LITHIUM TRANSPORT IN GUINEA PIG ISOLATED JEJUNAL MUCOSA

Birch N J, Coleman I P L and Karim A R, Biomedical Research Laboratory, School of Applied Sciences, The Polytechnic, Wulfruna St, WOLVERHAMPTON WV1 1LY.

Absorption *in vitro* of lithium in rat small intestine was found to be time and concentration dependant and uptake by the tissue was passive (Birch *et al* 1983, 1984a). Similar findings were reported for isolated epithelial cells from guinea pig small intestine (Birch *et al* 1984b). Subsequently, lithium permeation in guinea pig small intestine was shown not to follow saturation kinetics (Karim *et al* 1984).The present investigation reports the characteristics of lithium transport in the guinea pig isolated jejunal mucosa; its mechanism and site of permeation in a three compartment model.

Guinea pig isolated jejunal mucosa was prepared (Lauterbach 1977) by separating it from the underlying tissue layers and incubated between two flux chambers (exposing an area of 0.2 cm^2) at 37°C in Krebs-Tris medium, gassed with oxygen at both sides. ^{3}[H]-Polyethylene glycol (PEG) was measured by liquid scintillation and lithium determined by atomic absorption spectroscopy (IL 357).

Viability was confirmed by a steady potential difference during incubation, active transport of glucose, post-incubation histological examination and minimal lactate dehydrogenase release. Artifical porosity was checked using ^{3}[H]-PEG permeation.

The transmucosal fluxes and tissue uptake of lithium in the absorptive mucosal (M) to serosal (S) and secretory (S to M) directions were linearly related to the lithium concentration. Furthermore, the uni-directional fluxes both in the absorptive and secretory direction were similar. Total lithium transport after 45 minutes into serosal or mucosal compartments was 3-4 times greater than that found in the tissue. The plasma membrane of epithelial cells offers greater resistance to the movement of lithium than whole epithelium indicating that majority of ions pass via 'pores' in the epithelium rather than intracellularly.

Permeation of lithium correlated with that of PEG suggesting that movement of lithium in either direction occurred via same PEG permeable, extracellular pathway. Confirmation for this route was obtained using solutions of high osmolarity, which collapsed the tight junctions (Madara 1983): Lithium absorption was reduced ($P<0.02$). Clinically, this observation may be relevant to the pharmacokinetics of lithium absorption. Passive permeability of electrolytes and small ions is largely determined by the paracellular pathway (Schultz 1981).

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CHARACTERISATION OF EX-VIVO LUMINOL-AMPLIFIED CHEMILUMINESCENCE ACCOMPANYING THE INITIATION OF FOOT-PAD OEDEMA IN THE KOCH MODEL

E.J. Dowling*, M.K. Jasani[1], D.V. Parke, R.F. Peters[1] and A.M. Symons, Department of Biochemistry, University of Surrey, Guildford, Surrey and [1]Ciba-Geigy Pharmaceuticals Division, Horsham, West Sussex RH12 4AB

In the Koch model, the inflammatory response induced by the subplantar inoculation of Freund's complete adjuvant (FCA) is accompanied by in vivo formation of malondialdehyde, prostaglandins and substances that ex vivo give rise to intense chemiluminescence (Dowling et al, Proceedings this meeting).

The possible occurrence and relative contributions of singlet oxygen, hydrogen peroxide (H_2O_2) and free hydroxyl radicals (OH·) to the observed ex vivo chemiluminescence was investigated in the present study. Foot-pad inflammation was induced in male Wistar rats (200-250g) previously immunised to FCA (Bullock et al, 1983). Following challenge, the FCA-inoculated and contralateral non-inoculated hind paws were amputated post-mortem at the ankle. The plantar skin was dissected and reflected backwards exposing the underlying inflamed tissue. The dissected paw was immersed in a solution of luminol (5-amino 2,3 dihydro 1,4 phthalazinedione, 800 ng) in phosphate buffered saline (PBS, 4 mls), contained in a scintillation vial, and chemiluminescence was measured using a scintillation counter (LKB Wallac 1216) set in the out of coincidence mode. The samples were counted for 1 min at 4°C.

The presence and relative contribution of H_2O_2 and OH· radicals was determined by measuring luminol-amplified chemiluminescence (LAC)in the presence of catalase (5400 IU) and mannitol (10 µm) respectively. Singlet oxygen was determined by measuring its spontaneous ultra-weak chemiluminescence in the presence of the enhancer DABCO (1,4-diazabicyclo 2,2,2 -octane, 330 µm), Deneke & Krinsky (1977). These measurements were carried out sequentially on the same paw, washing with PBS and checking the LAC with the initial value between each procedure.

Following challenge, LAC increased in a biphasic manner as shown in Figure 1a. Figure 1b shows that H_2O_2, OH· and singlet oxygen contributed variously to both phases. Peak contributions of H_2O_2 were seen during both the first phase at 4 h and the second phase at 8 h; that of OH· was seen between the two phases at 6 h; that of singlet oxygen was seen only during the second phase at 8 h.

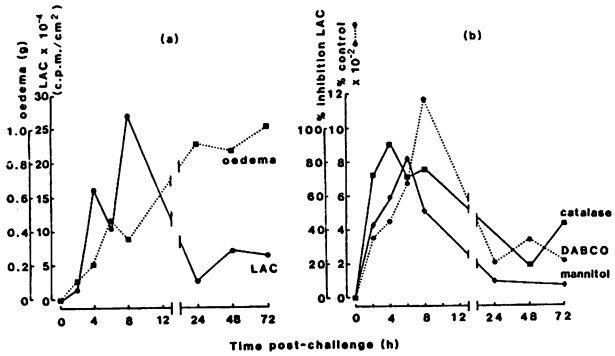

Figure 1. Comparison of time-course of development of a) luminol-amplified chemiluminescence (LAC) and foot-pad oedema and b) mean inhibition by catalase and mannitol of luminol chemiluminescence and chemiluminescence with DABCO (n = 4).

The data indicate that in this model of inflammation the initiation of foot pad oedema occurs in association with the production of at least three types of free radical. They suggest further that their contribution to luminol-amplified chemiluminescence occurs in a sequential manner: H_2O_2 : OH· : singlet oxygen.

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80P

CONTRIBUTION OF MUCUS TO LUMINAL ALKALINISATION IN AN ISOLATED RAT DUODENUM PREPARATION

S.P. Canfield & J.P.N. White, Department of Physiology, St. Mary's Hospital Medical School, London W2 1PG

There are numerous reports that the duodenum is able to dispose of an acid load in the lumen without damage to the mucosa. Much information on the mechanism involved has been obtained from in vitro studies using amphibian duodenum (Flemstrom et al, 1982). However, such preparations may not be appropriate to elucidate duodenal control mechanism in mammals. This work describes an original in vitro duodenum preparation from the rat and an investigation of the contribution of mucus to the apparent rate of luminal alkalinisation.

Distal duodenum was removed from female Wistar rats (180 - 200g) and flushed with warm saline. Tissues were suspended in 15 ml of HCO_3^- buffered saline (pH 7.4) maintained at 37°C and gassed with 95% O_2/5% CO_2. The lumen was perfused with an unbuffered saline (0.3 ml min^{-1}) gassed with 95% O_2/5% CO_2 (pH≏4.70). The perfusate was collected at room temperature and titrated at 15 minute intervals to pH 4.5 with 1mM HCL. The collected perfusate was continuously gassed with 95% O_2/5% CO_2 to ensure a steady pCO_2 in the solution. The rate of alkalinisation is expressed as µmol cm^{-1} h^{-1}. Mucus in the perfusate was assessed as hexose content by the orcinol method (Bradbury et al, 1980) following acid hydrolysis and is expressed as µg cm^{-1} h^{-1}. All drugs were added to the serosal buffering solution. Differences between means were considered significant if $P<0.05$ by the Mann-Whitney 'U' Test.

The rat duodenum showed a steady rate of luminal alkalinisation over several hours and had a mean value of 1.44 ± 0.16 µmol cm^{-1} h^{-1} (s.e. mean, n = 9). Replacement of the HCO_3^-/CO_2 buffer in the serosal saline with Hepes/O_2 caused a significant reduction ($P<0.001$) of the rate of alkalinisation to 0.54 ± 0.04 µmol cm^{-1} h^{-1} (n = 6). Mucus output in the perfusate was <25 µg cm^{-1} h^{-1} which is the limit of detection with the methods used.

During the experiment, mucus plugs developed within the duodenal lumen. These could be removed by 'agitation', rapidly compressing the outflow tubes several times. The presence of this mucus in the perfusate transiently increased the titre to give a mean rate of alkalinisation of 8.16 ± 0.82 µmol cm^{-1} h^{-1} (n = 6) and a corresponding mucus content of 544 ± 77 µg cm^{-1} h^{-1} (n = 6). Similar results were obtained when the mucus plug was removed by addition of carbachol (10^{-5}M). Rates of alkalinisation were also significantly increased ($P<0.01$) by addition of IBMX (5×10^{-4}M) to 3.58 ± 0.40 µmol cm^{-1} h^{-1} (n = 6) without any change in mucus output. Responses to IBMX were significantly larger ($P<0.05$) following removal of the mucus by agitation or carbachol than when the mucus was present.

The rat duodenum in vitro is able to alkalinise perfusing saline at a steady rate comparable with that found in amphibian duodenum in vitro (Garner et al, 1984) and can sustain this for several hours. The presence of mucus in the perfusate increased this rate considerably and this contribution can only be assessed by simultaneous measurement of the mucus content. Alkalinisation was increased by the addition of IBMX without any change in mucus content of the perfusate. The apparent effect of the presence of a mucus plug on the response to IBMX suggests that the mucus layer may represent a barrier to ionic exchange between tissue and perfusate and this is being further investigated.

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THE EFFECTS OF DIABETIC RAT AND HUMAN SERUM ON THE POLYMERISATION OF BRAIN TUBULIN

S.J. Beahon, [1]I.F. Casson and W.G. McLean, Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX and [1]Royal Liverpool Teaching Hospital, Prescot Street, Liverpool, L7 8XP

Known alterations in slow axonal transport and axon calibre in experimental diabetes have suggested that abnormalities in structure or function of axonal cytoskeletal proteins may contribute to the neuropathy (Sidenius and Jakobsen, 1982, Mayer et al., in press). This is supported by the observation that brain tubulin from diabetic rats polymerises less readily into microtubules than does tubulin from normal rats (Williams et al., 1982). We have addressed the question of whether or not serum from diabetic animals or human patients might contain factors which influence the function of microtubule proteins.

Microtubule proteins were prepared from pig brain by two cycles of assembly/disassembly (Shelanski et al., 1973). Rats were made diabetic by a single intravenous injection of 50mg/kg streptozotocin. Diabetes was defined as a fasting blood glucose level in excess of 11.1mM. Serum was obtained by tail vein puncture 14 days after injection of streptozotocin or, in the case of control rats, citrate buffer. Patients with diabetes (Type I or Type II) were selected from the diabetic clinic on route visit. Control subjects were patients in a non-diabetic ward. Neuropathy in the diabetic patients was diagnosed as absence of vibration sence at the ankle and absent ankle jerks on reinforcement and was confirmed in most cases by nerve conduction measurements. Serum was coded for use in a double-blind manner. Serum was dialysed for 24h at 4°C against a buffer pH6.8 containing 100mM PIPES, 0.5mM $mgSO_4$ and 1mM EGTA.

Maximum assembly of microtubules occurred within approximately 3 min, after an initial lag period of 10-40s. The microtubules could be depolymerised by cooling to 4°C and induced to undergo second and third cycles of polymerisation by rewarming. The effects of rat serum on lag time and maximum assembly were measured on the first cycle, while those of human serum on maximum assembly were measured on the third cycle.

The presence of dialysed serum from control animals increased the lag time by 251 $\pm$ 59% and reduced maximum assembly to 76 $\pm$ 12% of their values in the absence of serum. The effects of diabetic serum was considerably greater, at 447 $\pm$ 143% and 65 $\pm$ 5%, respectively (n = 8). The difference between controls and diabetics was significant at the $P < 0.001$ and $P < 0.01$ levels, respectively (by Mann-Whitney rank sum analysis). In the case of the human samples, the maximum assembly was reduced to 68 $\pm$ 15% by control serum (n = 6) and 42 $\pm$ 13% by diabetic serum (n = 11). That difference was significant at the $P < 0.005$ level. There was no significant difference between the effects of serum from diabetic patients with neuropathy and those without.

We conclude that high molecular weight substances in diabetic serum more markedly inhibit microtubule polymerisation. The nature of these substances is currently being investigated. Their ability to impair microtubules may be important in a number of the cellular defects, including neuropathy, which show up as complications of diabetes. It is unlikely however that they are the sole causative agents of neuropathy in the clinical situation.

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THE EFFECTS OF DRUGS ON THE QUANTITY OF FOOD CONSUMED BY MICE UNDER NOVEL AND FAMILIAR CONDITIONS

J.E. Hartley, R.J. Stephens and A.M. Thompson. Wyeth Research (UK) Ltd, Huntercombe Lane South, Taplow, Maidenhead, Berks.

Exposure to a novel environment induces signs of stress which include a reduction in food consumed by hungry animals (Hall, 1934). In the present study, this phenomenon was used in an attempt to develop a method for the evaluation of the effects of drugs on stress-dependent behaviour.

One set (familiarized) of female mice (18-25g) was individually housed for 5 days in small plastic cages with access to water and a dish of mash (equal parts pelleted diet and water). Another (naive) was kept for the same period in a stock cage with access to water and pelleted food. On the morning of the 6th day, after being fasted overnight, groups of 12 mice from each set were given various doses of a test-drug or vehicle 15 min before being presented with a weighed dish of mash. The quantity of food eaten/mouse over the following 30 min was recorded. Mice from the stock cage were transferred to individual small plastic cages at the beginning of the feeding session. Thus, in contrast to the other set, both food and cages were novel to these mice. Mice from both sets were used in each experiment and the design was such that the order (position) of the individual boxes was balanced with regard both to sets and treatments. Probit analysis was used to estimate the doses required to decrease or increase food consumption by 50% in both naive and familiarized mice. Exploratory locomotor activity of separate groups of satiated mice was counted automatically in open fields over a 2 min period starting 30 min after treatment with the test drugs.

Food consumption was 1.76g/30 min for familiarized mice and 0.88 g/30 min for naive animals (mean of 18 expts.) with s.e.ms of about 15% of the means. The putative anxiogenic agents leptazol (Shearman and Lal, 1979) and β CCE (File, 1982) caused a decrease in food consumed by both sets of mice (see table) at doses not markedly affecting motor activity. Haloperidol and d-amphetamine also attenuated eating equally in both sets but only at doses that modified locomotion significantly. Diazepam and clonidine, however, caused an increase in consumption but only in naive animals; relatively large doses of clonidine attentuated feeding by both sets of mice. These results show that the acute effects of anxiolytic agents on food consumption by fasted mice may be affected substantially by the degree of familiarization with the environment. It is concluded that the procedure may be of value in the evaluation of potential anxiolytic/anxiogenic agents.

Table 1. Effects of drugs on food consumption and motor activity in mice.

Drug	ED_{50} or 150 (mg/kg s.c) with 95% confidence limits		
	Food intake (naive)	Food intake (famil)	Motor activity
Haloperidol	(-).50 (.36;.68)	(-).26 (.16;.43)	(-).66 (.22;2.0)
D-amphetamine	(-).50 (.35;.73)	(-).53 (.38;.73)	(+).50 (.23;1.0)
Leptazol	(-) 34 (31;38)	(-) 36 (30;43)	(-) >50
βCCE	(-) 48 (22;104)	(-) 45 (9;220)	NSE 1-300
Diazepam	(+) 1.0(.66;1.6)	NSE.1-30	(-) >30
Clonidine	(+).02 (.002;.15)	(-).13 (.09;.19)	(-).42 (.17;1.1)
	(-).40 (.15;1.2)		

(-) decrease, (+) increase relative to control. NSE, no significant effect.

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THE EFFECT OF MEPTAZINOL ON RAT SKELETAL AND SMOOTH MUSCLE

F.A.WALI. Anaesthetics Unit,The London Hospital Medical College,Whitechapel, London E1 1BB,U.K.

Meptazinol (Wyeth,England) is a potent opioid analgesic drug in both man and animal species (Goode & White,1971;Paymaster,1976;Green,1983) with mixed agonist /antagonist activity.Recent developments in analgesia and anaesthesia have indicated that meptazinol differs from the standard opiate analgesics not only in its binding sites but also in its central effects,as it appears to affect cholinergic transmission in mice (see Green,1983;for a review see Stephens,Waterfall & Franklin,1978;Bill,Cowlrick,Fox,Todd,Ward,Wood & Wyllie,1981).

The present investigation was carried out to examine the effect and interactions of meptazinol at the rat phrenic nerve-hemidiaphragm neuromuscular junction and rat intestinal smooth muscle to see if meptazinol altered neurotransmitter release and/Or the responses produced by cholinergic and non-cholinergic agents.

The preparations were set up in separate organ baths (20 or 80 ml) containing Krebs-Henseleit solution maintained at 38± 2°C and bubbled with O_2 & C O_2 (19:1). The muscles were indirectly stimulated at a repetitive rate of 0.2-100 Hz with 5V and 0.5 ms pulse duration.The contractile responses produced either by nerve stimulation or by drug action were recorded isometrically using a force displacement transducer and a Washington pen recorder.

At the rat neuromuscular junction,meptazinol(3.7-370 µM) initially increased then decreased the twitch contractions in response to indirect stimulation.The mean increase in the twitch tension was 33± 1.3% (mean±s.e.,n=6,$P < 0.001$).This was followed by a 95% reduction in the twitch contractions in 4-5 min.Physostigmine (0.77 µM) slightly potentiated the effect of meptazinol.High concentrations of meptazinol(500 µM) blocked the twitch contractions in about 1-2 min.Meptazinol (370 µM) also blocked the contractions evoked by motor nerve stimulation at high frequencies,e.g 30 or 50 Hz,by 50-60%. If compared to the local anaesthetic lignocaine (372 µM),the neuromuscular blocking agents tubocurarine (12.7 µM) and atracurium (16 µM), meptazinol (370 µM) was the most effective twitch inhibitor in the rat skeletal muscle. Fentanyl (290 µM) and morphine(290 µM) ,unlike meptazinol(370 µM) always reduced the twitch contractions without initial stimulation (reduced by 35±1.2% and 27±1.6%,n=6,$P < 0.001$,respectively).

In the rat intestinal smooth muscle,meptazinol(370 µM) immediately reduced the spontaneous contractions by 30-40%.The contractures produced by ACh (55 nM-5.5 uM) or TEA (1.2-12 mM) were also reduced in meptazinol.A mean maximum contracture of 2.7±0.5 g was obtained with ACh (275 nM) and this was reduced in meptazinol by 35±3% (n=6,$P < 0.001$).Similar results were obtained with fentanyl and morphine.

In conclusion,although the ionic basis of meptazinol-induced increase in twitch tension was not further investigated,the results may indicate that this effect could be attributed to a cholinergic mechanism,i.e an effect on ACh release. In the rat ileum,the relaxation induced by meptazinol may be due to an action via opioid-receptor-dependent mechanism involving enhanced release of noradrenaline.

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INFLUENCE OF ASSAY BUFFER ON DISSOCIATION CONSTANTS OF DRUGS AT SUBTYPES OF β-ADRENOCEPTORS

G.A. McPherson, P. Molenaar, E. Malta & C. Raper, School of Pharmacology, Victorian College of Pharmacy, Parkville, Victoria 3052, Australia.

Dissociation constants (K_D values) for propranolol (PROP), betaxolol (BETAX), ICI 118,551, (-)-isoprenaline (ISO), salbutamol (SALB) and RO363 have been assessed from their ability to displace [^{125}I]iodocyanopindolol (^{125}ICYP) from guinea-pig left atrial (β_1) and uterine (β_2) membrane homogenates (McPherson *et al.*, 1984) suspended in either a 50 mM Tris-HCl or a complete ionic buffer (Krstew *et al.*, 1984). Both buffers contained EDTA (0.1 mM) and ascorbic acid (1 mM); only the ionic buffer contained GTP (0.1 mM).

Saturation studies using ^{125}ICYP (10 - 200 pM) revealed that the maximal density of β_2-adrenoceptor sites in uterine membrane homogenates was similar in both buffers whilst in atria the maximal density of β_1-adrenoceptor sites in the ionic buffer was approximately 48% of that in Tris buffer. For the six drugs the slope factors of their respective displacement curves were close to unity in both tissues and in both buffers. The mean pK_D values for the drugs are shown in Table 1.

Table 1. Mean K_D values (nM) for the six drugs at β_1- and β_2-adrenoceptor sites in ionic or Tris buffers

	β_1 (atria)		β_2 (uterus)	
	Tris	Ionic	Tris	Ionic
Antagonists				
PROP	2.7 ± 0.5	3.7 ± 0.4	2.3 ± 1.2	1.5 ± 0.3
BETAX	11 ± 3	6.6 ± 1.5	537 ± 210	988 ± 186
ICI 118,551	83 ± 18	172 ± 12	3.5 ± 1.5	3.4 ± 0.2
Agonists				
ISO	226 ± 50	379 ± 34	202 ± 62	985 ± 160
SALB	11300 ± 4000	5480 ± 1000	455 ± 124	1310 ± 88
RO363	37 ± 9	14 ± 2	293 ± 40	935 ± 184

Values are mean ± s.e.mean from 3 - 6 experiments.

The nature of the buffer used markedly altered the affinities of selective agonists but had little influence on the affinities of selective antagonists at β_1- and β_2-adrenoceptor sites. Thus for SALB, its relative β_2-adrenoceptor selectivity was approximately 25-fold in Tris but only 4-fold in the ionic buffer. For RO363 its relative β_1-adrenoceptor selectivity was approximately 8-fold in Tris and some 67-fold in the ionic buffer. It is therefore concluded that the relative β_1-/β_2-adrenoceptor selectivity of the agonists is dependent on the assay buffer used.

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DOPAMINE RECEPTORS ON NON-DOPAMINE NIGRAL NEURONS MAY MEDIATE DOPAMINE AGONIST INDUCED CHANGES IN LOCOMOTOR ACTIVITY

P. Jenner, E. Kelly & C.D. Marsden, MRC Movement Disorders Research Group, University Department of Neurology and Parkinson's Disease Research Centre, Institute of Psychiatry and King's College Hospital Medical School, Denmark Hill, London SE5, U.K.

Bilateral intranigral injections of dopamine agonists can increase (Jackson & Kelly, 1983) or decrease (Bradbury et al, 1983) locomotor activity in rodents. Such effects may reflect an action on dopamine cell body autoreceptors or on dopamine receptors located on nigral non-dopaminergic neurones. We report on the effect of intranigral injection of dopaminergic drugs on locomotor activity and on striatal dopamine turnover.

Female Wistar rats (151-175 g) received bilateral injections of drug or equivalent amount of sucrose into substantia nigra zona reticulata (A + 2.0, L + 2.0, V - 2.5). Locomotor activity was measured in photocell cages for a period of 30 min. Striatal HVA and DOPAC concentrations were determined 15 min post injection.

Bilateral injection of apomorphine (2.5 ug) reduced motor activity compared to sucrose-injected controls ($F\ [1,48] = 32.4$, $p < 0.01$, by two-tailed, two-way ANOVA with replicates) and reduced striatal HVA and DOPAC concentration (Table 1). Bilateral intranigral injection of dopamine (10 ug) slightly but non-significantly reduced locomotor activity ($F\ [1,96] = 5.86$, $p > 0.01$), while a higher dose (50 ug) induced hyperactivity ($F\ [1,60] = 32.0$, $p < 0.01$). Dopamine (10 or 50 ug) did not alter striatal HVA or DOPAC concentrations (Table 1). Intranigral injection of (±)-3PPP (2.5 ug) did not affect motor activity ($F\ [1,54] = 0.07$, $p > 0.05$) whereas (±)-3PPP (10 ug) enhanced motor activity ($F\ [1,60] = 11.2$, $p < 0.01$). Again neither dose altered striatal HVA or DOPAC concentrations. Injections of haloperidol (2.5 ug) into substantia nigra did not alter motor activity ($F\ [1,84] = 0.03$, $p > 0.05$) but increased the striatal DOPAC concentration (Table 1).

Table 1 Striatal HVA and DOPAC concentrations

	Apomorphine (2.5 ug)	Dopamine (10 ug)	Dopamine (50 ug)	(±)-3PPP (2.5 ug)	(±)-3PPP (10 ug)	Haloperidol (2.5 ug)
HVA	73 ± 6*	80 ± 7	105 ± 16	95 ± 9	87 ± 4	117 ± 11
DOPAC	73 ± 9*	96 ± 4	95 ± 6	84 ± 9	107 ± 4	138 ± 7*

Values are ± SEM and are expressed as a per cent of controls injected with the same weight for weight dose of sucrose. * $p < 0.05$ by Student's t test. Range of values following intranigral sucrose (0.1 - 50 ug) were: HVA between 0.84 and 1.12 ug/g wet weight striatum and DOPAC between 1.13 and 1.33 ug/g wet weight striatum.

The intranigral application of dopaminergic drugs can influence motor activity and striatal dopamine turnover. However the changes in motor activity did not correspond to alterations in dopamine turnover. We suggest that nigral dopamine receptors other than cell body autoreceptors, at least in part may be responsible for alteration in motor activity induced by the intranigral application of dopaminergic drugs.

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86P

$GABA_A$ AGONISTS POTENTIATE AND BACLOFEN ANTAGONISES THE L-5-HTP HEAD-TWITCH

S.L. Handley and L. Singh, Drug Mechanisms Research Group, Department of Pharmaceutical Sciences, University of ASton, Birmingham, B4 7ET.

We have previously reported the effect of drugs which modify central GABA transmission on the head-twitch induced by L-5-hydroxytryptophan (L-5-HTP) (Handley and Singh, 1984). We now examine the effect of GABA agonists on this behaviour.

Male MF1 mice (20-30g) received carbidopa (9.0 mg/kg; s.c.) 15 min before L-5-HTP (200 mg/kg; i.p.) Head-twitches were counted for 5-min starting 20 min after L-5-HTP. Each mouse was observed in parallel with a control animal (saline /carbidopa/L-5-HTP).

The GABA receptor agonists muscimol, imidazoleacetic acid (IMAA) and 3-aminopropanesulphonic acid (3-APS) (Hill and Bowery, 1981) were administered before L-5-HTP and the ED_{200} (the dose to double the control response) was calculated for each drug (Table 1). IMAA and 3-APS did not cause head-twitching when given alone at the ED_{200} for potentiating L-5-HTP. Muscimol caused a low incidence of head twitching which was insufficient to account for its potentiation of L-5-HTP.

Table 1. ED_{200} Values for $GABA_a$ receptor agonists. (95% confidence limits in brackets).

Drug	Time of Administration	ED_{200} mg/kg (i.p.)
muscimol	Simultaneously with L-5-HTP	0.26 (0.19 - 0.36)
IMAA	30 minutes before L-5-HTP	16.02 (9.93 - 25.85)
3-APS	15 minutes before L-5-HTP	83.45 (45.54 - 152.9)

The $GABA_b$ receptor agonist baclofen (Hill and Bowery, 1981) was administered (i.p.) simultaneously with carbidopa and ID_{50} (dose to halve the control response) was calculated to be 2.66 mg/kg (95% confidence limits: 3.86 - 1.84).

These results suggest that the stimulation of $GABA_a$ receptors potentiate the L-5-HTP head-twitch, which is in line with the action of $GABA_a$ receptor antagonist bicuculline, shown to reduce this behaviour (Handley and Singh, 1984). The $GABA_b$ agonist baclofen reduced this syndrome, supporting our previous suggestion that the inhibitory effect of GABA on the L-5-HTP head-twitch in the mouse may be related to an effect at these receptors (Handley and Singh, 1984).

We wish to thank Ciba-Geigy for the gift of baclofen.

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LOCUS COERULEUS LESIONS DO NOT AFFECT DIAZEPAM- OR α-ADRENERGIC-MODULATION OF OPERANT CONFLICT

S.L. Handley, S. Mithani & L. Singh, Drug Mechanisms Research Group, Department of Pharmaceutical Sciences, University of ASton, Birmingham B4 7ET.

Agonists and antagonists of α-adrenoceptors have well defined effects in animal models of fear and anxiety (Handley & Mithani, 1983; 1984 a,b,c) however the neuronal basis of these effects is as yet unknown. The noradrenergic Locus Coeruleus-dorsal bundle system (LC-db) has been implicated in the expression of fear-motivated behaviours (Redmond & Huang, 1979), although not all workers agree on this involvement (Mason & Fibiger, 1979). We have therefore investigated the effect of LC lesions on the effects of α-adrenoceptor ligands in an operant conflict model of anxiety.

Male Lister Hooded rats received 6-OHDA or vehicle bilaterally into LC. 6 rats which were subsequently shown to have sustained at least 80% depletion of hippocampo/cortical noradrenaline (hypothalamic noradrenaline and striatal dopamine concentrations were unaffected) and 8 shamoperated controls were trained on a VI_{30}sec schedule of reinforcement for condensed milk reward. The schedule was divided into four 5 min periods, the second being accompanied by reward-contingent footshock and the fourth being time-out. Rats were reinforced daily and drugs injected at weekly intervals commencing 21 days post-lesion.All rats successfully aquired the reinforcement schedule. Footshock-induced suppression of lever pressing was titrated to produce <6 lever presses/5 min for diazepam, clonidine and prazosin and later adjusted to 6-10 lever presses/5 min for yohimbine and phenylephrine. There were no significant differences in performance between lesioned and shamoperated rats during unpunished or time-out periods, or in the shock intensity required to produce the specified response suppression level in the punished period.

Table 1. Lever presses/rat/5 min during punished period (mean +/-sem).

drug/dose		lesioned	sham operated	
diazepam	2.5	31.0+/-5.1 (3.5 +/-1.2)	42.5+/-9.3(3.5 +/- 0.9)	ns
clonidine	0.025	8.5+/-2.3 (2.8 +/-0.7)	13.5+/-4.4(5.4 +/- 2.2)	ns
prazosin	0.05	8.3+/-1.9 (3.5 +/-1.7)	7.4+/-0.4(2.6 +/- 1.4)	ns
phenyl-ephrine	0.5	0.3+/-0.4 (6.6 +/-0.5)	1.1+/-0.36(6.9+/-1.0)	ns
Yohimbine	2.5	0.2+/-0.7 (6.3 +/-1.0)	1.0+/-0.9(5.9+/-1.2)	ns

All drugs were $p<0.05$ vs previous day's vehicle control (shown in brackets). ns:- drug effects on lesioned vs sham, $p>0.05$.

Table 1 shows that the antipunishment effects of clonidine and prazosin were not modified by the presence of LC lesions,neither were the punishment-enhancing effects of yohimbine or phenylephrine. These results suggest that an intact LC-db system is not essential either for normal performance, or for the expression of the effects of the above drugs, in this model of anxiety.

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88P

DECREASED GABA SYNTHESIS IN REGIONS OF RAT BRAIN FOLLOWING A SEIZURE

A.R. Green, Corinne Hayes, A. Metz, M.C.W. Minchin, Helen Williams & N.D. Vincent, MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford OX2 6HE

Following an electroconvulsive shock (ECS), bicuculline- or flurothyl-induced seizure there is a marked rise in seizure threshold lasting for over 3h and which appears to be associated with an increase in GABA function (Nutt et al, 1981). There is also an increase in the concentration of GABA in several brain regions following a convulsion (Bowdler & Green, 1982). However, since this change was not seen after a flurothyl-induced seizure, it seems unlikely that the rise in GABA concentration is directly responsible for the change in seizure threshold (Bowdler & Green, 1982). We have now examined the rate of GABA synthesis in the brain following a seizure in an attempt to define further the way that GABA function is altered by a convulsion.

GABA synthesis was measured by determining the rate of GABA accumulation in specific brain regions following administration of the GABA transaminase inhibitor amino oxyacetic acid (AOAA) as described by others (e.g. Bernasconi et al, 1982). Rats were either handled or given a single ECS (125V 1s through ear-clip electrodes) and 5min later injected with AOAA (10 mg kg^{-1} i.p.). They were killed 20, 40 or 60min later by a focussed high-intensity microwave beam (70W/cm^2) for 4s, the brain dissected into regions and tissue GABA concentration measured fluorimetrically as described elsewhere (Bowdler & Green, 1982).

Following AOAA injection, the GABA concentration rose linearly in hippocampus, striatum and cortex and the mean synthesis rates were calculated to be respectively 5.75, 4.70 and 3.40 μmole per g brain tissue (wet weight) h^{-1}. In confirmation of an earlier study, 5min following a convulsion the GABA concentration rose in all three regions (e.g. hippocampus, control: 1.95 ± 0.68 (4) μmole g^{-1}; ECS: 3.63 ± 0.60 (6) μmole g^{-1}). However, in the ECS-treated animals there was little further increase in GABA concentration in the 60min following AOAA. (Handled + AOAA: 7.55 ± 1.55 (6); ECS: 3.35 ± 0.28 (6); ECS + AOAA: 3.87 ± 0.96 (6); all expressed as μmoles g^{-1}). These data suggest an almost complete inhibition of GABA synthesis following a seizure. A similar inhibition was seen following a flurothyl-induced convulsion. Two hours after ECS the rate of GABA accumulation following AOAA had returned to normal values. The inhibition of synthesis was not due to increased concentrations of AOAA in the brain following the seizure producing an inhibition of glutamic acid decarboxylase since AOAA given at three times the dose (30 mg kg^{-1}) produced a similar accumulation of GABA to that seen after 10 mg kg^{-1}.

These data therefore demonstrate that 5min following a convulsion there is an almost total inhibition of GABA synthesis and that this change lasts for less than 2h.

AM is an Oxford Regional Health Authority Fellow in Clinical Psychopharmacology and NDV holds a MRC Research Studentship.

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GABA RELEASE IN RAT BRAIN FOLLOWING A SEIZURE

A.R. Green, M.C.W. Minchin & N.D. Vincent, MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford OX2 6HE

Five min following an electrically or drug-induced seizure GABA synthesis is almost totally inhibited in hippocampus, striatum and cortex (Green et al, 1985). We have now investigated possible reasons for this inhibition by studying GABA release in brain tissues taken from rats given a seizure.

Rats were handled or given a single electroconvulsive shock (ECS) (125V 1s through ear-clip electrodes). Thirty min later they were killed and brain regions dissected out and chopped on a McIlwain tissue chopper in two directions at 45° at 0.2 mm intervals. The slices were rinsed three times in oxygenated Krebs solution. Approximately 200 mg of tissue was incubated for 10min in oxygenated Krebs solution at 37°C, the supernatant withdrawn and replaced with Krebs solution containing high K^+ (40 mM) for a further 10min. The supernatants from both incubations were acidified with 50 µl HCl (1 M) and placed on a Dowex H^+ column. GABA was eluted with NH_4OH (1 M), dried under N_2 and the residue dissolved in HCl (0.002 M) for fluorimetric analysis of GABA (see Bowdler & Green, 1982). Spontaneous release of GABA in cortical slices was unchanged in rats given an ECS. In contrast, there was a significant decrease (30%) in K^+-evoked GABA release from the slices of ECS-treated rats (Table). An inhibition of K^+-evoked release was also seen in hippocampal (32%) and striatal (51%) slices prepared from rats given ECS.

In further experiments tissue samples prepared as above (except that the slices were 0.1 mm thick) were pre-incubated with 2 µCi [^{3}H]-GABA for 30min at 37°C and filtered (GF-A), placed in a superfusion system and the fractional rate constant determined prior to and during K^+-evoked release. In this situation no inhibition of [^{3}H]-GABA release was observed in the cortical slices prepared from ECS-treated rats (Table) compared with handled controls.

It appears, therefore, that following an ECS there is an inhibition of release of endogenously stored GABA and that this is not seen in slices preloaded with [^{3}H]-GABA. It is possible that the inhibition of release precedes and perhaps initiates the inhibition of GABA synthesis which is also seen at this time (Green et al, 1985), although we have been unable to detect any change in GAD activity following a seizure (Green, Minchin & Vincent, unpublished).

Table: Release of GABA in slices of cortex from rats given a single ECS

	Endogenous GABA release ($\mu mole\ g^{-1} h^{-1}$)		[^{3}H]-GABA release fractional rate constant ($min^{-1} \times 10^{-3}$)	
	Spontaneous	K^+ (40 mM)	Spontaneous	K^+ (40 mM)
Control	2.51 ± 0.36 (5)	7.72 ± 0.45 (5)	5.4 ± 0.4 (7)	14.9 ± 2.2 (7)
ECS	2.57 ± 0.61 (6)	5.43 ± 0.51 (6)*	5.6 ± 0.6 (7)	14.9 ± 2.8 (7)

Values are mean ± SD with the number of experiments in brackets. Different from control K^+-induced release: *$p < 0.001$ 2-tailed t-test.

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90P

AUTORADIOGRAPHIC LOCALISATION OF 5-HT AND IMIPRAMINE BINDING SITES IN THE RAPHE NUCLEI OF THE HUMAN BRAIN

J.M. Candy, R.H. Perry & M. Yates, MRC Neuroendocrinology Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE

High affinity binding sites for imipramine are present in the human brain and are thought to be associated with the neuronal uptake for serotonin (5-HT; Langer et al, 1981). In this communication we report the presence of a high density of binding sites for imipramine and 5-HT in the raphe nuclei of the human brain.

Blocks of tissue from the brain stem containing the raphe nuclei were obtained from 3 male patients (48, 53 and 54 years with 20, 10 and 12 h post mortem delay respectively) who had no clinical history of neurological or mental illness. The blocks were frozen in melting arcton (Freon 12, ICI) and stored at -70°C. Serial, coronal, 15 µm cryostat sections were cut, thaw mounted and air dried before storage at -30°C. Serial, triplicate sections were preincubated for 10 min at 4°C in a) 50 mM tris-HCl, pH 7.4 containing 125 mM sodium chloride and 5 mM potassium chloride for imipramine binding sites, b) 50 mM tris-HCl, pH 7.4 containing $5x10^{-5}$M pargyline and 1g/l ascorbic acid for 5-HT binding sites, and c) 50 mM tris-HCl, pH 7.7 for S_2 5-HT binding sites. The sections were then incubated for 60 min at 4°C in for assay a) 1.5 nM ^{3}H-imipramine (22.0 Ci/mmol, Amersham), assay b) 6 nM ^{3}H-lysergic acid diethylamide (LSD 46.7 Ci/mmol, NEN), and assay c) 2 nM ^{3}H-ketanserin, 64.6 Ci/mmol NEN), sections were also incubated in the presence of 10 µm imipramine, 1 µM 5-HT and 1 µM ketanserin respectively to define non-specifically bound ligand. The sections were washed for a total of 10 min for the imipramine and LSD ligands and for 5 min in the case of ketanserin, and then dipped in ice cold distilled water, rapidly dried under a stream of argon and exposed to tritium sensitive film (Ultrofilm, LKB) for 6 to 8 weeks. The autoradiographic localisation of imipramine binding sites revealed that the highest density of binding sites was associated with the dorsal raphe nucleus, raphe medianus and ventral aspect of the periaqueductal grey, a high density of binding sites was also associated with other raphe nuclei in the pons and medulla. LSD labels both S_1 and S_2 5-HT binding sites in the human brain (Cross, 1982) and the distribution of binding sites for this ligand was very similar to that observed for imipramine. Thus a high density of binding sites was observed in the raphe nuclei in agreement with the finding of Palacios et al (1983). The localisation of ketanserin binding sites which labels S_2 5-HT receptors (Leysen et al, 1982) revealed similar distribution to that for LSD with a marked localisation in the raphe nuclei, particularly the dorsal raphe nucleus and also the ventral aspect of the periaqueductal grey. This suggests that at least a proportion of the 5-HT binding sites in the human raphe nuclei are of the S_2 type in agreement with the finding of Slater and Patel (1983) in the rat.

These results demonstrate that high densities of both imipramine and 5-HT binding sites are associated with the raphe nuclei in the human brain and suggest that at least some of the effects of imipramine may be mediated by an effect on raphe cell bodies.

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APPARENT DESENSITISATION OF EXCITATORY AMINO ACID RECEPTORS IN RAT CORTEX

J.I. Addae and T.W. Stone, Department of Physiology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE.

Excitatory amino acid receptors in the central nervous system have been classified into three types, one of which is acted on by N-methyl-D, L-aspartic acid (NMDLA) (Watkins & Evans, 1981). In an attempt to develop an in vivo system to quantify the relative potencies of the amino acid agonists, we have looked at the effects of topical application of these agents on somatosensory evoked potential (SEP) in the rat cortex.

Male Wistar rats were anaesthetised with urethane and the contralateral forepaw representation on the somatosensory cortex exposed. A well of paraffin wax was constructed to hold 1ml of fluid over the exposed cortex. Solutions of various compounds were made in 0.9% NaCl and used to form a static pool of fluid at room temperature on the cortex, which was superfused continuously with 0.9% NaCl in between application of compounds. Monopolar recording of SEP from forepaw stimulation was made with a microelectrode at 500-800μm below the cortical surface. The effects of compounds were assessed by expressing the initial negative wave of the SEP (N_1) during the application of the compound as a percentage of the control value.

NMDLA in concentrations of 20-500μM applied for 2.5 min caused a decrease in N_1 with a steep dose-response curve. 500μM NMDLA completely abolished all components of SEP within 1 min, and if left on the cortex for a longer time the SEP returned to or beyond control level within 5 min. A second application of 500μM NMDLA within 5 min of the return of the SEP did not change the SEP. However 500μM NMDLA completely abolished SEP within 1 min if applied 60 min after the first application. Carbachol at 1mM for 2.5 min decreased N_1 by about 70% even if preceded by 500μM NMDLA for 5 min. The effect of NMDLA but not carbachol was prevented if preceded by 200μM 2-aminophosphonovaleric acid (APV), a specific NMDLA antagonist, applied for 15 min. D, L-homocysteic acid (DLH) produced a similarly steep dose response curve at concentrations of 0.2-10mM. It also exhibited apparent desensitisation. L-glutamic acid in concentrations of up to 20mM applied for 5 min, and quisqualic acid 4mM for 5 min did not change SEP. The lack of effect of L-glutamate and quisqualate may be due to the presence of an efficient inactivation mechanism for these amino acids (Lodge et al., 1980). This is supported by the finding that 100μM Cl-adenosine, an inhibitor of synaptic transmission, caused a 75% decrease in N_1, whilst 100μM adenosine which has an efficient uptake system did not alter SEP.

In our in vivo system NMDLA was about 10-fold as potent as DLH. However responses to both compounds appeared to indicate some kind of desensitisation of the receptors. In the hippocampus slice Fagni et al., (1983) demonstrated desensitisation to NMDLA but lack of any apparent desensitisation to DLH. The reason for this difference in DLH responses is not clear at present.

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THE EFFECT OF QUINOLINIC ACID AND THE KYNURENINES ON THE UPTAKE OF [^{3}H]-D-ASPARTIC ACID IN RAT BRAIN

J.H. Connick and T.W. Stone, Dept. of Physiology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 ORE.

Quinolinic acid is a member of the kynurenine pathway for the oxidation of tryptophan and its presence in the brain has recently been confirmed (Wolfensberger et al., 1983). Quinolinate has been shown to have powerful neurotoxic properties, which together with its excitant action on central neurones, appear to be mediated by the N-methylaspartate (NMA) class of amino acid receptors.

Schwarcz et al. (1984) have recently proposed a possible indirect action for these effects, and in an attempt to investigate this we have examined the effect of quinolinate and other kynurenines on the sodium dependent uptake of ^{3}H-D-aspartate.

The procedure used was essentially that of Davies and Johnston (1976). Male Wistar rats (250g) were decapitated and the cortex removed. 400μm slices were prepared using a McIlwain tissue chopper, and single slices placed in 2.5ml incubation medium; (KH_2PO_4, 1.25mM; $MgSO_4$, 0.92mM; KCl, 5.0mM; Glucose, 10.0mM; $NaHCO_3$, 25.9mM; NaCl, 123.9mM; $CaCl_2$, 3.1mM). The slices were gassed with 5% CO_2 in O_2 at 34°C, in the presence or absence of inhibitor, for 15 min before addition of ^{3}H-D-aspartate (10^{-8}M). They were then incubated for a further 8 min before being collected by filtration and washed with 10ml ice cold incubation medium. Activity was extracted in 1ml 10% TCA overnight and counted.

Table 1

Compound	Uptake as % Control	p value
Dipicolinic acid	85.25±0.95	<0.01
Kainic acid	82.50±1.34	<0.05
Kynurenine	129.73±10.38	n.s.
Kynurenic acid	87.75±11.69	n.s.
Anthranilic acid	91.50±5.24	n.s.
Nicotinic acid	137.50±14.50	<0.01
Quinolinic acid	94.50±7.60	n.s.
L-Aspartic acid	51.00±3.34	<0.01
D-Aspartic acid	53.13±2.70	<0.01

All values are expressed as mean ± s.e.m. of four separate determinations. All compounds were tested at 0.5mM.

It would appear that members of the kynurenine pathway have no significant effect on the uptake system of D-aspartate in brain cortex. This also implies that the uptake systems of L-glutamate and L-aspartate would be similarly unaffected. The only exception to this is nicotinic acid which apparently stimulates a 30% increase in uptake. The reason for this is at present unclear, and must await the results of release experiments. It is interesting to note that the quinolinate analogue, dipicolinate, inhibits uptake.

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NATURE OF THE FACILITATORY INFLUENCE OF GABA ON CENTRAL NORADRENERGIC TRANSMISSION

T. Dennis, and B. Scatton. Biochemical Pharmacology Unit, Synthélabo-LERS, 31 av. Paul Vaillant Couturier, 92220 Bagneux, France.

Recent biochemical evidence has shown that GABA exerts a facilitatory influence on central noradrenergic transmission. Thus, GABA mimetics given systemically or intracerebroventricularly increase noradrenaline synthesis and utilization rate and metabolite formation (Pycock & Horton, 1978 ; Andén et al., 1979 ; Scatton et al., (1982). However, the mechanism by which GABA modulates noradrenergic neuronal activity has not yet been elucidated. We have attempted to localize the anatomical site of this interaction by studying the effects of local micro-injections of muscimol in noradrenergic cell bodies (locus coeruleus) and projection areas on 3,4-dihydroxyphenylethylene glycol (DOPEG) levels in these areas. Moreover, the effect of a systemic administration of the GABA receptor agonist progabide (Kaplan et al.,1980) on hypothalamic DOPEG levels after ibotenic acid-induced lesion of the hypothalamus or acute cessation of noradrenergic impulse flow was also studied.

Experiments were performed on male Sprague-Dawley rats. For local injections, indwelling guide cannulae were implanted in the locus coeruleus (coordinates P : 0, L : 1.2, H : -4.4 at a posterior angle of 44° atlas of Pellegrino et al), in the nucleus accumbens (coordinates A : 2.2 (vs bregma) ; L : 1.2 ; H : -6.0, atlas of Paxinos and Watson) and in the hypothalamus (coordinates A : 6.0 ; L : 1.0 ; H : -2.0 ; atlas of König and Klippel). Ibotenic acid lesions were performed on chloral hydrate anaesthetised rats (400 mg/kg i.p.). 10 µg ibotenic acid in 0,5 µl phosphate buffer was infused into the hypothalamus. Free and total DOPEG were assayed by the radioenzymatic procedure of Dennis and Scatton (1982). Interruption of impulse traffic in noradrenergic neurons was achieved by a local injection of tetrodotoxin (50 ng) in the medial forebrain bundle (Scatton, 1982).

Local injection of high concentrations (1 µg) of muscimol into the locus coeruleus did not modify free or total DOPEG concentrations in the corresponding projection areas (septum, cortex and hippocampus) 1 h after infusion. In a similar manner, the local injection of muscimol (1 µg) into the nucleus accumbens or hypothalamus failed to affect DOPEG levels in these structures. Systemic administration of progabide (400 mg/kg i.p.) induced similar increases in hypothalamic total DOPEG levels in sham-operated and ibotenate-lesioned rats. Finally, progabide (400 mg/kg i.p.) no longer increased hypothalamic total DOPEG levels after acute interruption of noradrenergic impulse traffic.

The present results indicate that the facilitatory influence of GABA on cerebral noradrenergic transmission is not exerted via GABA receptors located in the vicinity of noradrenergic cell bodies or nerve endings. Moreover, this GABAergic influence depends upon ongoing noradrenergic nerve activity. The facilitatory GABAergic control of noradrenergic neurons may be mediated via GABAergic synapses involved in the transsynaptic neuronal circuits (the nature of which remains to be defined) regulating noradrenergic cell activity.

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94P

STEREOSPECIFIC BINDING OF L-TRYPTOPHAN TO RAT CORTEX - A LIGAND FOR TRYPTOPHAN UPTAKE SITES?

D.J.K. Balfour and M.E.M. Benwell, Department of Pharmacology and Clinical Pharmacology, University of Dundee Medical School, Dundee, DD1 9SY.

The facilitated uptake of L-tryptophan into serotonergic neurones is believed to be an important step in controlling the biosynthesis of 5-hydroxytryptamine (5-HT) in brain (Gerson et al 1974). Previously the properties of this process have been measured by kinetic analysis of the uptake of L-tryptophan by synaptosomes or slices. The purpose of the present study was to attempt to examine these carriers using a radioligand binding procedure employing ^{3}H-L-tryptophan.

The uptake of L-tryptophan was measured in a synaptosome fraction prepared from the cerebral cortices of male Wistar rats using the procedure described by Benwell and Balfour (1982). Membrane fractions for the binding studies were prepared from homogenates (50mg/ml) of cerebral cortex in 0.05M phosphate buffer (pH7.4) by a series of centrifugation and resuspension steps similar to those described by Romano and Goldstein (1980). L-tryptophan binding was measured by incubating the membrane suspension (approx. 1mg protein), in 1.5ml Eppendorf microtubes, with ^{3}H-L-tryptophan (50nM) in phosphate buffer (total volume 1ml) for 15 minutes at 35°C. Bound ^{3}H-L-tryptophan was separated from free by centrifugation (16,500gx 1 min) in an Eppendorf Microfuge. The pellet was washed rapidly with ice-cold buffer, the bottom of the tube cut off, and the tritium trapped in the pellet measured by scintillation counting. Non-specific binding was measured in the presence of 1mM DL-phenylalanine. The uptake and binding parameters were estimated using a computer-based curve fitting programme.

L-tryptophan binding to cortical membranes attained equilibrium within 5 minutes and was linear with protein over the range 0.2 $\pm$ 1.6 mg protein per incubation ($r = 0.98$; $p < 0.001$). Analysis of the binding data indicated a homogenous population of sites with a K_D for L-tryptophan of 14.4 $\pm$ 1.6μM and a B_{max} of 174 $\pm$ 7pmoles/mg protein. The K_m for L-tryptophan uptake into synaptosomes was 17.5 $\pm$ 1.3μM with a V_{max} of 664 $\pm$ 18pmoles/mg protein/min. The IC_{50} and K_I values for D-tryptophan were 199 $\pm$ 60 and approximately 285μM respectively. Large neutral amino acids competed effectively for both the binding sites and uptake system for L-tryptophan whereas acidic or basic amino acids appeared to have little affinity for either process. A significant correlation ($r = 0.95$; $p < 0.001$) was obtained between the effects of 18 amino acids (100μM) on L-tryptophan binding and uptake. Sodium cyanide (5mM) caused 98 percent inhibition of uptake but had no effect on binding. Neither tryptamine nor 5-HT had any effect on either process.

The study shows that L-tryptophan binds saturably and stereospecifically to rat cortical membranes and that the properties and pharmacological characteristics of these binding sites closely resemble those of the high-affinity carrier system for this amino acid present in synaptosomes.

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BLOCKADE OF AUDIOGENIC SEIZURE SUSCEPTIBILITY BY FOCAL INJECTION INTO THE INFERIOR COLLICULUS OF AN EXCITANT AMINO ACID ANTAGONIST

[1]C.L. Faingold, [2]B.S. Meldrum and [2]M.H. Millan, [1]Southern Illinois University School of Medicine, Springfield, Illinois, U.S.A. and [2]Department of Neurology, Institute of Psychiatry, Denmark Hill, London SE5 8AF

The inferior colliculus (IC) is an important site in the pathway for audiogenic seizure production in the Genetically Epilepsy Prone rat as shown by studies utilising electrical stimulation, focal lesions or focal injection of pharmacological agents. Recent studies suggest that an excitant amino acid plays a role in afferent transmission in the inferior colliculus (Faingold et al., 1984). Systemic administration of an excitant amino acid antagonist, 2-amino-7-phosphonoheptanoic acid (2-APH), reduces audiogenic seizure susceptibility in DBA/2 mice (Croucher et al., 1982).

Therefore, 2-APH was administered systemically to Genetically Epilepsy Prone Rats (University of Arizona, strain 9) to observe if this agent was an effective anticonvulsant against seizures in this model of epilepsy. A dose of 1 mM/kg of 2-APH administered intraperitoneally produced a significant reduction in the severity of audiogenic seizures in this strain of epilepsy prone rats. In order to investigate the site of action of this agent within the brain, we have studied the effect of focal injection of 2-APH into the IC of genetically epilepsy prone rats on audiogenic seizure susceptibility. Genetically Epilepsy Prone Rats were chronically implanted with guide cannulae over inferior colliculus (IC). The animals were tested at least a week after implantation with a mixed frequency sound (electric bell) at an intensity of 109 dB. The animals exhibited the complete pattern of maximal audiogenic seizure including wild running and clonus leading to tonic extension of the hind limbs. The following day they received focal infusions of 50 nM 2-APH in 0.5 µl buffer, given over 4 min, bilaterally into IC. In every case the sound induced seizure response was completely abolished. This anticonvulsant effect was fully established by 30 min; seizure susceptibility began to return 150 min after infusion. Complete recovery of the seizure severity did not occur until 270 min or more after the infusion. A reduction of motor activity was occasionally observed up to 60 min after 2-APH injection, but subsequently the anticonvulsant effect was evident without signs of altered activity.

These data suggest that the inferior colliculus may be a site of anticonvulsant action against audiogenic seizures of systemically administered excitant amino acid antagonists. These findings also raise the possibility that excessive action of an excitant amino acid in the inferior colliculus may be a mechanism underlying audiogenic seizure susceptibility.

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96P

INTRINSIC ACTIONS OF RO15-1788 AND CGS 8216: HYPERPHAGIA AND ANOREXIA INDUCED IN NON-DEPRIVED RATS CONSUMING A PALATABLE DIET

S.J. Cooper & Lorna Estall, Department of Psychology, University of Birmingham, Birmingham B15 2TT.

Benzodiazepine receptor antagonists include Ro15-1788 and CGS 8216 (Boast et al., 1983; Czernick et al., 1982; Hunkeler et al., 1981). Since benzodiazepine receptor agonists produce hyperphagia in nondeprived and food-deprived animals (Cooper, 1980), we investigated the possibility of intrinsic actions of these two compounds in a feeding test which has been shown to be particularly sensitive to drug-induced hyperphagic effects.

Sixty male nondeprived hooded rats (General strain, bred in our laboratory) were familiarised with a highly palatable diet (1 part sweetened condensed milk: 2 parts water) in 30 min daily sessions in a familiar environment (c.f. Locke et al., 1982). When intake had stabilised at asymptotic levels, drug test trials were initiated. In order to increase the sensitivity of the method to detect hyperphagic effects, rats were given access to the sweetened milk for 5 or 10 minutes before drug administration, as a partial presatiation procedure. For each drug tested, animals were allocated at random to six groups (n = 10 per group), and treated with different doses. In partially presatiated animals, the milk was restored 20-30 min following administration. To test for anorectic effects, rats were not presatiated: injections were given 30 min before the milk was first made available. Control animals were tested following corresponding vehicle injections. All injections were by the intraperitoneal route. The data were analysed using the analysis of variance and Dunnett's t test.

The benzodiazepine agonists, clonazepam (0.3125-5.0 mg/kg), midazolam (0.625-10.0 mg/kg), chlordiazepoxide (2.5-40.0 mg/kg), as well as phenobarbitone sodium (5.0-80.0 mg/kg), were consistent in producing significant hyperphagic effects. Maximum increases in milk consumption were in the range of 6.4-7.1 ml during a 30 min period, shown by rats that had been partially presatiated before drug administration. Clonazepam was the most potent compound tested and produced a maximum increase in consumption when given in a dose of 0.625 mg/kg.

Of the two putative benzodiazepine receptor antagonists tested, Ro15-1788 significantly <u>increased</u> milk consumption ($F_{5,54} = 2.81$, $p < 0.05$), with significant effects at 5.0, 10.0 and 20.0 mg/kg. The maximum effect, an increase in consumption of 5.1 ml occurred at 5.0 mg/kg. In contrast, CGS 8216 produced significantly <u>decreased</u> milk consumption ($F_{5,54} = 2.66$ $p < 0.05$). The maximum effect, a decrease in consumption of 5.7 ml, recorded in animals not presatiated, occurred at 20.0 mg/kg. The decrease in milk consumption could not have been due to a general suppression of ingestional responses, since CGS 8216 (2.5-40.0 mg/kg) had no effect on water consumption in thirsty rats. These data establish intrinsic actions of both Ro15-1788 and CGS 8216 in a test of palatability-induced food consumption in nondeprived rats. They confirm the importance of benzodiazepine receptor-mediated mechanisms in the control of ingestional responses. CGS 8216 may rank as a partial inverse agonist, with an apparent behaviourally-specific anorectic effect.

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SIMILAR ACTIONS OF A BENZODIAZEPINE AGONIST AND CONTRAGONIST ON INGESTIVE BEHAVIOUR

H.C. Jackson & R.D.E. Sewell, Division of Pharmacology, The Welsh School of Pharmacy, UWIST, Cardiff CF1 3XF, U.K.

FG 7142 (N'methyl-β-carboline-3-carboxamide) belongs to a class of compounds which have been described as benzodiazepine 'contragonists' since they appear to interact with benzodiazepine receptors but generally exert pharmacological effects opposite to those normally associated with benzodiazepines (Cowen et al. 1981; Dorow et al. 1983). In a recent report, however, FG 7142 was found to produce a decrease in body temperature in a similar fashion to a benzodiazepine agonist (Little et al. 1984). Both thermoregulatory and ingestive behaviours are closely associated with hypothalamic mechanisms. It was of interest in the present study, therefore, to investigate any possible appetitive effects of FG 7142 and to compare them with those of the benzodiazepine agonist diazepam.

Subjects were individually housed male Wistar rats (350-400g) which were allowed free access to powdered diet and tap water at all times. Experiments were carried out during the daylight period when the food and water intakes of control animals were minimal. All drugs were suspended in Tween 80 (2 drops/10 ml distilled water) and administered intraperitoneally in a dose volume of 1 ml/kg. Feeding jars and water bottles were weighed at the time of injection and after 1, 2 and 4 hours, enabling the calculation of mean group cumulative food and water intakes (g/kg rat weight ± SEM). Statistical comparisons were made using the Mann Whitney 'U'-test.

Animals were treated with diazepam or FG 7142 over the dose range 0.1 - 10 mg/kg i.p. and significant increases in both food and water intake were observed with 1.0 mg/kg of either agent compared to controls. However, a 10 mg/kg dose of either the agonist or contragonist induced locomotor depression which interfered with any possible stimulation of ingestive behaviour. Concurrent treatment with the benzodiazepine antagonist CGS 8216 (2-phenylpyrazolo[4,3-C]-quinolin-3[5H]one; 10 mg/kg i.p.) significantly reduced the increase in feeding and drinking induced by both diazepam (1 mg/kg) and FG 7142 (1 mg/kg) though CGS 8216 itself had no effects on food and water intake in doses up to 30 mg/kg i.p.

Thus FG 7142 appears to influence ingestive behaviours in a manner closely resembling diazepam. This finding lends support to the notion that benzodiazepine contragonists may, in some systems, produce qualitatively similar pharmacological changes as benzodiazepine agonists.

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COMPLEX INHIBITION OF [^{3}H]-5-HT BINDING BY SPIPERONE AND MIANSERIN

Pamela A. Blurton and M.D. Wood (Introduced by Christine Ennis), Wyeth Research (UK), Huntercombe Lane South, Taplow, Maidenhead, Berks. SL6 OPH

Evidence has accumulated to suggest that multiple 5-HT receptors exist in the CNS (Nelson et al, 1983). Recently the neuroleptic spiperone has been used to discriminate between two populations of 5-HT$_1$ binding sites (5-HT$_{1A}$ and 5-HT$_{1B}$ - see Nelson et al, 1983). The purpose of this study was to compare the abilities of spiperone and the 5HT-antagonist mianserin in discriminating between the two 5HT$_1$ sites in rat brain.

The binding of |^{3}H|-5-HT (2.0nM in inhibition studies; 0.05-20nM in saturation studies) to rat cortical membranes was performed essentially as described elsewhere (Nelson et al, 1983). The data were subjected to nonlinear least squares regression analysis (ALLFIT, Delean et al, 1978; LIGAND, Munson and Rodbard, 1980).

Radioligand binding studies for 5-HT provided evidence consistent with there being a single population of high affinity |^{3}H|-5-HT binding sites (k_D=5.83nM ± 0.42 (n=3); Bmax = 347 fmol/18mg tissue ± 8.7 (n=3)). Preliminary analysis suggested that mianserin and spiperone displayed a low affinity interaction with |^{3}H|-5-HT binding (IC_{50}=953 nM ± 64 (n=6); IC_{50}=965nM ± 110 (n=6), respectively). However, the slopes of the inhibition curves were significantly less than unity (Table 1) and nonlinear regression analysis revealed that the results obtained with both compounds were better described by two-site models (Table 1). The possibility that the two sites for mianserin were not identical to those for spiperone was suggested by the observation that, in paired experiments, the ratio of high to low affinity sites for the two compounds was significantly different. Furthermore, in the presence of a concentration of spiperone sufficient to block the high affinity spiperone-sensitive site (1000nM), inhibition of |^{3}H|-5-HT binding by mianserin was still consistent with the existence of two binding sites even though the maximum binding was reduced. Similar results were obtained for spiperone in the presence of a concentration of mianserin sufficient to block its high affinity site (250nM).

These results provide further evidence for the existence of multiple |^{3}H|-5-HT$_1$ binding sites. The sites discriminated between by spiperone and mianserin appear to be different thus supporting the concept of there being more than two |^{3}H|-5-HT$_1$ binding sites (Pazos et al, 1984).

Table 1. Inhibition of |^{3}H|-5-HT binding by spiperone and mianserin

	n	Slope	High affinity site		Low affinity site	
			IC_{50}	Bmax	IC_{50}	Bmax
M	6	0.65±0.02	75±20	42±4.6	2080±200	93±15
M+S	6	0.54±0.04	37±9.0	17±2.4	3670±470	52±5.9
S	5	0.36±0.02	41±8.1	63±10	47200±6500	69±11
S+M	5	0.34±0.02	48±14	46±8.4	95600±11000	56±98

Results were computed from inhibition curves for mianserin (M) and spiperone (S) in the presence and absence of S (1000nM) or M (250nM). IC_{50}'s in nM. Bmax in fmol/18mg tissue.

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QUANTITATIVE AUTORADIOGRAPHY OF [^{3}H] DYNORPHIN$_{1-9}$ BINDING SITES IN GUINEA PIG BRAIN

P. Slater, Department of Physiology, Medical School, University of Manchester, Manchester M13 9PT

The distribution of κ-type opiate receptors, labelled with [^{3}H]bremazocine, has been measured autoradiographically (Foote & Maurer, 1982; Patel & Slater, 1983). However, [^{3}H]bremazocine labels μ-, δ- and κ-sites equally well (Maurer, 1982) and for specific autoradiography it required the simultaneous blockade of μ- and δ -receptors . Binding studies have shown that dynorphin$_{1-9}$ is a selective κ receptor ligand (Corbett et al, 1980). In this study, quantitative receptor autoradiography has been used to visualise [^{3}H]dynorphin$_{1-9}$ binding sites in guinea pig brain sections. The distribution of [^{3}H]bremazocine binding sites (with μ- and δ-blockade) was also measured autoradiographically for comparison.

Slide mounted, cryostat-cut, serial sections (15μm) of female guinea pig brain were washed in 50 mM Tris HCl buffer (pH 7.4) at 2°C for 15 min. Some sections were covered with 0.15 ml of Tris buffer containing 4 nM [^{3}H]dynorphin$_{1-9}$ (25 Ci/mmol), bestatin (10μM), L-leucyl-L-leucine (2 mM), thiorphan (0.3 μM) and captopril (10 μM) and incubated at 2°C for 2 h. The sections were washed twice in fresh buffer (1 min, 2°C), dipped in ice-cold water, freeze dried and exposed to tritium film together with thin sections of tritium standards (Microscales, Amersham) for 12 weeks. A microdensitometer was used to analyse the images.

Other brain sections were incubated with 1 nM [^{3}H]bremazocine in buffer containing 100 nM of DAGO and DADLE (Patel & Slater, 1983) and exposed to film for 16 weeks.

Non-specific binding of both ligands was determined by co-incubating sections with 1μM of ethylketocyclazocine and scraping the sections into vials for liquid scintillation counting. Specific binding averaged 65% of the total for [^{3}H]-dynorphin$_{1-9}$ and 91% for [^{3}H]bremazocine.

[^{3}H]Dynorphin$_{1-9}$ bound to many brain areas, with low binding to white matter. The greatest densities of binding sites (fmol total binding/mg of tissue; mean of 6 sections) were recorded in the nucleus accumbens (2.0 ± 0.1), striatum (1.91 ± 0.03) and layers 5 and 6 of the cerebral cortex (1.9 ± 0.02). Medium concentrations of binding sites (1.0-1.3 fmol) were present in the globus pallidus, interpeduncular nucleus, substantia nigra (zona reticulata), the superficial layer of the superior colliculus and layers 1-4 of the cerebral cortex.

The regional distribution of [^{3}H]bremazocine binding sites bore a striking similarity to the distribution of [^{3}H]dynorphin$_{1-9}$ sites. There was a preferential binding of [^{3}H]bremazocine to the deeper (5, 6) layers of the guinea pig cerebral cortex (Foote & Maurer, 1982). Apart from a few patches in the antero-medial part of the striatum, neither ligand was concentrated in the discrete islands that is a characteristic of μ-type receptors in this brain area.

Under the experimental conditions used, [^{3}H]bremazocine binds specifically to κ receptors (Foote & Maurer, 1982). [^{3}H]Dynorphin$_{1-9}$ bound exclusively to the same sites but without μ- and δ-receptor blockade. Thus, [^{3}H]dynorphin appears to be a highly selective κ-receptor ligand.

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DOPAMINE ANTAGONIST ACTIVITY IS NOT A PREREQUISITE FOR INCREASING GASTRIC EMPTYING IN THE RAT WITH BENZAMIDES

B. McRitchie & W. Miner (Introduced by D.H. Turner), Beecham Pharmaceuticals Research Division, Coldharbour Road, The Pinnacles, Harlow, Essex. CM19 5AD

Metoclopramide is a stimulant of upper gut motility which possesses dopamine antagonist activity (Pinder et al 1976). In the guinea pig gastric emptying of radio-opaque pellets has been shown to be increased by metoclopramide after both peripheral (Ennis & Cox 1979) and central (Costall et al 1983) administration. Using a similar model in the rat, comparison has now been made of the effect of metoclopramide and BRL 20627, a novel benzamide which stimulates gastric motility in the apparent absence of dopamine blocking activity (McClelland et al 1983).

Male Wistar rats (250-500g), which had previously been fitted with a chronic gastric fistula, were used for these studies which permitted manipulation of the gastric contents with minimal stress to the animal. Animals were maintained on standard laboratory diet until immediately prior to the experiment. After gently lavaging the stomach free of food residue with warm water, 50 lead glass spheres of 1mm diameter were washed into the stomach via the fistula with 2mls of 25% w/v barium sulphate suspension (Micropaque DC), the barium sulphate being used to delineate the upper gut. Animals were then immediately dosed subcutaneously with either vehicle or test drug. One hour after dosing the location of the spheres was determined by X-ray fluoroscopy and the number having emptied from the stomach assessed by two independent observers.

The results (Table 1) show that both BRL 20627 and metoclopramide increased the gastric emptying of glass spheres in the rat over the dose range 1-10mg/kg, there being little difference in potency between the two drugs.

Table 1 Effect of BRL 20627 and metoclopramide on the gastric emptying of 1mm glass spheres in the rat

dose mg/kg sc	No. of spheres emptied in 1 hr. (mean ± s.e.m.) n	BRL 20627	n	metoclopramide
vehicle	20	4.4 ± 1.0	20	4.4 ± 1.0
0.2	7	12.0 ± 6.7	-	-
1.0	6	19.0 ± 5.7*	6	19.3 ± 8.6
5.0	6	46.7 ± 10.6***	6	36.3 ± 14.3*
10.0	10	25.4 ± 8.0***	9	38.4 ± 11.4*

*P<0.05; ***P<0.01 Mann-Whitney 'U' test

These results provide further evidence that BRL 20627 is similar to metoclopramide in enhancing gastric emptying in the rat. The mechanism of action of this effect cannot be deduced from these experiments but as BRL 20627 has been shown to lack significant dopamine antagonist activity (McClelland et al 1983) it is unlikely that such a property is contributing to the effect.

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IRREVERSIBLE LABELLING OF THE TRH RECEPTOR IN RAT PITUITARY MEMBRANES AND GH_3 CELLS

A.K. Keenan, F. Martin and M. Treacy, Department of Pharmacology, University College, Belfield, Dublin 4.

It is well known that TRH increases prolactin biosynthesis in, and secretion from rat pituitary. The clonal rat pituitary tumor cell line (GH_3 cells) mimics these pituitary cell responses. The purpose of the present work was to use both of these systems to study the molecular characteristics of these TRH-mediated responses. To this end we have (a) quantified the binding of ^{3}H-TRH to pituitary and GH_3 cell membranes (b) solubilised the TRH receptor and (c) irreversibly crosslinked ^{3}H-TRH to its receptor using a bifunctional crosslinking agent.

Pituitary homogenates were prepared from estrogen-treated and control adult female rats. GH_3 cells were grown in suspension in Dulbecco's modified Eagle's medium (DMEM) supplemented with 15% horse serum and 2.5% foetal calf serum and were maintained in a humidified atmosphere of 7.5% CO_2/92.5% air. The cells were used at a density of approximately 3.0×10^7 cells/64 mm petri dish. Specific binding of ^{3}H-TRH (under equilibrium conditions) was defined as that displacable by excess TRH and was measured in cell homogenates and whole cell preparations. Solubilised receptors were assayed by filtration following precipitation with PEG and by gel filtration on Sephadex G-200. The crosslinking agent used was disuccinimidyl suberate.

Under equilibrium conditions, using ^{3}H-TRH (L-2,3,4,5-^{3}H-proline, 102 Ci/m.mol) as ligand, whole GH_3 cells and GH_3 cell homogenates were shown to contain 1.35 ± 0.19 and 1.36 ± 0.12 p.moles receptor per mg protein respectively. K_D values were 2.3 ± 0.2 and $4.1 \pm 0.4 \times 10^{-8}$ M. Pituitary homogenates from non-estrogenised rats contained 120 ± 4.0 f.moles ^{3}H-TRH binding sites/mg protein (K_D $1.07 \pm 0.8 \times 10^{-8}$ M) while pituitary homogenates from estradiol benzoate-treated rats contained significantly greater ($p<0.05$) numbers of such sites (625 ± 11.5; K_D $1.14 \pm 0.4 \times 10^{-8}$ M).

Crosslinking of the ^{3}H-TRH-receptor complex using 1 mM disuccinimidyl suberate (20-30 min, ice-bath) was investigated in homogenates and solubilised preparations from pituitaries and GH_3 cells. After such treatment of homogenates from either source, a significant fraction of the ^{3}H-TRH ligand could no longer be displaced under dissociating conditions. The presence of the crosslinking reagent did not affect ^{3}H-TRH-receptor binding in this preparation as judged by levels of specific binding. However the crosslinker appeared to impair ^{3}H-TRH binding to the solubilised receptor to the extent that crosslinking could not be effectively studied.

Further studies are under way to solubilise the receptor which has been crosslinked in the homogenate.

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THE FROG OPTIC TECTUM IN VITRO IS A USEFUL PREPARATION FOR STUDIES ON THE PHARMACOLOGY OF CENTRAL TRANSMITTERS

Lucia Sivilotti (introduced by A. Nistri), Department of Pharmacology, Saint Bartholomew's Hospital Medical College, Charterhouse Square, London E.C.1M 6BQ.

The amphibian optic tectum is a key area involved in processing visual inputs and integrating them with accessory sensory information. Its main input is from the contralateral retina with different classes of optic nerve fibres terminating in distinct tectal layers (Chung et al, 1974). This laminated organization of the tectum makes it suitable for field potential analysis. In the frog tectum in vivo four waves evoked by optic nerve stimulation (Chung et al, 1974) represent post-synaptic excitatory events elicited by separate classes of optic nerve fibres. Data on the pharmacology of these responses are however scarce, even if a dis-homogenous laminar distribution of markers for putative transmitters, including acetylcholine and GABA, has been found (Oswald and Freeman, 1980). Here I describe a convenient preparation to study the pharmacology of this brain area with simple electrophysiological techniques.

Frogs (Rana temporaria) kept in a refrigerated aquarium on a 12:12 hours light-dark cycle were decapitated under tricaine anaesthesia (0.1%). After severing the optic nerves the brain was dissected out in cold Ringer solution (NaCl 111; KCl 2.5; $NaHCO_3$ 10; $NaH_2PO_4.2H_2O$ 0.1; $CaCl_2$ 2; glucose 4 mM, gassed with O_2/CO_2, 95/5%). The preparation was pinned to the bottom of a 2 ml bath, the dura was removed and the optic nerves drawn into stimulating suction electrodes. Oxygenated Ringer (5-10 ml/min; composition as above) was continuously superfused onto the preparation at 7°C (bath temperature monitored with an electronic thermometer). All drugs were added to the perfusion medium. DC-coupled recordings were obtained from the tectum by means of glass micropipettes (3M NaCl or KCl; 2-8 MΩ). The evoked field potentials were amplified and either displayed on a storage oscilloscope for photography or digitized by a transient data recorder and played back on a chart recorder.

During recordings from the tectal surface two negative-going waves were observed peaking at 56 ± 4 and 112 ± 12 ms (mean ± S.E.M., n = 11) after the stimulus artifact. These two waves, corresponding to the u_1 and u_2 waves described by Chung et al (whose nomenclature is adopted here), reversed at different depths in the tectum, and displayed paired pulse potentiation. The peak amplitudes of u_1 and u_2 were 0.6 ± 0.1 and 0.4 ± 0.08 mV respectively. Maintaining the preparation at 7°C helped to ensure excellent viability for many hours, presumably reduced the uptake systems for putative transmitters (v. infra) and allows a direct comparison with data obtained from the frog spinal cord at similar temperature (Nistri, 1983). Nevertheless, synaptic fatigue often developed when stimulating frequencies > 0.2 Hz were used. Controlled warming-up of the preparation up to 24°C decreased the latency and increased the amplitude of both waves. GABA (0.5 - 1 mM) had no effect at 20°C but induced a threefold reversible increase in the amplitude of the u_1 wave and a doubling of the u_2 wave at 7°C. This effect (not mimicked by 2 mM glutamate or 12 mM K^+) was little antagonized by bicuculline (10 - 100 μM).

A comparison of the effects of GABA and other putative transmitters on the optic tectum and the spinal cord of the frog might aid our understanding of regional differences in transmitter action in the CNS.

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THE BENZODIAZEPINE RECEPTOR ANTAGONIST RO 15-1788 HAS ANXIOGENIC ACTIVITY IN FOUR ANIMAL TESTS OF ANXIETY

Sandra E. File and Sharon Pellow. MRC Neuropharmacology Research Gp. Dept. Pharmacology, The School of Pharmacy, University of London, UK.

Ro 15-1788 is a benzodiazepine (BDZ) antagonist that can antagonise the effects both of agonists and 'inverse agonists' acting at BDZ receptors. Ro 15-1788 also has clear intrinsic activity, including an anxiogenic action (10mg/kg) in the social interaction test (File et al, 1982). Corda et al (1983) failed to find anxiogenic activity at 2mg/kg in the punished drinking test; however this is likely to be too low a dose and so in the present study more appropriate doses were used. The effects of Ro 15-1788 were also investigated on water intake in a novel environment, since Hoffman and Britton (1983) found anxiogenic activity (8mg/kg) in a modified open-field test.

In the punished drinking test, rats were deprived of water for 23h. On day 1 they were familiarised with the apparatus for 15 min, allowed a further 2h water in their home cages, and deprived for a further 23h. On day 2 rats were given a 6-min test. After the first lick, 1 min unpunished drinking followed so that each rat served as its own control for direct drug effects on water intake. A 5-min test followed where after every 20 licks the rat received a 0.5sec, 0.17mA electric footshock. Measures were: latency to lick, no. of licks in unpunished and punished periods, no. of shocks received. The novelty drinking test was identical to day 1 of the above procedure. Latency to lick and to no. of licks in each 5 min of the 15-min test were recorded. In each test rats were randomly allocated to the following groups: control, Ro 15-1788 (10, 20 or 30mg/kg). Rats received one i.p. injection 20 min before testing.

Ro 15-1788 (10-30mg/kg) had no effect on latency to drink or unpunished drinking. However at 20 and 30mg/kg there was a significant decrease in the number of shocks taken. In the novelty drinking test Ro 15-1788 (10mg/kg) significantly reduced the number of licks in the first 5 min of the test; this was no longer present at 20-30mg/kg. This reduction was followed by a compensatory increase in intake during the second 5 min, so that total drinking in 15 min did not differ from controls. The enhanced response to novelty (10mg/kg) in the first part of the test is consistent with a report using an open field novelty procedure (Hoffman and Britton, 1983) and with the anxiogenic effect in social interaction at this dose (File et al, 1982). That the proconflict effect was observed not at 10 but at 20-30mg/kg was surprising since at these doses Ro 15-1788 tends to have BDZ-like activity. In conclusion, anxiogenic-like activity has been observed with Ro 15-1788 in four different procedures: the social interaction test, the punished drinking test, and two different measures of response to novelty.

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EFFECTS OF TWO LIGANDS FOR PERIPHERAL BENZODIAZEPINE BINDING SITES ON ANXIETY AND STRESS IN RAT

Sandra E. File and Sharon Pellow. MRC Neuropharmacology Research Gp., Dept. Pharmacology, The School of Pharmacy, University of London, UK.

Ro 5-4864 is an atypical 1,4-benzodiazepine (BDZ) and PK 11195 an isoquinoline carboxamide derivative, both of which have high affinity for peripheral-type BDZ binding sites but not for 'classical' CNS BDZ sites (Braestrup and Squires, 1977; LeFur et al, 1983). The latter authors proposed that Ro 5-4864 was an agonist and PK 11195 an antagonist there. This study investigated whether PK 11195 could antagonise the behavioural effects of Ro 5-4864, and whether it had any intrinsic activity alone.

Ro 5-4864 (20 mg/kg) had an anxiogenic action in the social interaction test; this was not reversed by PK 11195 (30, 60 or 90 mg/kg). Ro 5-4864 (10 mg/kg) had proconflict activity in the Vogel punished drinking procedure; this was not reversed by PK 11195 (30 or 60 mg/kg). Alone, at 90 mg/kg, PK 11195 had an anxiogenic effect in the social interaction test, but (30 and 90 mg/kg) not in the punished drinking test.

PK 11195 (30, 60 and 120 mg/kg), like high doses of BDZs, significantly increased basal plasma corticosterone levels in the rat, as measured in the home cage, by fluorimetric assay. Ro 5-4864 (20 and 60 mg/kg) had no effect. However, both compounds, at doses that had anxiogenic activity (Ro 5-4864 20 mg/kg; PK 11195 60-120 mg/kg) enhanced the elevation in plasma corticosterone levels produced by the stress of exposure to a novel environment (a brightly-lit hole-board). This effect is similar to that produced by the anxiogenic BDZ receptor antagonist, Ro 15-1788 (File and Pellow, 1984). In contrast, BDZs decrease this stress response (File, 1982).

That PK 11195 does not antagonise the anxiogenic activity of Ro 5-4864 suggests, along with other behavioural, biochemical and electrophysiological evidence (see Pellow and File, 1984 for review) that these actions of Ro 5-4864 are not mediated via peripheral-type BDZ sites. The overall pattern of results with PK 11195 in behavioural tests is not easy to classify; but these effects occur at doses far higher than those that would be necessary to maximally occupy peripheral BDZ binding sites (LeFur et al, 1983).

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STRAIN DIFFERENCES IN BEHAVIOURALLY-INDUCED ANTINOCICEPTION AND MORPHINE ANALGESIA IN MALE MICE

R.J. Rodgers and Jill I Randall*, Pharmacoethology Laboratory, Postgraduate School of Studies in Psychology, University of Bradford, Bradford, West Yorkshire, BD7 1DP, U.K.

A wide variety of environmental stimuli are known to activate endogenous pain inhibitory mechanisms(Watkins & Mayer, 1982). Although many such stimuli are of dubious ecological significance to the species concerned(eg footshock in rats),it has more recently been reported that social conflict engenders a potent opioid analgesia in recipients of attack(Rodgers & Hendrie, 1983). In view of reported differences in opiate receptor characteristics in several mouse strains(Reith et al, 1981), the present study examined the nociceptive reaction of BKW and DBA/2 mice to both social conflict(Experiment 1) and morphine(Experiment 2).

Adult male mice(20-50g) of the BKW(Bradford University colony) and DBA/2(Bantin & Kingman, Hull) strains were used. Animals were housed in groups of ten in a temperature-controlled room($24\pm1^{o}C$), in which a reversed LD cycle was operative. Food and water were freely available. All testing was conducted during the dark phase of the LD cycle. In both studies, baseline tail-flick latencies(TFL) were established 2 hours prior to experimental manipulation. In Experiment 1, nine animals of each strain were individually introduced into the home cages of 'stimulus' conspecifics(aggressive males) and, following exposure to a predetermined number of attacks, were reassessed for TFL at 0, 10, 20, 40 and 60 minutes post-encounter. In Experiment 2, ten animals of each strain were randomly allocated to one of two conditions:- saline(10ml/kg, i.p.) or morphine sulphate(5mg/kg). All animals were reassessed for TFL at 30, 60, 90 and 120 minutes post-injection.

Results of both studies were subjected to repeated measures analyses of variance. Despite an absence of significant strain differences in baseline TFL, statistical analysis indicated that the two strains differed markedly in nociceptive response to both social conflict and morphine. In Experiment 1, DBA/2 mice showed a very potent analgesia immediately after encounters($p<0.001$)and they remained significantly analgesic for 40-60 minutes following social conflict. In contrast, BKW mice displayed a significant reduction in TFL($p<0.01$) which was still evident at 60 minutes post-encounter. In Experiment 2, DBA/2 mice exhibited a strong analgesic reaction to morphine($p<0.001$), an effect which returned to control values between 90-120 minutes following injection. On the other hand, BKW mice showed only a weak analgesic response to morphine($p<0.05$)which had a duration of 30-60 minutes. Importantly, in this study, saline-treated BKW mice(but not DBA/2) evidenced a significant 'sensitization' to repeated TF testing, suggesting that the 'hyperalgesic' profile of this strain in Experiment 1 was probably unrelated to the experience of attack.

Together, present data extend the literature on strain differences in opioid sensitivity(endogenous & exogenous) and point to repeated testing as a potentially important variable in analgesiometric procedures.

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PHARMACOLOGICAL SIMILARITY BETWEEN THE 5HT-D-RECEPTOR ON THE GUINEA PIG ILEUM AND THE $5HT_2$ BINDING SITE

G. Engel, D. Hoyer, H. Kalkman, M.B. Wick, Preclinical Research Sandoz Ltd., CH-4002 Basle, Switzerland

In the isolated guinea-pig ileum serotonin (5HT) induces contraction via pre- and postjunctional 5HT receptors (Gaddum & Picarelli, 1957). One receptor subtype which is located prejunctionally on nerves of the myenteric plexus is designated 5HT-M receptor and the other one on the smooth muscle is termed 5HT-D. Peroutka and Snyder (1979) proposed another classification of 5HT receptors ($5HT_1$ versus $5HT_2$) on the basis of radioligand binding studies in the CNS. It was the aim of the present investigation to compare the affinities of various 5HT antagonists to the $5HT_2$ binding sites labelled by [^{3}H]-ketanserin with their antagonistic potency on 5HT-D mediated contraction in the guinea-pig ileum. Strips of the longitudinal muscle layer prepared from the distal part of the ileum of young (150-200 g) guinea-pigs were mounted in 10 ml organ bath filled with a Tyrode solution. The experiments were carried out in the presence of 1 µmol/l atropine to rule out constrictor effects mediated via 5HT-M receptors. A concentration of 1 µmol/l 5HT causing approximately half maximal contraction was chosen for experimental use. When given at 10 min intervals the response to 1 µM 5HT became constant after 2 to 4 applications and remained stable for at least two hours. Addition of 1 µmol/l of the 5HT-M antagonist ICS 205-930 (Donatsch et al., 1984) did not influence the contractile response. The inhibition constants for the different antagonists were measured using a fixed concentration of 5HT and increasing concentrations of the antagonists. The dose of the antagonists causing 50% inhibition of the contraction (IC_{50}-value) are shown in Table 1. [^{3}H]-ketanserin binding to homogenates of rat cortex membranes was performed by standard procedures. The pK_D-values are listed in Table 1.

Table 1: Inhibition constants and $5HT_2$ affinities of antagonists (means ± s.e.)

Compound	-log (IC_{50}) mol/l	pK_D
Ketanserin	8.16 ± 0.07 (6)	8.86 ± 0.08 (4)
Methysergide	8.04 ± 0.03 (4)	8.62 ± 0.08 (4)
Metergoline	8.15 ± 0.08 (5)	9.03 ± 0.09 (3)
Ketotifen	7.08 ± 0.09 (5)	6.90 ± 0.06 (3)
Domperidone	5.74 ± 0.29 (5)	6.42 ± 0.04 (3)
Quipazine	5.64 ± 0.15 (5)	6.19 ± 0.04 (3)
Propranolol	5.25 ± 0.55 (6)	5.45 ± 0.07 (4)
[5HT -log EC_{50}:	5.60 ± 0.06 (6)	5.53 ± 0.08 (5)]

Regression line $y = 0.833\ x + 0.79$, $r = 0.974$, $p < 0.001$.

Due to the low receptor reserve in the preparation (in older animals one cannot detect a 5HT-D response at all) serotonin itself fits in the correlation very well. In conclusion, the data demonstrate a highly significant correlation between the potencies of 7 antagonists plus 5HT itself on the smooth muscle 5HT receptor and the affinities for the $5HT_2$ binding site. Therefore it can be assumed that the postjunctional 5HT-D receptor has pharmacological properties in common with $5HT_2$ receptors.

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VASOPRESSIN ALONE PRODUCES PASSIVE AVOIDANCE BEHAVIOUR IN A TYPICAL MEMORY EXPERIMENT

I.S.Ebenezer (introduced by J.W.Thompson) Department of Anatomy, Medical School, University of Newcastle upon Tyne, Framlington Place, Newcastle upon Tyne.

Experiments conducted over the past 20 years have suggested that the neurohypophysial hormone, vasopressin, is involved in memory processes (see e.g. de Wied, 1983). In the majority of these experiments 'memory' was operationally defined as the refusal of an animal to enter a box in which it has once experienced an electric shock. Vasopressin enhances such 'passive avoidance behaviour'. Recently, Ettenberg et al. (1983) have reported that vasopressin itself has aversive effects. It therefore seems possible that, during a passive avoidance experiment, an animal given vasopressin is, in effect, faced with two unpleasant experiences (i.e. vasopressin and the electric shock) rather than one, and that it is this which improves the efficiency with which the animal acts. The present study was designed to find out whether vasopressin alone is a sufficient stimulus to produce passive avoidance behaviour.

Sixteen female Wistar rats (wt.180-220g) were used. They were randomly divided into 2 equal groups. One group received saline (0.9% w/v) while the other received arginine-8 vasopressin (AVP) (4.0IV/kg). This dose of AVP is within the dose range used by other workers (Ettenberg et al. 1983). Saline and AVP were administered subcutaneously.

The apparatus consisted of a rectangular perspex box divided into 2 compartments (18x25x25cm and 10x25x25cm) by a wall with a 6x8cm transparent sliding door. The interior of the small compartment was black and unlit, while the walls and ceiling of the large compartment were transparent and illuminated by a 100W bulb placed 50cm above the large compartment.

The experiment protocol was as follows:
Day 1: Exploration: The door between the 2 compartments was opened, the rat placed in the 'light' compartment and allowed to explore the box for 5 mins. Rats naturally seek out dark enclosed spaces, and they therefore tend to spend more time in the 'dark' compartment.
Day 2: Learning: The rat was put into the 'light' compartment, left until it entered the 'dark' compartment, then removed, injected and replaced in the 'dark' compartment. The transparent sliding door was closed so that the rat was unable to re-enter the 'light' compartment. The rat was then left in the 'dark' compartment for 20 mins.
Day 3: Retention: The rat was placed for 3 min in the 'light' compartment with the sliding door open. Three measures were taken: (a) time for the rat to enter the 'dark' compartment, (b) total time spent in the 'light' compartment, (c) total number of crossings between the two compartments.

The results showed that rats treated with AVP (a) displayed significantly ($p<0.01$) longer latencies to enter the 'dark' compartment, (b) spent a significantly ($p<0.01$) longer time in the 'light' compartment and (c) made a greater number of crossings ($p<0.01$) between the 2 compartments. The rats treated with vasopressin therefore displayed passive avoidance of the 'dark' chamber. This suggests that passive avoidance experiments may not be suitable techniques on which to base the claim that vasopressin improves memory.

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ENDOGENOUS ACETYLCHOLINE MEDIATES INOSITOL PHOSPHOLIPID HYDROLYSIS INDUCED BY DEPOLARIZING STIMULI IN CEREBRAL CORTEX SLICES

I. Batty, D.A. Kendall & S.R. Nahorski, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester.

There is much evidence that exogenous agonists stimulate inositol phospholipid breakdown by interacting with specific receptors in rat cerebral cortex slices (Brown et al. 1984; Daum et al. 1984). In an attempt to determine whether one could determine responses to endogenously released transmitters, inositol phospholipid hydrolysis was examined following depolarizing stimuli.

Inositol phospholipid hydrolysis was assayed in rat cerebral cortex slices by monitoring the accumulation of ^{3}H-inositol phosphates (^{3}H-IP) in the presence of 5 mM LiCl after incorporation of ^{3}H-inositol into phospholipid (Brown et al. 1984). Acetylcholine (ACh) released from the slices into the surrounding medium was estimated using a radioreceptor assay. This method used rat cardiac membranes, ^{3}H-oxotremorine as ligand and possessed a limit of detection of 2 pmol acetylcholine.

Depolarization-evoked by K^+ and veratrine was accompanied by increased ^{3}H-IP accumulation with maximal responses observed at 30 mM and 30 μM respectively. Higher concentrations of K^+ and veratrine produced submaximal responses and also suppressed the incorporation of ^{3}H-inositol into phospholipid. The cholinesterase inhibitor physostigmine (50 μM) markedly enhanced (> 4-fold at maximum) response to these depolarizing stimuli and shifted the dose-response curve of K^+ to the left.

Pretreatment of slices with submicromolar concentrations of the muscarinic antagonist atropine suppressed the enhanced response observed with physostigmine and either K^+ or veratrine. On the other hand, the accumulation of ^{3}H-IP in the absence of physostigmine was resistant to block by atropine, prazosin, ketanserin or mepyramine (all 1 μM) and was unaffected by the presence of the peptidase inhibitor bacitracin (10^{-5} M). Reduction of extracellular calcium to 10-30 μM suppressed the atropine-sensitive but not the atropine-insensitive response to K^+ (17 mM).

Veratrine and high K^+ markedly stimulated ACh release in a dose-related manner in the presence of physostigmine and accumulation of the transmitter was linear for at least 45 min. Pretreatment of slices with tetrodotoxin (1 μM) completely abolished both the inositol phospholipid response and release of ACh stimulated by veratrine but not high K^+.

The results provide evidence that in the presence of physostigmine depolarization of cortical slices by elevated K^+ or veratrine stimulates inositol phospholipid hydrolysis by the interaction of released acetylcholine at muscarinic receptors. It is not clear, however, whether the much smaller atropine-insensitive response in the absence of physostigmine is a direct result of membrane-depolarization or release of an unknown mediator.

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DOPAMINE D_2 RECEPTORS REGULATE SPONTANEOUS AND VERATRINE-EVOKED RELEASE OF ENDOGENOUS DOPAMINE FROM STRIATAL SLICES

S.R. Nahorski & J. Strupish, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester.

The analysis of catecholamine release from brain tissue in vitro is usually studied by assaying efflux of radioactive transmitter following prelabelling with the amines or their precursors. However, there is little reason to assume that labelled amines taken up by brain slices distribute homogenously with releasable endogenous pools, and indeed we provide evidence in the accompanying study (Herdon et al. 1985) that release of newly accumulated ^{3}H-dopamine does not reflect endogenous release from striatal slices. In the present experiments, we have examined the possibility that dopamine D_2 receptors regulate release of endogenous dopamine from rat striatal slices.

Cross-chopped striatal slices (0.35 x 0.35 mm) (8-10 mg protein) were superfused with Krebs-Bicarbonate buffer (0.5 ml/min) containing 10 μM nomifensine to block dopamine re-uptake. Dopamine in superfusate (2 min) fractions was assayed by HPLC/electrochemical detection and spontaneous release (6-10 pmol/fraction) could be markedly (3-4 fold) elevated by depolarizing the slices with a 4 min pulse of the alkaloid veratrine (5 μM). Removal of extracellular Ca^{++} reduced spontaneous and veratrine-evoked release by 40% and 80% respectively. Pretreatment of slices with tetrodotoxin (1 μM) totally abolished veratrine-stimulated dopamine release but did not significantly alter spontaneous overflow.

When slices were superfused in Krebs containing 1 μM of the dopamine D_2 agonist pergolide, both spontaneous and veratrine-evoked release of dopamine was significantly suppressed. On the other hand, the D_2 antagonist (-)-sulpiride (1 μM) increased spontaneous and evoked release and totally reversed the effects of pergolide. The effects of sulpiride were clearly stereospecific in that almost maximal responses were observed at 100 nM (-)-sulpiride whereas 300 nM (+)-sulpiride was virtually ineffective. A careful analysis of the action of this antagonist on spontaneous overflow of dopamine revealed that (-)-sulpiride rapidly increased release. Maximal response to 300 nM (-)-sulpiride (2-3 fold of Ca^{++} dependent release) was observed 5-7 min after addition to the superfusion medium. This action of sulpiride was Ca^{++} dependent but not suppressed by tetrodotoxin (1 μM).

The present results provide evidence that dopamine D_2 receptors can regulate depolarization-evoked endogenous dopamine release from rat striatal slices. Although this qualitatively supports previous data using prelabelling techniques, the present studies also suggest that unlike the latter approach (e.g. Lehmann et al. 1983), spontaneous release of endogenous dopamine appears to be under the influence of feedback control through dopamine D_2 receptors. These data again emphasise the importance of assaying endogenous catecholamine release from brain slices.

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110P

PREPARATION OF ANTI-HALOPERIDOL ANTIBODIES

R. Juszczak & P. G. Strange, Department of Biochemistry, The Medical School, Queen's Medical Centre, Nottingham NG7 2UH, U.K.

An antibody specifically directed against the D_2 dopamine receptor would be of great value for studies on the receptor, particularly in purification. One approach to this is the antiidiotypic route where a first antibody (Ab-1) is raised against a receptor-specific drug. A second antibody (Ab-2), the antiidiotype is then raised aginst Ab-1 and under favourable circumstances Ab-2 may be an anti-receptor antibody. We are applying this approach to the D_2 dopamine receptor using haloperidol as the receptor-specific drug.

Haloperidol was derivatised with carboxymethylhydroxylamine and the derivative was linked to human serum albumin by a carbodiimide coupling method. The haloperidol-albumin conjugate was used to immunise two female rabbits (Californian, New Zealand White cross). Anti-haloperidol antibodies were detected in the rabbits by [^{3}H]haloperidol binding and after booster injections antisera could be obtained which gave 50% binding of [^{3}H]haloperidol at antiserum dilutions of 1/150-1/5000. The properties of the two antisera were characterised by displacement of [^{3}H]haloperidol (1nM approx.) binding and IC_{50} values (concentrations of substance that displace half the [^{3}H]haloperidol binding) were determined (Table 1).

Table 1 Displacement of [^{3}H]haloperidol from rabbit antisera by various substances.

displacing substance	IC_{50} (M) rabbit-1	rabbit-2
(+)-Butaclamol	$>10^{-4}$	1.6×10^{-5}
(-)-Butaclamol	$>10^{-4}$	$>10^{-4}$
Domperidone	7.1×10^{-5}	$>10^{-4}$
Dopamine	$>10^{-4}$	$>10^{-4}$
Haloperidol	2.8×10^{-9}	4.2×10^{-9}
Mianserin	3.9×10^{-5}	$>10^{-4}$
Spiperone	1.4×10^{-6}	1.2×10^{-4}

The two antisera clearly differ in their binding specificity. Anti-haloperidol antibodies have been purified from the antiserum of rabbit-1 by affinity chromatography on a spiperone-sepharose column. These purified antibodies are being used for immunising further rabbits with the aim of obtaining an anti D_2 receptor antiserum.

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EFFECT OF D_2 DOPAMINE RECEPTOR ON INOSITOL PHOSPHOLIPID METABOLISM IN ANTERIOR PITUITARY

S. H. Simmonds and P. G. Strange, Department of Biochemistry, The Medical School, Queen's Medical Centre, Nottingham NG7 2UH, U.K.

The mechanism of action of the D_2 dopamine receptor is not clearly understood although inhibition of adenylate cyclase and modulation of Ca^{2+} ion concentration/inositol phospholipid metabolism have been considered. The mammotroph cells of the anterior pituitary provide a useful system for studying D_2 receptor mechanism and we have previously characterised the binding properties of the D_2 receptors in bovine anterior pituitary (Simmonds and Strange, 1984). In the present report we have investigated the linkage of the D_2 receptors to inositol phospholipid metabolism using a dissociated anterior pituitary cell preparation (Schofield and Saith, 1981). Inositol phospholipid metabolism was assessed from the accumulation of [^{3}H]inositol monophosphate as described by Berridge et al. (1982).

The accumulation of [^{3}H]inositol monophosphate in unstimulated cells was low and unaffected by dopamine agonists. Cells were therefore stimulated with TRH and the TRH-analogue, DN1417, giving a four fold stimulation of [^{3}H]inositol monophosphate accumulation (DN1417 E_{50} 2.2μM). When dopamine agonists were applied prior to the stimulatory substance approximately half the stimulation was inhibited in a dose dependent manner by low concentrations of agonist (IC_{50}, apomorphine, 120nM; dopamine, 13μM).

The inhibitory effects of low concentrations of agonist could be prevented by D_2 receptor selective substances such as (+)-butaclamol (K_i, 1.1nM) or spiperone (K_i, 57pM) whereas (-)-butaclamol was essentially inactive. Thus D_2 receptors on anterior pituitary cells inhibit the stimulated accumulation of [^{3}H]inositol monophosphate and this may be important for the mechanism of action of the receptor.

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RU 24969 A 5-HT_1 AGONIST, STIMULATES INOSITOL PHOSPHOLIPID BREAKDOWN IN RAT BRAIN SLICES

P.P. Godfrey, S.J. McClue, M.C.W. Minchin & Michelle Young, MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford OX2 6HE

5-Hydroxytryptamine (5-HT) stimulates the breakdown of inositol phospholipids in rat brain slices (Berridge et al, 1982; Brown et al, 1984) and it has been suggested on the basis of inhibition studies that this effect is mediated by 5-HT_2 receptors (Conn & Sanders-Bush, 1984). There are few selective agonists for the two main 5-HT receptor sub-types but we now report that RU 24969, a suggested 5-HT_1 specific agonist (Hunt & Oberlander, 1981; Green et al, 1984), is capable of inducing the breakdown of inositol phospholipids in the brain.

Male Sprague-Dawley derived rats were decapitated and the cerebral cortex was dissected and cross-chopped at 350 μM intervals. The slices were pre-incubated in Hepes-buffered Krebs solution at 37°C for 30min, rinsed and then incubated with approximately 5 μCi/ml [^{3}H]-inositol for a further 45min. They were then rinsed with Krebs solution containing 10 mM LiCl and 50 μl portions of the packed slices were distributed into 250 μl of the same solution. After 10min RU 24969 was added for a further 30min when the incubation was terminated by the addition of chloroform:methanol (2:1). Antagonists were added prior to the 10min pre-incubation period. The tissue extract was separated into two phases by the addition of chloroform and water and the aqueous phase was mixed with a slurry of Dowex-1 in the formate form. The resin was extensively washed with 5 mM unlabelled inositol and the inositol phosphates were eluted with 1 M ammonium formate/0.1 M formic acid.

RU 24969 increased the production of inositol phosphates over the basal level in a dose-related fashion, approaching a maximum effect at 1 mM when there was an 88% increase in inositol phosphate production. Ketanserin, a 5-HT receptor antagonist selective for the 5-HT_2 sub-type, caused no rightward shift of the RU 24969 dose-response curve at a concentration of 1 μM. Neither were maximal concentrations of RU 24969 antagonised by 5 μM (-)-propranolol, which is a more potent antagonist at 5-HT_1 than at 5-HT_2 receptors. Similarly, mepyramine, a histamine H_1 antagonist, atropine, a muscarinic antagonist, and prazosin, an α_1 antagonist, did not inhibit the response to RU 24969 (all present at 5 μM). However, iprindole and amitriptyline (5 μM) both inhibited the RU 24969-induced phosphoinositide response by 84% and 100% respectively. Furthermore, removing Ca^{2+} from the Krebs solution and raising the Mg^{2+} concentration to 10 mM depressed the RU 24969 response so that at 1 mM it was 20% of control.

These results suggest that RU 24969 may induce inositol phospholipid breakdown by an indirect effect involving exocytotic release, and that an antidepressant-sensitive receptor such as the 5-HT receptor described by Quach et al (1982) may be involved.

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ASSOCIATION BETWEEN DOWN-REGULATION OF CORTICAL 5-HT_2 RECEPTORS AND WANING OF A RELATED BEHAVIOURAL RESPONSE

N. Koshikawa, R.W.J. Smokcum & J.D. Stephenson, Dept. of Pharmacology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF.

Marley & Wozniak (1984) described a syndrome in rats produced by administration of a monoamine oxidase inhibitor with an inhibitor of 5-HT uptake. The syndrome included wet-dog shakes (WDS), other myoclonic features, muscle fasciculation, enhanced blood pressure, ECoG and spinal cord reflex changes. It peaked at around 90min and was almost completely absent at 6h although the features of the syndrome could be abolished at any time by pirenperone demonstrating their dependence on 5-HT_2 receptors.

We have sought to correlate the time course of the syndrome with changes in cortical 5-HT_2 receptor numbers using (^{3}H)-ketanserin as the ligand (Leysen et al, 1982) Groups of 3 rats were given phenelzine 18h (46.8mg/kg i.p.) and 90 min. (11.7mg/kg i.p.) before paroxetine (11.6mg/kg i.p.) or citalopram (5mg/kg i.p.). The aspect of the syndrome quantified was the total number of WDS occurring in 5 min periods for either 3 or 6 h after injection of the 5-HT uptake inhibitor, at which time rats were killed for determination of 5-HT_2 binding sites.

In 6 groups each of 3 rats which received paroxetine after phenelzine the mean number of WDS/group at the time of maximum effect (90min) was 28/5 min this reducing (P=0.01) to 15/5 min at 180 min. The Bmax for (^{3}H)-ketanserin binding to cortices from rats killed at 180 min was significantly smaller (P= 0.0001, ANOVA) than that obtained in rats receiving saline instead of paroxetine, 11.63 $\pm$ 0.39 fmoles/mg tissue (mean $\pm$ s.e. mean) compared to 16.43 $\pm$ 0.50; the Kd was unchanged. Similar results have been obtained with citalopram, the reduction in Bmax being slightly smaller at 180 min (17.82 $\pm$ 1.02 to 14.8 $\pm$ 0.76, P = 0.02) than that obtained with paroxetine. Control experiments in which rats received saline (0.9% w/v NaCl solution) instead of phenelzine and/or 5-HT reuptake inhibitor have established that neither phenelzine alone nor a 5-HT reuptake inhibitor alone changed binding parameters from those obtained in rats receiving only saline.

To determine whether or not this 30% reduction in number of 5-HT_2 binding sites after phenelzine and paroxetine was of pharmacological significance and might have contributed to the waning of the WDS, rats were given a second injection of paroxetine at 180 min and the WDS quantified for a further 180 min. The second injection did not significantly increase WDS above the number obtained at the time this second injection was administered.

The results show that rapid down-regulation of 5-HT_2 receptors can be achieved within 3 h by combining an antidepressant inhibiting monomine oxidase with one inhibiting 5-HT reuptake and that this down-regulation is sufficient to attenuate an evoked response.

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ETHYLCHOLINE MUSTARD AZIRIDINIUM: INHIBITION OF RAT BRAIN SYNAPTOSOME CHOLINE UPTAKE AND CHOLINE ACETYLTRANSFERASE

E.K.Pedder & A.K.Prince, Dept. Pharmacology, King's College, London WC2R 2LS.

Incubation of intact synaptosomes with ECMA (30 μM) inhibits Na^+ dependent choline uptake and intrasynaptosomal ChAT. Na^+ dependent uptake is completely inhibited within 5 min. In marked contrast, 1h is necessary for 45-50% inhibition of ChAT, an effect antagonised by hemicholinium-3 (HC-3) in a dose dependent manner (Pedder & Prince, 1984). We therefore investigated further the reaction of ECMA with intrasynaptosomal ChAT.

P_2 preparations from rat cerebral cortex were pre-incubated (10 mg original tissue) in 2 ml Tris-Krebs (118 mM Na^+, 37°C, 5 min or 2h), incubated with ECMA, washed (5% v/v x 3, Na^+ free Tris-Krebs containing 236 mM sucrose, 0°C), rewarmed (Na^+ Tris-Krebs or Na^+ free Tris-Krebs, 37°C, 5 min), incubated with ^{3}H-choline (1 μM, 1 μCi, 4 min), then filtered (GF/C discs) and counted for tritium. ChAT activity was measured (Fonnum, 1975) after intact synaptosomes (P_2 preparations) had been incubated with ECMA (10 mg original tissue, 200 μl Na^+ Tris-Krebs, 37°C) washed (5% v/v x 3, Na^+ Tris-Krebs) and solubilised (glass-teflon homogenisation, 5000 rpm; then 0.5% Triton X-100, 1h, 0°C).

In fresh P_2 preparations 30 μM ECMA caused complete inhibition (irreversible by washing) of Na^+ dependent choline uptake within 5 min, Na^+ independent uptake was unaffected after 1h. HC-3 (40 μM, 5 min) completely inhibited total uptake (Na^+ dependent plus Na^+ independent). Pre-incubation of fresh P_2 preparations for 2h without ECMA abolished Na^+ dependent choline uptake. Na^+ independent uptake was unchanged. Subsequent incubation with 30 μM ECMA (Na^+ Tris-Krebs, 37°C, 5 min or 1h) had no effect on this Na^+ independent uptake.

30 μM ECMA inhibited intrasynaptosmal ChAT in fresh P_2 preparations by 14.2 ± 0.3% in 5 min ($p < 0.01$) and by 41.6 ± 1.9% in 1h ($p < 0.01$). 40 μM HC-3 added with the ECMA prevented this latter effect. Thus the inhibition of ChAT by ECMA appears dependent on a functioning choline carrier.

Washing the synaptosomes (5% v/v x 3, Na^+ Tris-Krebs 0°C) after 5 min with ECMA only and then continuing the incubation for 55 min (without adding further ECMA) failed to reduce the inhibition (30 ± 1.2%, $p < 0.01$) to that apparent after the initial 5 min. HC-3 (40 μM) added after 5 min of 1h incubations with ECMA similarly failed to reduce inhibition to 5 min values. Thus the greater part of the inhibition after 1h is by ECMA entering the synaptosomes in the first 5 min of incubation.

In P_2 preparations that had been pre-incubated for 2h, and thus exhibited no Na^+ dependent uptake of choline, 30 μM ECMA caused no inhibition of intrasynaptosomal ChAT in 5 min, but 23 ± 2.5% ($p < 0.05$) in 1h. 40 μM HC-3 added with the ECMA again prevented this effect. Thus HC-3 sensitive Na^+ independent uptake may contribute appreciably to the inhibition of intrasynaptosomal ChAT during 1h incubations with ECMA. Alternatively, in synaptosomes pre-incubated for 2h, the carrier subserving ACh synthesis is reduced in activity in relation to choline but not to ECMA.

A Na^+ independent choline uptake mechanism transporting ECMA without being inhibited would contrast with the response of the sodium dependent mechanism and would suggest a means of distinguishing between the two.

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THE REACTION OF O-ACETYLETHYLCHOLINE MUSTARD AZIRIDINIUM WITH RAT BRAIN CHOLINE ACETYLTRANSFERASE

E.K.Pedder & A.K.Prince, Dept. Pharmacology, King's College, London WC2R 2LS

ECMA is an irreversible inhibitor of Na^+ dependent choline uptake. Nevertheless it appears to be concentrated in synaptosomes, by a hemicholinium-3 sensitive mechanism, sufficiently to inhibit ChAT (Pedder & Prince, 1984). ACh is not selectively accumulated by cholinergic nerve terminals and a free hydroxyl is important in ligand interaction with choline carriers (Simon et al, 1975). We therefore tested the O-acetyl derivative of ECMA in rat brain synaptosome preparations.

Acetyl-ECMA was prepared from O-acetylethylcholine mustard hydrochloride by dissolving in distilled water (20 mg/ml) and allowing to cyclise for 10 min at room temperature. The conversion to aziridinium, as measured by thiosulphate titration, was 87.5 ± 3.9%. Choline uptake was measured in P_2 preparations of rat cerebral cortex preincubated (10 mg original tissue) in 2 ml Tris-Krebs (118 mM Na^+, 37°C, 5 min) then with or without 0.5 mM eserine (5 min) as required, incubated with ECMA or acetyl-ECMA (5 min) then with ^{3}H-choline (1 µM, 1 µCi, 4 min), filtered (GF/C discs) and uptake corrected for that at 0°C. ChAT was assayed (Fonnum, 1975) in P_2 preparations or in pre-solubilised samples (glass teflon homogenisation, 5000 rpm; then 0.5% Triton X-100, 0°C, 1h) that had been preincubated (10 mg original tissue, 200 µl Na^+ Tris-Krebs, 37°C, 5 min), incubated with eserine (0.5 mM, 5 min) if required, then with ECMA or acetyl-ECMA. Intact P_2 samples were washed (5% v/v x 3, Na^+ Tris-Krebs, 0°C) and solubilised before assay.

Without eserine, choline uptake was inhibited by acetyl-ECMA in a dose dependent manner. However the IC_{50} was approx 10 µM compared with 3 ± 0.3 µM for ECMA. Choline uptake was inhibited by eserine (0.5 mM; 27.7 ± 2.4%, $p < 0.01$). Acetyl-ECMA (1 to 30 µM) caused no additional inhibition. In contrast ECMA produced marked additional inhibition, IC_{50} identical to that in the absence of eserine. Thus 0.5 mM eserine does not prevent ECMA from inhibiting choline carriers and therefore seems unlikely to prevent the acetyl-derivative. Acetyl-ECMA therefore seems an intrinsically poor inhibitor of choline carriers, requiring de-acetylation by hydrolysis to be effective. Hydrolysis is not complete in 5 min.

ChAT from pre-solubilised synaptosomes was not inhibited by eserine (0.5 mM, 1h). With or without eserine, acetyl-ECMA (300 µM, 1h) caused inhibition (52%, $p < 0.001$ & 40%, $p < 0.001$) comparable with that caused by 300 µM ECMA (40%, $p < 0.001$ & 44.8 ± 2.4%, $p < 0.001$). In intact synaptosomes without eserine, acetyl-ECMA (30 µM, 1h) caused inhibition (46 ± 1.7%, $p < 0.001$) comparable with that caused by 30 µM ECMA (41.6 ± 1.9%, $p < 0.01$). However in the presence of eserine (0.5 mM) acetyl-ECMA caused no inhibition of ChAT in intact synaptosmes, although inhibition by ECMA (42%, $p < 0.001$) remained comparable to that in the absence of eserine. Thus 1h hydrolysis completely converts acetyl-ECMA into ECMA, which inhibits intrasynaptosomal ChAT as well as choline uptake.

Since eserine does not prevent ECMA from reaching intrasynaptosomal ChAT by uptake, it seems unlikely that it would prevent acetyl-ECMA. That acetyl-ECMA did not inhibit intrasynaptosomal ChAT, therefore, suggests its uptake by synaptosomes is poor. That acetyl-ECMA seems a poor substrate and inhibitor of choline carriers compared with ECMA is compatible with known carrier selectivity.

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INTRAVASCULAR FLUID COMPOSITION FOLLOWING BLOOD REPLACEMENT WITH FLUOSOL-DA IN THE RAT

K.C. Lowe & Denise C McNaughton* (Introduced by P.N.R. Usherwood), Department of Zoology, University of Nottingham, Nottingham NG7 2RD; *Department of Surgery, Addenbrooke's Hospital, Cambridge CB2 2QQ.

Emulsified perfluorocarbons (PFC) have been proposed as red blood cell substitutes and the effects of transfusion with such preparations have been studied in several species (Lowe, 1984). The most widely tested emulsions are Fluosol-DA (F-DA) and Fluosol-43 (FC-43; Green Cross, Japan); however, results from in vitro studies have revealed cytotoxic effects of these emulsions although the active agent(s) has not been identified (Bucala et al., 1983; Lowe et al., 1984). Since no data from corresponding in vivo studies has been published, the present experiments have examined changes in intravascular fluid composition following blood replacement with F-DA in the rat with emphasis on alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) concentrations.

Continuous, isovolaemic, exchange-transfusion was performed on 16 conscious female Sprague-Dawley/OLA rats (body weight: 250-330 g) according to methods described previously (Lowe et al., 1982; Hardy et al., 1983). Perfusion at $1ml.min^{-1}$ for 40 min. reduced the mean haematocrit to < 2%; mean (+ s.e.m.) arterial blood pressure, heart rate and respiration rate during perfusion were unchanged at 87 + 6 mm. Hg, 416 + 9 $beats.min^{-1}$ and 93 + 2 $breaths.min^{-1}$ respectively. Animals breathed 80-90% O_2 throughout the experiments. Intravascular fluid composition at the end of perfusion (+40 min.) and 6h. later was as follows:

	Initial	End of perfusion (+40min.)	6h. Post-Perfusion
Haematocrit (%)	36.3±1.4 (16)	1.4±0.2 (16)*	1.8±0.2 (16)*
Fluorocrit (%)	-	17.9±1.0 (16)	17.4±1.0 (16)
Total protein ($g.l^{-1}$)	71.9±3.2 (7)	4.2±1.1 (7)*	21.0±3.5 (7)*
Albumin ($g.l^{-1}$)	37.9±2.1 (7)	2.1±0.7 (7)*	11.3±2.4 (7)*
Na^+ ($m.mol.l^{-1}$)	143.9±0.8 (8)	137.9±1.9 (8)**	143.8±2.1 (8)
K^+ ($m.mol.l^{-1}$)	4.6±0.2 (8)	4.2±0.2 (8)	5.9±0.3 (8)*
Total Ca^{2+} ($m.mol.l^{-1}$)	2.3±0.1 (8)	1.8±0.1 (7)***	2.0±0.2 (7)
Glucose ($m.mol.l^{-1}$)	8.2±0.5 (5)	13.4±0.8 (5)*	7.3±1.4 (5)
Urea ($m.mol.l^{-1}$)	4.0±0.6 (5)	2.9±0.3 (5)	9.8±1.7 (5)**
ALP ($I.U.ml^{-1}$)	0.32±0.06 (5)	0.03±0.01 (5)*	1.56±0.52 (5)***
LDH ($I.U.ml^{-1}$)	0.45±0.07 (11)	0.23±0.04 (11)***	4.12±1.01 (5)*

Values are mean + s.e.m.; ( ) = no. observations; *P<0.01, **P<0.02, ***P<0.05

As expected, near total transfusion with F-DA produced decreases in blood cells and plasma proteins, together with an increase in the amount of F-DA in the circulation, as assessed by the fluorocrit. However, the changes in K^+, ALP and LDH post-perfusion provide direct evidence of cytotoxic effects of F-DA in vivo. Moreover, it is likely that the uraemia which occurred after perfusion was due to decreased urea excretion caused by impaired renal tubular function, as has been seen previously in progressive cardiogenic shock (Gorfinkel et al., 1972).

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WHAT IS THE VALUE OF ANIMAL TOXICOLOGY STUDIES BEYOND 6 MONTHS?

C.E. Lumley & S.R. Walker, Centre for Medicines Research, Carshalton, Surrey

The Centre for Medicines Research has established a toxicology databank containing comprehensive data from repeated dose animal safety evaluation studies provided by pharmaceutical companies (Lumley & Walker 1984). A comparative analysis of short and long-term toxicity tests has been carried out in order to determine what new findings, if any, become apparent in studies of longer than 6 months. Thirteen companies within the UK have provided toxicological data for 32 pharmaceutical compounds studied in the rat, dog or primate, resulting in 45 case studies for which both short ($\leq$ 6 months) and long-term (> 6 months) tests had been carried out. A case study is defined as one compound administered to one species by the same route for more than one time period. Results of the analyses of these data are summarised in the following table:

No. Of Studies	Results
20/45	All effects were seen within 6 months.
9/45	Although new effects were seen in the long-term studies no 6 month data are available. Therefore no conclusions can be drawn as to whether these effects would have been apparent by 6 months. In 6 cases the same or higher doses were used in the long-term as compared to the short-term study.
7/45	All findings after 6 months were not new effects but were extensions of those seen in the short-term studies.
3/45	The findings after 6 months were considered to be of no toxicological significance, i.e. shivering; colouration of gut contents.
2/45	The new findings had been seen in an alternative species within 6 months (liver weight increase; glycosuria and urinary casts). In both cases the same or higher doses were used in the long-term compared to the short-term study.
4/45	The new effects seen after 6 months did not influence the progression of the compounds to market or termination of research. In 3 of these cases the same or higher doses were used in the long-term compared to the short term study.

These data do not appear to support the need for animal toxicity studies of longer than 6 months duration, apart from those designed to investigate carcinogenicity. Although a relatively small number of compounds have been assessed, further case studies are currently being collected from Europe and the USA.

In addition to this information the databank also contains the results from 93 studies carried out for a maximum duration of 6 months or less and from 7 long-term studies for which no comparable short-term data are available. Analysis of the total databank has demonstrated that major effects which are occasionally not observed until after 6 months have been elicited within 6 months in other studies. Study design, including dose levels used, number of animals employed and parameters measured, is a major factor in determining whether or not effects are seen within 6 months.

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