ON THE ELECTROCHEMICAL DISCRIMINATION OF 5-HYDROXYINDOLES FROM URIC ACID IN THE RAT BRAIN IN VIVO

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In vivo voltammetry (IVV) is a promising technique for monitoring local activity in serotonergic systems in freely moving animals (Marsden, 1984). Carbon paste electrodes for the purpose are simple to fabricate, stable over long periods and robust. Two problems have arisen in their use in practice (i) slow voltammetric sweep rates have been used (1-2 mins) to improve resolution, which is less than ideal for the timescale of real behavioural responses and results in depletion of electroactive species (ii) even at these slow sweep rates uric acid is not resolved from the 5-hydroxindoles, and pharmacological evidence has been adduced that the major part of the baseline voltammetric signal at the 5-hydroxindole potential is due to uric acid (O'Neill et al 1984; Mueller et al., 1985). The use of voltammetry to monitor changes in 5HT release, or indeed changes in extracellular uric acid levels, will depend upon the ability to distinguish between 5-hydroxyindole and uric acid contributions to the signal. We have investigated a modified form of chronoamperometry, using positive followed by negative pulses, so that this discrimination can be made routinely with a sampling time of about twenty seconds.

In our hands, linear sweep voltammetry at carbon paste electrodes in pure solution confirmed that uric acid oxidised at the same potential as 5HIAA, but the use of cyclic voltammetry confirmed other reports that uric acid could not be re-reduced; 5-hydroxindoles in contrast showed a reduction peak at -250 mV. The oxidation of ascorbic acid showed an interaction with that of dopamine and DOPAC, with abolition of the reduction peak, but that of uric acid was simply additive with that of 5HT and 5HIAA, and the reduction peak was not abolished. For in vivo studies, electrodes and cannulae were fabricated and implanted as described by O'Neill et al (1984) except that a silver/silver chloride reference electrode, (in 0.9% NaCl) and a skull screw as auxilliary electrode, both on the dural surface, were used and equithesin was used as anaesthetic. After recovery from surgery, IVV was carried out using equipment as described by O'Neill et al (1984); a square wave voltage profile was applied consisting of 4 seconds successively at each of the following potentials: 0, +150, 0, +300, -300, 0, +500, -300, 0 mV, current being sampled each 200 msec. Administration of 5HTP (50 mg/kg, ip.) or 5HT (20 nmoles adjacent to the electrode) increased the signal at +300 and -300 mV. Uric acid (20 mg/kg, ip.) increased the signal at +300 mV but not that at -300 mV. Furthermore, Haloperidol (0.5 mg/kg ip.) was shown to increase the peak at +500 mV.

It is concluded that use of biphasic chronoamperometry should enable the contribution of 5-hydroxindoles to the in vivo electrochemical signal to be distinguished from that of uric acid. HVA can potentially be measured in the same sweep. Depletion of electroactive species is reduced. The rapid sampling made possible by this technique should be advantageous for experiments in which detailed behavioural studies are to be carried out contemporaneously in the freely moving animal.

We thank Robert O'Neill for helpful advice.

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N,N DIPROPYL-5-CARBOXAMIDOTRYPTAMINE (DP-5CT), AN EXTREMELY POTENT AND SELECTIVE 5-HT_{1A} AGONIST

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Recently the electrically stimulated guinea-pig ileum was described as a model for 5HT1A receptors (Fozard & Kilbinger, 1985). We have employed this model to determine agonist or antagonist activity of a series of putative 5HT1-selective compounds.

Segments of ileum from guinea-pigs (300-400 g) were suspended under a tension of 1.0 g in a Krebs solution of 37°C and intramural nerves were stimulated electrically with square wave pulses(4 msec, 0.2 Hz) Contractions of the longitudinal muscle were measured isotonically (HSE B368 levertransducers). Test compounds were added in a cumulative concentration schedule. The concentration required to reduce the twitch response by 50% (IC50%) is given in the table. In radioligand binding experiments the affinity for 5HT1A, 5HT1B and 5HT1C binding sites of rat brain cortex was determined, using the ligands (3H)-8OH-DPAT (1A), (125I)-CYP in presence of isoprenaline (1B) and (3H)-mesulergine (1C) (Hoyer et al, 1985). The results are summarised in the table.

Table

	pIC50%	SD	(n)	pKD: 1A	1B	1C
RU 24969	4.5 +/-	0.4	(4)	8.2	8.4	6.5
BEA 1654	< 4			7.5	5.8	4.0
5CT	7.3	0.3	(6)	9.7	8.5	6.2
DP-5CT	7.0	0.2	(4)	9.5	4.4	3.7
(-)8OH-DPAT	5.1	0.2	(4)	8.0	4.8	4.7
(+)8OH-DPAT	6.0	0.6	(6)	8.9	5.1	4.6

Abbreviations: RU 24969 = 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)1H-indole, BEA 1654 = 1-(3-acetylamino-phenyl)piperazine, 5CT = 5-carboxamidotryptamine and 8OH-DPAT = 8-hydroxy-2-dipropylamino-tetralin.

The active compounds all induced a complete inhibition of the twitch response. The putative irreversible 5HT1A antagonist N-(2-chloroethyl)-N-n-propyl-8-methoxy-2-aminotetralin (Fozard & McDermott, 1985) (10^{-7} M) inhibited the reduction in twitch amplitude, whereas the selective α_2-antagonist BDF 6143 (Armah & Cohnen, 1982) (10^{-8} M) was inactive. In this small series of agonists, only the 5HT1A receptor affinity was found to correlate (r=0.96), albeit the pharmacological response occured at concentrations 3 orders of magnitude higher than predicted by the binding. The relatively limited stereoselectivity of the antipodes of 8OH-DPAT is in agreement with observations by Hjorth et al (1981). The most interesting finding of the present study is however the extreme 5HT1A selectivity of the agonist DP-5CT.

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AN INVESTIGATION OF THE INFLUENCE OF CENTRAL 5-HT FUNCTION ON CLONIDINE-INDUCED HYPOACTIVITY IN MICE

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Clonidine administered at low dose to rodents induces hypoactivity, a distinct form of sedation (Drew et al, 1979; Heal et al, 1981). Although this behaviour is believed specific to α_2-adrenoceptors (Drew et al, 1979; Heal et al, 1981) little is known about the possible influence of other neurotransmitters. Kostowski et al (1981) reported that in rats the locomotor activity depressant effect of clonidine was prevented by lesioning 5-hydroxytryptamine (5-HT) neurones in the raphé. We have, therefore, now examined the effects on clonidine hypoactivity in mice of acutely manipulating brain 5-HT function. Adult male C57/Bl/6 Ola mice (Olac, Bicester) weighing 24-30 g were used. Drugs were injected intraperitoneally and pretreatment times are given in parentheses with the dose. No drug used produced overt sedation at the time of clonidine injection. After administration of clonidine (100 μg/kg) hypoactivity was rated (0-3) using 5 behavioural parameters (passivity, tactile responsiveness, posture, gait and body sag) as previously described (Heal et al, 1981). The selective 5-HT re-uptake inhibitor zimeldine (1 or 10 mg/kg; 60 min) had no effect on sedation responses. These were similarly unaffected by the non-selective 5-HT agonist quipazine (0.25 or 2.5 mg/kg; 30 min). However, the 5-HT_1 agonist RU 24969 (0.2 or 1 mg/kg; 30 min) produced a modest dose-dependent increase in clonidine hypoactivity.

The non-selective 5-HT antagonist methysergide (1 or 10 mg/kg; 30 min) had no effect on clonidine hypoactivity at low dose but produced a moderate enhancement at high dose. However, metergoline (0.2 or 1 mg/kg; 30 min) produced a marked dose-dependent increase in this response. The selective 5-HT_2 antagonists ritanserin (0.1 or 1 mg/kg; 30 min) and ketanserin (0.1 or 1 mg/kg; 60 min) given before clonidine also both produced a moderate dose-dependent increase in hypoactivity. At high dose, ß-adrenoceptor antagonists also have 5-HT_1 receptor antagonist properties (Nahorski & Willcocks, 1983). While pindolol (10 mg/kg; 10 min) had no effect on clonidine responses (-)-propranolol (20 mg/kg; 45 min) slightly attenuated this response. However, this latter effect is unlikely to be due to 5-HT_1 antagonism since the inhibition was equally potent at low dose (2 mg/kg). Groups of mice were pretreated with desipramine (DMI; 5 mg/kg) and 60 min later were injected intracerebroventricularly with 5,7-dihydroxytryptamine (5,7-DHT; 50 μg) or saline ascorbate vehicle (2 μl). Clonidine hypoactivity was marginally enhanced by 5,7-DHT lesioning when tested 14 days later. Mean percentage 5-HT depletion ± S.D. was: cortex 65 ± 6.4; mid/hindbrain 64 ± 7.5: n = 9.

In conclusion, 5-HT agonists and antagonists, with the exception of metergoline, produced only modest changes in clonidine hypoactivity in mice. Furthermore, lesioning with 5,7-DHT produced only a marginal increase in this response, suggesting that central 5-HT function has only a very modest influence on this behaviour.

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CIRCADIAN VARIATION IN THE SENSITIVITY OF RAT FRONTAL CORTICAL NEURONES TO IONTOPHORESED 5-HT

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Neurones in the suprachiasmatic nucleus of the hypothalamus, hippocampus and thalamus show a circadian variation in their sensitivity to iontophoresed 5-hydroxytryptamine (5-HT) (Brunel & de Montigny, 1980; Mason, 1984). This study was undertaken to see if a similar circadian variaton in sensitivity to iontophoresed 5-HT is present in the frontal cortex.

Male Wistar rats were kept on a 12 hr light:12 hr dark (LD) lighting cycle (lights-on 08.00-20.00 hrs) or under continuous light (LL, 200 lux) for 8-12 weeks. Extracellular recordings were made from the frontal cortex (1.0-4.0 mm anterior to bregma) of rats anaesthetised with urethane (1.3-1.5 g/kg, i.p., supplemented when necessary) at times corresponding to both the resting and active phases of an unanaesethetised rat's circadian cycle. Effects of iontophoresed 5-HT creatinine sulphate (20 mM, pH 4.0) and GABA (20 mM, pH. 4.0) were observed on neurones recorded for periods of 30 min to 10 hours in experiments of up to 30 hours duration. Neuronal sensitivity was evaluated from the iontophoretic charge (ejection current (nA) time(s)) required to obtained a 50% reduction in firing rate (I.T50); a low I.T50 product reflecting a high sensitivity.

Frontal cortical neurones showed a circadian variation in their sensitivity to 5-HT. The period of maximum sensitivity occurred at 19.00-22.00 hrs (I.T50: 98$\pm$9 nC, n=33 cells, 9 rats; mean $\pm$S.E) and minimum sensitivity at 06.00-10.00 hrs (I.T50: 351$\pm$20 nC, n=41 cells, 8 rats; $P<0.01$ with respect to 19.00-22.00 hrs). No circadian variation in sensitivity to iontophoresed GABA was apparent (I.T50 19$\pm$1 nC, n=42 cells at 06.00-10.00 hrs; 16$\pm$1 nC, n=53 cells at 19.00-22.00 hrs).

Exposure to continuous lighting can induce a disruption of circadian rest activity behaviour in some individual animals (Mason, 1984). Recordings from frontal cortical neurones in rats exposed to continuous light showed either (i) no evidence of a circadian variation in 5-HT sensitivity (I.T50: 447$\pm$21 nC, n=9 cells at 06.00-10.00 hrs; 411$\pm$17 nC, n=10 cells at 19.00-22.00 hrs; 3 rats) or (ii) a variably phase-shifted circadian variation in sensitivity (5 rats) compared with LD exposed rats, consistent with free-running circadian behaviour.

The circadian system has recently been implicated in the aetiology of mental illness (Wehr et al, 1983) and circadian variation in sensitivity to 5-HT may have significance for the timing of drug therapy targeted on the serotoninergic system.

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EFFECT OF MINAPRINE ON ISCHEMIA-INDUCED AMNESIA AND ABNORMALITY IN ELECTROENCEPHALOGRAM IN MONGOLIAN GERBILS

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Bilateral occlusion of the common carotid arteries produces cerebral ischemia in the mongolian gerbil (M. gerbil) (Kahn, 1972, Payan and Conrard, 1977) and loss of neurological function. The neurological dysfunction is accompanied by a deterioration of memory functions. Minaprine is a new atypical antidepressant drug (Bizière et al. 1982) which has proven to be effective for the treatment of senile dementia in recent clinical trials in Italy (Passeri et al. 1985). The purpose of study was to examine the effect of minaprine on ischemia-induced amnesia and on the abnormalities in electroencephalogram (EEG) in M. gerbils.
Male M. gerbils (60-80 g Charles River, Japan) were used. Bilateral carotid arteries were clamped for 5 min, after which the clips were removed. For recording the EEG, electrodes were chronically implanted in the frontal cortex and dorsal hippocampus (P:2.5, L:2.5, H:2.0) according to the stereotaxic coordinates of Del Thiessen's brain atlas. The M. gerbils were trained in a conventional step-down type passive avoidance apparatus. Training and the passive avoidance tests were carried out 2 and 3 days after ischemic treatment, respectively. The response latency in non-ischemic M. gerbils was 55.0 $\pm$ 5.0 s and that in the ischemic group was 5.7 $\pm$ 1.1 s***. When minaprine in a dose of 50 mg/kg p.o. was administered 30 min before occlusion, it significantly improved the latency (51.4 $\pm$ 4.1 s^{+++}). Minaprine at low doses (1-5 mg/kg p.o.) significantly improved the memory deficit when administered before (5 mg/kg, 42.2 $\pm$ 7.4 s^{+}) and after (2 mg/kg, 22.2 $\pm$ 6.5 s^{+}) the training session and before (2 mg/kg, 29.7 $\pm$ 7.7 s^{+}) test session. EEG was recorded 1 h before ischemic treatment, for 6 h after that, as well as during 1 h, 2 and 7 days.

Immediately after 5 min occlusion, the EEG in the frontal cortex and hippocampus was completely flat. One to 2 h after occlusion, theta waves started to recover in the hippocampus. About 6 h after occlusion, the EEG had returned to the normal pattern. However, 2 days after occlusion, there was a reduction in the amplitude of hippocampal theta waves. With administration of minaprine (50 mg/kg p.o., 30 min before occlusion), the hippocampal theta waves appeared within 30 min in all occlusion-treated animals. The reduction in the amplitude of hippocampal theta waves 2 days after occlusion was also prevented when minaprine was injected before occlusion. These data suggest that minaprine may be effective for ischemia-induced neuronal dysfunction and could be beneficial in vascular-related dementia. Cerebral protecting effects, demonstrated in other experiments on gasping, KCN lethality and hypoxia may relate to this prevention. Furthermore, the improvement of acquisition, consolidation and recall by low doses of minaprine may relate to the dopaminergic stimulating effect, since minaprine has central dopaminergic stimulating effects, at low doses (Bizière et al. 1984)

*** $p < 0.002$ vs non-ischemic animals. $^{+++}$ $p < 0.002$, $^{+}$ $p < 0.05$ vs saline injected ischemic animals (Mann-Whitney U test)

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THE CONCENTRATION-RESPONSE RELATIONSHIP FOR CARBACHOL ACTIVATION OF SINGLE CHANNELS AT THE FROG ENDPLATE

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Measurement of the concentration-response relationship at equilibrium for the nicotinic receptor is greatly complicated by the problems of desensitisation and channel block at high concentration of agonist. We have studied the activation of nicotinic ion channels by carbachol (CCh, concentration range 50 μM to 10 mM) by recording single channel currents from the skeletal neuromuscular junction of *Rana temporaria* (membrane potential -110 mV, temperature 12°C). Under these conditions currents from a single channel occur in bursts of high activity separated by long, silent desensitised periods (Sakmann, Patlak & Neher, 1981, Ogden and Colquhoun, 1984). The fraction of time for which a channel was open, p_O, was recorded during bursts of high channel activity, after exclusion of silent desensitised intervals. The results are compared with those recently presented for acetylcholine (ACh) by Ogden (1985).

The value of p_O increased from 8% at 100 μM CCh to a maximum of 51% at 500 μM, then declined at concentrations higher than 1 mM to 20% at 10 mM. Previous studies have shown that the decline of p_O is due to block of the open ion channel by free CCh (Ogden & Colquhoun, 1985).

The results were fitted by least squares to a reaction scheme with two sequential binding steps (microscopic dissociation constants K_1, K_2) followed by channel opening (equilibrium constant β/α) and then block of the open channel by free CCh (equilibrium constant K_B).

$$R \underset{}{\overset{K_1/2}{\rightleftharpoons}} AR \overset{2K_2}{\rightleftharpoons} A_2R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2R^* \overset{K_B}{\rightleftharpoons} A_2R^*A$$

A good fit was obtained with the following values (corresponding values for ACh in parentheses): $K_1 = K_2 = 880$ μM (ACh, 77 μM), $\beta/\alpha = 9.4$ (ACh, 32) and $K_B = 2.6$ mM (ACh, 1.5 mM). These results suggest that CCh is a rather strong agonist which would be capable of opening 90% of ion channels at high concentration in the absence of desensitisation and channel block, though its affinity is low.

The data imply that CCh has a 21-fold lower potency than ACh (at low concentration). This is a result partly of the lower equilibrium constant for channel opening, which contributes a factor of about 2, but mainly of the 11-fold lower binding affinity of CCh for the receptor.

Our observations also suggest that individual activations of the channel by CCh should contain fewer openings and briefer interruptions than are seen with ACh. This is in agreement with observations by Colquhoun & Sakmann (1985).

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SPONTANEOUS COUPLING OF THE β-ADRENERGIC RECEPTOR TO N_s IN MAMMALIAN CARDIAC MEMBRANES

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The alkylating reagent, N-ethylmaleimide (NEM) is a valuable tool for investigating the interaction between the ß-adrenergic receptor (ßAR) and the stimulatory nucleotide regulatory protein, N_s (Korner et al, 1982). As previously described for turkey erythrocytes, S49 lymphoma cells and rat lung, NEM alone did not affect the number of ßAR, but there was a 50-60 % reduction in the number of ßAR when a ß-agonist was concomitantly present (Vauquelin & Maguire, 1980; Severne et al, 1985). This decrease in receptor number was completely abolished in the presence of GTP.

In the present study we investigated the effect of NEM (in the absence of agonist) on ßAR in membranes prepared from the left ventricle of reserpinized (2 mg/kg 18 h before sacrifice) cat, guinea pig and rat. The concentration of noradrenaline in these membrane preparations was less than 0.1 nM. Membranes were incubated with increasing concentrations of NEM (10^{-5} - 10^{-3}M) for 10 min (30°) in the absence or presence of GTP (10^{-8} - 10^{-3}M). Aliquots of preincubated membrane suspension were then incubated with ^{125}I-Pindolol (IPIN) for 18 min (30°C). Specific binding (>95 %) was defined by $5 \cdot 10^{-5}$M isoprenaline. The effects of NEM (10^{-4}M) ± GTP (10^{-3}M) on the number of ßAR are shown in Table 1.

Table 1

	% Change in number of ß-adrenergic receptors		
Preincubation	Cat	Guinea pig	Rat
NEM (10^{-4}M)	-17 ± 7	-23 ± 8	-17 ± 4
NEM (10^{-4}M) + GTP (10^{-3}M)	+1 ± 3	-1 ± 8	+3 ± 6

Mean values ± s.d. (n=6-7)

The NEM induced reduction in the number of ßAR, as well as the capacity of GTP to abolish this reduction, were concentration dependent with a maximal effect at 10^{-4} and 10^{-3}M, respectively.

It is concluded that NEM (in the absence of a ß-agonist) reduces the number of ßAR by about 15-30 %. This effect is abolished in the presence of GTP. The effect of NEM on cardiac ßAR is compatible with a model wherein (part of) the receptors are able to undergo spontaneous coupling with N_s.

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NORADRENALINE RELEASE FROM THE ANOXIC RAT HEART: MECHANISMS OF RELEASE AND METABOLIC PREREQUISITES

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Stop flow ischaemia in the Langendorff perfused rat heart causes noradrenaline (NA) overflow, detected during reperfusion, after ischaemic periods of 10 minutes or longer which can be diminished by 80% by prior treatment with neuronal NA uptake blockers (e.g. desipramine, DMI) suggesting that such release occurs via "reversed transport" over the NA uptake carrier (Schömig et al, 1984). The following experiments were performed to determine the particular metabolic conditions required for such a release since perfusion at 5% of normal flow (with a normoxic perfusate containing substrate) is sufficient to prevent this increased overflow (Dart & Riemersma, 1985).

Rat hearts were perfused (Langendorff method) at constant flow (5-6ml/g/min) with a modified Krebs-Henseleit solution at 37°C, pH 7.4. The perfusate initially contained 5.5mM glucose and 1.8mM pyruvate with PO_2 > 500mmHg. After stabilisation and collection of control samples perfusion continued with PO_2 < 1mmHg but unchanged flow. Anoxic perfusate was produced by gassing with 95% N_2/5%CO_2 with subsequent addition of sodium dithionite (0.5mM). Experiments were performed in which the perfusate contained no substrate, no substrate and DMI (100nM) or 11mM glucose. Samples for NA estimation were assayed using HPLC separation and electrochemical detection. Results are shown in the Table.

NA Concentration in Effluent (pmol/ml)

Anoxic perfusate containing	n	Control	Anoxia			
			10	20	30	40(min)
No substrate	6	<0.5	<0.5	7.3±0.7	15.1±1.6	10.5±0.5
No substrate + DMI	6	<0.5	<0.5	2.5±0.3	3.1±0.2	5.3±0.9
11mM glucose	6	<0.5	<0.5	<0.5	<0.5	<0.5

Values are mean ± sem

These results show that anoxic mediated NA release is significantly depressed by DMI ($p < 0.05$) and that anaerobic metabolism of glucose is sufficient to prevent this release. These results further support the hypothesis that NA release does not occur by exocytosis but by carrier mediated efflux - the carrier being identical with that normally responsible for neuronal NA uptake. The severe conditions necessary for such release suggest that in vivo it would be restricted to areas of extreme flow reduction thus causing differential catecholamine stimulus within the ischaemic territory - a potentially potent arrhythmic stimulus.

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THE EFFECT OF THE PROPRANOLOL METABOLITE NAPHTHOXYLACTIC ACID ON CARDIAC β-ADRENOCEPTORS

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Propranolol is extensively metabolised in vivo, one of the main urinary metabolites being naphthoxylactic acid (NLA) (Walle and Gaffney, 1972; Walle et al, 1979). Plasma concentrations of NLA in renal failure have been shown to be up to 37 times that in healthy controls (Stone and Walle, 1980). The high NLA levels in patients on long term propranolol therapy and potential NLA accumulation in patients with severe renal disease indicates the need to establish whether this metabolite is associated with biological effect.

This project examined the effect of NLA on cardiac β-adrenoceptors utilising two methods, the inhibition of the inotropic effect of isoprenaline on isolated rat atria (Ogg et al, 1985) and secondly the ability of NLA to inhibit the binding of (^{3}H)-dihydroalprenolol (DHA) to rat ventricle membrane preparations (Kunos et al, 1980). The activity of NLA (10^{-7} - 10^{-4}M) towards the β-adrenoceptor was compared with that of propranolol (10^{-11} - 10^{-6} M) in both techniques. From dose-response curves produced the ED_{50} values were determined and Schild plots obtained. The results for the isolated tissue experiments are shown in Table 1.

Table 1 - The inhibition of the inotropic effect of isoprenaline
mean ± SEM

Drug	Slope of Schild Plot	pA_2	Log Affinity Constant (K_2)
Propranolol	0.98 ± 0.15	9.12 ± 0.33	9.32 ± 0.27
NLA	0.94 ± 0.13	5.76 ± 0.34	6.19 ± 0.16

For radioligand binding the DHA concentration was 8.35 x 10^{-8} M and homogenate equivalent to 0.3mg protein was used. The membranes were incubated for 20 min and the bound and free drug separated by vacuum filtration, specific binding being determined with L-alprenolol as the competing ligand. Scatchard analysis of the specific binding gave a clear linear relationship (Kd = 1.36 ± 0.1 x 10^{-9} M; Bmax = 172.6 ± 52.5 x 10^{-12} mol/mg protein) and in the presence of NLA, showed no evidence of multiple binding sites. Table 2 shows the IC_{50} values determined from the inhibition curves and the respective calculated Ki and pA_2 values.

Table 2 - Inhibition of (^{3}H)-dihydroalprenolol binding (nM)
mean ± SEM

Drug	IC_{50}	pA_2	Ki
Propranolol	1.54 ± 1.08	9.38 ± 0.34	0.99 ± 0.66
NLA	210000 ± 64500	4.05 ± 0.27	131000 ± 39900

The difference in order of potency of the two assessment procedures is probably not unexpected in view of the grossly different nature of the systems used. It is reasonable to suggest that there will be relatively little effect from NLA on the overall β-blockade produced by the parent drug, since in both assays its potency was very much lower than that of propranolol itself.

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AMIODARONE INHIBITION OF HEPATIC OXIDATIVE DRUG METABOLISM: AN ACUTE AND CHRONIC STUDY

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Amiodarone (AD) interacts with warfarin, phenytoin and other drugs possibly due to inhibition of hepatic metabolism. We examined the effects of AD on hepatic oxidative drug metabolism (HODM) in Wistar rats, using an in vivo aminopyrine breath test (ABT) (Desmond et al, 1980) and on the level of hepatic microsomal cytochrome P-450 (Omura & Sato, 1964).

In an acute study control ABT, following i.p. injection of 1 μCi/kg of ^{14}C-aminopyrine, were recorded in each group 24h. prior to dosing with AD (increasing doses) for 3 days and 1h., 48h., 2 weeks, 4 weeks and 6 weeks following dosing the regimen below. In a chronic study control ABT were recorded 24h. prior to dosing with AD (100 mg/kg/day i.p. for 1 month) and at weekly intervals during and after the treatment for a total period of 8 weeks. The results are summarised in Table 1 (means ± SEM).

Table 1 Effect of AD on ABT ($t_{½}$ in min) and P-450 content (as nmol/mg protein)

Acute study (3 days i.p.)	Control	1 h.	48 h.	2 w.	4 w.	6 w.	(P-450)
Group I : saline	46.8±1.6	46.7±5.1	47.1±2.3	46.81±1.9	47.3±2.6	48.3±1.7	(0.31±0.01)
Group II : 50 mg/kg/day	44.9±0.9	50.2±0.9**	57.5±1.3**	48.1 ±4.8	48.1±2.1	47.2±2.3	(0.33±0.08)
Group III : 100 mg/kg/day	43.0±0.4	56.3±1.5**	63.4±1.8**	55.8 ±1.9**	46.3±3.1	48.5±2.6	(0.11±0.02)**
Group IV : 200 mg/kg/day	46.8±0.6	89.5±7.1**	68.7±3.4**	57.9 ±2.7**	50.9±2.8	50.6±4.2	(0.07±0.01)**

Chronic study (1 month i.p.)	Control	1 w.	2 w.	3 w.	4 w.	5 w.	6 w.	7 w.	8 w.
Group V : 100 mg/kg/day (Weeks 1 to 4)	40.6± 0.9	57.1± 3.2*	47.5± 1.6*	48.8± 1.8*	48.7± 1.9*	42.1± 2.0	40.7± 1.0	40.2± 1.1	40.9± 2.0
P-450	(0.31± 0.06)	(0.05± 0.01)**			(0.03± 0.01)**				(0.03± 0.01)**

Significantly different from values (n = 6) before drug dosing. Student's t-test *$p < 0.05$ ** $p < 0.01$.

AD depresses the activity of HODM in a dose-dependent fashion and also has a long term inhibitory effect. Of interest during chronic AD dosing ABT returned to control values and there was no correlation between ABT and P-450 content,possibly due to a compensatory increase in extra-hepatic sites (e.g. kidney) of drug metabolism (Babany et al. 1985).

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INHIBITION OF GUINEA PIG LIVER MONOAMINE OXIDASE-B BY HORDENINE (N,N-DIMETHYL TYRAMINE)

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Hordenine is a selective substrate for the B form of monoamine oxidase (MAO) in rat liver (Barwell, 1985). In contrast the MAO activity in guinea pig liver mitochondria is inhibited by hordenine (Barwell et al. 1984). The different reactivity of rat and guinea pig liver MAOs towards hordenine could be due to differences in the MAO-A and MAO-B content of mitochondrial preparations or the kinetic properties of the enzymes. This has been investigated using the irreversible, selective inhibitor of MAO-A, Lilly 51641 (N-[2-(O-chlorophenoxy)-ethyl]-cyclopropylamine) (Squires, 1972) to determine the MAO-A and MAO-B content of guinea pig liver mitochondria and the effect of hordenine upon preparations containing only MAO-B.

Livers from three male guinea pigs weighing 500-600g were homogenised in 0.3M sucrose. Mitochondria were isolated by differential centrifugation and washed twice. MAO activity was assayed at pH 7.4 and 37°C using an oxygen electrode. The MAO-A and MAO-B content of mitochondria was determined by preincubation for 30 min at 37°C with various concentrations of Lilly 51641 and assaying the remaining activity with a saturating concentration of tyramine (10 mM). Preparations containing only MAO-B were obtained by treating mitochondria with a concentration of Lilly 51641 (10^{-5}M) which inhibited all of their MAO-A activity.

Titration of mitochondrial MAO with Lilly 51641 yielded a biphasic inhibition curve with a distinct plateau between 10^{-6}M and 10^{-5}M inhibitor. The plateau occurred when 48% of the total activity had been inhibited, indicating that the preparation contained essentially equal activities of MAO-A and MAO-B.

Hordenine was not oxidised by preparations containing only MAO-B activity, but it inhibited tyramine oxidation. Kinetic experiments revealed the type of inhibition. At 37°C, maximum inhibition occurred within two seconds of addition of hordenine and plots of percent inhibition against hordenine concentration were hyperbolic, indicating reversible inhibition. The form of Hanes plots (s/v against s) in the absence and presence of inhibitor was typical of noncompetitive inhibition and an inhibitor constant of 1.25 mM was determined from Dixon plots (1/v against I).

The results show that guinea pig liver contained a MAO-B, as defined by its sensitivity to a selective irreversible inhibitor. In this respect guinea pig was similar to rat (Barwell, 1985; Squires, 1972). However, the MAO-B forms in the liver of these species are not identical since hordenine is a substrate for the enzyme of rat liver but a noncompetitive reversible inhibitor of the guinea pig liver enzyme. Thus these results provide further evidence of the heterogenous nature of MAO types, as defined by their inhibitor sensitivities (Fowler et al. 1981).

Lilly 51641 was a gift from Lilly Research Laboratories, USA.

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INDUCTION OF DRUG METABOLISM BY PHENOTHIAZINE DRUGS: INFLUENCE OF LIPOPHILICITY

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Certain phenothiazine drugs have been established as inducers of drug metabolism. The capacity of a drug to attain sufficiently high concentrations within the smooth endoplasmic reticulum and result in a stimulation of the drug metabolising enzymes, has been discussed with reference to the drug's lipid solubility (Conney 1967). Perhaps therefore the potency of inducers is dependent upon their lipophilicity since in addition to governing their ability to penetrate the hepatic lipoidal barriers, lipophilicity may also influence their affinity for, and duration of binding to the enzymes. We have investigated the effects of pretreatment with phenothiazine drugs on the antipyrine breath test and assessed the influence of lipophilicity on the magnitude of the induced changes.

Groups of 8 GB-1 mice were each pretreated with one of the phenothiazine drugs; promazine (PZ), chlorpromazine (CPZ), triflupromazine (FPZ), promethazine (PMZ), perazine (PER) or prochlorperazine (PCP). The mice received three single daily doses (0.16 mmol/kg) given orally. Twenty four hours after the last dose of the phenothiazine, antipyrine (0.25 μCi, 0.8ng) was given i.p. and the animals housed in metabolism chambers designed for the collection of exhaled CO_2. Antipyrine half-life was determined from CO_2 exhalation rate kinetics over a seven hour period. Using a balanced crossover designed study, the control half-life of antipyrine was similarly determined either seven days prior to, or seven days after phenothiazine pretreatment. Apparent partition coefficients (Papp) of the drugs between octanol and pH 6.5 phosphate buffer were measured using shake flasks. Intrinsic log P (Pint) values represent partition coefficients corrected for the degree of ionization of the drugs, according to literature pK_a values.

	Antipyrine t1/2 (min)			% $^{14}CO_2$ exhaled			
	Control	Pretreat	% ΔK	Control	Pretreat	Log Papp	Log Pint
PZ	20.39	14.45	41.78	49.94	53.43	1.81	4.73
PMZ	17.96	10.05	78.25	47.78	53.07	2.09	4.69
PER	17.42	13.24	32.41	43.00	46.67	2.41	4.02
CPZ	19.00	10.84	79.05	45.04	46.81	2.53	5.33
FPZ	20.05	12.47	64.23	42.70	44.53	2.64	5.35
PCP	20.12	11.22	80.95	42.80	51.30	2.81	4.42

The results presented in the table indicate that all of the phenothiazines studied significantly decreased the half-life of antipyrine. Also the % $^{14}CO_2$ exhaled tended to be increased by phenothiazine pretreatment, although this was significant in only three of the six drugs studied, namely PZ, PMZ and PCP. This suggests that phenothiazines increase the clearance of antipyrine by both demethylation and non-demethylation pathways.

There were poor correlations between log Papp, and either changes in antipyrine half-life or elimination rate constant (K). Similarly log Pint values gave poor correlations with these parameters; in all cases $r<0.6$. In conclusion it appears that properties other than lipophilicity may be more important in determining the induction potencies of phenothiazines.

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(-) DEPRENYL: AN α-ADRENOCEPTOR ANTAGONIST ON THE RAT VAS DEFERENS

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(-) Deprenyl, a selective MAO-B inhibitor, inhibits contractions induced in the isolated rat vas deferens by tyramine (TA): an effect largely attributed to inhibition of the neuronal uptake of TA (Knoll, 1978). However, Finberg et al (1981) found the neuronal uptake blocking action of deprenyl (10μM) was not sufficient to explain its inhibiting effect on TA action. They suggested it might act by inhibiting active efflux of noradrenaline (NA) from the nerve ending following its release into the cytoplasm by TA. They also suggested that the lack of potentiation of NA responses in the presence of TA antagonism argued against a reduction of neuronal uptake. Deprenyl also possessed α-adrenoceptor blocking activity at higher doses, and they suggested that blockade of α-receptors might mask potentiation of NA in the presence of deprenyl. Our study provides evidence that deprenyl inhibits responses to indirectly acting amines by a dual action: inhibition of neuronal uptake and block of α-adrenoceptors.

Vasa deferentia from Sprague-Dawley rats (200-300g) were set up in Mg^{2+} -free Tyrodes bubbled with 95% O_2: 5% CO_2. Some rats received chronic treatment with guanethidine (injected i.p. 25mg/kg per day 5 days a week for 6 weeks) in order to destroy adrenergic nerves (Burnstock, et al., 1971).

Exogenous TA, octopamine (OA) and NA produced concentration-dependent contractions of the vasa. Half-maximal contractions were produced by 20μM TA, 30μM OA and 3μM NA. Deprenyl (5μM) inhibited responses to TA>OA>NA (Table 1). Chronic guanethidine pretreatment completely abolished responses to TA and markedly potentiated (about x15) responses to NA, with no effect on responses to OA; ie. NA (0.15μM) and OA (30μM) produced half-maximal contractions of the denervated vasa. Deprenyl (5μM) inhibited responses to NA and OA in denervated vasa by an increased amount (Table 1).

Table 1

	% Inhibition of Responses to		
	NA	TA	OA
Deprenyl (5μM)	32.6±1.7* n=12	79.3±1.4 n=4	61.2±4.2* n=4
Denervation + Deprenyl 5μM	70.9±2.2** n=8	--------	72.6±1.5** n=4

± S.E.M., t-test: *$p<0.001$; ** not significant

Thus in denervated vasa, deprenyl showed a small increase in inhibition of OA-induced responses ($P<0.05$) but a much larger increase in inhibition of NA-induced contractions ($P<0.001$). This unmasking of the deprenyl inhibition of NA-responses by denervation is evidence that, at this concentration (5μM), deprenyl has an α-adrenoceptor antagonistic activity as well as its neuronal uptake blocking activity. Each of the two activities will also inhibit responses to TA and OA. In untreated vasa the neuronal uptake blocking activity will tend to enhance NA action. Thus the two activities counteract each other, resulting in a smaller inhibition of responses to NA than to TA or OA.

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POTENTIATION OF THE GABA ANALOGUE MUSCIMOL BY PHYSIOLOGICALLY OCCURRING STEROIDS

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Selye (1941) showed that steroid hormones had CNS effects, by inducing anaesthesia in rats. It appeared that a saturated steroid molecule, oxygenated at the C_3 and C_{17} or C_{20} or C_{21} was required for good anaesthetic potency. This was particularly true for the pregnane group of progesterone metabolites (Selye 1942). These observations led to the development of steroid anaesthetics for clinical use, and in particular alphaxalone (Child et al 1971). Alphaxalone may mediate its anaesthetic effects via its modulation of the $GABA_A$ receptor complex (Harrison & Simmonds 1984). We have recently been examining the effects of two endogenous steroid metabolites similar in structure to alphaxalone on muscimol responses at the GABA receptor.

Slices of rat cuneate nucleus superfused with Krebs medium at 20°C (Simmonds 1980) were used to examine the effects of two 5β-pregnane derivatives, 5β-pregnan-3α-ol-20-one (epipregnanolone) and 5β-pregnan-3β-ol-20-one (pregnanolone), on the depolarizing responses of dorsal funiculus fibres to the $GABA_A$ receptor agonist muscimol.

Both epipregenanolone and pregnanolone produced a dose dependent potentiation of muscimol responses in the 0.3-10μM concentration range. Epipregnanolone produced a far greater leftward shift in the muscimol dose-response relationship than pregnanolone (Table 1), and appeared to be approximately equi-potent with alphaxalone in potentiating muscimol responses.

	POTENTIATION AS - LOG (LEFTWARD SHIFT)			
Steriod (μM)	0.3	1	3	10
Alphaxalone	0.27 ±0.040 (10)	0.33 ±0.037 (13)	0.39 ±0.120 (3)	0.46 ±0.115 (3)
Epipregnanolone	0.23 ±0.036 (4)	0.33 ±0.076 (5)	0.50 ±0.046 (5)	0.47 ±0.081 (3)
Pregnanolone	0.07 ±0.066 (4)	0.14 ±0.050 (5)	0.16 ±0.063 (5)	0.18 ±0.021 (3)

We therefore conclude that epipregnanolone and pregnanolone appear to have a similar action to alphaxalone at the $GABA_A$ receptor complex and that this interaction may be responsible for their anaesthetic property. The circulating concentrations of epipregnanolone required for hypnosis in the rat (Gyermek et al 1968) correlate well with those for enhancement of muscimol action at the $GABA_A$ receptor.

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EFFECT OF BACLOFEN ON THE RELEASE OF ENDOGENOUS 5-HT FROM MOUSE CORTEX SLICES

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The release of 5-hydroxytryptamine (5-HT) from slices prepared from rat brain cortex preloaded with [^{3}H]-5-HT has been shown to be inhibited by the $GABA_B$-receptor agonist baclofen (Bowery et al, 1980; Schlicker et al, 1984). This effect, however, only occurred in the presence of quite high concentrations of baclofen; Schlicker et al (1984), for example, obtaining a maximum inhibition of around 30% in the presence of (-) baclofen (1mmol/l). We have now investigated the effect of the (±)-baclofen on the K^+-evoked release of endogenous 5-HT from slices of mouse cortex and the effect of longer term administration of baclofen on this response.

Mice were killed and frontal cortex dissected out on ice and chopped on a McIlwain tissue chopper in two directions at 45° at 0.3 mm intervals. The slices were rinsed in Ca^{2+}-poor oxygenated Krebsbicarbonate buffer. Four tubes containing approximately 20 mg of tissue were incubated for 15 min in the oxygenated Krebs solution at 37° C, centrifuged at 30 s at 1000 x g and resuspended in Ca^{2+}-containing Krebs-bicarbonate buffer for a further 5 min. Ten µl of high K^+ solution (final concentration: 35mmol/l) as added to two tubes and 10 µl of K^+ -free buffer to the other tubes. Incubation was continued for a further 20 min, the tubes centrifuged for 30 s and 200 µl of supernatant removed for assay of 5-HT by high performance liquid chromatography as described by Molyneux and Clarke (1985). Pargyline and fluoxetine (50µ mol/l of each) were present throughout the incubation. (±)-Baclofen was present in the Ca^{2+}-containing buffer in appropriate experiments.

The K^+-evoked release of 5-HT was inhibited by (±)-baclofen added in the range 10 nmol/l-10 µmol/l in a concentration-dependent manner, release being totally inhibited by 10 µmol/l. (±)-Baclofen inhibited 5-HT release with an IC_{50} of 0.9 µmol/L. Pretreatment of mice for 14 days with baclofen (10 mg/kg)(i.p. once daily) reduced the sensitivity of the inhibitory response to baclofen. Twenty-four hours after the final dose cortical slices were prepared as above. The concentration of (±)-baclofen required to inhibit K^+-evoked release of 5-HT was increased in the slices prepared from the longer term baclofen-treated mice.

These data demonstrate that baclofen is much more potent than originally suggested in inhibiting 5-HT release and further suggest that the $GABA_B$-receptor involved in the control of 5-HT release becomes becomes subsensitive after longer term treatment with baclofen.

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ELECTROPHYSIOLOGICAL STUDY OF PYRIDAZINE-GABA DERIVATIVES WITH GABA-A ANTAGONIST PROPERTIES

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It has recently been shown, on biochemical and physiological basis, that an arylamino-piridazine derivative of GABA, SR 95103 [2-(carboxy-3'-propyl)-3-amino-4-methyl-6-phenylpyridazinium chloride] possesses antagonistic activity on GABA-A receptors (Chambon et al, 1985).

In the present study, we have analysed this property on a wider variety of neuronal categories (central and peripheral neurons, neuroendocrine cells). Furthermore, a new compound has been tested : SR 42641, the p-chlorophenyl derivative of SR 95103. Electrophysiological results were obtained from three different rat nervous structures : neurons in the central nervous system, sensory neurons in the dorsal root ganglion and neurosecretory fibres in the neural lobe of the hypophysis.

GABA-induced responses were recorded from isolated neurohypophysis as transient increases in the extracellular concentration of K^+ ions, measured by means of ion-sensitive microelectrodes. Continuous perfusion of SR 95103 or SR 42641 decreased GABA-induced responses reversibly, as did bicuculline. SR 42641 was approximately an order of magnitude more potent than SR 95103.

In vivo microiontophoretic studies were performed in the cuneate nucleus of the urethane-anaesthetized (1.5 g/kg) adult male rat (200-300 g). Twenty-six cells were recorded extracellularly upon either spontaneous or glutamate-excited baseline spike firing (10 to 30 spikes/sec). Expelling currents of up to 100 nA were passed through micropipette barrels filled with aqueous solutions of GABA 0.2 M and SR 95103 0.1 M, and with 165 mM NaCl solutions of bicuculline methochloride 5 mM and SR 42641 5 mM.

Both SR 95103 and SR 42641 reversibly antagonized GABA-induced inhibition of firing in a dose-dependent fashion. These effects paralleled those of bicuculline but the latter, like SR 42641 were less often found to elicit baseline firing exaltation per se than SR 95103.

Intracellular recordings were performed from lumbar (L4-L5) dorsal root ganglia in vitro. The study was performed on large neurons (known to display large responses to GABA) at the surface of the ganglion (in order to limit diffusion barriers). Perfusion of SR 95103 (1 to 10 µM ; 8 cells) or SR 42641 (0.1 to 0.25 µM ; 9 cells) had no effect on membrane potential and resistance. Both compounds and bicuculline (0.7 µM ; 10 cells) rapidly decreased GABA-induced responses (within 5 mn). This effect was reversible. Responses were also antagonized by picrotoxin (2 µM), the recovery time being markedly longer with this latter antagonist. Dose-response curves for GABA (K_a value = 23 µM) were shifted to the right in a parallel manner by bicuculline (K_B = 0.33 µM $\pm$ 0.08 s.e.m. ; n = 10), SR 95103 (K_B = 1.3 $\pm$ 0.1 ; n = 5) and SR 42641 (K_B = 0.10 $\pm$ 0.02 ; n = 9).

In conclusion, SR 95103 and SR 42641 display antagonistic properties, most probably of the competitive type, at GABA-A receptor sites in various preparations. These compounds offer a number of advantages : they represent a new class of GABA-A antagonists with rapid action and easy washout properties ; SR 95103 is easily solubilized in water and both new products are very stable in solution. The selectivity of these GABA-A antagonists towards other receptor sites (already observed on biochemical basis) is now investigated on the preparations described in the present issue.

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EFFECT OF THE NOVEL NON-BENZODIAZEPINE HYPNOTIC ZOLPIDEM ON CYCLIC GMP LEVELS AND MONOAMINE METABOLISM IN THE RAT BRAIN

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Zolpidem (N,N,6-trimethyl-2-(-4-methylphenyl) imidazo-[1,2a] pyridine-3-acetamide hemitartrate) is a novel non benzodiazepine hypnotic with rapid onset and short duration of action (Arbilla et al, 1985; Langer et al, 1985; Nicholson and Pascoe, 1985). Although structurally different from the benzodiazepines, zolpidem interacts in vitro with central benzodiazepine receptors in rat brain membranes (Arbilla et al, 1985; Langer et al, 1985). Here, we report the effects of zolpidem on cerebellar cyclic guanosyl 3',5' monophosphate (cGMP) levels and on biochemical indices of cerebral noradrenaline, serotonin and dopamine metabolism in the rat and mouse.

Cyclic GMP levels were measured by radioimmunoassay (RIA Kit, Amersham). The rate of utilization of noradrenaline and dopamine was estimated by measuring the disappearance of the amines after inhibition of tyrosine hydroxylase.Monoamines and their metabolites were assayed by reversed phase high-performance liquid chromatography with electrochemical detection.

Zolpidem diminished markedly the levels of cerebellar cGMP in the rat (ED_{50}=0.7 mg/kg ip). This effect was rapid in onset (less than 15 min), of short duration (less than 1 hour) and was antagonized in a competitive manner by the benzodiazepine antagonist Ro 15-1788 (30 mg/kg ip). When given in combination with muscimol (in a dose of 0.5 mg/kg ip which by itself did not alter cerebellar cGMP content) zolpidem potentiated the diminution of the cyclic nucleotide levels induced by the GABA mimetic.

Zolpidem (up to 30 mg/kg ip) failed to alter the rate of utilization of noradrenaline or the levels of its metabolites in the rat brain. However, similarly to the benzodiazepines, the compound (10-30 mg/kg ip) diminished serotonin synthesis in the hippocampus, striatum and frontal cortex. At high doses (30-100 mg/kg ip), zolpidem also decreased the rate of utilization of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the rat striatum. Like benzodiazepines, zolpidem (10 mg/kg ip) prevented the increase in DOPAC concentrations induced by haloperidol (0.2 mg/kg ip) in both striatum and frontal cortex and the increase in DOPAC levels in the frontal cortex induced by electric footshock stress in rats (ED_{50}=2 mg/kg ip)and BALB/C mice. This latter effect was antagonized by co-administration of Ro 15-1788 (30 mg/kg ip).

Biochemical experiments have demonstrated that benzodiazepines decrease cerebellar cGMP levels as a result of their interaction with specific benzodiazepine recognition sites with subsequent facilitation of GABAergic transmission (Mao et al, 1975). The diminution by zolpidem of cerebellar cGMP levels and the antagonism of this effect by Ro 15-1788 support the view that this drug interacts in vivo with the benzodiazepine-GABA receptor complex. The similarity of the effects of benzodiazepines and zolpidem on cerebral serotonin and dopamine metabolism and the reversal of these biochemical alterations by Ro 15-1788 is also consistent with this hypothesis.

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ANTAGONISM BY ZOLPIDEM OF STRESS-INDUCED INCREASE IN DOPAC LEVELS IN THE NUCLEUS ACCUMBENS, AS DEMONSTRATED BY VOLTAMMETRY

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Stressful conditions (e.g. electric footshock, conditioned fear) are known to cause a selective activation of dopaminergic neurons projecting to the frontal cortex and nucleus accumbens in rodents (Thierry et al, 1976; Lavielle et al, 1978; Herman et al, 1982). These data taken together with the fact that stress-induced changes in dopamine metabolism are antagonized by benzodiazepines (Lavielle et al, 1978) implicate the meso-cortico-limbic dopaminergic neurons in emotional states. In the present study, we have used the technique of in vivo voltammetry with carbon fiber electrodes to measure the changes in extracellular 3,4-dihydroxyphenylacetic acid (DOPAC) levels in the ventral tegmental area (VTA) and nucleus accumbens of the rat in response to various peripheral stresses (handling, tail pinch, electric footshock). We have also studied the effect of diazepam and zolpidem, a new non benzodiazepine hypnotic agent possessing anxiolytic properties in animal models (Arbilla et al, 1985; Langer et al, 1985), on the tail-pinch induced alteration of extracellular DOPAC levels in the nucleus accumbens.

Male Sprague Dawley rats (240 g) were chronically implanted with carbon fiber electrodes (8 um in diameter) using a micromanipulator designed to implant electrodes in unanaesthetized and unrestrained rats. The carbon fiber electrodes were pretreated with a triangular current according to Gonon et al (1983). Under our conditions, oxidation peaks corresponding to the oxidation of ascorbic acid and DOPAC were recorded at -50 and +100 mV, respectively in the nucleus accumbens and VTA. Inescapable electric footshock [trains of 10 shocks (1.5 mA) of 160 msec duration with a 160 msec interval delivered every 10 sec for 10 min] or mild tail pressure (for 7 min) provoked a large increase in the height of the DOPAC (but not ascorbic acid) oxidation peak in both the nucleus accumbens and VTA, the effect being the most pronounced in the former brain region (+60-70 % above baseline at 1h after stress). Very mild stress caused by handling of the animals elicited a significant short lasting (15 min) increase (+ 15 %) in the amplitude of the DOPAC oxidation peak in nucleus accumbens. Systemic administration of zolpidem (5 mg/kg ip) to non-stressed rats failed to affect, whereas diazepam (5 mg/kg ip) reduced slightly the height of the DOPAC oxidation peak in the nucleus accumbens. The tail-pinch induced increase in extracellular DOPAC observed in the nucleus accumbens was totally antagonized by pretreatment with zolpidem or diazepam (5 mg/kg ip).

The above data confirm the view that meso-limbic dopaminergic neurons are activated in response to stress. The antagonism of this effect by the hypnotic/anxiolytic agent zolpidem is consistent with the anxiolytic potential of this compound demonstrated in the conflict drinking model (Zivkovic et al, 1985). The technique of in vivo voltammetry should be useful in correlating behavioural responses with specific regional changes in extracellular DOPAC.

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QUISQUALATE INHIBITS THE EFFECT OF N-METHYL-D,L-ASPARTATE ON THE SOMATOSENSORY EVOKED POTENTIAL

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N-methyl-D,L-aspartic acid (NMDLA), a selective excitatory amino acid receptor agonist, has been shown to depress the somatosensory evoked potential (SEP) in a concentration dependent manner, an effect which is blocked by 2-aminophosphonovaleric acid (Addae & Stone, 1985a). The effect of NMDLA and other excitatory amino acids on the SEP have also been shown to exhibit apparent desensitisation of the receptors (Addae & Stone, 1985a; 1985b). To examine further this phenomenon of apparent desensitisation, we have looked at the interaction between some of the excitatory amino acid receptor agonists.

The area of cortex representing the contralateral forepaw of urethane anaesthetised male Wistar rats was exposed. Solutions of compounds were made in 0.9% NaCl and used to form a static pool over the exposed cortex at room temperature. Monopolar recording of the SEP from forepaw stimulation was made with a glass microelectrode at 400-800μm below the cortical surface. The effects of compounds were assessed by expressing the change in amplitude of the initial negative wave of the SEP (N_1) as a percentage of the control value.

Topical application of 0.5mM quisqualate for 5 min caused a 25±2% (mean s.e.mean) decrease in N_1; 0.5mM NMDLA completely abolished N_1 within 1 min, whilst 1.0mM carbachol applied for 2.5 min decreased N_1 by 67±6%. A 5 min application of 0.5mM quisqualate led to a complete inhibition of the effects of 0.5mM NMDLA on the SEP, whilst the effect of 1.0mM carbachol was not significantly changed ($p = 0.16$, Mann-Whitney test). When preceded by 0.25mM quisqualate applied for 2 min, 0.5mM NMDLA slightly changed N_1 (22±2% decrease in 3 experiments and 29±6% increase in 4 experiments). This inhibitory effect of 0.25mM quisqualate on 0.5mM NMDLA was still observed in the presence of 0.1mM bicuculline, but was blocked by 5mM pentobarbitone, which has been reported to be a selective quisqualate and kainate antagonist (Teichberg et al., 1984; Harrison, 1985).

The inhibitory effect of quisqualate on NMDLA is not likely to be due to a non-specific inactivation of cortical neurones because of the absence of an effect of quisqualate on carbachol. It is also unlikely to be an effect mediated through inhibitory interneurones, since it was still observed in the presence of bicuculline. Quisqualate has been observed to block kainate in the goldfish retina (Ishida and Neyton, 1985) and in our system (unpublished finding). Together with our previous reports of apparent desensitisation in the rat cerebral cortex (Addae & Stone, 1985a; 1985b), we conclude that quisqualate is able to act on the NMDLA receptor/effector mechanism to cause a decrease in sensitivity of the NMDLA system. Quisqualate is probably not acting via the same site and mechanism as NMDLA since the two agonists are distinguished by pentobarbitone.

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THE ANTAGONISM OF N-METHYL-D-ASPARTATE BY KETAMINE AND AMINO-PHOSPHONATES: QUANTITATIVE STUDIES IN THE RAT SPINAL CORD IN VITRO

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The dissociative anaesthetic ketamine has been reported to selectively antagonise excitatory amino acid action at one class of excitatory amino acid receptor, the N-methyl-d-aspartate (NMDA) receptor, in the spinal cord of the cat and rat (Anis et al, 1983) and in slices of rat neocortex (Harrison and Simmonds, 1985). In the latter quantitative study, the Schild plot for ketamine was found to have a slope of approximately 1, as did the Schild plots for the competitive antagonists, 2-amino-5-phosphonovalerate (APV) and 2-amino-7-phosphono-hepanoate (APH). However, combination studies with the antagonists by Harrison and Simmonds (1985) suggested that antagonism of NMDA by ketamine occurred at a site distinct from that for APV and APH. In an attempt to clarify this contradiction, we have quantitatively studied the antagonism of NMDA by (±)APV, (±)APH and ketamine using the neonatal rat spinal cord in vitro.

Hemisected neonatal rat spinal cords were prepared for the recording of ventral root depolarisations as previously described (Evans and Watkins 1978). Dose-response (d-r) curves (n=5-9) were obtained for NMDA in either antagonist-free medium or medium containing one of a range of concentrations of each of the antagonists. For each d-r curve, depolarisations were normalised as percentage of maximal response and data was pooled for each concentration of each antagonist to obtain mean d-r curves. ED_{50}s for each curve were compared with the control value (1.8×10^{-5}M) to calculate dose ratios for antagonism which were subjected to Schild regression analysis. Values of pA_2 and Schild slope obtained in this study are tabulated below (Table 1), with reported values from the rat cortical slice included for comparison.

While the pA_2 values estimated for APV and APH in the cord were in good agreement with those of Harrison and Simmonds (1985) in the cortex, and the Schild slopes of 1 were consistent with competitive antagonism, our ketamine pA_2 value was markedly higher than their value and the Schild slope of 0.6 was inconsistent with competitive antagonism. Our results, from a different mammalian preparation, support their observations that APV and APH are competitive antagonists of NMDA and that ketamine acts at a site distinct from the NMDA receptor. An understanding of the mechanisms underlying the functional antagonism of NMDA by ketamine would help in resolving the relevance of NMDA antagonism to the psychotomimetic effects of ketamine. (Berry and Lodge, 1985).

TABLE 1. pA_2 values (Schild regression slopes) for NMDA antagonism by APV, APH and ketamine in the neonatal rat spinal cord and rat cortical slice in vitro

	(±)APV	(-)APV	(±)APH	Ketamine
Neonatal rat cord	5.0 (1.0)	5.3[b]	5.1 (1.0)	6.0 (0.6)*
Rat cortical slice (a)	4.9[b]		5.2 (1.0)	4.8 (1.0)	5.0 (1.0)

*slope sig <1, p=0.003
(a) All values from Harrison and Simmonds (1985).
(b) Estimated from experimental data assuming (+) isomer to be inactive (Evans et al, 1982).

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THE ANTICONVULSANT β-KAINATE DEPRESSES ENDOGENOUS GLUTAMATE EFFLUX IN RAT BRAIN SLICES

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β-kainic acid is a structural isomer of α-kainate, with the C2 carbon in the D-configuration. It has been shown to possess anticonvulsant properties when injected i.c.v. or i.p. in mice (Collins et al., 1984; Turski et al., 1985). When tested by electrophysiological methods, however, β-kainate shows no antagonist activity at either N-methyl aspartate, quisqualate or kainate receptors (Stone & Collins, 1985). We have therefore investigated the effect of β-kainate on the efflux of endogenous amino acids from brain slices. The release of amino acids was determined using a sensitive HPLC technique (Turnell & Cooper, 1982).

400μm hippocampal slices were prepared (Connick and Stone, 1985) and preincubated for 30 min in oxygenated Krebs solution at 37°C, before being transferred to chambers containing 1 ml normal Krebs solution (2mM KCl), or High Potassium Krebs (containing 44mM potassium), with or without β-kainate for 15 min. Aliquots were then removed and frozen at -20°C for later analysis by HPLC using pre-column derivatization with o-phthalaldehyde. Slices were homogenized and the protein concentration determined by the method of Lowry et al. (1951) with BSA as standard.

β-kainate has little or no effect on the efflux of endogenous amino acids when applied to slices in normal Krebs medium. 1mM β-kainate produced a potent and selective depression of K^+ evoked glutamate release (Table 1).

Table 1: Results are expressed as mean % of K^+ induced release ± s.e.mean (n). Statistical significance was assessed using a paired t test. *$p<0.05$.

Conc. β-kainate	Aspartate	Glutamate	GABA
250μM	88.46 ± 13.0 (3)	120.7 ± 7.1 (3)	109.8 ± 9.9 (3)
1mM	67.90 ± 21.0 (5)	49.0 ± 6.9* (4)	99.3 ± 7.3 (5)
5mM	116.70 ± 13.3 (5)	57.3 ± 25.6* (5)	79.7 ± 14.7* (5)

It is possible that the depression of glutamate release is of importance in the anticonvulsant properties of β-kainate. A reduction of stimulated excitatory amino acid release might prevent the spreading excitation seen in epileptic seizures and thus prevent or suppress a convulsion. The dose of β-kainate necessary to prevent seizures in mice (0.33-1.0μmol in 10μl i.c.v.) is consistent with an active dose of 1mM on glutamate efflux.

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REDUCTION OF [^{3}H]-D-ASPARTATE BINDING TO GLUTAMATE UPTAKE SITES IN STRIATUM OF HUNTINGTON'S DISEASE PATIENTS

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A marked similarity exists between the lesions of rat corpus striatum following intrastriatal injection of some glutamate analogues and the striatal lesions of Huntington's disease (HD). This has led to the suggestion that glutamate (or an analogue) may be involved in the pathological processes of the disease. Whilst post-mortem neurochemical studies of HD have described some changes in glutamate metabolism (Carter,1984), these studies have been hampered by the lack of a suitable marker of glutamate-releasing neurones in human brain. We have recently described, using human brain, the binding of [^{3}H]-D-aspartate to the high-affinity glutamate uptake system, which may provide a marker of glutamate-releasing terminals in the striatum (Cross et al,1985). In the present study we have examined this marker in brains from controls and subjects with HD.

Brains were obtained at autopsy from patients with HD (n=15; age = 63 ± 3y; p.m. delay = 38 ± 8h) and a group of controls (n=14; age = 64 ± 3y ; p.m. delay = 43 ± 6h). A total particulate fraction of dissected brain regions was used to study the sodium-dependant binding of [^{3}H]-D-aspartate as described previously (Cross et al, 1985).

Table 1 [^{3}H]-D-Aspartate binding in control and HD brain

Brain region	[^{3}H]-D-Aspartate bound (fmol/mg) Control	HD
Caudate	707 ± 117	196 ± 38**
Putamen	808 ± 100	322 ± 55**
Cortex (BA4)	426 ± 34	418 ± 47
Hippocampus	377 ± 34	257 ± 33*

*P<0.05; **P<0.01

A large and highly significant reduction in [^{3}H]-D-aspartate binding was recorded in the HD striatum. A less significant reduction was found in the hippocampus with no change in the frontal cortex. In the caudate nucleus, saturation analysis revealed no change in affinity but a large decrease in the maximum binding capacity in HD.

This finding suggests that high-affinity glutamate uptake sites are sharply reduced in the striatum in HD, possibly reflecting some loss of cortico-striatal glutamate-containing terminals. As inhibitors of glutamate uptake may be neurotoxic in the rat (McBean & Roberts,1985), the loss of glutamate uptake sites in HD may be relevant to the pathogenesis of the striatal lesion.

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EFFECT OF NORADRENALINE ON POTASSIUM-EVOKED RELEASE OF ENDOGENOUS AMINO ACIDS FROM RABBIT HIPPOCAMPAL SLICES

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Catecholamine fluorescent fibres entering the hippocampus originate primarily in the locus coeruleus (Pickel et 1974). Ionophoretically-applied noradrenaline (NA) depresses the firing of hippocampal neurones as does stimulation of locus coeruleus (Segal and Bloom 1974a, b). In this study the effect of NA upon K+-evoked release of glutamate, aspartate and GABA from hippocampal slices was investigated.

Transverse slices (250μm) cut at 70° to the longditudinal axis were superfused (0.5ml/minute) with artificial cerebro-spinal fluid (ASCF) oxygenated with 95% O_2-5% CO_2 at 37°C. Initial experiments showed the release of glutamate, aspartate and GABA to stabilize at a basal level within 30 minutes. Three, two minute aliquots of superfusate were collected between 45-51 minutes and then the slices were superfused for 4 minutes with ACSF containing 40mM K+. 2 minute aliquots continued to be collected up to 59 minutes. Varying concentrations of NA were added to the stock ACSF and the slices were superfused with this solution throughout. The aliquots of superfusate were collected and freeze-dried prior to analysis by HPLC. The analytical method involved pre-column derivatization with O-phthaldialdehyde, followed by separation on a C_{18} reversed-phase chromatography column with fluorescence detection.

The addition of NA to the superfusion medium for the duration of the experiment produced a concentration dependent increase in both basal (Table 1) and evoked (Table 2) release of GABA and aspartate while glutamate release was not affected.

Table 1. Effect of NA on Basal Release (Mean ± s.e. mean) pmoles/mg wet weight tissue/2 min.

		n	Aspartate	Glutamate	GABA
	Control	14	4.4 ± 0.7	5.7 ± 0.5	3.9 ± 0.4
NA	10^{-12}	8	8.3 ± 2.4	5.5 ± 0.7	3.7 ± 0.5
	10^{-10}	8	4.5 ± 0.9	4.8 ± 0.6	7.6 ± 1.2 **
	10^{-8}	8	7.0 ± 0.9 *	4.4 ± 0.5	10.1 ± 0.8 ***
	10^{-7}	8	7.3 ± 1.2 *	4.2 ± 0.5	9.7 ± 1.2 ***

Table 2. Effect of NA on Evoked Release (Mean ± s.e. mean) pmoles/mg wet weight tissue/2 min.

	Control	14	6.7 ± 0.8	22.5 + 1.2	7.4 ± 0.7
NA	10^{-12}	8	7.9 ± 0.5	20.5 ± 1.4	6.4 ± 0.4
	10^{-10}	8	7.0 ± 1.0	19.5 ± 1.1	10.0 ± 1.0 *
	10^{-8}	8	10.5 ± 1.2 *	19.4 ± 1.5	12.7 ± 0.8 ***
	10^{-7}	8	11.1 ± 1.6 *	18.7 ± 1.6	11.1 ± 0.7 ***

* p <0.05 ** p <0.01 *** p <0.001 Students t-test

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SOLUBILISATION OF AN EXTRAJUNCTIONAL GLUTAMATE BINDING PROTEIN FROM RAT BRAIN MEMBRANES

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Electrophysiological studies have permitted division of excitatory amino acid receptors into 3 principle subtypes, namely those activated by N-methyl-D-aspartate (NMDA), quisqualate (QA) and kainate (KA) (Watkins & Evans, 1981). Although binding studies have yielded data broadly in accord with this description (Foster & Fagg, 1984) they have revealed little of the molecular interactions that underly generation of the physiological response. To achieve this, a prerequisite is to solubilise the receptors such that they retain their fundamental characteristics found in the intact membrane. In this study, we have investigated L-[^{3}H] glutamate binding to proteins solubilised from synaptic membranes (SPMs) under conditions where the junctional regions are probably not disrupted.

Rat whole brain SPMs were solubilised with 1% Triton X-100 or potassium cholate (pH 7.5), followed by affinity chromatography on glu$_3$-loaded glass fibre (Julliard et al, 1973). Specific binding of L-[^{3}H] glutamate was determined in phosphate buffer using an ultrafiltration procedure; the solubilised protein had a K_d = 0.9 uM and B_{max} = 35.1 nmol/mg protein.

The glutamate binding seen in these preparations was only minimally sensitive to inhibition by NMDA or KA, but was rather more sensitive to QA. More potent inhibitors however, included L-cysteic acid, L-cysteine and L-cysteine sulfinic acid, each with K_d s of approx 30-60 uM. This site was not related to the enzyme glutamine synthetase, since the potent inhibitor L-methionine-DL-sulfoximine was devoid of activity. Although different procedures have been employed in this study, the solubilised binding protein exhibits strong similarities to the site isolated by Michaelis et al (1983). It has been reported that NMDA sites are highly enriched in postsynaptic densities (Fagg & Matus, 1984). Thus, it may be that physiological excitatory amino acid receptors are resistant to solubilisation under conditions which are thought to disrupt extra-junctional membranes, or that the pharmacological characteristics of these proteins become modified following solubilisation.

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A SIMPLE METHOD FOR RECORDING EXCITATORY AMINO ACID-EVOKED DEPOLARISATIONS OF RAT CORTEX IN VITRO

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Harrison and Simmonds (1985) have recently described a method for recording excitatory amino acid-evoked DC depolarisations of slices of rat cerebral cortex in vitro. Their method enabled them to study quantitatively agonist/antagonist interactions at the receptor for which N-methyl-D-aspartate (NMDA) is a selective agonist. This test method has proved difficult to replicate. We have therefore developed a method which combines the cortical/corpus callosum wedge preparation of Harrison and Simmonds (1985) with the simple superfusion method of Evans and Watkins (1978), used routinely to study excitatory amino acid receptors in the neonatal rat spinal cord in vitro. In order to validate the usefulness of this method we have initially estimated the agonist potencies of NMDA, quisqualate and L-glutamate and obtained a pK_B value for the antagonism of NMDA by (±)2-amino-7-phosphonoheptanoate (APH) for comparison with published data and our own unpublished observations in the neonatal rat spinal cord.

Wedges (0.5mm thick) of rat brain, comprising cerebral cortex and corpus callosum, were prepared from male Wistar rats, 250-300g, according to the method of Harrison and Simmonds, and preincubated for 3-4h in a Mg^{2+} free, oxygenated Krebs solution. For DC recording, the cortical portion of a wedge was placed between 2 layers of medium-wetted nappy liner on a perspex superfusion bath. The corpus callosum was placed on the wick of an Ag/AgCl electrode and insulated from the superfusate with a 1:1 w/w mixture of petroleum jelly/liquid paraffin. A similar reference electrode was placed in contact with the nappy liner with the bath inclined at 45°C. Mg^{2+} free medium was dripped onto the top of the nappy liner, at a rate of 2 ml min^{-1}. The potential difference between the electrodes was continuously recorded directly on a Bryans BS272 Y-T recorder. Drugs were dissolved in medium for superfusion.

Prior to the routine addition of TTX, 3×10^{-7}M, to the superfusate, cortical slices were spontaneously active. Spontaneous depolarisations were also abolished by 1mM Mg^{2+} or 20 µM APH, suggesting an NMDA-receptor mediated mechanism. 90% of preparations were viable and responsive to agonist application. A single dose-response curve was obtained from each preparation to the application of increasing concentrations of agonists for 30s at 10 min intervals, until a maximum response was obtained (n=7-14). Dose-response curves were also obtained to NMDA in the presence of varying concentrations of APH (2×10^{-5} to 5×10^{-4}M, n=6 per concentration). Data was normalised as % maximal response and pooled to estimate mean ED_{50} values for control agonist dose-response curves, and NMDA in the presence of APH. Dose-ratios for NMDA antagonism by APH were estimated from ED_{50}s and a Schild regression analysis performed.

Control agonist ED_{50}s in the cortex and neonatal rat spinal cord (Wheatley and Collins, unpublished observations) respectively were: NMDA, 1.45×10^{-5}M and 1.8×10^{-5}M; quisqualate, 4×10^{-6}M and 1×10^{-5}M; L-glutamate, 7.6×10^{-4}M and 8.9×10^{-4}M. Schild regression analysis of the antagonism of NMDA by APH gave a pK_B value of 5.11 (slope = 1.0). This value is comparable with pA_2 or pK_B values obtained in the cortex by Harrison and Simmonds (1985) of 4.8 and neonatal rat spinal cord of 5.1 (Wheatley and Collins, unpublished observations). These results suggest that the method is valid for the quantitative study of excitatory amino acid antagonist/agonist interactions in an adult rat cortical preparation, and add further to the pool of quantitative data on excitatory amino acids and antagonists.

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ACTION OF SCH23390 ON NEURONAL RESPONSES TO DOPAMINE IN THE RAT SUBSTANTIA NIGRA SLICE

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It has recently been suggested that SCH23390 (R-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepine-7-01) is a selective antagonist at D_1 receptors linked to adenylcyclase (Iorio et al, 1983). In an in vitro preparation of the substantia nigra it has been shown that antagonism of dopamine response by dopamine antagonists can correlate with their potency as D_2 receptor ligands (Pinnock, 1984).

In the present experiments the in vitro actions of SCH23390 as a dopamine antagonist in the substantia nigra were examined. The techniques utilized in these experiments were the same as those described previously (Pinnock, 1984). Dopamine neurones were characterised by their extracellular waveform and response to dopamine. Drugs were dissolved in the bathing medium and applied to the slice until plateau responses were obtained. SCH23390 was initially dissolved in 50% acetone, 50% H_2O and then diluted to a maximum concentration of 100 µM in artificial CSF made up as follows: NaCl, 124; KCl, 2; KH_2PO_4, 1.25; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 25; glucose, 11; all concentrations are mM.

As previously reported (Pinnock, 1983) dose response curves to dopamine were obtained. Extracellularly recorded firing of neurones was inhibited by dopamine, threshold doses were in the 0.3 to 10 µM range. 100% inhibition of firing was produced by 10 to 300 µM dopamine. 3 µM SCH23390 had no effect on neuronal firing (n=4) while 10 µM SCH23390 caused firing to increase by 50 to 100% (n=6). 30 and 100 µM SCH23390 caused firing to accelerate to the point at which firing became irregular, or spikes disappeared into the background noise (depolarization inactivation). 3 µM SCH23390 had no effect on inhibitory responses to dopamine (n=4). 10 µM SCH23390 reduced the response to dopamine by a small amount, the mean dose ratio for dopamine before and after 10 µM SCH23390 being 1.7 ± 0.24 (mean and SE, n=6). Since 30 and 100 µM SCH23390 disrupt neuronal firing leaving no working baseline, the actions of dopamine could not be quantitatively assessed in these conditions.

Since SCH23390 does not cause hyperprolactinaemia (Iorio et al, 1983) considered to be a D_2 effect (Kebabian and Calne, 1979) and IC_{50} for inhibition of dopamine sensitive adenylcyclase is 0.01 µM (Iorio et al, 1983) the dopamine receptors in the substania nigra bear more resemblance to those involved in prolactin secretion than D_1 receptors related to stimulation of adenylcyclase.

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DOPAMINERGIC DENERVATION DOES NOT AFFECT [^{3}H]-SCH 23390 BINDING IN THE RAT STRIATUM: SIMILARITIES TO PARKINSON'S DISEASE

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SCH 23390 [(R)-(+)-8 chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol-hemimaleate] is a potent and selective antagonist of the adenylate cyclase coupled D1 dopamine receptor (Billard et al., 1984). ^{3}H-SCH 23390 has been described as a selective D1 antagonist ligand labelling a single population of sites in the rat striatal membranes (Billard et al., 1984) and in the human putamen tissues (O'Boyle, 1985). In Parkinson's disease, classically thought to involve a dopaminergic deficiency associated with a degeneration of nigro-striatal dopaminergic pathways, a subsentivity of D1 adenylate cyclase activity was observed by Shihuya (1979). However, the binding of ^{3}H-SCH 23390 was unchanged in the postmortem putamen from Parkinsonian patients (Pimoule et al., 1985). To eliminate the possibility that antemortem drug treatment interfered with the binding results, we examined the binding of ^{3}H-SCH 23390 in the striatum of rats which had received an unilateral injection of 6-hydroxydopamine (6-OHDA) in the substantia nigra.

Rat striatal membranes were prepared by homogenisation and centrifugation in Tris-HCl buffer (50 mM, pH 7.5) at 48,000 x g for 10 min. The final pellet was resuspended in 50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 2 mM $CaCl_2$, pH 7.4. Aliquots of the membrane preparation (0.15 mg prot./ml) were incubated with ^{3}H-SCH-23390 (0.03 - 3 nM ; 80 Ci/mmol, provided by Dr. L.C. Iorio, Schering Corp. Bloomfield, N.J., U.S.A.), in a total volume of 1 ml. Nonspecific binding was defined as residual binding observed in the presence of 1 µM SCH 23390. Membranes were collected by vacuum filtration over GF/B filters after 20 min incubation at 37°C. Specific binding represented 80 % of the total radioactivity at 0.9 nM. Five weeks after an unilateral injection of 8 µg 6-OHDA (provided by B. Scatton) into the substantia nigra, the rats were sacrificed.

In the rat striatum, ^{3}H-SCH 23390 binding is saturable (Kd = 0.65 ± 0.19 nM ; Bmax = 557.8 ± 67.4 fmol/mg prot, n = 3), reversible and stereospecific. Competition curves are consistent with the view that ^{3}H-SCH 23390 labelled a single population of sites, with the pharmacological profile of D1 receptors.

Injection of 6-OHDA in the substantia nigra produced a 96 % decrease of the dopamine levels in the striatum (control : 6.01 ± 0.74 µg/g tissue ; 6-OHDA : 0.204 ± 0.137 µg/g tissue, $p < 0.001$). Under these conditions, however, ^{3}H-SCH 23390 saturation characteristics were not changed (control : Kd = 0.75 ± 0.16 nM ; Bmax = 507.7 ± 56.2 fmol/mg prot, n = 6 ; 6-OHDA : Kd = 0.84 ± 0.17 nM ; Bmax = 412.8 ± 65.4 fmol/mg prot, n = 6).

We reported previously (Pimoule et al., 1985) that when compared to controls (40.6 ± 2.5 fmol/mg prot., n = 12), ^{3}H-SCH 23390 binding was unchanged in the Parkinsonian putamen (41.4 ± 1.6 fmol/mg prot., n = 8). No changes were observed in the binding characteristics of ^{3}H-SCH 23390 in the rat striatum following a degeneration of the dopaminergic system. It is likely that drug treatment of Parkinsonian patients did not affect these binding data.

One possible interpretation of our results is that the receptors labelled by the D1 antagonist ligand ^{3}H-SCH 23390 are not innervated. Alternatively, data with this antagonist ligand could reflect the density of D1 receptors, but perhaps not the agonist sensitivity of D1 adenylate cyclase activity.

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ARE THERE DOPAMINE RECEPTORS IN THE RAT SUPERIOR COLLICULUS?

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In vivo administration of [^{3}H]-spiperone and [^{3}H]-N-n-propylnorapomorphine to rats identifies binding sites in the superior colliculus with characteristics of dopamine receptors (Chivers et al 1984). We now report the in vitro characterisation of specific [^{3}H]-spiperone binding sites in the rat superior colliculus and attempts to demonstrate innervation of these sites.

In membrane preparations of male Wistar rat superior colliculus, the binding of [^{3}H]-spiperone (0.15 nM) was displaced by the incorporation of 10^{-7}M (+)-butaclamol, 10^{-5}M haloperidol, 10^{-5}M apomorphine and 10^{-5}M (±)-sulpiride, but not by 10^{-7}M (-)-butaclamol, 10^{-7}M prazosin, 10^{-5}M propranolol, 10^{-7}M ketanserin and 10^{-7}M cinanserin. Equilibrium analysis of [^{3}H]-spiperone binding (0.03-1.0 nM) defined by 10^{-5}M (±)-sulpiride showed binding in the superior colliculus to be saturable and of high affinity. The number of binding sites (Bmax) in the superior colliculus was approximately 10% of that found in the striatum (1.9 ± 0.16 pmol/g tissue compared to 24.8 ± 0.8 pmol/g tissue) but no difference was found in the equilibrium dissociation constants (K_D). Inhibition constants (Ki) for the displacement of [^{3}H]-spiperone by (±)-sulpiride, (+)-butaclomol and haloperidol were found to be the same in both superior collicular and striatal membrane preparations.

Table 1 Ki values for neuroleptics displacing [^{3}H]-spiperone binding

		Superior colliculus	Striatum
(±)-sulpiride	(10^{-10} - 10^{-5}M)	26.2	88.0
(+)-butaclamol	(10^{-10} - 10^{-5}M)	1.0	1.3
haloperidol	(10^{-10} - 10^{-5}M)	1.7	2.0

The uptake of [^{3}H]-dopamine into synaptosomal preparations of the superior colliculus was approximately 20% of that occurring in striatal synaptosomes. [^{3}H]-dopamine uptake into striatal synaptosomes was reduced by 56% in the presence of $5x10^{-7}$M nomifensine (controls 291.8 ± 19.4 pmol/mg protein/hour; nomifensine 128.3 ± 13.9 pmol/mg protein/hour), but no such reduction of [^{3}H]-dopamine uptake was observed in superior collicular synaptosomes (controls 63.5 ± 9.2 pmol/mg protein/hour; nomifensine 63.6 ± 9.4 pmol/mg protein/hour).

HPLC analysis of dopamine and its metabolites revealed low superior collicular concentrations of dopamine and DOPAC (dopamine 0.94 ± 0.13 ng/mg protein; DOPAC 0.07 ± 0.03 ng/mg protein) compared to striatal levels (dopamine 148.4 ± 17.9 ng/mg protein; DOPAC 21.9 ± 6.1 ng/mg protein). HVA was present in higher concentrations; 3.53 ± 0.79 ng/mg protein in the superior colliculus which compared to a striatal level of 11.71 ± 2.04 ng/mg protein. The HVA + DOPAC/dopamine ratio was much higher in the superior colliculus (3.8) than in the striatum (0.2).

The rat superior colliculus contains a small population of specific [^{3}H]-spiperone binding sites with some characteristics of dopamine receptors. Small amounts of dopamine and its metabolites are present and the large metabolic ratio indicates a high turnover rate. No evidence for the innervation of these specific [^{3}H]-spiperone binding sites could be provided from the study of [^{3}H]-dopamine uptake.

Chivers et al (1984) J.Pharm.Pharmacol. 36, 484.

EFFECTS OF CHRONIC ADMINISTRATION OF TETRABENAZINE RESERPINE OR CHLOPROMAZINE ON RAT STRIATAL DOPAMINE FUNCTION

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Tetrabenazine, a monoamine depleting agent, is used to treat hyperkinetic movement disorders but may also interact directly with brain dopamine receptors (Reches et al 1983; Hong et al 1985). Consequently, repeated treatment with tetrabenazine may induce dopamine receptor supersensitivity. We now examine the effects of oral treatment of rats for 6 months with tetrabenazine in comparison with reserpine and chlorpromazine on brain dopamine function.

Male Wistar rats (180g $\pm$ 3g at the start of the experiment) received either tetrabenazine (5.5-7.5 mg/kg/day), reserpine (0.25-0.35 mg/kg/day) chlorpromazine hydrochloride (34-42 mg/kg/day) via their daily distilled drinking water for 6 months. Age-matched control animals received distilled water alone.

All drug treatments reduced spontaneous locomotor activity after 1 month. Tetrabenazine caused increased activity after 6 months treatment; reserpine-treated animals showed normal locomotor activity after 6 months. Chlorpromazine treatment increased activity at 6 months (Table 1). Striatal dopamine concentrations were decreased after 1 and 6 months of tetrabenazine intake; reserpine administration decreased dopamine levels after 1 and 3 months of treatment. Chlorpromazine had no effect on striatal dopamine concentrations. The number (Bmax) of specific striatal ^{3}H-spiperone (0.02-1.0 nM; defined using 10^{-5}M ($\pm$)-sulpiride) binding sites was increased after 1 and 6 months treatment with tetrabenazine and chlorpromazine; reserpine administration had no effect. Bmax for specific ^{3}H-piflutixol (0.05-2.0 nM; defined using 10^{-6}M cis-flupenthixol in the presence of 3 x 10^{-5}M ($\pm$)-sulpiride) binding was unchanged by the drug treatments. Striatal dopamine (50 µM)-stimulated adenylate cyclase activity was unchanged by all drug treatments at 1 month. Enzyme activity was reduced by chlorpromazine and reserpine treatment, but not by tetrabenazine, at 6 months.

Table 1 The behavioural and biochemical effects of 6 months drug administration

Drug treatment	Locomotor activity (counts/60 min)	Striatal DA (µg/g tissue)	^{3}H-spiperone Bmax (pmol/g tissue)
Control	644 $\pm$ 73	15.7 $\pm$ 1.4	23.4 $\pm$ 1.7
Tetrabenazine	1187 $\pm$ 99*	8.2 $\pm$ 2.6*	31.2 $\pm$ 2.5*
Reserpine	607 $\pm$ 67	13.7 $\pm$ 1.7	29.4 $\pm$ 1.8
Chlorpromazine	836 $\pm$ 52*	19.4 $\pm$ 0.8	34.8 $\pm$ 2.50*

* $P < 0.05$ compared to controls; Student's t'test

In the dose used chronic administration of tetrabenazine may result in the development of brain dopamine receptor supersensitivity.

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D_2 RECEPTOR REGULATION OF ENDOGENOUS DOPAMINE RELEASE FROM STRIATAL SLICES: EFFECT OF UPTAKE INHIBITORS AND SYNTHESIS INHIBITION

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A large number of studies have demonstrated that the depolarisation-induced release of radiolabelled catecholamines from brain slices can be regulated by autoreceptors (Chesselet, 1984), but autoreceptor regulation of endogenous catecholamine release has been little studied. We have recently shown that both spontaneous and veratrine-induced endogenous dopamine (DA) release can be regulated by D_2 receptors (Nahorski & Strupish, 1985). However, these experiments were carried out in the presence of 10 μM nomifensine, which as well as blocking DA uptake may also have a releasing effect (Bowyer et al. 1984), resulting in an artificially high level of spontaneous DA release. We have therefore repeated the studies using 0.1 μM GBR 12921, a very potent and specific DA uptake inhibitor with no releasing action below 1 μM (van der Zee et al. 1980). In addition, since newly synthesised DA makes up a large component of both spontaneous and veratrine-induced release (Herdon et al. 1985a,b), we have also examined the effects of synthesis inhibition on the autoreceptor regulation of release.

Rat striatal slices were prepared and superfused as described previously (Nahorski & Strupish, 1985; Herdon et al. 1985b) and DA in superfusate fractions quantified by HPLC with electrochemical detection. Spontaneous DA release in the presence of 0.1 μM GBR 12921 was 4-fold lower than in the presence of 10 μM nomifensine, again suggesting DA-releasing properties of the latter drug. However, in the presence of GBR 12921 the DA agonist pergolide (1 μM) still reduced both spontaneous and veratrine (10 μM)-induced release by 25-35%. On the other hand, the elevation in spontaneous release by the D_2 antagonist l-sulpiride (1 μM) observed in the presence of nomifensine was not seen in the presence of GBR 12921, although sulpiride could still increase veratrine-induced release by 30-80% and block the effects of pergolide. In the presence of 50 μM alpha-methyl-p-tyrosine (alpha-MpT), which inhibits tyrosine hydroxylation by over 90% (Nahorski & Strupish, this meeting), sulpiride was no longer able to increase spontaneous DA release (in the presence of 10 μM nomifensine) but could still potentiate veratrine-induced release. However, pergolide could still reduce both spontaneous and veratrine-induced DA release in the presence of alpha-MpT, and this reduction was seen whether either 10 μM nomifensine or 0.1 μM GBR 12921 were present.

These studies confirm that autoreceptors can regulate both spontaneous and veratrine-induced endogenous DA release from rat striatal slices. The finding that the sulpiride-induced increase in spontaneous release seen in the presence of nomifensine was not observed if release was reduced either by synthesis inhibition or by the substitution of GBR 12921 for nomifensine suggests that a certain threshold of autoreceptor occupation by endogenous DA is necessary for this action of sulpiride. However, the fact that pergolide was effective in reducing DA release in the presence of either uptake inhibitor as well as when synthesis was inhibited indicates that autoreceptors can regulate even very low levels of release and do not selectively influence only newly-synthesised release.

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EFFECT OF DOPAMINE, NOMIFENSINE AND TRH INFUSION ON TRH-STIMULATED PROLACTIN SECRETION IN RATS

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We have previously shown that reversal of the dopaminergic control of basal prolactin secretion, with the dopaminergic antagonist domperidone, can impair prolactin secretory responses to TRH in ovariectomised rats (Gilna & Martin, 1983). We now report on the effects of infusing dopamine, nomifensive and TRH on the acute TRH induction of prolactin secretion in the ovarietomised rat.

Groups of oestradiol-primed ovariectomized adult rats were anaesthetized with urethane (0.8 ml. 100 g^{-1}, 14% solution (Wt/V in saline)) and cannulae implanted in the jugular vein and carotid artery. One hour later a basal blood sample (arterial (100 µl)) was taken and TRH (50ng in 200 µl saline) administered intravenously. A further blood sample was taken 5 min post TRH administration. Dopamine (400 µg. ml^{-1} at 2 ml. h^{-1} for 30 min), nomifensine (200 µg. ml^{-1} at 2 ml. h^{-1} for 30 min) or TRH (50ng. ml^{-1} at 2 ml. h^{-1} for 2 h) was then infused. Further basal and 5-min post TRH blood samples were taken (as above) at 30 min intervals both during and up to 2h after the infusion ended. Plasma was separated and prolactin levels determined by radioimmunoassay. Differences arising during and post-infusion were investigated using a two-way analysis of variance.

Dopamine infusion caused a significant reduction in the increment in TRH induced prolactin secretion both during infusion and 60 and 90 min post-infusion (pre infusion increment: 7.5 ± 2.3(6) ($\bar{x}$ ± SEM (n)) ng rat prolactin RP-3. ml^{-1}; during infusion: 2.1 ± 0.2(6), $p<0.005$; 30 min post infusion: 5.1 ± 1.4(6); 60 min post infusion: 2.4 ± 1.3(6), $p<0.01$; 90 min post infusion: 2.0 ± 0.9, $p<0.005$). Nomifensive infusion also caused significant reductions in TRH induced prolactin secretion both during infusion ($p<0.005$) and 60 min post infusion ($p<0.05$). Neither dopamine or nomifensive significantly reduced basal plasma prolactin levels during or post-infusion. This may be at least partly due to the animals being oestrogen primed in order to facilitate TRH responsiveness.

TRH infusion, as expected, significantly increased basal prolactin levels but at the end of the 2 h infusion, levels had fallen to <50% of their peak value (86.6 ± 25.0(3) at 60 min of infusion vs. 37.9 ± 3.5(3) at 120 min of infusion). Thirty minutes post infusion both normal basal and normal acute TRH response prolactin levels were seen but 90 min post infusion both basal and acute TRH response levels were markedly reduced (control basal: 15.9 ± 3.9(3); 90 min psot infusion basal; 3.2 ± 0.2, $p<0.01$; and control TRH 80.5 ± 10.4(3); TRH response 90 min in post infusion: 8.6 ± 1.3, $p<0.005$).

These experiments highlight the sensitivity of acute TRH stimulation of prolactin secretion to factors which perturb basal prolactin secretion.

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EFFECTS OF EXTRACELLULAR CATIONS AND DIBUTYRYLCAMP ON DOPAMINE AND OCTOPAMINE INHIBITION OF IDENTIFIED HELIX NEURONES

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Dopamine is an inhibitory transmitter in the central nervous system of Helix aspersa and the dopamine induced hyperpolarization (H response) is potassium mediated (Kerkut et al 1969). The aim of the present study was to investigate whether this potassium response is calcium dependent and whether it is mediated by cAMP. A dopamine dependent adenylate cyclase has been isolated from Helix pomatia (Osborne 1977). The octopamine H response of Helix neurones is also potassium mediated (Bokisch 1984). Since the actions of octopamine have been shown to be mediated through a specific adenylate cyclase in other systems (Nathanson & Greengard 1973), the possible involvement of cAMP in the octopamine H response of Helix neurones was also examined.

Microelectrode recordings were made from cells in the isolated suboesophageal ganglionic mass of Helix. Cells F-5/6 and E-15/17 were used for the dopamine and octopamine H responses respectively (Kerkut et al 1975). Cells were voltage clamped at membrane potential using a Dagan 8100 single electrode clamp. Drugs were applied either directly into the bath containing the nervous system or ionophoretically. All experiments were repeated at least five times and the values given are from typical experiments.

The dopamine response was affected by the extracellular concentrations of both sodium and calcium. However the dopamine induced potassium current was not calcium dependent since it was potentiated in calcium free saline, for example, the dopamine induced current increased from a control value of 0.85 nA to 1.54 nA. In sodium free saline the dopamine induced current was depressed from 1.23 nA to 0.54 nA in a typical experiment. This dependence of the dopamine H response on both extracellular sodium and calcium agrees with observations on Aplysia neurones (Chesnoy-Marchais & Ascher 1983). Sodium free saline also increased the rate of desensitization of the Helix dopamine H response. Bath addition of 1 μM dibutyrylcAMP (dbcAMP) induced an outward current in cells F-5/6 and the dopamine response was potentiated following addition of dbcAMP. For example, control currents of 0.81 nA were enhanced by dbcAMP to a maximum of 3.42 nA. Full recovery to control value occurred after 2 hours. A similar modulation of dopamine excitation has been observed following intracellular injection of cAMP into Lymnaea neurones (Akopyan et al 1980). Bath addition of 1 μM dbcAMP mimicked the effect of 4.2 μM octopamine on cells E-15/17. The octopamine response was potentiated following addition of 1 μM dbcAMP with a similar time course to that observed for dopamine. In a typical experiment, the octopamine current of 0.35 nA was potentiated to 0.96 nA.

It is concluded that the dopamine H response of cells F-5/6 is not calcium dependent but may be mediated through cAMP. Similarly the octopamine H response of cells E-15/17 may be linked to cAMP.

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A COMPARISON OF CLONIDINE AND κ-OPIOID INDUCED DIURETIC EFFECTS

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Following parenteral administration, clonidine has been shown to induce diuresis in rats (Puri, 1980; Miller, 1980). The mechanism of action of clonidine-induced diuresis is not clear. However, recent evidence suggests that this diuretic response might be attributable to an inhibition of vasopressin release from the neurohypophysis (Puri and Sur, 1985), as has been suggested for k-opioid induced diuresis (Leander, 1983). An attempt has therefore been made to ascertain whether similar mechanisms are involved in mediating clonidine and k-opioid induced diuresis in conscious rats.

Male Alderley Park rats (~200g) were used throughout. In conscious, saline (0.9%) loaded (4 ml/100g, p.o.) rats, clonidine (0.5 mg/kg, s.c., n=8) induced a significant diuresis (27.0 ± 0.6 ml) over 6 hours, compared to controls (11.7 ± 0.3 ml). A similar diuretic response (24.3 ± 0.9 ml) was observed after administration of the k-opioid agonist tifluadom (3.5 mg/kg, s.c., n=8). Pretreatment with the selective α_2-adrenoceptor antagonist idazoxan (RX 781094, 0.5-1.0 mg/kg, s.c.) which abolished clonidine-induced pressor responses, had no effect on the clonidine or tifluadom induced diuresis, (32.0 ± 1.0 and 24.3 ± 2.2 ml respectively), indicating a lack of α_2-adrenoceptor involvement in this response. Clonidine-induced diuresis was also unaffected (28.0 ± 1.2 ml) by pretreatment with naloxone (10 mg/kg, s.c.) which was effective in preventing tifluadom-induced diuresis (11.3 ± 1.0 ml).

As reported previously (Blackburn et al., 1985), bilateral adrenal demedullation abolished k-opioid induced diuresis, indicating a peripheral rather than purely neurohypophyseal component. Nevertheless, clonidine-induced diuresis was unaffected in rats, which had been bilaterally adrenal demedullated seven days previously.

Whilst tifluadom-induced diuresis does not involve α_2-adrenoceptors, but does involve opioid receptors and is, at least in part, dependent upon an intact adrenal medulla in the conscious rat, there is no evidence that clonidine-induced diuresis involves α_2 or opioid receptors nor is dependent upon a functional adrenal medulla. Hence, different mechanisms appear to be involved in mediating clonidine and tifluadom-induced diuresis in rats and, while plasma vasopressin levels have yet to be measured, it is possible that clonidine, but not tifluadom, inhibits the neurohypophyseal release of vasopressin and so induces diuresis.

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SODIUM REGULATION OF OPIOID BINDING IN NG 108-15 NEUROBLASTOMA X GLIOMA HYBRID CELLS AND 7315c PITUITARY TUMOR CELLS

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Sodium has been shown to regulate opioid binding sites in neural membrane preparations by increasing the agonist dissociation rate (Blume, 1978). However, the cellular location at which sodium exerts its regulatory effect on binding has not been determined. We report here the effects of varying the sodium concentration (at constant ionic strength) on binding at μ and δ opioid receptors, and the effects on opioid binding of the sodium ionophore, monensin. Treatment with monensin results in an increase in intracellular sodium without a change in extracellular concentration (Motulsky and Insel, 1983). The effects of sodium and monensin have been examined in NG 108-15 cells, which have a homogeneous population of δ opioid receptors, and in 7315c pituitary cells, which have μ receptors (Frey and Kebabian, 1985).

The specific binding of [^{3}H]etorphine to membrane preparations or intact cells was determined at 37° in a Krebs-HEPES medium, as described previously (Werling, Zarr, Brown and Cox, 1985). Under each treatment condition, binding of [^{3}H]etorphine concentrations from 0.1 to 25 nM was measured. The pooled results of replicate experiments were analyzed by non-linear curve fitting procedures. Replacement of sodium in the Krebs-HEPES by potassium resulted in an apparent approximately two-fold increase in the number of etorphine binding sites (B_{max} increased from 260 to 430 fmol/mg protein) in NG 108-15 cell membranes, with no change in the affinity of binding. Similar effects were observed in intact NG 108-15 cell suspensions when sodium in the extracellular medium was replaced by N-methylglucamine (N-MG). In contrast, replacement of sodium by potassium in 7315c cell membrane incubates resulted in a two-fold increase in etorphine affinity (K_D decreased from 1.6 to 0.71 nM) without any change in the number of binding sites. In intact 7315c cell suspensions, sodium replacement by N-MG also resulted in an increase in agonist affinity. Thus both δ and μ opioid receptors are affected by sodium. Sodium produced a modest lowering of agonist affinity at all the μ sites in 7315c cells, but caused a major reduction in agonist affinity at about 50% of the δ sites observable in the absence of sodium, resulting in an apparent reduction in the number of δ binding sites in NG 108-15 cell membranes.

When monensin (30 μM) was added to intact NG 108-15 cell suspensions in Krebs-HEPES, [^{3}H]etorphine binding was significantly reduced (by about 60%). However, when sodium in the medium was replaced by N-MG, monensin was without effect. Monensin also reduced [^{3}H]etorphine binding in intact 7315c cells provided sodium was present in the extracellular medium. The ionophore had no effect of [^{3}H]etorphine binding by membrane suspensions prepared from NG 108-15 cells, and only slightly reduced binding in 7315c membranes. Thus the effects of monensin on opioid binding are dependent both on the presence of sodium and on the integrity of the cell membrane. The results suggest that sodium acts at an intracellular site to regulate agonist binding at both δ and μ receptors.

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THE EFFECTS OF σ-OPIOID RECEPTOR AGONISTS ON URINE-OUTPUT IN THE RAT

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Opioid receptor agonists produce marked effects on urine output in the rat (Leander, 1983); κ-agonists being diuretic and μ-agonists antidiuretic. In their original classification of opioid receptors, Martin et al.(1976) postulated a further subtype - the σ-receptor - activation of which produces psychotomimetic effects. We have thus studied the effects of putative σ- receptor agonists on urine output in the water-loaded rat.

Adult rats (180-230g), PVG hooded, were starved overnight and then water-loaded (25ml/kg) immediately prior to subcutaneous administration of opioid drug, given alone or mixed with an opioid antagonist, either naloxone or naltrexone. Urine output was then recorded hourly for 5h after injection.

The prototype σ- agonists, SKF10,047 and cyclazocine, produced diuresis at low dose-levels (0.625 - 10mg/kg s.c.), an effect which was reduced by naltrexone 5mg/kg s.c.. At higher doses, SKF10,047 (40-80 mg/kg s.c.) and cyclazocine (10-40 mg/kg s.c.) produced antidiuretic effects. Antidiuresis was also seen with two other psychotomimetic opioids, pentazocine and dextrorphan, as well as with the μ-agonist fentanyl (0.02 - 0.2mg/kg s.c.) (Table 1). However, whereas the antidiuresis produced by fentanyl, 0.2mg/kg s.c., was completeley abolished by naloxone, 1mg/kg s.c, the antidiuretic effects of the psychotomimetic opioids were either unaffected or only slightly reduced by naloxone, 5mg/kg s.c., leaving a naloxone-resistant component. Phencyclidine, 0.25-4mg/kg s.c. a non-opioid hallucinogenic drug, which is suggested to interact with a binding site similar to the σ- receptor (Zukin & Zukin, 1981) produced only a naloxone-insensitive diuresis.

TABLE 1. Antidiuresis produced by opioid receptor agonists and effect of naloxone.

Drug	Dose (mg/kg s.c.)	Mean Urine Output (ml ± s.e.) at 2h After Dosing: Control	Drug Alone	Drug plus Naloxone 5mg/kg
SKF10,047	40	4.9 ± 0.7	1.4 ± 0.2*	2.0 ± 0.4*
SKF10,047	80	6.1 ± 0.1	1.5 ± 0.1*	1.4 ± 0.3*
Cyclazocine	40	5.6 ± 0.1	1.4 ± 0.2*	3.1 ± 0.4*
Pentazocine	40	5.3 ± 0.2	1.0 ± 0.4*	3.4 ± 0.4*
Dextrorphan	80	5.4 ± 0.2	3.9 ± 1.0	2.1 ± 0.5*
Fentanyl	0.1	5.4 ± 0.4	0.0 ± 0.0*	5.1 ± 0.4 (Naloxone 1mg/kg 5.4 ± 0.5)

* Significant from control at $p<0.05$

The naloxone-reversible diuresis seen with low doses of SKF10,047 and cyclazocine may be κ-receptor mediated; but the naloxone-insensitive antidiuretic effects seen with higher doses of psychotomimetic opioids may reflect an interaction with the so-called σ-receptor.

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CYCLO-OXYGENASE INHIBITORS AFFECT MORPHINE DEPENDENCE IN GUINEA PIG ISOLATED ILEUM

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A model of opiate dependence can be consistently induced and measured in vitro using the guinea pig isolated ileum preparation (Collier et al. 1981). When this tissue is incubated with morphine (Mor) a phenomenon, that may be a model of dependence, can be observed and measured as a contraction of the longitudinal muscle of the myenteric plexus on the application of the opiate antagonist naloxone. Using this model, the effects of the cyclo-oxygenase inhibitors indomethacin (Ind) and mefenamic acid on the inducton of opiate dependence and withdrawal have now been investigated.

Segments of ileum were incubated for 22-24 hours at room temperature (20-25°C) in Krebs bicarbonate buffer solution containing hexamethonium (70μM) and test drugs. This solution was constantly perfused through the bath using a multi-channel peristaltic pump. The tissues were tested in baths at 37°C. The withdrawal response to 1μM naloxone was measured as a percentage of the maximum response to acetylcholine (ACh) on the same tissue.

RESULTS

INCUBATION MEDIUM	TEST MEDIUM	RESPONSE TO NALOXONE (%MAX.RESPONSE TO ACh)
1μM Mor	1μM Mor	40.4 ± 3.4 (6)
1μM Mor	1μM Mor + 1.3μM Ind	7.0 ± 2.6 (6)
1μM Mor + 1.3μM Ind	1μM Mor + 1.3μM Ind	5.0 ± 2.5 (6)
CONTROL	CONTROL	7.7 ± 3.7 (6)

The ilea incubated with morphine but tested in the presence of morphine and indomethacin showed a significantly reduced response to naloxone in comparison to paired ilea that were tested in the presence of morphine alone. This suggests therefore that indomethacin inhibits the expression of the withdrawal response rather than preventing the development of the phenomenon. Preliminary experiments with the alternative cyclo-oxygenase inhibitor mefenamic acid (10μM) have given similar results to those with indomethacin.

These experiments may indicate a role for prostaglandins in the expression of opiate withdrawal in this preparation.

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EFFECT OF OPIATE RECEPTOR ANTAGONISTS ON ALCOHOL-INDUCED INCREASE IN ENDOGENOUS DOPAMINE RELEASE FROM RAT CORPUS STRIATUM IN VITRO

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We have previously demonstrated that alcohol treatment in vivo results in a time-dependent increase in the basal secretion of endogenous dopamine (DA) from the rat corpus striatum (CS) measured in vitro (Holman & Snape, 1985). Striatal DA release can also be increased by endogenous opioid peptides (Chesselet et al, 1983). In addition, the opiate antagonist, naloxone has been reported to reverse alcohol-induced coma (Ducobu, 1984) and to prevent increased striatal DA turnover following alcohol pretreatment (Barbaccia et al, 1980). The present study was carried out to assess whether alcohol acts directly to increase DA release or indirectly through an endogenous opiate peptide link.

Male C/D rats were killed by decapitation and the striata rapidly dissected. The tissue was chopped into blocks (1.0 mm^{-3}) and then incubated in a modified Tyrodes solution (pH=7.4) containing Hepes buffer (5mM), pargyline (350uM) and l-DOPA (4uM). The endogenous DA concentrations in ten consecutive 15 min samples were assayed by HPLC with electrochemical detection (Mefford, 1980). In control experiments the basal secretion of DA declined gradually from an initial value of 0.97±0.06 ng/mg tissue to 0.78±0.05 ng/mg tissue. The addition of alcohol (75mM) to the incubation media at the start of an experiment resulted in a time- and calcium-dependent increase in the efflux of DA up to maximum of 213±13.0% of the control. The increase in DA release was reduced with lower doses of alcohol, but was still significantly greater than the control in the presence of 25 mM alcohol (154±9.0%). A high dose of naloxone (50uM) was required to prevent the alcohol-induced increase in DA release; while 25uM naloxone was ineffective. ICI 174864, a specific antagonist at delta-opiate receptors, produced dose-dependent (0.5-10.0 uM) inhibition of the increase DA release elicited by the presence of alcohol (75mM). When tissues were treated with either naloxone (50uM) or ICI 174864 (10.0uM) alone, not in combination with alcohol, basal DA secretion did not differ significantly from the control.

The results suggest that alcohol is not acting directly on the DA terminals to increase endogenous DA release from the striatum. However, since the increase in release can be blocked by ICI 174864, the alcohol's effects appear to be mediated indirectly through the release of an endogenous delta opioid agonist, possibly Met- or Leu-enkephalin.

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ENHANCEMENT OF A DELAYED HYPERSENSITIVITY REACTION IN MICE BY THE LIPOXYGENASE INHIBITOR AA861

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Induction of paw oedema in mice by a delayed hypersensitivity reaction to methylated bovine serum albumin (MBSA) has been shown to be inhibited by NSAIDs administered at the time of antigen challenge, and immunosuppressive agents given at the time of sensitisation (Cashin et al, 1979). Since LTB_4 is a potent chemotactic and chemokinetic agent and may also modulate T-cell activity (Gualde et al, 1985) the effect of the selective lipoxygenase inhibitor AA861 (Ashida et al, 1983) has been evaluated in this model and compared to both NSAIDs and immunomodulators.

Groups of 10 mice (MF1 ♂ 20-25g) were sensitised on day 0 by i.d. injection of equal volumes of 0.5% w/v MBSA and Freunds Complete Adjuvant. On day 8 they were challenged by subplantar injection of 0.02 ml 1% w/v MBSA in one hind paw and an equal volume of sterile saline in the other. 24 h later paw volumes were measured by H_2O displacement plethysmography and the oedema expressed as the % increase in volume of the challenged paw compared to the control paw. Drugs were administered (10 ml kg^{-1} p.o.) on days 0-4 (sensitisation) or days 7-9 (elicitation).

Table 1 Effect of drugs on MBSA induced paw oedema

1 Sensitisation day 0-4			2 Elicitation day 7-9		
Treatment	$mgkg^{-1}$	% paw swelling	Treatment	$mgkg^{-1}$	% paw swelling
Control		$^{1}76.6\pm8.1$	Control		$^{3}83.5\pm6.9$
		$^{2}90.5\pm4.2$			$^{4}99.9\pm5.8$
Azathioprine	100	$^{1}17.8\pm2.9^{c}$	Indomethacin	2	$^{4}52.9\pm9.3^{c}$
Cyclophosphamide	30	$^{1}23.8\pm10.5^{c}$	BW755c	30	$^{3}83.4\pm8.0$
Dexamethasone	5	$^{1}35.5\pm16.0^{a}$		100	$^{3}64.5\pm10.6$
AA861	30	$^{2}93.9\pm9.8$	AA861	10	$^{4}139.3\pm11.0^{b}$
	100	$^{2}109.2\pm8.3$		30	$^{3}108.1\pm6.4^{a}$
				100	$^{3}46.9\pm11.0^{a}$

All values $\pm$ s.e.m. (n=10), significant difference from control[1,2,3,4]

$p<0.05^{a}$; $<0.01^{b}$; $<0.001^{c}$; Student's t-test

Enhancement of the paw oedema by the lower doses of AA861 given during the elicitation phase may reflect increased cyclooxygenase activity following the inhibition of lipoxygenase. At the higher dose AA861 inhibited the response as did indomethacin suggesting that it may not be selective for lipoxygenase at higher concentrations. In contrast, at the doses used, BW755c was inactive. Administration of AA861 during the sensitisation phase had no effect on development of the reaction whilst the immunomodulators and dexamethasone significantly reduced the response. The findings suggest that lipoxygenase metabolites may be of little importance in induction and expression of the inflammatory response in this model and inhibition of lipoxygenase may further increase production of pro-inflammatory prostaglandins.

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STUDIES ON TWO NOVEL TRH ANALOGUES

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To differentiate the central from the endocrine actions of thyrotrophin-releasing hormone (TRH, Glp-His-ProNH$_2$), two novel TRH analogues (Glp-Nva-ProNH$_2$ and Kpc-Leu-ProNH$_2$; Nva = norvaline, Kpc = L-6-keto-pipecolic acid) have been synthesized, with effects believed to be limited to the central nervous system (Szirtes et al.1984). To study these central effects, the TRH analogues were injected in 0.5 µl saline in the periaqueductal grey region (PAG) of male Sprague-Dawley rats (180-200g) and their ability to induce wet-dog shaking (WDS) compared with TRH (Widdowson et al. 1983). Injection sites were confirmed histologically. The analogues' ability to displace ^{3}H-TRH (Amersham) from specific receptors on 15 µM coronal sections of rat brain was investigated. The stability of the two analogues to degradation by rat brain peptidases was studied by HPLC (Griffiths et al. 1985). Conformational preferences for the analogues were calculated by a gradient minimization technique incorporating a solvent effect based on the Onsager reaction field (Finn et al. 1984).

Both Glp-Nva-ProNH$_2$ and Kpc-Leu-ProNH$_2$ significantly stimulated WDS after PAG injection:- 1 µg Kpc-Leu-ProNH$_2$ 89.4 ± 10.7 WDS/30 min; 1 µg Glp-Nva-ProNH$_2$ 68.6 ± 15.1 WDS/30 min; 1 µg TRH 63.5 ± 17.4 WDS/30 min (n = 6-8 ± S.E.M.). Binding studies revealed an IC_{50} for Glp-Nva-ProNH$_2$ of 100 nM and Kpc-Leu-ProNH$_2$ of 5 µM; for TRH a value of 20 nM was obtained. HPLC studies showed relatively rapid degradation of the analogues by soluble and particulate rat brain peptidases to the respective deamidated forms (as a result of action by proline endopeptidase) and to the respective diketopiperazines (via pyroglutamyl aminopeptidase). Computer-predicted conformations of the two analogues (with multidimensional scaling) suggest that they fit two of the three preferred TRH conformations (P, $Y_{1,3}$ and $Y_{2,3}$):- Kpc-Leu-ProNH$_2$ with $Y_{1,3}$ and Glp-Nva-ProNH$_2$ with P. Neither of these conformations was preferred by RX 77368 (Glp-His-3,3 dimethyl ProNH$_2$), a hyperactive TRH analogue, for which the conformation $Y_{2,3}$ was calculated.

The results suggest that both novel TRH analogues can act centrally to induce WDS, presumably via binding to TRH receptors. Their activity was not related to enhanced stability to brain peptidase activity but may be due to their ability to adopt conformations similar to those preferred by TRH. A combination of experimental techniques with theoretical global minimization for prediction of conformation may provide a new means of designing neuropeptide analogues.

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AUTORADIOGRAPHIC LOCALIZATION OF VIP RECEPTORS IN GUINEA PIG AND HUMAN LUNG

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Vasoactive intestinal peptide (VIP)-immunoreactive nerves have been localized in the lung of several species including man (Dey et al., 1981) and pharmacological studies indicate that VIP exerts important biological actions on structures in the lung innervated by nerves containing this peptide (Said, 1984). Using [^{125}I]VIP, high and low affinity binding sites have been demonstrated in lung membrane preparations from several species and recently the autoradiographic localization of VIP receptors has been reported for rat lung (Leroux et al., 1984). We have applied a similar autoradiographic technique to localize binding sites for VIP in guinea-pig and human lung.

Preliminary binding studies on membrane preparations and lung slices showed that [^{125}I]VIP binding was displaced by native VIP. Competition experiments showed that unlabelled VIP was more potent than PHI at displacing labelled VIP whilst VIP fragments 1-10 and 16-28 were ineffective. The displacement curve for VIP was shallow and consistent with the presence of a second, low affinity binding site.

For autoradiographic studies, cryostat sections of lung from guinea-pig and human (obtained at lobectomy and microscopically normal) were mounted on microscope slides and incubated at a radioligand concentration of 30pM for 3h at 37^{o}C in 50mM Tris-HCl buffer (pH 7.4, 37^{o}C) containing 5mM $MgCl_2$, 2% polypeptide, 500 KI units ml^{-1} Trasylol and 0.5mg ml^{-1} Bacitracin. Non-specific binding was determined by parallel incubation of slices in the presence of 1μM unlabelled VIP. Under these conditions non-specific binding represented 10% of total binding for guinea-pig and 34% for human lung.

Autoradiograms showed specific labelling over discrete structures in both guinea-pig and human lung. Labelling was present over submucosal glands in the trachea of guinea-pig and intrapulmonary bronchi of human. Airways were also labelled, with the epithelium of these structures having a higher grain density than the smooth muscle. Labelling over smooth muscle of bronchioles was sparse compared with that over the muscle in larger airways. Vascular smooth muscle was also labelled in both guinea-pig and human lung as were the walls of the alveoli.

With the exception of the alveolar walls, the localization of VIP receptors on structures in the lung is consistent with known functional effects of the peptide and with the reported pattern of VIP-like immunoreactive innervation. It is difficult to explain the presence of VIP receptors on the alveolar walls in the absence of any reported functional effects of the peptide on the cells of the alveolar walls.

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