex vivo TRH release and post incubation tissue levels. (TRH expressed as pg/mg protein Mean±s.e. (n=11).)

		Nuc. Accumbens	Septum	Ventral Cord	Dorsal Cord
TRH Release:	Control	59± 8	188±39	91± 8	104±15
	PCPA	50± 9	149±21	55± 7*	153±21
TRH Level:	Control	145±18	534±83	458±55	271±30
	PCPA	74±10*	416±66	428±38	303±33

*p<.01 Student's t-test.

Following PCPA, no change in TRH release was seen in the nucleus accumbens although the level in the incubated tissue was reduced (-49%). In contrast, the ventral spinal cord showed a reduction in TRH release (-40%) but not in tissue level. In the hypothalamus, dorsal spinal cord and septum no significant changes in TRH release or levels were observed, though both values tended to decrease in the septum. We have shown previously (Lighton et al, 1984) that similar PCPA treatment does not alter noradrenaline levels in the hippocampus, but PCPA-induced changes in catecholamines in the accumbens and cord cannot be excluded in the present study.

These results thus provide confirmatory evidence that TRH co-exists with 5HT in ventral spinal cord neurones and suggest the existence of a more complex relationship in the accumbens where TRH production but not release may be regulated by 5HT.

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IN VIVO MEASUREMENTS OF DOPAC, 5HIAA AND 5-HT IN SPECIFIC BRAIN REGIONS BY INTRACEREBRAL DIALYSIS

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The recently developed technique of brain dialysis combined with HPLC with electrochemical detection (Zetterström et al, 1983) provides a sensitive in vivo method for monitoring endogenous extracellular amine and metabolite levels in the brain. The present study compares the levels of 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxytryptamine (5HT) and 5-hydroxyindoleacetic acid (5HIAA) in rat striatum and frontal cortex.

Male Wistar rats (260-300 g) were anaesthetized with chloral hydrate (600 mg/kg i.p.) for measurements made under anaesthesia and with halothane for implantation of dialysis loops prior to measurements made in the unanaesthetized rat. Dialysis loops, consisting of a folded dialysis tube (length 1.00 mm, diameter 0.25 mm) were implanted stereotaxically into the striatum and frontal cortex and perfused with physiological saline at a rate of 1 or 2 µl/min. Samples were collected at 25 minute intervals, and the perfusates assayed for DOPAC, 5HT and 5HIAA by HPLC with electrochemical detection (carbon paste working electrode at 0.65 V). Separation was performed on a Spherisorb 5ODS reverse phase column using 0.1 m acetate-citrate buffer pH 4.75 containing 10% methanol. Recovery in vitro of DOPAC, 5HIAA and 5HT by the dialysis loops using a flow rate of 2 µl/min was 6.00%, 7.30% and 6.00% respectively and at 1 µl/min, 8.00%, 7.75% and 8.00%.

Table 1 shows the different levels of DOPAC, 5HT and 5HIAA in the striatum and frontal cortex in the anaesthetized rat. The extracellular concentrations of DOPAC, 5HIAA and 5HT in the striatum were estimated to be 3.46×10^{-6}M, 1.02×10^{-6}M and 4.46×10^{-8}M respectively, while in the frontal cortex they were 5.04×10^{-8}M, 4.34×10^{-7}M and 6.18×10^{-8}M.

Table 1. DOPAC, 5HIAA and 5HT in perfusates from striatum and frontal cortex of anaesthetized rats. (Values (pmols/50 µl) corrected for recovery.)

COMPOUND	STRIATUM	FRONTAL CORTEX
DOPAC	170.96±12.43 (4)	2.52±0.36 (6)
5HIAA	50.94± 1.44 (4)	11.57±0.036 (6)
5HT	2.23± 0.19 (4)	3.09±0.15 (6)

In the unanaesthetized rat the basal levels, in particular that of 5HT, were higher than in the anaesthetized animals. This emphasises the difference in results that may be obtained with anaesthetized and unanaesthetized rats and indicates that anaesthetics may modify amine release.

The results demonstrate the application of the brain dialysis technique, in combination with HPLC and electrochemical detection, to obtain a direct comparison of in vivo extracellular levels of DOPAC, 5HIAA and 5HT, in different brain regions in freely moving and anaesthetized animals.

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DIURNAL VARIATION IN BEHAVIOURAL RESPONSES TO 5-HT RECEPTOR STIMULATION

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Levels of 5HT in rodent brain show a circadian variation (Hillier and Redfern, 1977), but to our knowledge no study of postsynaptic activity over 24h has been carried out. 5 methoxy N,N-dimethyltryptamine (5MDMT), which acts as a direct agonist at central 5HT receptors and which induces a number of behaviours used as indices of central 5HT activity, was used in this study to investigate possible variation in behavioural responses following stimulation of central 5HT receptors at different times of day.

Head twitch and the 5HT syndrome were induced by i.p. injection of 5MDMT in male CFLP mice (28-38g) kept in a controlled environment under a 12h light - 12h dark cycle. The number of head twitches was counted between 1 and 4 minutes after injection, and the intensity of the 5HT syndrome was rated immediately thereafter. When different groups of 8 mice were injected with 10mg/kg 5MDMT at 1.5 hourly intervals throughout the 24h cycle, a clear diurnal variation in head twitch response was evident, with peak activity occurring midway through the light period (peak value 15.1±2.0, trough value 4.5±1.1; ANOVA for 17 groups: $F = 6.4$ 16/118 DF, $p<0.01$). In contrast there was no variation in the 5HT syndrome, or in any individual component of it, over 24h. In order to study this phenomenon further, dose- and time-response curves were obtained at the two times corresponding to the peak and trough of the 24h rhythm in head twitch activity. The dose related head twitch response (Table 1) shows a significant parallel shift to the right when measured mid-dark as compared to mid-light.

Table 1. Head twitch and 5HT Syndrome Following 5MDMT

	Dose mg/Kg i.p.	2	4	8	16	32	64
mid-light	Head Twitch	2.9±0.7	7.0±1.0	10.1±1.0	13.3±1.2	18.0±1.4	19.2±2.8
	Syndrome	3(1-4)	6(5-7)	8.5(7-9)	10(8-10)	10(-)	10(9-10)
mid-dark	Head Twitch	2.0±0.4	4.0±0.9	5.3±0.4	9.1±0.9	13.4±1.1	13.4±2.5
	Syndrome	2(1-5)	5(4-8)	8.5(6-10)	9.5(7-10)	10(9-10)	10(7-10)

Head Twitch: mean±sem; Syndrome: median (range); n = 8.

Dose response curves to the 5HT syndrome at the two time points were identical. This observation made it unlikely that pharmacokinetic differences accounted for the 24h rhythm of head twitch, but to confirm this the time course of the response was assessed by measuring the number of head twitches and the intensity of the 5HT syndrome for alternate minutes for 21 minutes following injection of 5MDMT, 8mg/kg. The time courses were identical at the mid-light and mid-dark points. In view of this, the most likely explanation of these findings is that the diurnal variation in head twitch response reflects a diurnal rhythm in central 5HT receptor function. Twenty-four hour variation in receptor number has been reported for other neurotransmitter systems in rodent brain (Kafka et al., 1981) ; further work will be required to ascertain whether the same phenomenon accounts for our results. It also remains to be demonstrated whether the clear separation which exists between these two behavioural manifestations in terms of susceptibility to diurnal fluctuation indicates that two distinct 5HT pathways are involved, or whether diurnal oscillation is introduced into the head twitch response at some point distant from the initiating 5HT receptors.

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THE ANTIDEPRESSANT DOTHIEPIN INDUCES DOWN-REGULATION OF CORTICAL 5-HT_2 RECEPTORS AFTER SUBCHRONIC ORAL TREATMENT IN THE RAT

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Dothiepin down-regulates both β-adrenoceptors (Buckett & Thomas, 1982) and the noradrenaline-stimulated adenylate cyclase (Buckett & Diggory, 1982) after subchronic dosing in rats for up to 21 days. Since down-regulation of serotonin receptors in rat cortex is also a feature of antidepressant drug action (Peroutka & Snyder, 1980) it was of relevance to examine dothiepin for effects on both subtypes of serotonin receptor after subchronic treatment.

Male Sprague-Dawley rats (200 ± 25g wt) were dosed orally twice daily for 24 days with dothiepin HCl (30 $mgKg^{-1}$) or 0.9% saline (control). 24h after the last dose, the frontal cortex was rapidly dissected out, homogenized (4°C) in 40 vol 50mM Tris-HCl (pH 7.7, 25°C), centrifuged (40,000g, 15 min). This procedure was repeated, followed by pre-incubation (37°C, 15 min) and centrifugation. Final pellet resuspension was in 100 vol Tris buffer contg 4mM $CaCl_2$. 10μM pargyline and 0.1% ascorbate. Tissue aliquots equivalent to 18mg original wet wt were incubated (15 min) with 1-20nM ^{3}H-5HT and 0.25-5nM ^{3}H-spiperone (in triplicate with 10μM 5HT and 1μM d-LSD to define specific binding) and rapidly filtered (GF/B), washed with buffer (5 x 4 ml) and filters counted. Binding parameters were estimated using a least-squares linear regression programme and values for K_D and B_{max} generated from individual analyses were pooled and compared (t-test) to obtain means ±s.e. A comparison of Scatchard and Woolf plots was also an objective of this study, since it has been suggested that the latter yields improvements in data analysis (Keightley & Cressie, 1980).

Scatchard analysis of ^{3}H-spiperone binding revealed a significant reduction ($P<0.01$) in $5HT_2$ receptors in rats treated with dothiepin (B_{max} 9.69 ± 0.74 (dothiepin; n = 16); 14.24 ± 0.90 (control; n = 8) $fmol\ mg^{-1}$) without affinity changes (K_D 1.54 ± 0.17; 1.63 ± 0.14 nM). Woolf plots also indicated significant ($P<0.01$) reduction (B_{max} 8.51 ± 1.00 (dothiepin; n = 16); 14.01 ± 1.12 (control; n = 8) $fmol\ mg^{-1}$) with no change in affinity (K_D 1.58 ± 0.15; 1.61 ± 0.20 nM) but better mean correlation coefficients (r = 0.98 vs 0.91). ^{3}H-5HT binding results did not show changes in S_1 receptors after drug treatment when analysed by the methods of Scatchard (B_{max} 14.35 ± 1.22, K_D 8.0 ± 1.35 (dothiepin; n = 12); B_{max} 16.93 ± 1.46, K_D 10.8 ± 2.24 (control; n = 5) or Woolf (B_{max} 14.86 ± 1.43, K_D 8.55 ± 1.46 (dothiepin; n = 12); B_{max} 15.46 ± 2.27, K_D 9.07 ± 1.99 (control; n = 5), the latter yielding better correlation (r = 0.96 vs 0.88).

These results extend to dothiepin the finding that antidepressants down-regulate rat cortical $5HT_2$ receptors (Peroutka & Snyder, 1980). In contrast, the dothiepin pretreatment does not affect ^{3}H-5HT binding in common with some, but not all, antidepressant drugs. Functional effects mediated via 5HT receptors include syndromes induced by the agonist 5-methoxy-dimethyltryptamine and the precursor 5-hydroxytryptophan, both of which are attenuated by dothiepin pretreatment (Buckett & Luscombe, 1983). Data analysis appears better achieved using the Woolf plot, thus confirming for a neurotransmitter system the improvements reported for hormone receptor assays using oestradiol-17β (Keightley & Cressie, 1980).

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EFFECT OF CINANSERIN ON THE RELEASE OF GLUTAMATE FROM RAT CEREBELLAR SLICES

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Previous work has shown that the amino acid glutamate is released from cerebellar slices in a Ca^{++} dependant fashion in response to a depolarizing pulse of potassium (Davies & Leighton, 1984). Studies performed using synaptosomal preparations from the cerebellar molecular layer strongly suggest that the glutamate released in response to depolarizing stimuli originates from the parallel fibre terminals (Sandoval & Cotman, 1978). Autoradiographic methods have demonstrated serotonergic projections to the cerebellar cortex that appear to synapse with the granule cells (Chan-Palay, 1977), and exogenous 5-HT has been shown to inhibit the release of glutamate from cerebellar slices in a concentration dependant manner (Davies & Leighton, 1984).

This study investigated the effect of the 5-HT antagonist cinanserin on the K^+-evoked release of glutamate in both the presence and the absence of an inhibitory concentration of 5-HT.

Cerebellar slices (250 µm) were superfused with oxygenated artificial CSF. Where appropriate,5-HT and/or varying concentrations of cinanserin were included in the perfusing solution for the duration of the experiment. Release of glutamate was evoked by a 4 minute pulse of CSF containing 35 mM K^+. The analysis of glutamate released was performed using an HPLC method with fluorescence detection.

Cinanserin was found to have a biphasic effect on the inhibition of glutamate release produced by 5-HT. At a concentration of 10^{-4} M, cinanserin potentiated the effect of 5-HT, as the concentration of cinanserin was decreased (1×10^{-6} - 1×10^{-10} M) an antagonism of the inhibitory effect of 5-HT was observed (Table I).

In the absence of 5-HT cinanserin at a concentration of 10^{-4} M inhibited the K^+-evoked release of glutamate. When the concentration of cinanserin was reduced to 10^{-8} M the K^+-evoked release of glutamate was markedly potentiated (Table I). It is possible that this potentiation resulted from an antagonism by cinanserin of a serotonergic inhibitory influence affecting the release of glutamate from the cerebellar slice preparation.

Table I Effect of cinanserin on K^+-evoked release of glutamate

K^+ (mM)	n	5-HT(M)	Cinanserin(M)	Glutamate release*	Glutamate release as % of control
35	10	-	-	167.64 ± 26.70	100
35	8	-	10^{-4}	49.86 ± 23.06[a]	29.7
35	8	-	10^{-8}	410.24 ± 55.75[b]	244.7
35	6	10^{-12}	-	60.27 ± 14.26	100
35	7	10^{-12}	10^{-4}	20.95 ± 4.63[a]	34.8
35	7	10^{-12}	10^{-6}	112.36 ± 30.22	186.4
35	7	10^{-12}	10^{-8}	258.46 ± 21.30[b]	428.9
35	6	10^{-12}	10^{-10}	233.29 ± 32.74[b]	387.1

* The evoked release of glutamate is expressed as the total increase in release (pmoles/mg of tissue) above the pre-stimulation level. (a) $p<0.01$ (b) $p<0.001$ Students t-test.

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REDUCTION OF NEUROLEPTIC-WITHDRAWAL INDUCED SUPERSENSITIVITY FOLLOWING SIMULTANEOUS ADMINISTRATION OF 5-HYDROXYTRYPTOPHAN

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Although much work has been performed to investigate the action of neuroleptics upon central dopamine turnover and receptor sensitivity to dopamine, little is as yet known about the action of neuroleptics upon the other neurotransmitter systems which influence dopaminergic neurones. The ability of neuroleptics to enhance the synthesis and utilization of 5-hydroxytryptamine (5HT)(Rastogi et.al., 1981) is of particular interest as other investigators have indicated that 5HT may modulate the release of striatal acetylcholine (Vizi et al., 1981) and dopamine (Ennis et al., 1981).

The present study was undertaken to investigate the effect of 5-hydroxytryptophan (5HTP) before, during and after exposure to the neuroleptic α-flupenthixol(αFPT). Male TO mice (WNSM strain) were housed in temperature controlled, sound-proofed cabinets maintained on a 24 hour light-dark cycle. All experimental work was performed between 2nd and 6th hours of darkness and, where appropriate, neuroleptic-induced supersensitivity was produced with α-flupenthixol in the drinking water for 10 days (20 mg/l, equivalent to approximately 2,8 mg/kg/day). Climbing was assessed using a 20 cm high cylindrical frame made of stainless steel mesh. Apomorphine was injected sc 10 min prior to scoring and 5HTP was given in the drinking water (50 mg/l equivalent to a daily intake of approximately 7 mg/kg). The mice were observed at 6 sec intervals for 20 min and climbing, rearing, grooming or gnawing behaviours were noted. Spontaneous climbing activity was assessed for 30 min using the same apparatus placed behind coloured film to reduce light intensity. No animal was exposed to the climbing apparatus more than once.

5HTP (50 mg/l) was found to reduce apomorphine-induced climbing activity in a time-dependent manner but had no effect on spontaneous climbing. The action of 5HTP upon αFPT-induced supersensitivity was assessed during the ten day treatment period by measuring spontaneous climbing. Although there was no significant difference in water intake between mice on αFPT and those on αFPT + 5HTP, spontaneous climbing was significantly higher in the presence of 5HTP. Also, following the withdrawal of αFPT the level of spontaneous climbing was found to be higher in those mice receiving αFPT alone. Although both groups of mice demonstrated a transient supersensitivity to apomorphine (0,25 mg/kg sc) following αFPT withdrawal, the magnitude of the response obtained in the mice which received αFPT alone was approximately four times that observed in mice which received αFPT with 5HTP. When 5HTP was given in the drinking water only after withdrawal of αFPT, apomorphine-induced climbing (0,25 mg/kg sc) was significantly reduced by the 3rd day ($p<0.05$).

It therefore appears that 5HTP is able to reduce both apomorphine-induced climbing activity in normal mice and the level of supersensitivity to apomorphine following withdrawal of αFPT. When 5 HTP is given together with αFPT the mice adapt to the presence of the neuroleptic more rapidly and the level of supersensitivity observed following withdrawal is reduced.

Although the results may be explained by 5HTP acting upon the post synaptic DA-receptor to reduce its sensitivity to agonists and so facilitate a compensatory elevation in dopamine release, a direct action upon the dopaminergic neurone cannot be excluded.

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ANTAGONISM OF INHIBITORY BUT NOT EXCITATORY EFFECTS OF 5-HYDROXYTRYPTAMINE IN THE RAT HIPPOCAMPAL SLICE

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The inhibitory effects of 5-hydroxytryptamine (5-HT) on neurones in most regions of the mammalian CNS have been found to be poorly antagonised by 5-HT receptor blocking agents (Peroutka et al, 1981; Segal, 1981). The present study examined the effects of known concentrations of such agents on the excitatory and inhibitory responses of the rat isolated hippocampal slice to 5-HT.

Transverse hippocampal slices (350μ thick) from male rats were completely submerged in physiological solutions at 34°C. Population spikes were recorded in the pyramidal cell body layer of the CA1 region by stimulation of the stratum radiatum. A population spike which was 50% of the maximum amplitude was chosen to study the effects of 5-HT.

Perfusion of 5-HT onto the hippocampal slice caused an initial small transient increase in the population spike, followed by a much larger decrease which was usually sustained for the duration of 5-HT perfusion. Upon returning to the control saline, the population spike increased above the control amplitude before slowly decreasing to the control level. In approximately 25% of cases a secondary population spike appeared after washout of the 5-HT. The temperature of the bathing medium was found to alter the decrease in the population spike brought about by 5-HT. In five experiments carried out at 28°C, 10^{-5}M 5-HT caused only a 6 ± 3% decrease in the population spike, compared with a 46 ± 7% decrease at 34°C. The 5-HT mediated initial increase and washout increase were not affected.

The depression of the population spike produced by 10^{-5}M 5-HT was completely antagonised by pretreatment of the preparation with 10^{-5}M cyproheptadine for 1 hour. The block was only partly developed 30 minutes after commencing perfusion with cyproheptadine. The increases in the amplitude of the population spike were not affected by this concentration of cyproheptadine. Similar selective antagonistic effects were observed with the perfusion of 7.5×10^{-6}M ketanserin for 1 hour prior to the application of 10^{-5}M 5-HT.

Pretreatment with 10^{-5}M imipramine for one hour caused a 43 ± 9% antagonism of the decrease of the population spike mediated by 10^{-5}M 5-HT (n = 6) and a 57 ± 6% antagonism of the population spike decrease caused by 5×10^{-6}M 5-HT (n = 3). Imipramine (10^{-5}M) had no effect on the increases produced by 5-HT.

That the inhibitory and excitatory effects of 5-HT showed a differential sensitivity to temperature and 5-HT receptor antagonists is indicative that these effects are mediated through different pharmacological receptors. These antagonists and imipramine have a much higher affinity for the 5-HT_2 receptor than for the 5-HT_1 receptor (Peroutka et al, 1981; Leysen et al, 1982). This suggests that the inhibitory responses may be mediated through 5-HT_2 receptors. This effect of imipramine may be important in chronic studies as we have previously reported that chronic imipramine treatment produced a selective reduction in the inhibitory effect of 5-HT in the slice (Anwyl & Rowan, 1983).

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FEASABILITY STUDY OF A SEROTONIN RADIOIMMUNOASSAY KIT ASSEMBLED FROM COMMERCIALLY AVAILABLE MATERIALS

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Serotonin is a neurotransmitter substance of major clinical importance (Osborne, 1982). Current methods of analysis is based on enzymatic-isotopic or fluorometric procedures for large numbers of samples, both of which are time consuming and involve the use of carcinomic organic solvents. The alternative method, which is very sensitive, involves the use of HPLC and electrochemical detection. Unfortunately in this method only a single sample can be analysed at once, which is not ideal when very many samples need to be routinely analysed. Radioimmunassay for serotonin would be ideally advantageous because it is both sensitive and specific, and capable of measuring many samples simultaneously.

Antibodies to serotonin were first raised in 1967 by Ranadive and Sehon (1967), coupling serotonin to BSA with formaldehyde, but their avidity was too poor for radioimmunoassay. Greater avidity was achieved using a diazo linkage on the sixth position of the indole and radioimmunoassays based upon it, (Kellum and Jaffe, 1976, Peskar and Spector, 1973). However, such assays were not widely used because, although having a sensitivity of 100 pg, their titre was never more than 1:50. Recently a novel approach with a sensitivity of 50 pg was published (Delaage and Puizillout, 1981) in which all the serotonin in the assay was coupled to BSA, which allowed the use of a high specific activity iodine radiolabelled tracer. However, although successful, this method proves too labourious for routine use.

The aim of our tests was to judge the performance of a radioimmunoassay kit assembled from commercially available materials so that, in the event of it proving a workable stop-gap, it could easily be reproduced elsewhere. An immediate limitation was that the only available antiserum (Immuno nuclear corporation), was that suited to histochemistry, raised to formaldehyde conjugation to BSA (Steinbusch et al., 1978). This was compared to one raised by the same method in our own laboratory, using tritiated serotonin (13Ci/mmol), and the performance of the kit prepared compared to measurements of the same samples on HPLC.

A titre of both antibodies showed a total binding of counts of only 6%, thus neither possessed a titre in the accepted sense, but could be used at a working dilution of between 1:50 and 1:100, being the lowest dilution yielding highest binding. Dose displacement by 'cold' serotonin standards was found over the range 10^{-10} -10^{-6} g/tube. Due to the low total binding, although both assays had a sensitivity of 100 pg, they also had a low precision, so would only be capable of gross quantative estimations. However, when compared with HPLC measuring the serotonin content of biological extracts, the assay was found not to be sensitive enough to detect the indolamine while HPLC had no difficulty in detecting and descriminating between several neurotransmitters.

In conclusion, it has to be reported that while a radioimmunoassay kit capable of generating a standard curve can be assembled from commercial materials, it it not sensitive enough to make measurements in tissue extracts, and only capable of the grossest estimations. This is a consequence of the low avidity of the available antiserum, which is unable to give a titre, resulting in sensitivity at the cost of poor precision.

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MICRO ANALYTICAL HPLC METHOD FOR THE DETERMINATION OF PYRIMETHAMINE AND ITS 3-N-OXIDE METABOLITE IN BIOLOGICAL FLUIDS

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Pyrimethamine/sulphonamide combination therapy is a first choice in areas of chloroquine resistant Plasmodium falciparum malaria. The pharmacokinetics of pyrimethamine are poorly understood due to the lack of selectivity of previous analytical methods. We have developed a micro HPLC method for the determination of pyrimethamine and its 3-N-oxide metabolite in mouse plasma and urine. This animal is a widely used experimental model for the study of malaria. The assay has been designed to quantitate selectively and sensitively both drug and metabolite in a sample of less than 50 µl.

To samples of plasma or urine (10-45 µl) contained in microcap tubes (1.5ml) were added internal standard, proguanil (0-200ng as a methanolic solution) and ammonia solution (500 µl). The mixture was extracted twice with ethyl acetate (800 µl) by vortex mixing. After separation, the combined organic phases were evaporated to dryness under N_2 (45°C) and reconstituted in methanol (20 µl). Chromatography was performed on a Partisil ODS 10 µm particle size reversed phase column. The mobile phase consisted of water, acetonitrile and methanol (55:35:10 v/v) containing octanesulphonic acid (0.013M) buffered to pH 3.5 and flowing at 1.8 mls/min. Retention times for pyrimethamine, pyrimethamine 3-N-oxide, and internal standard were 9, 6 and 13 minutes respectively. The assay was free from chromatographic interference both from endogenous material, and the antimalarial drugs chloroquine, amodiaquine, cycloguanil, primaquine, sulphadiazine and sulphadoxine. The minimum detectable level of pyrimethamine extracted from a 20 µl sample was 330 ng/ml and for pyrimethamine 3-N-oxide, 600ng/ml. The coefficients of variation (c.v.) of inter and intra assay variability were below 6% at 10.0 µg/ml in plasma, and below 8% at 10.0 µg/ml in urine. The corresponding c.v. values for the 3-N-oxide were below 6% at 5.0 µg/ml in plasma, and 12% at 2.0 µg/ml in urine. Recoveries of pyrimethamine its 3-N-oxide and internal standard were 91.2 ± 5.9%, 82.0 ± 5.1% and 79.0 ± 6.4% respectively. All calibration curves showed linearity (r = 0.99) for pyrimethamine and its 3-N-oxide in both plasma and urine.

The assay was applied to the analysis of plasma and urine samples derived from a pilot pharmacokinetic study conducted in mice dosed with pyrimethamine base (75mg/kg i.p.). A concentration of pyrimethamine of 12.3 µg/ml was attained 1 hour post dose, which declined monoexponentially, with a half life of 7.9 ± 5.9 hours. Pyrimethamine 3-N-oxide attained a peak plasma level of 2.0 µg/ml at 2 hours, falling below detectable levels by 26.5 hours. Urinary excretion of pyrimethamine over 96 hours was 41.8 ± 22.2 µg or 2.3% of the dose. Excretion of pyrimethamine 3-N-oxide was 7.0 ± 5.0 µg or 0.4% of the dose.

This selective and sensitive micro assay is well suited to the analysis of small volumes of plasma and urine, such as those obtained in the chemotherapy of experimental rodent malaria.

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EFFECT OF A PERFLUOROCARBON EMULSION, FLUOSOL-DA, ON HISTAMINE RELEASE FROM RAT PERITONEAL MAST CELLS IN VITRO

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Fluosol-DA 20% (Green Cross Corporation, Japan) is an emulsion of perfluorocarbons which has been used as a blood substitute in several species (Lowe, 1983). However, the immunological effects of Fluosol-DA have not been investigated in detail. In the present experiments, we have examined the effect of Fluosol-DA 20% on histamine release from rat peritoneal mast cells in vitro.

The perfluorocarbons of Fluosol-DA 20% are emulsified by mixing with 2.7% (w/v) of detergent, Pluoronic F-68, and 0.4% (w/v) yolk-phospholipids (Naito and Yokoyama, 1978). When 1 volume of Fluosol-DA 20% was extracted with 2 volumes of methanol and 2 volumes of petrol ether (40°C to 60°C fraction), the perfluorocarbons partitioned into the petrol phase and the phospholipids into the methanol phase: Pluoronic F-68 was present in both phases. The methanol phase was washed four times with an equal volume of petrol and the petrol phase with an equal volume of methanol. Solvent was removed from both extracts by evaporation under a stream of nitrogen and the solid residue resuspended in 2ml of Mast Cell Medium (MCM; Smith et al., 1979) by vortex-mixing.

Suspensions of peritoneal mast cells (1×10^5 ml^{-1} in MCM) were prepared from adult female Wistar rats as described in Smith et al. (1979). After incubation of the cells for 15 minutes at 23°C with different concentrations of the methanol- or petrol extracts of Fluosol-DA 20%, histamine release into the medium was determined using a fluorimetric assay (Sullivan et al., 1975). A progressive increase in histamine release occurred as the proportion of methanol-extract in the incubation was increased. When the concentration of methanol-soluble material in the incubation was approximately equivalent to that present in unfractionated Fluosol-DA 20%, 25% of the total histamine content of the cells was released. In contrast, the same concentration of petrol-extract caused 7.5% release. When mast cells were incubated with 100µg.ml^{-1} concanavalin A and 1×10^{-6}M lyso-phosphatidylserine, histamine release was 38.5%; basal release was 1.5%.

These results show that methanol-soluble components of Fluosol-DA 20% can cause degranulation of rat mast cells in vitro. The active agent could be Pluoronic F-68, or a constituent of the yolk-phospholipid stabiliser. We note that phospholipids extracted from several tissues contain up to 2% lyso-phospholipids (e.g., Rouser et al., 1970). The concentration of phospholipids in Fluosol-DA 20% is approximately 5mM: any lyso-phospholipid contamination to an extent greater than 0.2% implies that the lyso-phospholipid concentration in the bloodstream of perfused animals will be above the level of 10µM previously shown to cause mast cell lysis (Smith et al., 1979; Martin and Lagunoff, 1979). Whatever the mechanism by which histamine is released, it could contribute to the hypotension which eventually follows severe blood replacement with Fluosol-DA 20% (Lowe et al., 1982).

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THE EFFECTS OF SOME LOCAL ANAESTHETICS AND OTHER DRUGS ON CALCIUM-INDUCED LUMINESCENCE OF AEQUORIN

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The photoprotein aequorin,extracted from the jelly-fish Aequorea aequorea , (Shimomura,Johnson & Saiga,1962) is used as an indicator of the free calcium ions (Ashley & Ridgway,1970;Baker,Hodgkin & Ridgway,1971;Blinks,Prendergast & Allen, 1976;Kamaya,Ueda & Eyring,1977;Baker & Schapira,1980).

In the present study,the effects of some local anaesthetics and other drugs on the calcium-induced luminescence of aequorin was investigated in-vitro and the results were compared with those previously reported by Kamaya et al.(1977) and Baker & Schapira (1980) in the same preparation.

Purified aequorin (0.1 µL) was injected into a 500 µm diameter porous cellulose acetate capillary containing 0.5 M KCl, 20 mM phosphate (pH 7.2) and a variety of Ca-EGTA buffers. The trapped aequorin was superfused with the same buffer solutions which sometimes contained the anaesthetic and test solutions.Changes in the baseline aequorin luminescence induced by the drug action were recorded using a photomultiplier as described by Baker & Schapira (1980).

Superfusing the trapped aequorin with a mixture of Ca (4 mM) and EGTA (10 mM) (buffered ionized calcium concentration of about 100 nM),the local anaesthetic drug lignocaine (5.5 mM) increased the aequorin luminescence by 89± 6% (mean±s.e., n=6,$P < 0.001$).Tetracaine (2.5 mM) produced no significant change in the aequorin light output.High concentrations of tetracaine (25 mM) reduced the aequorin glow by 38± 3% (n=5, $P < 0.01$). Glycerol (0.435 mM) reduced the light output by 50± 5% whereas atracurium (10 µM) only slightly reduced the aequorin flash (17± 3%, P: N.S.).On the other hand,tetraethylammonium (2.4 mM),gamma-aminobutyric acid (0.1 mM) and caffeine (0.52 mM) all had no apparent effect on the light output of aequorin. The effect of the anaesthetic drugs and glycerol was concentration-dependent and the above concentrations were chosen due to their pronounced effects. On a log-log scale,the effect of the drugs used was to shift the line relating calcium concentration to aequorin consumption roughly in parallel manner,either to the left (excitation) or to the right (inhibition).

Analysis of the results showed that lignocaine and tetracaine produced opposite effects on aequorin luminescence,the former increased whereas the latter decreased the light output from aequorin.Unlike the results reported by Kamaya et al.(1977) who only observed the inhibitory effects of anaesthetics,the present results showed that anaesthetics can either excite or inhibit the aequorin flash depending on the concentration and the type of drug used.It is unlikely that anaesthetic drugs act directly on the calcium ions but it is possible that they can alter the sensitivity of aequorin reaction to ionized calcium as reported by Baker & Schapira (1980).

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THE EFFECT OF CIMETIDINE AND MEPYRAMINE ON HISTAMINE STIMULATED cAMP ACCUMULATION IN RABBIT CEREBRAL CORTICAL SLICES

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Histamine H_1- and H_2- receptors mediate histamine-stimulated cAMP accumulation in slices of guinea-pig hippocampus and cerebral cortex (Palacios et al, 1978; Hill et al, 1981). The H_1 actions, in contrast with the direct H_2 action, appears to be indirect and to require prior stimulation of the cyclase by a directly acting agonist, such as adenosine or histamine (H_2) itself. Kakiuchi & Rall (1968) have shown that histamine produces a large stimulation of cAMP accumulation in rabbit cerebral cortical slices and the present study was undertaken to characterize the nature of the receptors involved.

Experiments on rabbit (New Zealand White, 2.5 Kg) cerebral cortical slices were performed essentially as described previously (Hill et al, 1981) Histamine (0.1mM) elicited a 30 ± 5 fold stimulation of cAMP accumulation above basal levels, ED50 19 ± 6 µM. The selective H_2-receptor antagonist, cimetidine, caused a parallel shift of the dose response curve to histamine to higher agonist concentrations, consistent with competitive antagonism (K_B 1.5 ± 0.3 µM, Schild slope 1.1 ± 0.1). In contrast, in the presence of adenosine (0.1mM), the response to histamine was modified in a complex fashion by cimetidine, such that the response to histamine in low concentrations remained essentially unaltered while those to the amine in concentrations above 10µM appeared to be inhibited competitively (apparent K_B 1.1µM). The H_1-receptor antagonist, mepyramine (1 µM) inhibited the response to histamine in a similar complex fashion in the presence or absence of adenosine. In both cases the mepyramine-sensitive component was shifted to higher agonist concentrations by approximately one order of magnitude. In the presence of adenosine (0.1mM) the cimetidine-insensitive component of the response to histamine was further inhibited by 1µM mepyramine.

The specific H_2-receptor agonist impromidine produced a maximum response of only 31 ± 2% (ED50 0.05µM) of the response to 0.4mM histamine although studies with histamine and impromidine (1µM) in combination did not indicate that impromidine was acting as a partial agonist. Dimaprit, however, was without effect at concentrations up to 1mM, while 2-thiazolylethylamine (TEA), a selective H_1-agonist, produced only a weak response (ED50 > 1mM) consistent with its potency on H_2 receptors.

In the presence of a supramaximal concentration of impromidine (1µM), histamine and TEA further elevated the response to impromidine. In these conditions the relative potency of TEA (ED50 56µM) became comparable with the value expected for stimulation of H_1 receptors. Furthermore, in the presence of impromidine, mepyramine (1µM) appeared to competitively inhibit the responses to both histamine and TEA without affecting the response to impromidine alone. However the K_B values obtained (0.04 and 0.03µM respectively) were much larger than would be expected for antagonism of typical H_1-receptors.

These results suggest that there are two components in the response to histamine in rabbit cerebral cortical slices. The first component appears to have the characteristics of an H_2 receptor, while the second, mepyramine-sensitive, component requires prior stimulation of adenosine or histamine H_2 receptors to produce its effect.

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THE INDUCTION OF METHAEMOGLOBIN BY HYDROXYAMINOPROPIOPHENONE

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Several aminophenones have been shown to produce methaemoglobin when administered orally to bitches (Bright and Marrs, 1983). 4-aminopropiophenone (p-amino-propiophenone, PAPP) is not an effective methaemoglobin producer when added to blood in vitro (Tepperman and Bodansky, 1946). It has been reported by Graffe et al (1964) that the active metabolite of PAPP is the N-hydroxy derivative, 4-(N-hydroxyamino)propiophenone (HAPP) but the site at which the conversion takes place is obscure since PAPP is reported to be more effective in eviscerated than normal rats (Tepperman and Bodansky, 1946).

HAPP was added to beagle bitch blood in vitro at six different concentrations between 1.5 and 18 μmol l^{-1}. Over the succeeding 70 min, 300 μl aliquots of blood were removed, the methaemoglobin level in them being estimated using an IL 282 CO-oximeter. HAPP was an effective methaemoglobin producer: when added to give a final concentration in blood of 18 μmol l^{-1} a peak level of methaemoglobin of 35% was attained. Over the concentration range used the initial reaction rate was approximately proportional to the concentration of HAPP in the blood.

2 μmol kg^{-1} of PAPP or HAPP, dissolved in 1 ml dimethylsulphoxide were injected on separate occasions into the jugular veins of 4 beagle bitches. Blood samples were taken from the cephalic vein over the succeeding 240 min for methaemoglobin estimation on the IL CO-oximeter. Both materials were effective methaemoglobin producers PAPP producing a peak level of 14.8 ± 1.1%* while HAPP produced a peak level of 19.3 ± 1.2%*. Peak levels occurred somewhat later with PAPP (60.5 ± 2.6 min*) than with HAPP (49 ± 2.6 min*). With PAPP the time taken to reach half peak (HT) was 10.3 ± 1.6 min* while with HAPP it was 6.9 ± 0.7 min*. After peak levels had been attained the rate of decline was such that the % methaemoglobin had fallen to half peak levels at approximately the same time after dosing with both materials (168 ± 5 min for PAPP* and 161 ± 6 min for HAPP*).

The results in vitro show that unlike PAPP, HAPP is an effective methaemoglobin former. The fact that its activity in vivo is slightly more rapid than that of PAPP suggests that the former is the active methaemoglobin producer after administration of the latter.

* $\bar{x}$ ± SEM.

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EFFECT OF NABUMETONE ON RAT GASTRIC MUCOSAL 6-KETO-$PGF_{1\alpha}$ PRODUCTION AND THE RELATIONSHIP TO ITS LACK OF GASTRIC IRRITANCY

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Nabumetone (4-[6-methoxy-2-naphthyl]-butan-2-one), a novel compound, was reported to lack, unlike most non-steroidal anti-inflammatory drugs, gastric mucosal irritancy in the rat (Boyle et al, 1982). It was further suggested that the lack of gastric damage was due, in part, to the non-acidic nature of nabumetone coupled with weak cyclo-oxygenase inhibitory activity as demonstrated in a bovine seminal vesicle (BSV) preparation. We now report experiments with nabumetone on rat gastric mucosal cyclo-oxygenase activity ex vivo and in vitro using radio-immunoassay for 6-keto-$PGF_{1\alpha}$ (Melarange & Rashbrook, 1983). Indomethacin and the dual lipoxygenase/cyclo-oxygenase inhibitor, BW 755C were also tested.

Drugs were administered orally in 0.5% methyl cellulose to groups (n=4-6) of male Wistar rats (150-200g; fasted 18h) using the following dose ranges: indomethacin (1-5mg kg^{-1}); nabumetone (20-80mg kg^{-1}); or BW 755C (1-100mg kg^{-1}). After 2h the animals were killed and their stomachs removed. Mucosal sections were prepared following removal of the overlying muscle and then assessed for the presence of 6-keto-$PGF_{1\alpha}$, as described previously (Melarange & Rashbrook, 1983). In further studies nabumetone or indomethacin were tested in vitro. Results were analysed using Student's 't' test and expressed as percentage inhibition compared with the control value.

Indomethacin had a marked effect on 6-keto-$PGF_{1\alpha}$ production ex vivo, the maximum inhibition, from 4.7 ± 0.2ng to 1.0 ± 0.2ng/section (79%; $p<0.001$), occurring with a dose of 5mg kg^{-1}. In contrast, an inhibition of only 47% ($p<0.001$) could be demonstrated with nabumetone at 40mg kg^{-1}. Moreover, a dose of 80mg kg^{-1} produced no further inhibition. BW 755C produced an inhibition of 66% ($p<0.01$) at 100mg kg^{-1}. Thus nabumetone at anti-inflammatory doses (Boyle et al, 1982) was significantly less active ($p=0.02$) in inhibiting gastric mucosal cyclo-oxygenase compared with indomethacin.

Results in vitro showed that nabumetone at a concentration of 200μM, which was the reported IC_{50} value in the BSV preparation (Boyle et al, 1982), inhibited 6-keto-$PGF_{1\alpha}$ production by 50% ($p<0.001$) whereas indomethacin (2.8μM) caused 80% inhibition ($p<0.01$) in the present experiments.

This study shows that nabumetone ex vivo has limited effects on cyclo-oxygenase activity compared with indomethacin. It is possible that nabumetone, by failing to reduce cyclo-oxygenase activity by greater than 50%, does not impair prostacyclin-mediated cytoprotection. The results obtained with BW 755C show that, as with gastric corpus tissue (Melarange & Rashbrook, 1983), this compound is an inhibitor of mucosal cyclo-oxygenase ex vivo which contrasts with previous results (Whittle et al, 1980).

In conclusion, the above results suggest that nabumetone, a new non-steroidal anti-inflammatory agent, may lack gastric mucosal irritancy partly because it is a weak inhibitor of cyclo-oxygenase. Additionally, nabumetone may lack the topical irritancy that might be associated with indomethacin or aspirin.

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MEASURING THE DISSOCIATION CONSTANT OF A PARTIAL AGONIST

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The equilibrium dissociation constant, K, of a partial agonist can be estimated very simply by comparing its log concentration response curve with that of a full agonist acting on the same receptors using the assumptions of Stephenson's Receptor Model (1956) in the following way: 1) The maximum response produced by the partial agonist (D2) is measured. 2) The concentration of full agonist (A) producing a response equal to D2 is estimated and then, 3) the response (R) produced by half this concentration of full agonist. 4) The concentration of partial (P) producing a response equal to R is then obtained. It is assumed that as (A/2) of full agonist produces the same response as P, they must have the same Stimulus, i.e. efficacy X occupancy. Although the occupancy produced by (A/2) is not known it is assumed to be such a small proportion of the receptors that the occupancy of (A/2) will be half that of (A). But (A) produces a response equal to the maximum produced by the partial agonist and the maximum of a partial agonist is assumed to correspond to 100% receptor occupancy. Therefore P corresponds to 50% receptor occupancy and is therefore equal to the dissociation constant of the partial agonist.

The dissociation constant of a partial agonist can therefore be estimated quickly 'by eye'. Alternatively computer programs are available that can fit logistic curves to the experimental points (De Lean, et al 1978; Barlow, 1983). If D = the maximum response, C + the ED50 and B = the 'slope factor' then

$$Y = D.X^B/(X^B + C^B) \qquad (1)$$

Where Y = response and X = concentration. If B1,C1,D1 refer to the full agonist and B2,C2,D2 refer to the partial agonist then K can be calculated as

$$A^{B1} = C1^{B1}.D2/(D1-D2) \qquad (2)$$

$$R = D1.(A/2)^{B1}/((A/2)^{B1} + C1^{B1} \qquad (3)$$

$$K^{B2} = P^{B2} = C2^{B2}.R/(D2-R) \qquad (4)$$

As Stephenson's Model makes no assumptions about the shape of the curve for a full agonist, B1 can take any value. On the other hand B2 is dependent on B1 and D2 as

$$[A] = \text{constant}.\ [P]/([P] + K) \qquad (5)$$

where [A] and [P] are the concentrations of full and partial agonist producing equal responses. To determine what combinations of B1,B2 and D2 would be consistent with the Stephenson Model an iterative computer program was used. This minimised the sum of squares of the deviations from a straight line fitted if values of [A] and [P] corresponding to responses equal to 10,20 90% of D2 were estimated and 1/[A] plotted against 1/[P]. It was found that if B1=1 then B2 is always equal to 1. If B1>1 then B2<B1 and varies with D2. Similarly if B1<1 then B2>B1 and varies with D2.

Therefore if B1 is not significantly different from 1, the best estimate of K is obtained by fixing B2=1. If B1<>1 fitting logistic curves with a suitable combination of B1,B2,D2 is more difficult. If instead the values of B2 and D2 are estimated without reference to B1 an estimate of K can nevertheless be obtained as described above. This is analogous to estimating the affinity constant of a competitive antagonist without assuming a parallel displacement of the full agonist's dose response curve by measuring the dose ratio at the 50% response level.

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HISTAMINE SECRETION FROM HUMAN SKIN SLICES INDUCED BY ANTI-IgE, CALCIUM IONOPHORE A23187, POLY-L-LYSINE AND COMPOUND 48/80

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Human cutaneous mast cells may possess structural and functional differences from their counterparts in other tissues. Because techniques to disperse skin mast cells are not yet available, in vitro studies are restricted to the use of skin slices. Following passive sensitization, human skin slices release histamine on challenge with anti-IgE (Greaves et al, 1972; Tharp et al, 1983). We have extended these observations by examining the ability of anti-IgE, calcium ionophore A23187, poly-L-lysine and compound 48/80 to release histamine from slices of human foreskin in vitro.

Specimens of foreskin, obtained from children aged 18 months to 8 years, were immediately immersed in tissue culture medium at 4^{o} and used within 24 hours. Duplicate samples of approximately 50 mg in weight were placed epidermis down onto a drop of Tissue-Tek II O.C.T. embedding compound and placed in a freezer until the edge of the O.C.T., but not the tissue, was frozen. Samples were then cut into 200 μm thick slices with a Sorvall TC-2 tissue chopper and washed twice with HEPES buffered salt solution (HBSS) at pH 7.2. Skin slices, suspended in 1 ml of HBSS, were challenged by incubation with goat anti-human IgE serum, A23187, poly-L-lysine (average m.w. 40,000) or 48/80 for 30 minutes at 37^{o}. The histamine content of the supernatant and tissue residue was determined radioenzymatically (Church et al, 1982) and corrected for spontaneous release.

Following passive sensitization for 30 minutes at 37^{o} with a 1 in 100 dilution of atopic serum, challenge with anti-IgE (1/1000 to 1/10 dilution) induced a concentration dependent release of histamine, maximum release being 14±2% (n=10). After passive sensitization with a higher concentration of atopic serum (1/10 dilution), the secretory response to anti-IgE was only concentration related over the entire 1/1000 to 1/10 dilution range in half of the specimens studied, the remainder showing high dose tolerance to anti-IgE. The histamine releasing ability of A23187 in human skin slices was similar to that observed in lung and adenoidal mast cells (Church et al, 1981; 1982) being dose-dependent over the range 0.1 to 3 μM with a maximum release of 25±3% (n=7). In contrast to human lung and adenoidal mast cells, poly-L-lysine and 48/80 induced histamine release from human skin slices. Poly-L-lysine (0.01 to 10 μM) induced a concentration dependent release of histamine, the maximum release being 27±3% (n=7). In preliminary experiments, 48/80 (0.03 to 10 μg/ml) induced histamine release ranging from 10 - 16% but its effects were poorly concentration related.

The effects of poly-L-lysine and possibly those of 48/80 on human skin slices suggest that human skin mast cells have some functional dissimilarity to mast cells in human lung and lymphoid tissue which warrant further investigation.

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OBSERVATIONS OF THE ANTI-INFLAMMATORY ACTION OF PUTRESCINE

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Recently in a search for endogenous anti-inflammatory substances in inflammatory exudates putrescine was shown to be anti-inflammatory against carrageenan induced oedema and adjuvant induced arthritis in the rat (Bird et al, 1983). Although we were able to demonstrate the anti-inflammatory action of putrescine against animal models we had no knowledge of its mode of action. In this communication we report on experiments designed to provide information on the action of putrescine at the cell level.

The effect of putrescine on rat neutrophil chemotaxis was investigated in vitro on agarose plates (Nelson et al, 1975) using N-formyl-methionyl-leucyl-phenylalanine (FMLP) at 10^{-6}M in Hanks physiological medium for 30 min at 37° prior to chemotaxis the cells subsequently showed decreased directed migration (A) and random migration (B). However the chemotactic differential A-B was similar to that of untreated control cells. Putrescine did not affect the viability of the cells as shown by trypan blue exclusion.

In other experiments guinea pig neutrophils suspended in Hanks physiological medium were incubated in the presence of putrescine at various concentrations for 10 min at 37°. The cells were stimulated by the addition of phorbal myristate acetate (PMA) [10^{-6}M] and the amount of superoxide anion produced measured by the reduction of cytochrome C (Babior et al, 1973). The superoxide anion produced from cells pretreated with putrescine was compared to amounts produced from untreated controls. It was found that with cells pretreated with putrescine [80mM] superoxide anion production was inhibited by 28% ($p<0.05$). Lower concentrations of polyamine were not inhibitory. The inhibition of superoxide anion production by putrescine relates to earlier studies where we found that putrescine inhibited superoxide anion formation in the xanthine oxidase catalysed conversion of hypoxanthine to uric acid and also protected lysosomes in vitro from superoxide anion induced damage (Kafy & Lewis, 1983). Putrescine at low concentrations does not stabilise or labilise lysosomes. It appears that the protective effect of putrescine is due to its scavenging action against superoxide anion.

We have some preliminary evidence that neutrophils may possess ornithine decarboxylase activity. Ornithine (^{3}H) was incubated at 37° for 1h with rat neutrophils in Hanks physiological medium. The cells were recovered by centrifugation and after washing lysed by freezing and thawing. The lysates were examined for amines by the dansylation method of Seiler & Askar (1971). The dansylated amines were separated by tlc using several solvent systems and the presence of putrescine (^{3}H) detected. The possibility that neutrophils may convert ornithine to putrescine in situ is under further investigation.

Present work suggests that the anti-inflammatory activity of putrescine is mediated at least in part by its scavenging action on superoxide anion.

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LACK OF EFFECT OF CHRONIC ANTIDEPRESSANT ADMINISTRATION ON STRIATAL DOPAMINE AUTORECEPTOR SENSITIVITY

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A number of antidepressant treatments have been shown to reduce the sensitivity of dopamine autoreceptors (Serra et al. 1979). However recent biochemical studies have failed to demonstrate such a subsensitivity of dopamine autoreceptors in rat striatum. The present study examines the effect of chronic administration of imipramine and amitriptyline on a model of striatal dopamine autoreceptor function.

Striatal tissue obtained from 200-250g male Wistar rats was chopped into 0.5mm x 0.5mm prisms and incubated in Krebs-Henseleit buffer containing pargyline (10^{-6}M), ascorbic acid (0.2mM) and (^{3}H)-dopamine (10^{-7}M) for 20 min at 37°C. The slices were washed 3 times in Krebs-Henseleit and resuspended in 0.5ml buffer. 50µl aliquots of the tissue suspension were placed in 250µl superfusion chambers and superfused with Krebs-Henseleit containing 0.2mM ascorbic acid and 0.03mM EDTA at a flow rate of 0.4ml/min. 4 min. superfusate fractions were collected after a 20 min. equilibration period. The tissue was depolarized by the addition of a 4 min. pulse of 25mM KCl. The released radioactivity was measured by liquid scintillation spectrometry. Animals receiving chronic drug treatments were given twice daily intraperitoneal injections of saline, imipramine or amitriptyline (both 10mg/kg) for 14 days and were sacrificed 3 days after the last injection. In each experiment four animals were used per group and each determination was performed in quadruplicate.

Apomorphine (10^{-7}M) reduced the K^{+} evoked release of radioactivity by 78%. Addition of imipramine or amitriptyline to the superfusing medium was without effect on either basal or K^{+} evoked release of radioactivity. 14 day administration of imipramine or amitriptyline had no effect on the ability of apomorphine to inhibit the K^{+} stimulated overflow of radioactivity.

These results, in agreement with those of Holcomb et al. 1983, suggest that the striatum is not the site of the functional dopamine autoreceptor subsensitivity observed following chronic antidepressant treatment.

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HYPOTHERMIC EFFECTS OF BENZODIAZEPINE AGONISTS, ANTAGONISTS AND CONTRAGONISTS

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FG 7142 is a benzodiazepine contragonist which lowers seizure threshold in animals (Little and Nutt, 1984) and causes anxiety in man (Dorow et al., 1983). We studied its effects in mice and found that it lowered body temperature. The actions of the benzodiazepine antagonist Ro 15-1788 (Nutt et al., 1982) on this hypothermic effect of FG 7142, and that of the water soluble benzodiazepine flurazepam, were therefore investigated to determine whether the changes were mediated through benzodiazepine receptors.

Male CD1 mice, 30-35 g, were used and all drugs were given by the intraperitoneal route. Body temperatures were measured by rectal probe (inserted 2 cm). All experiments were started between 9.30 and 10.00 a.m. and the ambient temperature was maintained at 22°C. FG 7142 and Ro 15-1788 were suspended in Tween 40 (1 drop in 10 ml) and flurazepam was dissolved in distilled water. Control animals received Tween injections. Handling mice in order to weigh and code slightly raised body temperature so these procedures were carried out 15 min before drug administration and the temperatures measured immediately before injection. Table 1 shows the latter temperatures and those 30 min later.

Table 1

Treatment	Temperature °C: Preinjection	Temperature °C: 30 min post-injection	P value (Mann-Whitney 'U' test)
FG 40	38.1 ± 0.11	35.1 ± 0.42	P < 0.001
FG 40 + Ro 10	38.0 ± 0.1	37.5 ± 0.27	
FG 40	38.4 ± 0.01	35.8 ± 0.06	P < 0.001
FG 40 + Ro 20	38.4 ± 0.01	38.1 ± 0.02	
FLZ 40	38.5 ± 0.1	36.3 ± 0.22	P < 0.05
FLZ 40 + Ro 10	38.1 ± 0.14	37.0 ± 0.19	
FLZ 40	38.5 ± 0.01	35.5 ± 0.01	P < 0.001
FLZ 40 + Ro 20	38.6 ± 0.01	36.9 ± 0.03	
Tween	38.2 ± 0.1	37.4 ± 0.29	P > 0.1
Tween + Ro 10	38.5 ± 0.14	37.7 ± 0.26	
Tween	38.4 ± 0.1	38.2 ± 0.01	P > 0.1
Tween + Ro 20	38.3 ± 0.02	38.2 ± 0.01	

At 40 mg kg^{-1} FG 7142 (FG 40) and flurazepam (FLZ 40) lowered body temperature and both effects were significantly reduced by Ro 15-1788. Ro 15-1788 alone did not alter body temperature at 10 (Ro 10) or at 20 mg kg^{-1} (Ro 20). To our knowledge this is the first time that a benzodiazepine agonist and a contragonist have been shown to produce pharmacological changes in the same direction, both of which were antagonised by Ro 15-1788.

We thank Ferrosan for FG 7142 and Roche for Ro 15-1788.

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Little and Nutt (1984) British Pharmacological Society Meeting, London, C48.
Nutt et al. (1982) Nature, 295, 436-438.

FURTHER STUDIES ON BENZODIAZEPINE RECEPTOR-MEDIATED KINDLING

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In a previous communication to the Society we described the phenomenon of kindling produced by the benzodiazepine receptor "contragonist" or "inverse agonist", the β-carboline FG 7142 (Little & Nutt, 1984). After repeated administration, an initially proconvulsant dose reliably produced generalised seizures. We now report further studies on this phenomenon.

Male CD_1 mice (30-35 g) were treated with FG 7142 (FG) $40mgkg^{-1}$, Ro 15-1788 or vehicle (1 drop of Tween 40 per 10 ml distilled water). All drugs were given i.p. in a volume of $10mlkg^{-1}$, once daily unless stated otherwise.

FG, given daily for 12 days produced kindled seizures in 6/8 mice. Seizures were still seen when a further dose was given after a 7 day drug free period. A single dose of FG did not lead to sensitization as only a single mouse convulsed with a repeat dose on day 12. Kindling was shown not to be due to repeated vehicle injections since a single injection of FG after 12 days of vehicle produced no seizures. Furthermore, a vehicle injection in FG kindled mice produced no seizures.

The benzodiazepine antagonist Ro 15-1788, which blocks effects of both benzodiazepine antagonists and contragonists (Nutt et al. 1982) was given with FG. Owing to its shorter biological life Ro 15-1788 ($10mgkg^{-1}$) was given concurrently with the FG and repeated 30 mins later. This treatment fully antagonized the proconvulsant effects of FG. During this combined treatment no kindling was observed, which strongly suggests FG kindled via an action at the BDZ receptor. In addition a further dose of FG alone 7 days later also did not produce any seizures, showing covert sensitization had not occurred. In mice kindled with FG for 12 days, Ro 15-1788 (as above) given with the FG on day 19 prevented fully the expression of the previously kindled seizures. Finally the duration of kindling was found to last at least one month since 5/8 mice convulsed when rechallenged with FG on day 42 (30 days after their last dose).

Treatment and Duration		Challenge and Day		Result	P value
FG	12 d	FG	d 19	6/8	-
FG	1 d	FG	d 12	1/8	<0.02
FG	12 d	Vehicle	d 13	0/8	<0.01
Vehicle	12 d	FG	d 13	0/8	<0.01
FG + Ro	12 d	FG	d 19	0/8	<0.01
FG	12 d	FG + Ro	d 19	0/8	<0.01
FG	12 d	FG	d 42	5/8	<n.s.

Statistical analysis by Fisher's exact test.

These data suggest that FG kindling is a benzodiazepine receptor mediated phenomenon. Interestingly, Ro 15-1788 has recently been shown to inhibit electrically kindled seizures (Robertson & Riives, 1983), suggesting perhaps that this phenomenon involves activation of an endogenous beta-carboline-like compound.

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THE EFFECTS OF NIFEDIPINE ON ANTIGEN-INDUCED CONTRACTION OF SENSITISED GUINEA-PIG ISOLATED TRACHEA

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Antigen-induced contraction of airways smooth muscle involves two main events:- 1) mediator release and 2) smooth muscle contraction. Both these processes are calcium dependent. Calcium entry blockers such as nifedipine have modest effects on human allergic asthma, but have been reported not to affect antigen-induced contraction of sensitised guinea-pig trachea (Henderson et al., 1983). To attempt to resolve this discrepancy we have re-examined the effects of nifedipine on contractions of sensitised guinea-pig tracheal rings.

Male Dunkin-Hartley guinea-pigs (300-400g) were actively sensitised using ovalbumin (OA) at 100µg i.p. and 100µg s.c. After at least 3 weeks they were killed by a blow to the head and exsanguinated. The trachea was removed, rings were cut and suspended in an organ bath containing modified Tyrode solution at 37°C pH 7.4. The bathing solution contained indomethacin ($3x10^{-7}$g/ml) to suppress prostaglandin and thromboxane synthesis (Weichman et al, 1983). Control contractions were obtained to 10^{-7}g/ml carbachol (CCh). The CCh-induced contraction immediately before the test contraction was used as a standard (100%) against which the test contraction was compared. Ovalbumin (10^{-6}g/ml) was then added after 20 minutes preincubation with either nifedipine or its solvent DMSO. In the presence of DMSO the OA-induced contraction averaged 96±4% (n=10) of the previous CCh standard contraction. Nifedipine concentration-dependently reduced the OA-induced contraction (table 1) with a 50% inhibitory concentration of $2.2x10^{-8}$g/ml.

To examine the mechanism of action of this anti-anaphylactic effect of nifedipine we also examined its effect on pre-existing OA-induced contractions, and on contractions of the trachea induced directly by the mediators histamine (10^{-5}g/ml) or leukotriene D_4 (LTD_4, 10^{-7}M). Pre-existing OA-induced contractions were inhibited by nifedipine. Under these conditions nifedipine was more potent than when added before antigen challenge, suggesting that little of the anti-anaphylactic effect of nifedipine in this model is due to inhibition of mediator release. In addition nifedipine attenuated histamine or LTD_4 contractions whether administered before or during contraction. Thus in this model, nifedipine exerts the majority of its action against smooth muscle contraction and not against mast cell mediator release.

Standard CCh (10^{-7}) contraction	Treatment	Antigen-induced (OA, 10^{-6}) contraction	% of standard	n
1.75 ± 0.3g	DMSO	1.71 ± 0.21g	95.7 ± 2.7	10
0.97 ± 0.13g	N $3x10^{-9}$g/ml	0.88 ± 0.15g	89.6 ± 6.2	3
1.76 ± 0.17g	N $3x10^{-8}$g/ml	0.76 ± 0.16g	41.8 ± 4.3*	5
1.57 ± 0.19g	N $3x10^{-7}$g/ml	0.54 ± 0.13g	36.3 ± 8.4*	4
1.05 ± 0.23g	N $3x10^{-6}$g/ml	0.19 ± 0.04g	22.7 ± 7.2*	5

Table 1 Effect of DMSO or nifedipine (N) on the ovalbumin-induced contraction of isolated tracheal rings from actively sensitized guinea-pigs. Results are expressed as means (± SEM) and * indicates $P<0.05$ as compared to DMSO pretreatment

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DIFFERENTIAL EFFECTS OF ADMINISTRATION OF HALOPERIDOL, SULPIRIDE OR CLOZAPINE FOR 12 MONTHS ON RAT STRIATAL FUNCTION

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Chronic administration of classical neuroleptic drugs to rodents causes striatal dopamine receptor supersensitivity and an increase in striatal acetylcholine content despite continued drug intake (Clow et al, 1979; Murugaiah et al, 1982). The atypical neuroleptics sulpiride and clozapine may not induce tardive dyskinesia so we now examine the effects of chronic treatment with these agents, in comparison to haloperidol, on striatal dopamine function in rats.

Male Wistar rats received either haloperidol (1.4-1.6 mg/kg), sulpiride (102-109 mg/kg) or clozapine (24-27 mg/kg) via their daily distilled drinking water for 12 months. Age-matched control rats received distilled water alone.

After 12 months stereotypy induced by apomorphine (0.125 mg/kg sc) was inhibited in haloperidol-treated rats whereas higher doses (0.5-1.0 mg/kg sc) caused an exaggerated stereotyped response. No component of apomorphine-induced stereotypy was altered by chronic sulpiride or clozapine treatment (Table 1). Haloperidol, but not sulpiride or clozapine, treatment for 12 months increased the number (Bmax) of specific striatal ^{3}H-spiperone (SPIP, 0.5-4.0 nM; defined using 10^{-5}M (-)-sulpiride) binding sites by comparison to control rats. Bmax for ^{3}H-N,n-propylnorapomorphine (NPA, 0.05-2.0 nM; defined using 10^{-6}M (±)-6, 7-ADTN) binding was increased after 12 months treatment with haloperidol or sulpiride, but not with clozapine. Bmax for ^{3}H-piflutixol (PFT, 0.08-1.3 nM; defined using 10^{-6}M *cis*-flupenthixol in the presence of 3×10^{-5}M (±)-sulpiride) binding was increased by chronic clozapine treatment but not by haloperidol or sulpiride treatment. Striatal dopamine (50 uM)-stimulated adenylate cyclase activity was increased by 12 months treatment with sulpiride, but not by haloperidol or clozapine. Striatal acetylcholine content was increased in rats treated for 12 months with haloperidol or clozapine but not with sulpiride.

Table 1 ^{3}H-SPIP, ^{3}H-NPA and ^{3}H-PFT binding, dopamine (50 uM)-stimulated AC activity and basal striatal acetylcholine (ACh) content in rats treated continuously for 12 months with haloperidol, sulpiride or clozapine

Treatment	APOM stereotypy	Bmax (pmoles/g tissue) ^{3}H-SPIP	^{3}H-NPA	^{3}H-PFT	AC (pmoles cAMP/2.5min/ 2mg tissue)	ACh content (nmol/g)
Control	2.8±0.3	13.8±1.2	8.1±0.3	88±5	32.3±1.9	19.9±2.1
Haloperidol	3.8±0.2**	26.4±2.2*	11.0±0.3*	83±3	25.2±4.7	35.7±3.3*
Sulpiride	3.3±0.4	17.2±1.4	13.9±0.4*	102±5	54.1±3.1*	26.5±2.6
Clozapine	3.0±0.4	15.3±0.9	7.2±0.9	105±2*	51.9±6.6	29.5±3.4*

* $p < 0.05$ vs control rats, Student's t or Mann Whitney u-test. N = 3-8

Chronic administration of the atypical neuroleptics sulpiride and clozapine does not cause changes in striatal function identical to those produced by haloperidol.

Clow, A. et al (1979) Nature 278, 59
Murugaiah, K. et al (1982) Nature 296, 570

POORLY LIPOPHILIC SUBSTITUTED BENZAMIDE DRUGS SHOW ENHANCED AFFINITY FOR THE CHAPS SOLUBILISED RAT STRIATAL DOPAMINE D_2 RECEPTOR

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We recently reported that the zwitterionic detergent CHAPS solubilises the rat striatal D-2 receptor (Jenner et al, 1984). Dopamine agonist and antagonist drugs showed a decreased ability to displace specific ^{3}H-spiperone binding upon solubilisation, with the exception of sulpiride which was more potent on the solubilised receptor. Substituted benzamide drugs, such as sulpiride, also differ in the sodium ion dependence of their interaction with D-2 receptors (Stefanini et al, 1980). We now report on the ability of a range of substituted benzamide drugs to displace the specific binding of ^{3}H-spiperone to the membrane bound and solubilised D-2 receptors and the effect of 120 mM NaCl on this displacement.

The solubilisation of D-2 sites from rat striatal membrane and the determination of ^{3}H-spiperone binding were performed as previously described (Jenner et al, 1984) except that binding assays were performed in the presence or absence of 120 mM NaCl. Displacing drugs were incorporated in concentrations between 10^{-11} -10^{-5}M. Lipophilicity was assessed from the true retention factor (log k) determined using reverse phase HPLC.

In both striatal membrane and solubilised preparations the ability of the substituted benzamide drugs to displace specific ^{3}H-spiperone binding was enhanced by the addition of 120 mM NaCl. For example, sulpiride was approximately 10 fold more active in displacing ^{3}H-spiperone in both preparations in the presence of 120 mM NaCl than in cation free buffer (Table 1)

The lipophilic substituted benzamide drugs YM 09151-2 and clebopride were less effective in displacing ^{3}H-spiperone after solubilisation of the receptor (Table 1). However, the less lipophilic substituted benzamide drugs showed an enhanced affinity for the solubilised receptor. Some correlation exists between the lipophilicity (log k) and displacement of the ^{3}H-spiperone binding such that the lower lipid solubility, the greater the enhancement of affinity at the solubilised receptor.

Table 1 The inhibition constants (Ki) for displacement of ^{3}H-spiperone binding and lipophilicity (log k) of some substituted benzamide drugs

	Ki (nM)		Ratio	
	(a) Membrane	(b) Soluble	b/a	log k
YM 09151-2	0.072	2.32	31.3	4.08
Clebopride	1.3	15	11.5	3.45
Metoclopramide	62	53	0.86	2.44
Alizapride	44	31	0.70	2.11
(-)-Sultopride	7.1	3.8	0.54	2.25
(±)-Sulpiride(+NaCl)	53	15	0.28	1.24
(±)-Sulpiride(-NaCl)	580	135	0.23	1.24

In conclusion there appears to be a lipid barrier close to the D-2 receptor binding site that reduces the interaction of less lipophilic substituted benzamide drugs. This barrier is removed upon solubilisation with CHAPS. The sodium dependency is retained.

Jenner, P. et al (1984) Br.J.Pharmac. London meeting. C81
Stefanini, E. et al (1980) Brain Res. 198, 229

ANALYSIS OF THE CONTRACTILE RESPONSES OF HUMAN UMBILICAL BLOOD VESSELS TO 5-HT AND OXYGEN

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There is no evidence for adrenoceptor-mediated constriction of human umbilical arterial smooth muscle but 5-hydroxytryptamine (5HT) and O_2 are potent constrictors (McGrath & Stuart-Smith, 1982). We now report an analysis of the 5HT receptor in this tissue, a quantitative assessment of the constriction to oxygen and a comparison of the effects of each on the umbilical artery & vein.

Cords were collected immediately after birth and transferred to the laboratory in cold Kreb's bicarbonate saline at low PO_2. Isometric tension was recorded *in vitro* from longitudinal strips of artery and circular strips of vein, at 37°C, bubbled with O_2 4-5%, CO_2 6-7%, balance N_2, to mimic conditions *in utero*, e.g. O_2 20mmHg, pH 7.3. Cumulative concentration/response curves to 5HT were constructed, repeated in the presence of antagonists, dose-ratios were calculated at the EC50 level and pA_2 values were estimated by linear regression of log(DR-1) versus -log[antagonist]. Concentration/response curves to oxygen were constructed non-cumulatively since responses were not maintained.

5HT produced concentration-related contractions (10nM-1μM) in both tissues (pD_2: artery 7.3; vein 7.2). The receptor was characterised in the artery. Methysergide (10nM-1μM) and phentolamine (1μM-100μM) produced parallel rightward shifts of the 5HT concentration/response curve: methysergide (pA_2 8.5, slope 0.78); phentolamine (pA_2 6.0, slope 1.06). Neither antagonist contracted the tissue. These values are similar to those found in rabbit aorta (Apperley et al, 1976). Noradrenaline or adrenaline (1-10μM) produced contractions in some but not all preparations: these were blocked by methysergide (1μM) but not phentolamine (1μM) suggesting that they were mediated through 5HT receptors.

In the artery, oxygen (40-300mmHg) produced contractions related to log (partial pressure) with a decline in the response above 300mmHg. The threshold for contraction, estimated by extrapolation, was influenced by PCO_2. At PCO_2 of 50mmHg the O_2 threshold was 43 ± 10mmHg (n=6) (mean + s.e. mean) and at CO_2 30mmHg, it was 69 ± 4mmHg (n=6). In the presence of indomethacin (0.1 or 1μM) no contraction occurred at any oxygen tension across the range tested (40-500mmHg), whereas sensitivity to 5HT remained. In paired controls tested with the indomethacin solvent ethyl alcohol (0.9 or 9mM), sensitivity to O_2 was unaffected except in 2 out of 8 preparations where it was increased. In contrast, there was no significant contraction to any level of O_2 in the vein (6 veins responsive to 5HT).

The results indicate a specific 5HT receptor, present in the artery and vein, whose activation could restrict umbilical blood flow. Contraction to oxygen is present only in the artery suggesting that activation of this system by rising PO_2, following birth, could play a part in cessation of blood flow from the fetus while allowing flow to continue from the placenta to the baby. Activation of either system *in utero* could restrict umbilical blood flow.

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Apperley, E. et al. (1976) Br. J. Pharmac. 58, 211-221
McGrath, J.C. & Stuart-Smith, K. (1982) Br. J. Pharmac. 76, 258P

OPIATE RECEPTOR ANTAGONISTS ATTENUATE SWEETENED MILK CONSUMPTION IN MALE AND FEMALE RATS OF THE ROMAN STRAINS

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Endogenous opioid peptides appear to be involved in the control of food and water intake (Morley et al., 1983; Sanger, 1981). Opiate receptor antagonists typically reduce the consumption of food and water, although differences occur when comparisons are made across species. The present experiment addresses the possibility that differences may occur between genetically-differentiated animals of the same species in their response to the anorectic action of opiate receptor antagonists. We examined the effects of various drugs in both male and female rats of the Roman strains, Roman High-(RHA), Roman Low-(RLA) (Broadhurst & Bignami, 1965) and Roman Control-(RCA) Avoidance rats, respectively.

Subjects were 30 male and 30 female rats, 10 of each sex drawn from each of the 3 Roman strains maintained in our colony. They were housed in same-sex, same-strain pairs with free access to standard food pellets and water, and maintained on a 12h light : 12h dark cycle. Each day, each animal was transferred individually to a test cage and given 30 min access to sweetened condensed milk mixed with water (2 parts water : 1 part milk) (Locke et al., 1982). Daily intake stabilised over a 10-day familiarisation period, before drug tests were begun. Each animal served as its own control, and was tested following drug administration 3 times per week. Milk consumption was converted to ml/100 g body weight before statistical comparisons were carried out using the analysis of variance. The drugs tested were naloxone (0.01 - 10 mg/kg males; 0.01 - 30 mg/kg females), diprenorphine (0.01 - 1 mg/kg, both sexes), WIN 44,441-3 (0.01 - 10 mg/kg, both sexes), Mr 2266 BS (0.01 - 3 mg/kg, both sexes) and ICI 154129 (30 mg/kg, females). All injections were administered s.c., 15-20 min before the milk consumption test.

Naloxone, diprenorphine and Mr 2266 BS produced significant dose-related reductions in milk consumption in all 6 groups (3 strains x 2 sexes). WIN 44,441-3 reduced consumption at 10 mg/kg in RCA and RHA animals, but had no effect on consumption in RLA rats. ICI 154129 (30 mg/kg) had no effect on consumption in female rats of the 3 strains.

These results indicate differences amongst opiate receptor antagonists in their effects upon food ingestion in non-deprived animals. Naloxone, diprenorphine and Mr 2266 BS produced effects which did not differ markedly across strains and the two sexes, and when compared with each other. WIN 44,441-3 had a suppressant actions in two strains but not in a third. This may explain inconsistent reports in the literature with this compound. The lack of effect of ICI 154129 suggests a lack of involvement of delta receptors.

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TASTE AND THE ANTIDIPSOGENIC EFFECTS OF OPIATE RECEPTOR ANTAGONISTS IN THE THIRSTY RAT

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Levine et al (1982) reported that taste (sweet, salt, sour) enhanced the anti-dipsogenic effect of naloxone in water-deprived rats. They suggested that naloxone suppresses drinking behaviour due to alterations in taste perception. In our study we examined this issue using a number of different opiate receptor antagonists (naloxone, naltrexone, diprenorphine, WIN 44, 441-2, WIN 44, 44-3).

Subjects were adult male hooded rats (General strain) bred in our colony. They were housed individually with free access to standard food pellets and were maintained on a 12 h light: 12 h dark cycle at 21°C. All animals were adapted to 24 h water-deprivation on alternate days. Separate groups of animals were familiarised with drinking water, 0.9% saline and 0.005M sodium saccharin solution, respectively, as the sole available fluid in a 30 min period following 24 h water-deprivation. Ten animals were tested at each dose in each flavour condition. All injections were administered s.c. 15-20 min before the drinking test. Food was removed during the test.

Generally, under control conditions, there were differences in the baseline level of consumption: saline > saccharin > water. Naloxone (0.1-10 mg/kg), naltrexone (0.1-3 mg/kg) and diprenorphine (0.1-1 mg/kg) produced significant dose-related reductions in fluid consumption. The two isomers of WIN 44, 441 (0.3-10 mg/kg) had no effect on fluid consumption in any condition, except for a non-stereo-specific effect on saccharin drinking. Analyses of variance of the data for 30 min fluid consumption failed to reveal significant overall flavour condition x drug dosage interactions, in the cases of drugs which did significantly affect fluid consumption. These results therefore do not support the generality of the proposition that the effects of opiate receptor blockade on overall fluid intake are necessarily due to changes in taste perception.

Although Ostrowski et al (1981) reported that WIN 44, 441-3 (2 mg/kg) significantly reduced water intake, our results and those of Leander & Hynes (1983) failed to confirm the effect.

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OPIOID ANTAGONISTS, BY AN ACTION ON THE BRAIN, PREVENT ENDOTOXIN CAUSING THE DISAPPEARANCE OF CIRCULATING WHITE CELLS AND PLATELETS

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Opioid antagonists can prevent an endotoxin or diamorphine induced fall in white blood cell (WBC) or platelet counts in mice (Wright, 1981[a]). The system was used to assess the potency of a variety of opioid antagonists and compare intraperitoneal with the intracerebral administration.

All drugs tested, were dissolved and diluted tenfold to the appropriate concentration immediately before each experiment with sterile non-pyrogenic physiological saline, the dose being expressed in terms of free base. The drugs were given intraperitoneally, intravenously and intracerebrally along the mid-line deep into the region of the forebrain to 6-week old inbred CBA/CA mice. Intracerebral injection was confirmed microscopically by injecting control animals with indian ink. The opioid antagonist was given 5 min before the intravenous challenge injection of Escherichia coli lipopolysaccharide which brings about the virtual extinction of WBCs and platelets from the circulation (for method see Wright 1981[b]). The changes in these counts were measured in time matched control groups given saline instead of the opioid antagonist or endotoxin. Two experiments (n = 6x2) determined the lowest concentration of antagonist which inhibited the endotoxin effect, see table. None of the partial agonists produced a fall in circulating cells when given intracerebrally.

Table 1 The minimal inhibiting dose of antagonists Kg^{-1} preventing endotoxin induced changes in mice.

Drug	Route	
	intraperitoneal*	intracerebral
Naloxone	1mg	10µg
Naloxone methiodide	NE	10µg
Nalmefene	0.1mg	10µg
Nalorphine	0.1mg	10µg
Diamorphine	1mg	1µg
Meptazinol	1µg	1µg

* ip substantially same as iv NE = Not effective

All the drugs, apart from meptazinol, were much more effective when given intracerebrally. This suggests that the antagonist effect is exerted in the brain perhaps by reduction of opioid induced central excitation which disinhibits other neurones containing enkephelin (Glazer et al 1983).

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THE EFFECTS OF DELTA-SLEEP INDUCING PEPTIDE (DSIP) IN RELATION TO OPIOID RECEPTORS

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It has been shown that delta-sleep inducing peptide (DSIP) is capable of inducing an increase in the amount of sleep (Monnier and Schoenenberger, 1977). We have been able to confirm these observations in rats using electroencephalographic and electromyographic criteria taken over 3 hour recording periods. The animals were maintained on a 12 hour light/12 hour dark cycle and recordings were taken over the first 3 hours of the dark (waking) phase and between the eighth and eleventh hours of the light (sleeping) phase. DSIP, at an optimal dose of 6 nmol induced a 35.5 ± 6.7% (mean ± s.e.) increase in total sleep time (n=11), with proportionate changes in both REM and non-REM sleep stages, when given intracerebroventricularly during the dark (waking) phase. Moreover it was found that the opiate antagonist, naloxone (0.1mg/kg s.c.), given 20 minutes prior to the DSIP, significantly reduced or abolished the somnogenic effect of this peptide.

These results would suggest the involvement of opioid receptors in the response induced by DSIP. At present there are no data to indicate that DSIP affects opioid receptors directly. This possibility has been investigated in vitro by using the following field stimulated assay preparations: the guinea-pig ileum (for μ- and k-receptors), the mouse vas deferens (for δ-receptors) and the rabbit vas deferens (for k-receptors) (Oka et al, 1980). Field stimulation of 1cm lengths of each tissue was carried out using 0.5ms electrical pulses at supramaximal voltages (50-65V) and at a frequency of 0.1Hz. The induced- contractions were measured isometrically. The guinea-pig ilea were bathed in modified Krebs-Henseleit solution ($\frac{1}{2}Mg^{2+}$ and $\frac{1}{2}Ca^{2+}$) at 37°C, and the mouse and rabbit vasa deferentia were bathed in Krebs-Henseleit solution containing no magnesium at 34°C.

DSIP (10^{-10} to 10^{-4}M) did not cause any significant change in the magnitude of the electrically-induced contractions of either the guinea-pig ileum (n=8), the mouse vas deferens (n=8) or the rabbit vas deferens (n=8). Neither was there any significant change in the ability of morphine (10^{-8} to 10^{-6}M) to reduce the stimulation-induced contractions of the guinea-pig ileum (n=8) or that of a similar reduction induced by D-Ala^2-D-Leu^5-Enkephalin (10^{-8} to 10^{-6}M) in the mouse vas deferens (n=8), when either of these drugs were added concommitantly with DSIP (10^{-10} to 10^{-4}M).

The possibility that DSIP may induce its effect indirectly by modulation of opioid transmitter release has also been investigated. Guinea-pig striatal slices were superfused with DSIP 10^{-8} - 10^{-4}M in modified Krebs-Henseleit solution, and the release of met-enkephalin was measured by RIA (sensitivity > 50pg) of the superfusate (Bull and Tyers 1981). The results showed that DSIP did not evoke the release of met-enkephalin, nor did it affect the potassium-evoked release of this peptide.

In conclusion it would appear that DSIP does not act directly on either u-, δ- or k-opioid receptors in vitro, and neither does it act by modulation of opioid neurotransmitter release, at least in terms of met-enkephalin. The ability of naloxone to block the somnogenic response to DSIP, would suggest that DSIP, considered in relation to the above results, may induce its effect by activating receptors, as yet undefined, in a multineuronal pathway in which there is at least one opioid-controlled link.

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COMPARISON OF THE ANTINOCICEPTIVE EFFECTS OF CENTRALLY ADMINISTERED CALCITONINS AND CALCITONIN GENE-RELATED PEPTIDE

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Rosenfeld et al (1983) have demonstrated that alternative processing of the RNA transcribed from the calcitonin gene produces tissue specific forms of mRNA. Thyroid mRNA encodes a precursor to the hormone calcitonin whereas neural mRNA encodes a novel peptide termed calcitonin gene-related peptide (CGRP). The distribution of CGRP suggests that peptide may be involved in processing of painful stimuli. Several forms of calcitonin produce antinociception when administered centrally (Pecile et al, 1975; Bates et al, 1983). In this communication we compare the antinociceptive effects of centrally administered CGRP, human calcitonin (hCT) and salmon calcitonin (sCT).

Groups of mice (CFLP, ♂, ♀, 25-35g) received sCT (10 $U.kg^{-1}$ = 0.6 $nmol.kg^{-1}$). hCT (0.01-10 $nmol.kg^{-1}$) or CGRP (0.01-10 $nmol.kg^{-1}$) in 10µl of vehicle (50 mM Tris, 100 mM NaCl in 1% BSA, pH 7.4) by the intracerebroventricular (i.c.v.) injection technique of Haley and McCormick (1957). Ten minutes later the animals received, i.p., 0.3ml of a solution containing 1% acetic acid in 0.9% NaCl. The rate of consequent abdominal constrictions (constrictions per minute, c.p.m.) was determined between the 10th and 14th minute after injection. The possibility of peptides modifying locomotor or investigative behaviour was also assessed by the rotating drum and hole board tests.

The statistical validity of the results was assessed using Student's 't' tests, groups of mice under test being compared to an internal control group receiving vehicle alone.

The control abdominal constriction rate (3.12±0.22 c.p.m., n=22) was significantly reduced by i.c.v. hCT at 5 and 10 $nmol.kg^{-1}$ (36.6±8.5, 27.5±11.4% inhibition of internal control respectively, n=11-12, $P<0.05$). CGRP at 1, 5 and 10 $nmol.kg^{-1}$ i.c.v. reduced the control constriction rate (3.45±0.15, n=25) by 25.1±7.0, 34.9±9.0 and 33.3±9.4% respectively (n=19-20, $P<0.01$). Doses below those shown were without effect and maximal doses of peptides did not significantly modify locomotor or investigative behaviour.

In a further experiment significant inhibition of abdominal constriction was observed 20, 60 and 120 min. after sCT (10 $U.kg^{-1}$ ≃0.6 $nmol.kg^{-1}$, i.c.v) whereas antinociceptive effects of CGRP and hCT (10$nmol.kg^{-1}$) were only evident 20 minutes after administration.

We have previously reported a minimum antinociceptive dose of 0.1 $U.kg^{-1}$ (≃6 $pmol.kg^{-1}$) sCT in this model. The marked potency and duration of action of sCT as compared to hCT has been reported for the hypocalcaemic action of the hormones and may be associated with its higher receptor affinity and binding half-life.

In summary,we have demonstrated that central administration of relatively high doses of both hCT and CGRP can produce transient antinociception in mice, these results support the suggestion of Rosenfeld et al, 1983, that CGRP might play a role in central processing of painful stimuli.

The sCT and hCT were donated by the Armour Pharmaceutical Corporation and Ciba-Geigy respectively, CGRP was purchased from Merseyside Laboratories.

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EFFECT OF THE OPIOID-ANTAGONIST ICI 154,129 ON FOOD INTAKE IN RATS

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There is now considerable evidence implicating endogenous enkephalins in the regulation of appetite (see Morley et al. 1983). Opioid agonists increase, and opioid antagonists decrease, food and water intake in animals under certain experimental conditions. Furthermore, central injections of enzyme-resistant enkephalin analogues have been shown to increase food intake in freely-feeding rats (McLean & Hoebel, 1980; Tepperman et al. 1981). In the present study the appetitive effects of ICI 154,129 (N,N-Bisallyl-Tyr-Gly-Gly-ψ-(CH_2S)-Phe-Leu-OH) alone or in combination with various opioid agonists have been investigated. This drug has been reported to act as a selective antagonist at δ(enkephalin)-receptors in both in vitro and in vivo studies (Gormley et al. 1982; Shaw et al. 1982). Male Wistar rats (250-300g) were implanted with intracerebroventricular cannulae and allowed at least 7 days recovery from surgery before use. Animals were allowed free access to powdered rat diet and water. Food intake was measured at 1, 2 and 3 hours after drug administration and cumulative intakes/kg rat were calculated. Mean group values were compared using the Mann-Whitney U test and assuming significance at P values of 0.05 or less. In experiments when the δ-antagonist was given alone animals were injected with either saline or ICI 154,129 (10 or 100µg/rat) at the onset of the dark period so that any decrease in food intake would be observed. Interaction studies with levorphanol (1mg/kg i.p.), Mr 2033 ((±)-α-5,9-dimethyl-2-(L-tetra-hydrofurfuryl)-2'-OH-6,7-benzomorphan; 1mg/kg i.p.) and D-ala^2-D-leu^5-enkephalin (DADL; 10µg i.c.v.), however, were carried out in the light period when the food intake of control animals was minimal. Rats were injected with agonist alone or in combination with ICI 154,129 (10µg i.c.v.). Appropriate control injections were administered where applicable. Animals were pretreated with levorphanol and Mr 2033 (60 min). DADL was given at the same time as ICI 154,129.

Rats treated with 100µg ICI 154,129 ate significantly less than control animals though the effects of the antagonist were short-lasting and occurred mainly in the first hour after injection. ICI 154,129 (10µg) did not produce a significant decrease in food intake and therefore was used as a submaximal dose in the interaction studies. The decrease in feeding produced by ICI 154,129 agrees with the anorexia induced by other opioid antagonists (Sanger et al. 1983). DADL, levorphanol and Mr 2033 all produced a significant increase in cumulative food intake compared to controls over the test period. ICI 154,129 had no effect on the feeding induced by levorphanol (μ) or Mr 2033 (κ). The increase in food intake induced by DADL (δ) however was significantly decreased by ICI 154,129 so that animals treated with DADL and the δ-antagonist ate similar amounts to the control animals.

The present findings indicate a selective action of ICI 154,129 on δ-opioid receptors in the rat feeding model and lend further support to the notion that an endogenous enkephalin/δ-receptor system may be involved in the control of appetite.

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DI-ISOPROPYLFLUOROPHOSPHATE ENHANCES ENTRY OF ALFENTANIL INTO MOUSE BRAIN

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We have shown that the irreversible anticholinesterase agent, di-isopropylfluorophosphate (DFP) potentiates the antinociceptive activity of alfentanil but has no effect on the activity of morphine or fentanyl (Kitchen and Green, 1983). Since alfentanil is an extremely rapid and short-acting opioid the possibility of a pharmacokinetic interaction must be considered to explain this effect. We have accordingly studied this drug interaction further by examining the effects of DFP on the distribution of radiolabelled alfentanil.

Male albino mice (CD 1 strain, 25-30 g) received either DFP (1 mg/kg) or 0.9% saline subcutaneously. This was followed 55, 50 or 30 minutes later by a subcutaneous injection of radiolabelled alfentanil, fentanyl or morphine respectively. Two doses of each opioid were studied and each injection contained 7 μCi of [^{3}H]-labelled opioid. Animals were killed by decapitation at times of peak antinociceptive activity (5 minutes for alfentanil, 10 minutes for fentanyl, and 30 minutes for morphine). Trunk blood was collected into heparinised tubes and the brains rapidly removed. Brains were dissected over ice into eight regions as described by Glowinski and Iversen (1966). Tissues and plasma (20 μl) were solubilised in 1 and 0.5 ml of Soluene-100 respectively, and [^{3}H] measured by liquid scintillation counting.

Table 1. Effect of DFP on Brain/Plasma ratios for [^{3}H]-alfentanil

	BRAIN/PLASMA RATIOS x 100			
	Alfentanil 200 μg/kg		Alfentanil 400 μg/kg	
Brain Region		+ DFP (1 mg/kg)		+ DFP (1 mg/kg)
Cerebellum	42.3 ± 3.1	61.4 ± 4.3	62.3 ± 6.8	131.0 ± 11.3
Pons and Medulla	41.9 ± 4.1	52.6 ± 4.7	56.1 ± 6.5	120.7 ± 8.5
Hypothalamus	56.5 ± 3.3	111.1 ± 10.6	79.2 ± 20.0	347.3 ± 60.8
Striatum	45.5 ± 4.1	76.5 ± 5.5	61.7 ± 6.4	189.3 ± 20.6
Mid-brain	45.6 ± 4.0	69.2 ± 6.3	59.9 ± 5.7	166.1 ± 32.0
Hippocampus	47.2 ± 2.2	76.8 ± 7.9	62.7 ± 6.8	155.4 ± 12.8
Hind cortex	38.8 ± 3.7	51.5 ± 5.0	55.8 ± 5.7	99.4 ± 3.6
Frontal cortex	42.7 ± 4.1	60.7 ± 8.0	57.4 ± 5.7	112.2 ± 3.2

Each value is the mean ± s.e. mean of at least six observations.

DFP treatment significantly increased levels of alfentanil in all brain regions (Table 1). The magnitude of the increase varied from 1.6-fold in the hind cortex to 3.2-fold in the hypothalamus. In contrast, DFP pretreatment had no significant effect on the levels of fentanyl or morphine in the brain. The enhanced entry of alfentanil into the brain of DFP-treated mice probably accounts for the increased antinociception observed with this opioid. If this effect extends to reversible anticholinesterases used post-operatively, doses of alfentanil may require modification in patients who receive these drugs.

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THE EFFECT OF KETAMINE ON THE RESPONSE TO FIELD STIMULATION AND THE UPTAKE OF (^{3}H)-NORADRENALINE BY THE MOUSE VAS DEFERENS

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Ketamine has been widely used as both an induction anaesthetic and as a supplement during anaesthesia (McCarthy et al., 1965; Wessels et al., 1973). Its use is frequently associated with cardiovascular effects including increases in cardiac output, pulse rate and arterial pressure (Johnstone, 1976). Evidence would now suggest that these sympathomimetic effects of ketamine result from a 'cocaine-like' action on the uptake of noradrenaline (Liao et al., 1979) however little quantitative information of the potency of the drug in this respect is available.

Vasa deferentia from TO strain mice were suspended in oxygenated Krebs solution. Twitch responses to electrical field stimulation (0.2Hz, 1ms, 64V) were recorded isometrically and the effects of ketamine hydrochloride were observed by constructing cumulative dose-response curves over the dose range 100 - 1000μM. Ketamine produced a dose-dependant inhibition of the twitch reponse to a maximum inhibition of 88 ± 6% of the control (mean ± SD; n = 8) and the concentration eliciting a half-maximal twitch inhibition (IC50) was 552 ± 60μM. Cimetidine (100μM), naloxone (60nM) and verapamil (20μM) all failed to elicit any significant change in the dose-response characteristics. Similarly the inhibitory potency of ketamine was unaffected by changes in the frequency of tissue stimulation over the range 0.2 - 10.0Hz. Yohimbine (100nM) significantly decreased the effects of ketamine on the twitch response, however, increasing the concentration of yohimbine (300nM) failed to cause any further reduction.

The effects of ketamine (30 - 500μM) on the uptake of [^{3}H]-noradrenaline was measured in intact vasa deferentia in the presence of oestradiol (3.7μM). Single vasa were incubated with 1- 7-,8-[^{3}H]-noradrenaline (100Ci/mmol; 10ng per incubation) at 37°C for 10min and the resulting uptake determined by liquid scintillation spectrometry of a catechol-containing extract of a deproteinized homogenate of the vasa. Ketamine inhibited [^{3}H]-noradrenaline uptake in a dose-dependant fashion over the concentration range 30 - 500μM, the concentration producing 50% inhibition of uptake being 86 ± 17μM.

The results supported a 'cocaine-like' mechanism of action of ketamine and provided no evidence that an interactions with opiate receptors, histamine H_2-receptors or calcium occur in this tissue. The non-competitive nature of the antagonism by yohimbine of the ketamine response would indicate that it is unlikely that ketamine acts upon prejunctional α_2-receptors and in addition the lack of stimulation frequency dependence also argues against a prejunctional site of action. The actions of ketamine on the uptake of noradrenaline while qualitatively similar to those for cocaine show a considerably lower potency. However, the IC50 value observed was well within the range of plasma concentrations found to produce anaesthesia in man (60 - 200μM; Zsigmond & Domino, 1980) and it is possible that inhibition of neuronal uptake of noradrenaline makes a more significant contribution to the drug's sympathomimetic effects than previous work might suggest.

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EFFECTS OF HOUSING DENSITY ON NOCICEPTION OF HEAT AND PRESSURE AND THE HYPERALGESIC ACTIONS OF NALOXONE AND Mr 1452

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Prolonged crowding differentially modifies analgesic actions of μ- and κ-opioid agonists in rats (Pilcher & Browne, 1982). This may reflect the possibility that these two receptor systems mediate nociception of different types of stimuli (Tyers, 1980). We now report the effects of housing density on nociception of heat and pressure and on the hyperalgesic actions of naloxone and the more selective κ-antagonist, Mr 1452.

Male hooded rats were raised from weaning in isolated (I), crowded (C) or noncrowded (NC) conditions as described previously (Pilcher & Jones, 1981). Analgesic testing was carried out between 10.00 and 12.00 h using standard paw pinch and tail immersion procedures. Nociceptive pressure thresholds of non-inflamed hind paws were determined using an Analgesy meter. Tail immersion tests were conducted at 50 (± 0.2)°C. Thresholds of NC rats were taken as the basis for comparison. All injections were s.c. in a volume of 1 $mlkg^{-1}$.

Isolation and crowding both raised pressure thresholds and the elevation by isolation was significantly greater than that by crowding (Table 1). The threshold for heat was raised by isolation but, in contrast, it was lowered by crowding.

Table 1 Nociceptive thresholds of differentially housed rats

Housing condition	Mean pressure threshold (g)	%difference from NC	Mean latency (sec)	%difference from NC
NC (n=60)	107.8 ± 0.52	-	5.21 ± 0.03	-
C (n=60)	125.0 ± 0.65	+16.0*	4.00 ± 0.03	-23.2*
I (n=60)	136.2 ± 0.73	+26.3*(†)	6.33 ± 0.04	+21.5*(†)

*$p < 0.001$; † difference from C; $p < 0.001$

Naloxone (0.5 - 5 $mgkg^{-1}$) and Mr 1452 (0.01- 10 $mgkg^{-1}$) produced bell-shaped dose-response curves with both stimuli. Naloxone- and Mr 1452-induced hyperalgesia to pressure was greater in I ($p<0.02$) and C ($p<0.01$) rats at all doses. In both groups Mr 1452 was more potent than naloxone, maximal effects occurring at 0.5 and 2.0 $mgkg^{-1}$, respectively. With heat, hyperalgesia induced by both antagonists was greater in I rats only ($p<0.05$, both cases), but whereas the maximal effect with naloxone again occurred at 2.0 $mgkg^{-1}$, Mr 1452 was less potent with this stimulus and hyperalgesia was greatest at 3.0 $mgkg^{-1}$.

Differences in the relative potencies of naloxone and Mr 1452 with heat and pressure are compatible with μ- and κ-agonist studies and support the notion that κ-receptors may selectively mediate non-heat nociception. However, differential effects on the two antagonists by crowding or isolation were not detectable here.

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TOFISOPAM ENHANCES THE ANTICONVULSANT ACTIVITY OF DIAZEPAM AGAINST SOME, BUT NOT ALL, CONVULSIVE AGENTS

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Tofisopam (TP) a new clinically active anxiolytic possesses an unusual neuropharmacological spectrum related to its 3,4 benzodiazepine structure. By itself TP has no anticonvulsant, myorelaxant or anticonflict activity in animals (Stenger et al., (1984) ; nor does it displace the binding of ^{3}H-flunitrazepam. Indeed TP has been reported to increase ^{3}H-flunitrazepam binding and to enhance the antipentetrazol activity of diazepam (Saano, 1982, Mennini et al., 1982). In a recent study TP was also shown to enhance the anxiolytic activity of diazepam in the Geller-Seifter conflict situation (Stenger et al., 1983). We report here the enhancement by TP of the anticonvulsant activities of diazepam against different convulsant agents.

Male mice (20-24 g, 10 animals per dose) were treated with TP (50 mg/kg p.o.) associated with different doses of diazepam (0.1-5 mg/kg p.o.). 30 min. later the ED_{100} dose of the convulsant was given. The results presented in the table are expressed as the median effective dose (ED_{50}) which inhibits the tonic convulsion (or the mortality in experiments with strychnine and isoniazide) by 50 %. The ED_{50} with associated 95 % confidence limits were calculated by the method of Bliss as modified by Carmines et al., (1980).

Table 1. Enhancement of the anticonvulsant activity of diazepam by tofisopam.

	ED_{50} for diazepam (mg/kg p.o.)		
Convulsive agent	diazepam	tofisopam + diazepam	P
Pentetrazol	0.43 (0.31 - 0.74)	0.23 (0.13 - 0.40)	<0.05
Bicuculline	0.61 (0.35 - 1.04)	0.52 (0.38 - 0.72)	NS
Picrotoxin	2.19 (1.45 - 3.21)	0.81 (0.48 - 1.33)	<0.001
Electroschock	1.03 (0.68 - 1.58)	0.47 (0.26 - 0.83)	<0.05
Isoniazide	1.27 (0.84 - 1.92)	0.42 (0.25 - 0.67)	<0.001
Nicotine	0.62 (0.52 - 0.75)	0.52 (0.31 - 0.84)	NS
Strychnine	1.03 (0.70 - 1.50)	1.45 (0.75 - 2.78)	NS

The enhancement of the anticonvulsant activity of diazepam against some but not all convulsive agents suggests that TP may be a useful tool for the differentiation of benzodiazepine receptor subtypes and their relationship with various properties of the benzodiazepines.

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CHLORDIAZEPOXIDE ENHANCES THE INHIBITION OF VTA DOPAMINERGIC NEURONES PRODUCED BY NUCLEUS ACCUMBENS STIMULATION

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It is now well documented that electrical stimulation of the nucleus accumbens produces an inhibition of firing of dopaminergic neurones in the ventral tegmental area (VTA). This inhibition is blocked by picrotoxin indicating that it is mediated by GABA.

However, recent work (Yim, & Mogenson, 1980) has raised the possibility that GABA antagonists may mask, rather than abolish, this inhibition, as they produce an increase in the basal firing rate of the cells (Wang, 1981). It was therefore suggested that dopamine, not GABA, may be the inhibitory neurotransmitter involved following antidromic stimulation. Benzodiazepines are known to potentiate the inhibitory effect of GABA on CNS neurones (Geller et al, 1978). In the present investigation we have studied the effects of chlordiazepoxide on the inhibition of VTA neurones produced by nucleus accumbens stimulation.

Male Wistar rats (150-200g) were anaesthetised with chloral hydrate and maintained on 1-2% halothane. Extracellular single unit recordings were made with a single glass micropipette inserted into the VTA. The position of the recording electrode was later confirmed by histology.

Dopaminergic cells were identified using the criteria originally used for cells in the substantia nigra (Guyenet & Aghajanian, 1978). Type I cells, having a slow random firing pattern (<8Hz) with wide duration action potentials (3-4mS), were used. Square-wave stimuli of 0.5 mS pulse width were delivered at 1 Hz through a bipolar electrode in the nuclues accumbens. The current used was just above that required to produce consistent inhibition (2.0-3.0 mA). No cells included in this study showed antidromic spikes (cut off point 4.0 mA). Inhibitions were measured on a storage oscilloscope and from 64 or 128 sweeps of a 1000 ms duration post-stimulus histogram (PSTH).

Twelve cells were studied, having a duration of inhibition following stimulation of 173 ± 17 (s.e. mean) mS. Three minutes following 10 mg/kg chlordiazepoxide, introduced by the femoral vein, this inhibition duration was increased to 297 ± 36 ($P<0.01$). In 7 cases the basal firing rate of the cells was decreased by approximately 25% following chlordiazepoxide. The number of PSTH sweeps were therefore increased to maintain the number of sampled spikes for statistical validity. Furthermore, in 6 separate cells 0.1mg/kg haloperidol i.v. had no effect on either basal firing rate or duration of inhibition. Higher doses (up to 1 mg/kg) of haloperidol increased basal firing rate but did not effect the duration of inhibition.

This enhancement of inhibition produced by chlordiazepoxide provides further evidence for the involvement of GABA in the inhibition of the VTA produced on electrical stimulation of the nucleus accumbens.

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DO INTRAVENOUS BENZODIAZEPINES ENHANCE GABA-MEDIATED INHIBITION IN THE CEREBELLUM?

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Local application of benzodiazepines enhances GABA-mediated inhibition of Purkinje cells evoked by activation of basket and stellate interneurones by stimulation of parallel fibres, with little change in the firing rate of the cells (Geller et al, 1980). Intravenously administered benzodiazepines reduce simple spike firing of Purkinje cells and increase the duration of this evoked inhibition. However, it is necessary to control for changes in firing rate before any direct drug effect can be demonstrated.

Spontaneously active cells were recorded with tungsten microelectrodes (8-13 megohm impedence) in the cerebellar cortex of urethane-anaesthetised rats. Neurones were positively identified as Purkinje cells by the presence of complex spikes where possible, although they occurred infrequently. All cells tested were inhibited by bipolar stimulation (0.2-1.0msec pulses, 0.5Hz repetition rate) via insulated tungsten wires (0.5-1.0mm separation) and were therefore assumed to be Purkinje cells. The duration of inhibition was measured after construction of peristimulus-time histograms (PSTH) from 64 or 128 sweeps.

Firing rates were generally constant and ranged from <1Hz to 80Hz (mainly 5-20Hz) suggesting a depressant action of urethane. The duration of inhibition was greatest (100-200msec) at low firing rates (<5Hz) and smallest (20-50msec) at high firing rates (>40Hz). Analysis of the maximum duration of inhibition obtained during stimulus strength-response investigations on 92 cells indicated an exponential relationship between the mean firing rate during construction of the PSTH and maximum duration of inhibition. In a small number of cells which exhibited cyclic firing rates the same relationship between firing rate and duration of inhibition was observed for each cell.

The effect of benzodiazepines (diazepam 0.5-1mg/kg i.v.; CDZP, 1-5mg/kg; RU 32007, 8-nitro-6-(o-chlorophenyl)2,4-dihydro-2-(N-ethylpiperazin-1-yl) methylene-1H-imidazo[1,2a][1,4]benzodiaze-pine-1-one, 0.5-3 mg/kg i.v.) were studied on just suprathreshold responses to electrical stimulation. These drugs decreased the firing rate and increased the duration of inhibition but it is possible that the effect on inhibition was entirely a consequence of the altered firing rate. In three neurones with cyclic firing rates RU 32007 decreased firing and reduced or abolished cyclic firing but the relationship between firing rate and duration of inhibition was not altered. In some cells subsequent and often higher doses of RU 32007 did not decrease the firing rate further despite decreases in respiratory rate of the rats. In 4/8 of these recordings there were small increases in the duration of inhibition (8-35%) which were thus independent of firing rate. This effect lasted for only 8-15 min and may represent a weak direct enhancement of stimulus-induced inhibition.

These data suggest that there is little direct enhancement of this GABA-mediated inhibition by intravenously administered benzodiazepines in this preparation and the decrease in firing rate which they induce is not a consequence of enhancement of basket and stellate cell inhibition.

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STEREOTYPED BEHAVIOUR AND BODY TEMPERATURE OF RATS WITHDRAWN FROM SHORT TERM TREATMENTS WITH OXYPERTINE OR HALOPERIDOL

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Three week treatment of rats with oxypertine increased the specific binding of ^{3}H-spiroperidol in the corpus striatum and ^{3}H-WB4101 in the hippocampus (Fakouhi et al, 1982). We therefore sought correlates of these receptor effects.

Male Wistar rats (groups of 5, initial weights 120-150 g) were given oxypertine (35 mg/kg daily) or haloperidol (5 mg/kg daily) in drinking water for 10-20 days. After 3 days withdrawal, neuroleptic-treated rats and controls were given d-amphetamine (0.5 to 15 mg/kg, i.p.), caged individually for 40-60 min, then scored for behaviour over 1 min and decapitated. Thoracic cavity temperature was then taken by thermocouple. The behaviour rating scale (0-6, Creese & Iversen, 1975) was extended (7-8) to score backward locomotion, circling and loss of righting reflex. Alternatively apomorphine (0.1 to 1 mg/kg s.c.) was given; after 30 min behaviour was observed, then rectal temperature was measured. Ambient temperatures were 19-23°C throughout.

Amphetamine increased (max approx 2.6°C) thoracic cavity temperature of controls, in a dose-dependent manner; behaviour score increased to a mean of 7.5. Sensitivity to amphetamine, particularly at 5-10 mg/kg doses, was increased (approx 2-fold) in rats pretreated with oxypertine or haloperidol, but the maximum responses were unchanged (Table 1). There were no effects in the absence of amphetamine. Apomorphine lowered the rectal temperature of controls (max effect 2°C, 0.5 mg/kg) more markedly than of neuroleptic treated rats (haloperidol, max Δt 1°C, $P < 0.05$) although it did not elicit behaviour differences. Thoracic cavity temperature was affected like rectal temperature (oxypertine, Δt 1°C, $P < 0.01$).

TABLE 1:

Amphetamine	OXYPERTINE Δt (°C)	OXYPERTINE Δscore	HALOPERIDOL Δt (°C)	HALOPERIDOL Δscore
1.5 mg/kg	0.5 (0.3)	0 (- , 9)‡	-	-
5	1.0 (0.4)	1.6 (0.4, 20)	0.8 (0.4)	0.8 (0.4, 10)
7.5	1.1 (0.4)	1.7 (0.6, 20)	-	-
10	1.5 (0.6)	1.3 (0.6, 20)	1.6 (0.8)	2.8 (1.0, 10)
15	0.6 (0.7, 20)+	-	0.1 (0.5)+	0.3 (0.9, 19)‡

Mean response neuroleptic treated rats minus mean response controls (Δscore or Δt) quoted for each dose amphetamine; in parentheses: sum S.E. means neuroleptic treated and control groups; second figure (also applies to corresponding Δt where not indicated) n, usually equal for neuroleptic treated rats and controls. Two tailed t-test applied to original group means, increases significant ($P < 0.05$) unless marked+, Mann-Whitney U-test ‡

Thus thermoregulation may involve receptors, including DA receptors, capable of mediating supersensitivity. Stereotyped behaviour suppresses locomotor activity (Creese & Iversen, 1975) so that supersensitivity in amphetamine hyperthermia may not result from behavioural supersensitivity. Neuroleptic induced protection against apomorphine hypothermia, without overt behavioural effects, may support this. Central 5HT is also implicated in thermoregulation and in backward locomotion and circling. The greater incidence of these locomotor behaviours in rats after neuroleptic treatment may indicate 5HT receptor supersensitivity.

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THE EFFECTS OF GLYCOL SOLVENTS IN SOME SIMPLE TESTS FOR CNS ACTIVITY

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Propylene glycol (PG) and polyethylene glycol 400 (PEG) are commonly used to dissolve drugs with poor solubility in water prior to administration to laboratory animals. However little information (Budden et al, 1978; Singh et al, 1982; Zaroslinski et al, 1971) has been published on the pharmacological effects of such vehicles.

We have examined various concentrations of PG and PEG for pharmacological effect in mice following oral administration and, also, the effect of using a 40% solution of each glycol in water as vehicle on the pharmacological activity of midazolam maleate, a water soluble salt of a benzodiazepine, in a range of tests for CNS activity (leptazol and electroshock convulsions, inclined screen, rotarod, ethanol and barbiturate potentiation and acetylcholine induced writhing).

Using a dose volume of 10 ml/kg PEG at concentrations up to 80% had no effect on responses in any of the tests examined when compared with water treated controls. PG, however, had significant anticonvulsant, muscle relaxant and CNS depressant activity at concentrations as low as 40% but not 20%.

When midazolam maleate was dissolved in water, 40% PEG or 40% PG the activities shown in Table 1 were obtained.

Table 1 Activity of midazolam maleate in various vehicles

Test	ED_{50} in each solvent (mg/kg p.o.) Water	PEG	PG
Leptazol	3.7 ± 1.6	5.8 ± 1.6	1.0 ± 0.4
Electroshock	2.6 ± 1.0	1.8 ± 0.6	<0.25*
Inclined screen	56 ± 12	55 ± 12	11.0 ± 1.6*
Rotarod	4.8 ± 1.5	4.9 ± 1.9	1.7 ± 0.4*
Ethanol potentiation	20.0 ± 7.1	16.0 ± 1.5	6.1 ± 1.5
Barbiturate potentiation	5.6 ± 1.2	5.6 ± 1.6	1.3 ± 0.5*
Writhing	>40	>40	>40

n = 10/group, at least 3 groups per test.
*Significantly different from water, $p<0.02$.

These data indicate that using PG as a vehicle increased the apparent activity of midazolam maleate whereas the use of PEG as solvent produced similar results to those in water.

It is concluded that PEG is a preferable vehicle to PG for dissolving water insoluble compounds for CNS testing in mice as the latter solvent has activities which may modify those of the compound under test.

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DOPAMINE AUTORECEPTOR AGONISM DEFINED BY INHIBITION OF L-DOPA SYNTHESIS AND SPIPERONE-SENSITIVE EXPLORATORY LOCOMOTION

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Dopamine autoreceptor agonists inhibit locomotor behaviours in rats and mice (Di Chiara et al., 1976; Strombom, 1976). However this does not define the mechanism because locomotor behaviours can be suppressed by a wide range of drugs acting via other mechanisms (eg. α_2 agonism, sedation etc.). Dopaminergic specificity in locomotor models can be tested by reversal of the effect by dopamine antagonists at doses which are not themselves inhibitory. Additional confirmation that the type of activity is presynaptic and dopaminergic can be obtained by measuring in vivo inhibition of L-DOPA synthesis in nuc. accumbens or striatum using a method involving pretreatment of rats or mice with γ-butyrolactone (GBL) and the L-aromatic amino acid decarboxylase inhibitor NSD 1015. GBL inhibits impulse flow in dopaminergic neurones and thereby removes postsynaptic feedback.

We have compared the potencies of a range of drugs, including dopamine agonists and antagonists, an α_2 agonist and antagonist, an α_1 antagonist and anxiolytics/sedatives/hypnotics, to inhibit exploratory locomotor activity in mice and L-DOPA synthesis in rats.

An impressive correlation between the two measures was found only for dopamine agonists; the potency order was apomorphine, pergolide, N-(2-phenylethyl)-N-[2-(3-hydroxyphenyl)ethyl]propylamine (RU 24213), the N-(2-phenylethyl) analogue of (±) 3PPP, 2-dimethylamino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (TL-99), (+) 3PPP, and (±) 3PPP i.e. 3-(3-hydroxyphenyl)-N-n-propylpiperidine. The potency of (-) 3PPP, which inhibited L-DOPA synthesis only at 10 times the dose predicted from locomotor inhibition, was slightly anomalous. This may be due to a fine balance of agonist/antagonist properties in the molecule (Hjorth et al. 1983; Brown & Campbell, 1984). Other types of compound suppressed locomotor behaviour but did not inhibit L-DOPA synthesis; the potency order was clonidine, haloperidol, spiperone, prazosin, chlordiazepoxide, idazoxan (RX 781094), pentobarbitone-sodium, sulpiride, meprobamate. Furthermore the locomotor inhibitory effects of the dopamine agonists were in every case reversed by spiperone (0.02 mg/kg i.p.). In no case was a similar spiperone reversal of the inhibitory effect of a non-dopaminergic compound achieved. The α_2 antagonist RX 781094 reversed the inhibition caused by clonidine but was ineffective against dopamine agonists. Inhibition of new dopamine synthesis by α-methyl-p-tyrosine did not prevent the locomotor inhibitory effect of dopamine agonists demonstrating that their effect is not mediated only via suppression of synthesis; inhibition of release may also be involved.

The receptors involved in inhibition of dopamine synthesis appear to be very similar to those involved in locomotor inhibition. These are sensitive to low doses of dopamine agonists and are antagonised by low doses of spiperone. It is concluded that inhibition of exploratory locomotor activity which can be reversed by spiperone is diagnostic for dopamine autoreceptor agonists including those with some D_2 antagonism e.g. (-) 3PPP. In general it accurately predicts the potency of compounds in the more expensive and time consuming L-DOPA synthesis test.

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CHRONIC HYDROCORTISONE DOES NOT ALTER THE RESPONSE OF MICE IN CATECHOLAMINE-DEPENDENT BEHAVIOURAL MODELS

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Corticosteroids have been reported to change mental state, including the induction of depression (Carpenter & Bunney, 1971). After chronic hydrocortisone, however, mice responded normally in a putative behavioural model of depression (Porsolt test) despite a marked increase in 5-hydroxytryptamine (5HT) receptor sensitivity as assessed by the 5-hydroxytryptophan-induced head twitch model (Buckett & Luscombe, 1984). We have now investigated whether the lack of effect in the Porsolt test is due to the model being relatively insensitive to drug-induced changes in brain 5HT function (Porsolt, 1981), and/or due to a lack of change in cerebral catecholamine systems as assessed by the models of clonidine-induced behavioural hypoactivity and d-amphetamine-induced hyperactivity.

Male CD1 mice (15-17g; Charles River; n=10 per group) were chronically administered (p.o, b.i.d.) either hydrocortisone 21-hemisuccinate (10mg/kg) or deionised water (control group) for 2 weeks. Six hours after the final dose clonidine (0.1mg/kg i.p.)or saline was administered and the effect on behaviour was assessed 30 min later (Drew et al, 1977). A further group of mice was administered d-amphetamine (1 mg/kg ip) or saline 6h after the final dose of hydrocortisone or water, and activity was then measured by a Doppler activity recording system for 1 h. The acute effect of hydrocortisone (10mg/kg po) or deionised water administered 6 h prior to clonidine or d-amphetamine also was studied.

The behavioural hypoactivity induced by clonidine was not altered by either acute or chronic hydrocortisone administration (Table 1).d-Amphetamine-induced hyperactivity was similarly unaffected by chronic hydrocortisone administration.

Table 1 Effect of chronic and acute hydrocortisone (HC) administration on clonidine and d-amphetamine-induced behaviours

Length of pretreatment	Pretreatment/Test treatment: Water/Saline	Water/Drug	HC/Saline	HC/Drug
	d-amphetamine hyperactivity (mean ± SEM)			
Acute	278 ± 70	2122 ± 424	259 ± 83	2338 ± 502
Chronic	355 ± 78	1658 ± 320	332 ± 80	1710 ± 367
	Clonidine behavioural hypoactivity (mean score ± SEM)			
Acute	1.47 ± 0.10	7.00 ± 0.57	1.60 ± 0.19	6.80 ± 0.60
Chronic	1.30 ± 0.26	7.80 ± 0.57	1.40 ± 0.31	7.30 ± 0.54

Chronic hydrocortisone administration therefore does not modify the response of mice in either a noradrenaline-dependent or a dopamine-dependent behavioural model, in contrast to its induction of supersensitivity in a 5HT-dependent behavioural model (Buckett & Luscombe, 1984). The lack of effect of chronic hydrocortisone on brain catecholamine mechanisms may additionally explain its inability to induce "depression" in mice, as assessed by the Porsolt test.

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