GM-1 GANGLIOSIDE TREATMENT OF RATS WITH A PARTIAL UNILATERAL ELECTROLYTIC LESION OF THE SUBSTANTIA NIGRA

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The ganglioside GM-1 may promote functional recovery following unilateral hemitransection of the nigro-striatal pathway but not following a unilateral 6-hydroxydopamine lesion of the medial forebrain bundle (Toffano et al, 1984; 1986). Axonal sprouting has been suggested as the mechanism by which GM-1 produces its effect. We have investigated the effects of repeated GM-1 treatment on nigro-striatal dopamine function in rats with partial electrolytic lesions of the substantia nigra.

Male Sprague-Dawley rats (280-300 g) received unilateral electrolytic lesions (1 mA, 10 secs) of the dorsal substantia nigra (A - 3.6; L 2.2; V 7.2) (Pellegrino et al, 1979). All animals received daily injections of either GM-1(30 mg/kg i.p.) or vehicle for 3 days prior to surgery and for 34 days post-operatively. Rotational response to apomorphine (0.75 mg/kg s.c.) were measured at intervals post-operatively. Animals were killed on day 34 and striatal synaptosomal ^{3}H-dopamine uptake and striatal dopamine, DOPAC and HVA levels determined. The brain stem was fixed in buffered formalin for histological assessment of substantia nigra.

Table 1 Effects of GM-1 treatment on striatal dopamine function

Treatment	Side of brain	Total turns at day 31 (0.75 mg/kg) apomorphine	^{3}H-Dopamine uptake (pmol/mg/hr)	Dopamine (pmol/mg)
GM-1 (30 mg/kg i.p.)	Intact	113 ± 27	436.4 ± 67.4	538 ± 58
	Lesioned		268.7 ± 39.3*	367 ± 45*
Saline (1 ml/mg i.p.)	Intact	183 ± 24	446.5 ± 52.1	535 ± 52
	Lesioned		281.1 ± 25.5*	352 ± 35*

* $p < 0.05$ (intact compared with lesioned side); mean ± S.E.M.; n = 19

Rats treated with GM-1 showed lower rates of ipsiversive circling in response to apomorphine (0.75 mg/kg s.c.)(48-62%) when compared to control animals at 7, 10, 14, 18, 23 and 31 days post-operatively ($p < 0.05$, 2 sample t tests). In contrast, the decrease in striatal ^{3}H-dopamine uptake or dopamine content caused by the lesion was unaltered by GM-1 treatment compared to control animals. The area of the lesion was restricted to the substantia nigra. In GM-1 treated rats the tissue immediately surrounding the lesion site contained a lower glial cell density than in control animals. Also, in GM-1 treated rats a larger area within the lesion site was free from glial cell processes and lesion debris.

Repeated treatment with GM-1 ganglioside promoted partial functional recovery from apomorphine-induced rotation, but caused no recovery of striatal dopaminergic function as measured by biochemical parameters. Morphological studies of the substantia nigra suggest that a reduction of extent of damage at the lesion site by GM-1 may contribute to behavioural recovery.

Pellegrino L.J. et al. (1979) A Stereotaxic Atlas of the Rat Brain, Plenum, N.Y.
Toffano G. et al. (1984) Acta. Physiol. Scand. 122, 313-321.
Toffano G. et al. (1986) Int. J. Devl. Neurosci. 4, 97-100.

BRAIN DOPAMINE FUNCTION AND RESPONSES TO MPTP IN COMMON MARMOSETS TREATED WITH MPTP UP TO 18 MONTHS PREVIOUSLY

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Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to marmosets induces gross parkinsonism which reverses over the following 3-4 months (Jenner et al. 1985; 1986). Recovery is associated with increased dopamine turnover in the caudate-putamen and a recovery of mesolimbic dopamine content. We report on brain dopamine function in marmosets treated with MPTP up to 18 months previously and their response to further treatment with MPTP.

Common marmosets (250-350 g) were treated with MPTP (2-4 mg/kg ip) over a 5-7 day period to a cumulative dose of 6-22 mg/kg. Gross motor deficits developed which reversed over the following months. At 12 and 18 months following initial treatment some animals received MPTP (2-4 mg/kg ip) over a 5 week period to a cumulative dose of 75.2 - 83.6 mg/kg and were killed 3 months later.

Animals examined at 12-18 months following the initial MPTP treatment exhibited motor activity almost indistinguishable from that of control marmosets. However, caudate-putamen dopamine content was reduced to 5-8% of control values. There was a trend to increased dopamine turnover as judged by metabolite:dopamine ratio that was particularly marked in the putamen. In contrast, nucleus accumbens dopamine content and turnover was unaffected. Autoradiographic analysis showed a marked reduction in the specific binding of ^{3}H-mazindol in the caudate-putamen. The substantia nigra contained 20-30% of the control number of tyrosine hydroxylase immunoreactive cells.

Table 1 Dopamine parameters following MPTP treatment

Treatment	Putamen (ng/mg tissue)			Nucleus accumbens (ng/mg tissue)		
	DA	DOPAC	HVA	DA	DOPAC	HVA
Controls	13.4 ± 2.2	5.9 ± 0.2	12.3 ± 1.5	5.2 ± 0.2	4.6 ± 0.6	5.8 ± 0.5
MPTP	1.0* ± 0.6	1.6* ± 0.6	2.0* ± 1.0	4.5 ± 1.6	3.8 ± 1.0	4.4 ± 1.2
MPTP retreatment	0.6* ± 0.2	1.8* ± 1.0	1.2* ± 0.4	2.7* ± 0.5	2.2* ± 0.6	2.8* ± 1.0

n = 3 or 4; * $p < 0.05$ compared to control; ANOVAR followed by one way Dunnett's test.

Marmosets receiving further extensive treatment with MPTP exhibited an acute response to each administration but a clear parkinsonian syndrome did not develop. These animals showed no further reduction in caudate-putamen dopamine content although there was some reduction in the nucleus accumbens.

Motor function in the marmoset is restored following MPTP treatment despite persistent decreases in caudate-putamen dopamine content and extensive neuronal loss. Increased dopamine turnover and the recovery of nucleus accumbens dopamine content may contribute. The resistance of these animals to a further MPTP treatment may reflect the insensitivity of remaining nigral cells or the loss of dopamine terminals in caudate-putamen so preventing MPTP (or MPP^{+}) uptake.

Jenner P. et al. (1985) Brit. J. Pharmac. 84, 56P
Jenner P. et al. (1986) Brit. J. Pharmac. 89, 519P

CHARACTERISATION OF THE MUSCARINIC RECEPTOR SUBTYPE MEDIATING PILOCARPINE-INDUCED PURPOSELESS CHEWING BEHAVIOUR

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The induction of purposeless chewing behaviour in rats by the cholinergic agonist pilocarpine is mediated via central muscarinic receptors (Salamone et al, 1986). Based on the heterogeneous in vitro binding profile of the antagonist pirenzepine, two muscarinic receptor subtypes (M-1 and M-2) have been identified in rat brain (Hammer and Giachetti, 1982). We now report on an attempt in vivo to characterise the muscarinic receptor subtype mediating chewing behaviour. Since most compounds showing a selective effect on M-1 and M-2 sites do not penetrate into brain the experiments were carried out using intracerebroventricular administration.

Groups of male Wistar rats (200-350 g) (n = 6-12) received injections (µg/µl) of carbachol (12.5-50 µg), or pilocarpine (50-200 µg) or McN-A-343 (4-(m-chlorophenylcarbamoyloxy)-2-butnyl-trimethylammonium chloride) (50-200 µg) or AH 6405 (1,4,5,6 tetrahydro-5-phenoxy-pyrimidine) (100-200 µg) or vehicle through previously implanted stainless steel cannula into the lateral ventricle. Chewing behaviour was recorded as previously described (Jenner et al, 1986) over the time of peak agonist action as determined from preliminary experiments. In antagonist studies, N-methylscopolamine (5-20 µg) or oxyphenonium (10-40 µg) or 4-DAMP (4-diphenyl acetoxy-N-methylpiperidine) (40-160 µg) or AF-DX116 (11-2-(diethylamino)methyl-1-1 piperidinyl acetyl-5,11 dihydro 6H-pyrido 2,3-b1,4 benzodiazepine-6-one) (40-160 µg) or pirenzepine (80-320 µg) were administered ICV 30 min prior to administration of pilocarpine (4 mg/kg i.p.). Chewing behaviour was assessed for 5 min, 15 min after pilocarpine administration. Both agonist and antagonist studies were conducted in a cross over design basis.

Administration of carbachol (12.5-50 µg) or pilocarpine (50-200 µg) greatly enhanced the intensity of purposeless chewing compared to control ($p < 0.05$ Friedmans test). The putative M-1 ganglion stimulants McN-A-343 (50-200 µg) and AH 6405 (100-200 µg) had little or no effect on chewing behaviour. N-methylscopolamine (5-20 µg), oxyphenonium (10-40 µg) and the M-2 ileal selective antagonist 4-DAMP (40-160 µg) produced dose related inhibition of pilocarpine-induced chewing ($p < 0.05$ Friedmans test). The M-2 atrial selective antagonist AF-DX116 (40-160 µg) also inhibited chewing but the curve was U-shaped. The M-1 antagonist pirenzepine (80-320 µg) was ineffective.

Table 1 Inhibition of pilocarpine (4 mg/kg i.p.)-induced chewing

Antagonist	ICV dose range (µg)	Inhibition of pilocarpine-induced chewing ED_{40} (µg)
N-methylscopolamine	5 - 20	5
Oxyphenonium	10 - 40	18
4-DAMP	40 - 160	71
AF-DX116	40 - 160	80
Pirenzepine	80 - 320	> 320

These findings tentatively suggest that purposeless chewing behaviour induced by the cholinergic agonist pilocarpine is not mediated via the M-1 muscarinic receptor subtype.

Salamone et al. (1986) Psychopharmacology, 88, 467
Hammer R. & Giachetti A. (1982) Life Sci. 31, 2991
Jenner P. et al. (1986) Br. J. Pharmac. 88, 341P

PILOCARPINE-INDUCED PURPOSELESS CHEWING IS DEPENDENT UPON CENTRAL INTACT STORES OF 5-HT

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Purposeless chewing induced by pilocarpine administration to rats is inhibited by pretreatment with the catecholamine depletors tetrabenazine and reserpine (Jenner et al 1986). This finding suggest that the action of pilocarpine was dependent in part upon intact brain monoamine stores. We now report on the relative involvement of dopamine, noradrenaline and 5HT depletion in inhibiting pilocarpine-induced purposeless chewing.

Male Wistar rats (150-350 g) (n = 6) received one of the following pretreatments: tetrabenazine (10 mg/kg s.c., 30 min), reserpine (5 mg/kg i.p., 15 h), p-chlorophenylalanine (PCPA; 180 mg/kg i.p., 48 and 24 h) or α-methyl-p-tyrosine methyl ester (AMPT; 180 mg/kg i.p., 15 and 2 h), prior to administration of pilocarpine (4 mg/kg i.p.). Chewing behaviour was measured 15 min following pilocarpine administration as described previously (Jenner et al 1986). Immediately following behavioural assessment the animals were killed for the measurement of whole brain (minus cerebellum) concentrations of dopamine, noradrenaline and 5HT by HPLC using electrochemical detection (Weller et al 1986). In an additional experiment animals receiving PCPA (180 mg/kg i.p., 48 and 24 h previously) were again assessed behaviourally 15 min following pilocarpine (4 mg/kg i.p.) administration but killed at 2 h for biochemical examination.

Administration of pilocarpine (4 mg/kg i.p., 15 min previously) greatly enhanced the intensity of purposeless chewing compared to vehicle treated animals ($p < 0.05$). Pilocarpine (4 mg/kg i.p.)-induced purposeless chewing was markedly reduced by pretreatment with reserpine (5 mg/kg i.p., 15 h), tetrabenazine (10 mg/kg s.c., 30 min) or PCPA (180 mg/kg i.p., 48 and 24 h) (Table 1). In contrast, AMPT (180 mg/kg i.p., 15 and 2 h) pretreatment had no effect on pilocarpine-induced chewing. Whole brain dopamine noradrenaline and 5HT content was greatly reduced by reserpine and tetrabenazine treatment. In contrast, treatment with AMPT reduced dopamine and noradrenaline, but not 5HT, levels while PCPA only decreased the whole brain 5HT content when measured 15 min or 2 h following pilocarpine administration.

Table 1 Comparison of reduction in monoamine levels with inhibition of chewing

Treatment	% Reduction in brain monoamine			% Inhibition chewing
	NA	DA	5HT	
α-Methyl-p-tyrosine	77*	85*	0	0
p-Chlorophenylalanine	13	12	85*	25**
p-Chlorophenylalanine (2 h)	12	7	86*	40**
Reserpine	94*	93*	67*	34**
Tetrabenazine	68*	90*	75*	46**

* $p < .05$, Students t-test; ** $p < .05$, Mann-Whitney u-test

We conclude that the ability of the cholinergic agonist pilocarpine to induce purposeless chewing behaviour in rats appears at least to be partially dependent upon intact central stores of 5HT.

Jenner P. et al (1986) Br. J. Pharmac. 88, 341P.
Weller M.E. et al (1986) Neuropharmacology, 26, 347-354.

B.R. Stewart is an SERC CASE Award student in conjunction with Organon Laboratories.

THE MAUVE FACTOR OF SCHIZOPHRENIA AND PORPHYRIA: EFFECTS ON BEHAVIOUR OF RATS AND MICE

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The mauve factor, identified as 3-ethyl-5-hydroxy-4,5-dimethyl-Δ^3-pyrroline-2-one (HPL), is excreted in urine in 60-70% of acute schizophrenics as well as in acute porphyrics (Irvine, 1978). It has been suggested that this compound might be an endogenous psychotomimetic, although Gorchein and Rogers (1979) found it to have low pharmacological potency in the guinea pig ileum. We have thus examined effects of HPL on behaviour of rats and mice.

Rats received HPL by i.p. injections of 0.65 mmol/kg. Controls were given injections of the solvent. The animal's spontaneous activity was monitored for 5 min every 15 min over a period of 1.5 h commencing at 10 min following injection. Mice received HPL by i.p. injections at 2 dose levels, 0.98 mmol/kg and 1.95 mmol/kg; controls being given injections of saline. Behaviour of each mouse was examined by ethological procedures for a 5 min period in a neutral cage when encountering an unfamiliar animal from the same treatment group. All behavioural observations occurred at 50-60 min after injection. Acts and postures shown by the mice were recorded by two observers, one for each animal, using the ethological profile described by Mackintosh et al. (1977).

Rats treated with HPL showed lower levels of gross activity than controls ($P=0.05$) once habituated to the cage. No gross abnormalities of behaviour were apparent. In mice of the two groups treated with HPL ($n=32$), the mean frequencies ($\pm$SD) of two uncommon behaviours "head twitch" (Treated 0.41 ± 0.87; Control 0.07 ± 0.26, $P=0.05$) and "walk backwards" (Treated 0.35 ± 0.70; Control 0, $P<0.02$) were significantly greater than among control animals. No other differences in behaviour were apparent. Plasma HPL concentrations in mice given the higher dose of HPL amounted to 0.3 ± 0.1 mmol/l (mean$\pm$SD).

These experiments show that HPL can exert mild albeit significant effects upon behaviour of mice and rats after parenteral administration. However, these effects occurred at plasma concentrations that were 9-10 times greater than the human levels in acute porphyric attacks (Graham, 1978). It would appear extremely unlikely therefore that HPL is responsible for the psychiatric symptoms of acute porphyria or schizophrenia.

Gorchein, A. & Rogers, A.T. (1979) Experientia 35, 1078.
Graham, D.J.M. (1978) Monopyrroles in porphyria and related disorders, PhD Thesis, University of Glasgow.
Irvine, D.G. (1978) Life Sci. 23, 983-990.
Mackintosh, J.H. et al. (1977) Handbook of Psychopharmacology, vol 7, (Ed. Iversen, L.L. et al.) p 3-35, New York: Plenum.

THE POTENTIAL ANTISCHIZOPHRENIC ACTIVITY OF THE TETRACYCLIC NEUROLEPTIC ORG 5222 IN RATS AND MARMOSETS

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Trans-5-chloro-2,3,3a,12b-tetrahydro-2-methyl-1H-dibenzo[2,3:6,7]oxepino[4,5-c] pyrrole (Org 5222) is a tetracyclic dopamine antagonist structurally related to the antidepressant mianserin. It is also a potent serotonin antagonist and lacks cataleptogenic or extrapyramidal effects at doses which block dopamine receptors (Kelder et al, 1984).

The potential antischizophrenic activity of Org 5222 was assessed in classical tests and in situations of mesolimbic dopamine excess. In the rat Org 5222 caused dose-dependent catalepsy (1-100 mg/kg i.p., compared with 1-4mg/kg fluphenazine) and induced ipsilateral circling following unilateral intrastriatal injection (1-5µg, compared with 0.1µg fluphenazine) with apomorphine challenge. Relatively high doses of Org 5222 were also required to antagonise spontaneous locomotion on intra-accumbens injection (effects of 10-100µg equating to 5ng fluphenazine), or to antagonise the hyperactivity response to intra-accumbens amphetamine (effects of 100ng Org 5222 equating to 10ng fluphenazine). In contrast, when mesolimbic dopamine function was in excess, Org 5222 was potent and effective to suppress the resultant hyperactivity, without depressing locomotion below control values.

Both rats and marmosets were used in the intracerebral infusion studies (Barnes et al, 1987; Costall et al, 1982). Briefly, animals were prepared for persistent infusion, via osmotic minipumps, of dopamine (25µg/24h, 0.48µl/h) bilaterally into the nucleus accumbens. Rats responded with biphasic hyperactivity (227±23 compared to control values of 91±8.6, values in counts/60 min assessed in individual photocell cages): this response was dose-dependently suppressed by Org 5222 (0.025-0.1mg/kg i.p. b.d.) and by fluphenazine (maximum effect at 0.1mg/kg i.p. b.d.). In contrast to Org 5222, fluphenazine caused excess motor depression [locomotion reduced to a minimum of 87±9.3 counts/60 min using Org 5222 ($P>0.05$ compared to control), but to 11.6±2.0 counts/60 min using fluphenazine ($P<0.001$). In the marmoset, intra-accumbens dopamine infusion caused monophasic hyperactivity (286±23 counts/60 min, measured in photocell cages, compared to 72±8 counts/60 min for vehicle infused animals). This hyperactivity was suppressed by Org 5222 (0.0125 - 0.05 mg/kg i.p. b.d.) and fluphenazine (0.01 mg/kg i.p. b.d. maximally effective), but whilst Org 5222 returned activity to control values of 62±7 to 80±9 counts/60 min ($P>0.05$ compared with vehicle) fluphenazine depressed activity below vehicle levels, to 3±0.5 counts/60 min ($P<0.001$). Further, the suppression of responding caused by fluphenazine persisted for at least 42 days after ceasing treatment whilst marmosets withdrawn from Org 5222 continued to exhibit locomotion indistinguishable from that of vehicle treated animals (97±10 counts/60 min, $P>0.05$).

At high doses Org 5222 has properties similar to a classical neuroleptic but at lower doses this agent can inhibit a mesolimbic dopamine excess without causing motor impairment. Org 5222 may therefore present as a valuable antischizophrenic agent.

Barnes, N.J.G. et al. (1987) Neuropharmacology, in press
Costall, B. et al. (1982) Neuropharmacology, 21, 327-335
Kelder, J. et al. (1984) Pharm. Weekbl. Sci. Ed. 6, 261

ACTIVATION OF DOPAMINE-D_2 RECEPTORS INHIBITS THE RELEASE OF ENDOGENOUS DOPAMINE FROM SLICES OF RAT PREFRONTAL CORTEX

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Midbrain dopamine (DA) neurones innervate a wide variety of forebrain sites to differing degrees. For example, the DA content of rat caudate-putamen is some 100 times greater than that of the medial bank of prefrontal cortex (FCx) and serves to illustrate one aspect of the heterogeneity of DA neurones. Such heterogeneity also appears to be revealed in the relative densities and functional type of DA autoreceptors that each DA projection possesses. In this respect, those DA terminals within the caudate-putamen are thought to possess autoreceptors which regulate both DA synthesis and release whereas DA terminals in FCx do not appear to bear synthesis-modulating autoreceptors (see Wolf et al, 1986). Thus, slices of caudate-putamen are often used for mechanistic studies of endogenous DA release (e.g. Herdon & Nahorski, 1987), whilst FCx slices have only been used previously with radiolabelled DA loading (Plantjé et al, 1987).

The present study was therefore designed to test (a) the feasibility of monitoring endogenous DA release from rat FCx slices and (b) whether such release may be influenced by DA receptor activation.

Slices of FCx (400 x 400μm) were placed in polypropylene chambers at 37°C and superfused with an oxygenated Krebs-bicarbonate buffer containing 1μM nomifensine. The efflux of DA and its deaminated metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), were monitored by an HPLC-EC method as detailed previously (Kilpatrick et al., 1986). Protein content of the slices was assayed by the method of Bradford (1976).

A spontaneous release of DA was detectable (12 ± 1 fmol/mg protein/min) which could be elevated dose-dependently up to 70-fold in a Ca^{2+}-sensitive fashion by raising external K^+ concentrations over a 20-50mM range. Neither spontaneous nor K^+-enhanced DA outflow was influenced by supplying 50μM L-tyrosine as precursor. DA release evoked by 35mM K^+ could be reduced up to a 60% maximum inhibition by increasing concentrations up to 0.5μM of the selective D_2 agonist, quinpirole. This inhibitory action of quinpirole was readily and in many cases, completely offset by 1μM of the selective D_2 antagonist, (-)-sulpiride. On the other hand, cosuperfusion of 1μM SCH23390, a selective D_1 antagonist, did not significantly alter the DA release-depressant action of quinpirole.

The basal efflux of DOPAC (37 ± 5 fmol/mg protein/min) was enhanced 4-fold by supplying 50μM L-tyrosine in the superfusion medium and could also be elevated up to 10-fold in a dose-related manner by increasing external K^+ (20-50mM). However, the elevated release of metabolite did not coincide with but lagged behind that of DA. This may reflect deamination of released DA in non-neural tissue or a K^+-induced activation of terminal DA synthesis that is accompanied by intracellular DA metabolism.

This study therefore illustrates the feasibility of recording endogenous DA efflux from a relatively DA-poor region and provides evidence that D_2 receptors exist in FCx which are capable of regulating DA release.

Bradford, M.M. (1976) Anal. Biochem. 72, 248-254.
Herdon, H. & Nahorski, S.R. (1987) N.S. Arch. Pharmacol. 335, 238-242.
Kilpatrick, I.C. et al. (1986) J. Neurochem. 46, 1865-1876.
Plantjé, J.F. et al. (1987) Neuroscience 20, 157-168.
Wolf, M.E. et al. (1986) J. Pharmacol. Exp. Ther. 236, 699-707.

HISTAMINE AND STRESS-INDUCED ANTINOCICEPTION IN THE MOUSE

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Stimulation of histamine H_2-receptors in the rat raphe nucleus produced antinociception (Glick & Crane, 1978) whilst Terman et al (1982), in a study of a non-opioid model of stress-induced antinociception (SIA) in the rat, concluded that H_1-receptors were involved. We report an investigation of the involvement of histamine in two models of SIA in the mouse, only one of which is naloxone-sensitive (Hart et al., 1983).

Male LACA mice (25-35g) were allowed to acclimatise for 2h prior to experiments. In the naloxone-sensitive model of SIA the stress was a 3 min swim at 20°C with nociception assessed as the reaction time on a hot-plate (54°C). Reaction times for each mouse were determined three times; prior to drug or vehicle administration, immediately pre-swim and after a 2 min drying period post-swim: unstressed mice were treated in a similar manner. SIA was expressed as the difference between the post- and pre-swim values whilst drug effects were the difference between the last and first reaction times. Each treatment included at least four mice and reaction time values were analysed by Mann-Whitney U-test.

Histidine (400 mg/kg, i.p.) produced significant antinociception by itself and SIA in mice receiving histidine was 24s compared to 12.5s in saline treated animals. However, neither H_1 nor H_2 antagonists reduced SIA and, in fact, diphenhydramine (20 mg/kg), mepyramine (20 mg/kg), promethazine (20 mg/kg) and SKF 95282 (20 mg/kg) all produced a small but significant antinociception by themselves. In the presence of promethazine, cimetidine (100 mg/kg) and SKF 95282 (40 mg/kg) SIA was increased significantly.

In additional experiments, histamine (200 µg, i.c.v.) produced antinociception and SIA in mice treated with histamine was 29s compared to 12.5s in saline controls. Neither SKF 95282 (25 µg, i.c.v.) nor cimetidine (25 µg, i.c.v.) affected SIA whilst at higher doses both antagonists produced significant antinociception alone and a significant increase in SIA.

The naloxone insensitive model of SIA involved a 30s swim at 30°C followed by the injection of acetic acid (0.6%w/v, 1ml/100g, i.p.) with the abdominal constrictions counted 10-20 min after acetic acid injection. The count for saline treated, unstressed mice was 25.5 and that of swum animals was 5.5. Although histamine, 10 & 20 mg/kg, i.p., reduced the number of constrictions, it was impossible to study the involvement of histamine in this model of SIA for both H_1 antagonists (mepyramine, diphenhydramine, promethazine) and H_2 antagonists (cimetidine, SKF 95282) alone reduced the number of constrictions.

We conclude that although histamine is antinociceptive in the mouse, and appeared to potentiate SIA, there is no evidence from experiments with antagonists that histamine is involved in either the naloxone sensitive or the naloxone insensitive model of SIA.

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Glick, S.D. & Crane, L.A. (1978) Nature 273, 547
Hart, S.L. et al., (1983). Eur.J.Pharmac., 95,283.
Terman, G.W. et al (1982). Proc.West Pharmacol.Soc., 25,7.

[^{3}H]-MEPYRAMINE BINDING TO GUINEA-PIG AND RAT NUCLEUS ACCUMBENS INDICATES THE PRESENCE OF H_1-RECEPTORS

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We have previously reported that intra-accumbens histamine administration alters behavioural activity in rats and that these changes can be attenuated by pretreatment with the H_1-antagonist mepyramine (Bristow & Bennett, 1987). Since this data would suggest the presence of H_1-receptors in this region, we have investigated this proposal using ^{3}H-mepyramine binding, a ligand which binds selectively to H_1-receptors in guinea-pig and rat brain (Chang et al., 1979; Hill & Young, 1980).

Guinea-pig and rat cerebellum or nucleus accumbens were dissected, homogenized in 50 mM Na-K phosphate buffer (pH 7.5), centrifuged (50,000 g, 10 mins), resuspended in 9 vols buffer, snap frozen and stored at -20°C. Tissue aliquots (5 mg wet wt., guinea-pig cerebellum (100 µl), rat accumbens (40 µl); 2 mg wet wt. guinea-pig accumbens (40 µl)), were incubated in triplicate at 25°C for 30 mins with 1 nM mepyramine (26 Ci/mmol; cerebellum 50 µl, or nuc. accumbens 20 µl) and unlabelled inhibitor (100 µl or 40 µl) after which ice-cold buffer containing 1 µM non-radioactive mepyramine was added and samples filtered through Whatman GF/B filters.

Binding of different concentrations of ^{3}H-mepyramine (0.5-10 nM) in the presence or absence of 2 µM promethaziine (to define non-specific-binding) enabled calculation of dissociation constants (K_D) and binding site capacities (B_{max}). In addition, mepyramine, promethazine and SKF 93944 were tested over a range of concentrations for their ability to inhibit specific binding of ^{3}H-mepyramine (1 nM) in each region. In all cases slopes of inhibition curves were not significantly different from 1, and specific binding could be displaced to the defined non-specific level.

Tissue	K_D	B_{max}	K_i Mepyramine	K_i Promethazine	K_i SKF 93944
1. Guinea-pig cerebellum	0.77±0.04 (n=3)	15±3	1.5 ±0.1 (n=3)	1.7 ±0.3 (n=3)	4.8±1.3 (n=3)
2. Guinea-pig nuc. accumbens	0.84±0.18 (n=3)	3.2 ±0.1	1.61±0.25 (n=2)	2.69±0.29 (n=2)	13.5±5 (n=2)
3. Rat nuc. accumbens	0.99±0.15 (n=3)	1.34±0.03	3.67±0.8 (n=5)	2.06±0.02 (n=2)	15.1±2.8 (n=2)

K_D, K_i = nM; B_{max} = pmol/g wet wt.; n=no. of experiments (in triplicate); 12 x rat n.accumbens/experiment; 6 x guinea pig. n.accumbens/3 experiments.

The ability of H_1 antagonists to inhibit ^{3}H-mepyramine binding in rat accumbens is comparable with that previously described for whole rat brain, although the dissociation constant (K_D) is lower (Tran et al., 1978). These results thus provide evidence for the presence of H_1-receptors in the nucleus accumbens.

L.J. Bristow is a CASE student with SKF.

Bristow, L.J. & Bennett, G.W. (1987) Br.J.Pharmac. in press.
Chang, R.S.L., Tran, V.T. & Synder S.H. (1979) J.Neurochem. 32, 1653-1663.
Hill, S.J. & Young, J.M. (1980) Br.J.Pharmac. 68, 687-696.
Tran, V.T., Chang, R.S.L. & Snyder, S.H. (1978) Proc.Natl.Acad.Sci. U.S.A. 75, 6290-6294.

RELEASE OF [^{3}H]-NORADRENALINE BY THE PROTON-PUMP INHIBITOR OMEPRAZOLE

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Omeprazole (OMP) is a substituted benzimidazole that inhibits gastric acid secretion by inhibiting the gastric proton pump H^+-K^+ ATPase (EC 3.6.1.36). The inhibition OMP causes is not competitive. It has been suggested that, under low acidic conditions, OMP is converted into a sulphydryl reactive intermediate (a cyclic sulphenamide) that interacts with essential thiol groups on the pump. In our studies concerning the selectivity of its action we found that OMP evokes [^{3}H] noradrenaline ([^{3}H]NA) release from the rat vas deferens and in this preliminary report we have therefore explored the mechanism by which OMP might be evoking [^{3}H]NA release from this preparation.

Isolated vasa deferentia, which were prepared from Sprague-Dawley rats (200-300 g), were loaded with [^{3}H]NA (0.21-0.33 μM) and subsequently washed for 110 min as detailed previously (MacIntosh and Vohra, 1982). Thereafter, we collected at least three 5-min fractions before and six 5-min fractions with OMP present. Tissues were then removed and either digested to determine total radioactivity or they were homogenized in 0.4 M perchloric acid containing 0.05% Na_2EDTA + 0.1% sodium metabisulphite. We centrifuged the homogenate and used the supernatant to analyse the metabolic profile of the radioactivity. The profile of the released radioactivity and of the tissue radioactivity was determined using the double column chromatographic (Alumina and Dowex 50x4) procedure of Graefe et al. (1973). OMP was dissolved in 0.2-0.4 ml dimethylformamide (DMF) and then diluted as required with distilled water. For acid-treated (OMP-AT) and alkali-treated (OMP-BT) OMP, we incubated OMP with 0.1 N HCl and 0.2 N NaOH, respectively for 30 min at 37°C. After adding DMF, we adjusted both solutions to pH 5-6.

All three forms of OMP evoked a dose-related (1 μM - 0.5 mM) increase in ^{3}H outflow from the vas preload with [^{3}H]NA. OMP-AT was most potent and untreated OMP was the least potent in this regard. Different vehicles had no effect on the basal ^{3}H outflow. The ^{3}H overflow evoked by OMP-AT and by untreated OMP was blocked by neither tetrodotoxin (5 μM) nor 2 mM EGTA-Ca^{2+}-free Krebs solution, and it was unaffected by either cocaine (10 μM) or desipramine (3.3 μM).

Whereas >90% of tissue ^{3}H content consisted of unchange ^{3}HNA, the spontaneous outflow and the overflow evoked by either OMP-AT or untreated OMP consisted mostly (55-64%) of the deaminated metabolite [^{3}H]DOPEG, with about 17-20% being unchanged [^{3}H]NA.

These findings indicate that (a) acid treatment generates a more active form of OMP; (b) the release evoked by different forms is independent of the generation of action potentials and of the external Ca^{2+} concentration, and (c) OMP-AT and untreated OMP cause NA release intraneuronally. We suggest that the different forms of OMP evoke ^{3}HNA overflow intraneuronally either by altering proton concentration gradient across the vesicular membrane or by a tyramine-like action or by interacting with some thiol groups within the adrenergic neuron and/or the storage vesicles.

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Clissold, S.P. and Campole-Richards, D.M. (1986) Drugs 32, 15.
Graefe, K.H. et al. (1973) Biochem. Pharmacol. 22, 1147.
MacIntosh, C.M. and Vohra, M.M. (1982) Life Sci. 31, 2819.

THE MECHANISMS BY WHICH HISTAMINE EVOKES CATECHOLAMINE RELEASE FROM THE VAS DEFERENS AND THE ADRENAL GLAND OF THE RAT

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There are reports which indicate that, whereas histamine evokes catecholamine (CA) release in situ from the adrenal glands by an H_1-receptor mechanism (Staszewska-Barczak and Vane, 1965), it releases noradrenaline (NA) from sympathetic nerves by a tyramine-like action (MacIntosh and Vohra, 1982; Starke and Weitzell, 1978). It is, however, not clear whether this indicates a real difference in histamine's mechanism of action in the two systems or indicates differences attributable to other factors such as species and/or reflexly elicited sympatho-adrenal activity. We therefore studied the mechanism of histamine-evoked CA release in the two systems, using the isolated vas deferens and the isolated adrenal gland from the rat.

Stripped and longitudinally slit vasa deferentia were equilibrated at 37°C in Krebs containing 10 μM pargyline (an MAO inhibitor) (MacIntosh and Vohra, 1982). The left adrenal gland was isolated and perfused with Krebs solution at 35°C via the adrenal vein (Wakade, 1981). Catecholamines were determined on an HPLC coupled to an EC detector.

In both preparations histamine evoked a dose-related release of CA - the vas deferens released only NA and the adrenal gland released predominently adrenaline. Both H_1 and H_2 agonists caused NA release from the vas deferens and the release was unrelated to the relative potency of the analogue within each group. In contrast, only H_1 agonists evoked CA release from the adrenal gland and their activity profile -

histamine >> 2-ThEA ≥ 2-MH ≥ 2-PEA ≥ 4-MH >> dimaprit

- corresponded to their known relative potency as H_1-receptor agonists. Mepyramine (0.01-0.1 μM) did not affect the histamine-evoked release in the vas deferens but blocked that in the adrenal gland; the H_2-receptor antagonist cimetidine (35 μM) affected release in neither preparation. Omitting external calcium from and adding 2 mM EGTA to the bathing medium did not alter the release by histamine in the vas deferens but diminished that in the adrenal gland by >70%. On the other hand, 1 μM cocaine depressed the histamine-evoked release in the vas deferens by >80% but only slightly (<15%) reduced that in the adrenal gland. The release evoked by histamine in the adrenal gland was reduced by >75% when 1 mM verapamil was present in the normal Krebs. It was also reduced by ~60% when tetradotoxin (3 μM) was present, indicating that an increased permeability to Na^+ also results from this H_1-receptor stimulation.

From these results in the two preparations of the rat, we conclude that histamine causes release of NA from the vas deferens (sympathetic nerves) by a tyramine-like action, whereas the release of CA from the adrenal gland is mediated by exocytosis involving a mepyramine-sensitive, calcium-dependent H_1-receptor mechanism.

We thank the MRC of Canada and the New Brunswick Heart Foundation (Canada) for their financial support.

MacIntosh, C.M. and Vohra, M.M. (1982) Life Sci. 31, 2819.
Starke, K. and Weitzell, R. (1978) Naunyn-Schmiedebergs Arch. Pharm. 304, 372.
Staszewska-Barczak, J. and Vane, J.R. (1965). Br. J. Pharmacol. 25, 728.
Wakade, A.R. (1981) J. Physiol. 313, 463.

PHENYLEPHRINE AND NORADRENALINE EVOKE INCREASES IN SHORT CIRCUIT CURRENT AFTER α_2-ADRENOCEPTOR ANTAGONISM IN THE RAT JEJUNUM

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It has been reported that high concentrations (10^{-4}M) of phenylephrine evoke transient increases in short circuit current (SSC) after α_2-adrenoceptor antagonism in the rat jejunum in vitro (Williams, 1986). A transient increase in SCC evoked by β_2-adrenoceptor stimulation has also been reported (Dettmar et al, 1986). Williams (1986) suggested that the increase in SCC evoked by phenylephrine may be mediated through β_2-adrenoceptor stimulation.

We have investigated the effects of high concentrations (10^{-4}M) of phenylephrine (PHE) and noradrenaline (NA) on the rat jejunum in vitro after α_2-adrenoceptor antagonism, using a modified Ussing chamber. Tissues were obtained from male Wistar rats (200-240g) killed by cervical dislocation and exsanguination. Measurements were made of changes in SCC evoked by PHE and NA in the presence of idazoxan. A higher concentration of idazoxan was required to fully inhibit the decrease in SCC to NA than PHE (10^{-5}M & 10^{-6}M idazoxan respectively). Antagonists were added at 15 minutes, agonists at 30 minutes after tissue mounting. PHE and NA in the presence of idazoxan caused an immediate, transient increase in SCC (table 1) which was maximal within 1-2 minutes of addition. Cirazoline (10^{-6}-10^{-4}M) was without effect and was therefore tested as an antagonist. The increases in SCC evoked by PHE and NA were significantly inhibited by prazosin, corynanthine and cirazoline whereas propranolol, atropine, methysergide and tetrodotoxin had no significant ($p>0.05$) effect on these responses (Table 1).

TABLE 1 Changes in SCC (mean ± s.e.) induced by PHE (10^{-4}M) & NA (10^{-4}M) in the presence of various antagonists (n=6).

Agonist	Antagonist	SCC (μamps/cm^2)	*P
PHE	Idazoxan (10^{-6}M) only	+8.2 + 2.0	-
PHE	Idazoxan & atropine (10^{-6}M)	+5.1 ± 0.9	NS
PHE	Idazoxan & methysergide (10^{-5}M)	+6.4 ± 1.6	NS
PHE	Idazoxan & tetrodotoxin (10^{-6}M)	+5.5 ± 1.3	NS
PHE	Idazoxan & propranolol (10^{-6}M)	+6.3 ± 1.5	NS
PHE	Idazoxan & prazosin (10^{-7}M)	+0.6 ± 0.3	<0.01
PHE	Idazoxan & corynanthine (10^{-4}M)	+0.6 ± 0.3	<0.01
PHE	Idazoxan & cirazoline (10^{-5}M)	-0.9 ± 0.4	<0.01
NA	Idazoxan (10^{-3}M) only	+3.8 + 0.3	-
NA	Idazoxan & propranolol (10^{-6}M)	+3.3 ± 0.3	NS
NA	Idazoxan & prazosin (10^{-7}M)	+1.3 ± 0.2	<0.01
NA	Idazoxan & corynanthine (10^{-4}M)	+1.5 ± 0.2	<0.01
NA	Idazoxan & cirazoline (10^{-5}M)	+1.6 ± 0.4	<0.01

* Student's 't'-test for unpaired data: comparisons with agonist + idazoxan.

These results suggest the presence of an α_1-adrenoceptor mediated increase in SCC in the rat jejunum. The α_1-selective agonist cirazoline appears to act as an antagonist in this tissue. α_2-adrenoceptor mediated decreases in SCC appear to predominate over α_1-adrenoceptor mediated increases in SCC.

Dettmar, P. W. et al (1986) Br. J. Pharmacol. 88, 465P
Williams, R. J. (1986) PhD Thesis, Aston University.

Ca^{2+} ENTRY THROUGH VOLTAGE SENSITIVE K^+ CHANNELS CONTRIBUTES TO INOSITOL PHOSPHOLIPID HYDROLYSIS INDUCED BY K^+ IN GUINEA-PIG ILEUM

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The elevation of the extracellular K^+ concentration induces the hydrolysis of inositol phospholipids in guinea-pig ileum. We have hypothesised that this effect is caused by the release of neurotransmitters from enteric neurones (Watson and Downes, 1983). On the other hand, Best and Bolton (1986) have suggested that this hydrolysis is triggered by depolarisation, but that Ca^{2+} entry is not essential for this effect since the response to K^+ is not changed in the presence of a Ca^{2+} channel blocker, D600. In the present communication, we have investigated further the molecular events underlying the ability of K^+ to stimulate inositol phospholipid hydrolysis.

Dispersed cells of guinea-pig ileum longitudinal smooth muscle, isolated by collagenase treatment, or cross-chopped slices prepared as described previously (Watson and Downes, 1983), were labelled with [3H]inositol for 60 min before challenge with various stimuli in the presence of LiCl (10 mM). The reaction was stopped after 30 min by addition of $CHCl_3$:CH_3OH:HCl (100:200:2), and total inositol phosphates were analysed by Dowex anion exchange chromatography.

High K^+ (112mM) solution, in which Na^+ was replaced by the equimolar K^+, evoked a 309 $\pm$ 16 % increase in inositol phosphates in the dispersed cells. The dispersal of the smooth muscle cells may disrupt the close synaptic contacts of the neurones with the muscle cells, and makes it unlikely that the release of neurotransmitters contributes significantly to the response observed. This assumption is supported by the lack of effect of tetrodotoxin or atropine on the response to K^+ (not shown).

In cross-chopped slices (Table) and also dispersed cells (not shown) nifedipine inhibited the response to K^+ partially, but had no effect on the response to carbachol. These data demonstrate that the entry of Ca^{2+} through voltage sensitive channels contributes to the hydrolysis of the inositol phospholipids by K^+. The mechanism of the nifedepine-insensitive response is not clear, but might be Ca^{2+} influx due to Na^+-Ca^{2+} exchange resulting from the substitution of Na^+ with K^+.

Table: Effect of nifedipine (Nif) on carbachol (Carb)- or K^+-induced inositol phosphates (IPs) formation. The results are expressed relative to the control value and are shown as mean $\pm$ S.E.M. of three experiments performed in quadruplicate.

	IPs	p
Control	100	-
Nif (10uM)	92 $\pm$ 5	-
Carb (0.1mM)	357 $\pm$ 43	-
Carb (0.1mM) + Nif (10uM)	347 $\pm$ 38	n.s.
K^+ (112mM)	333 $\pm$ 29	-
K^+ (112mM) + Nif (10uM)	204 $\pm$ 25	< .01

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Best L. and Bolton T.B. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol. *333*, 78-82
Watson S.P. and Downes C.P. (1983) Eur. J. Pharmacol. *93*, 245-253.

PREJUNCTIONAL INHIBITION OF EXCITATORY JUNCTION POTENTIALS IN GUINEA-PIG VAS DEFERENS BY AMP

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The ability of adenosine 5'-monophosphate (AMP) to reduce the initial twitch component of the rat vas deferens to sympathetic nerve stimulation has been attributed to an agonist action at prejunctional P_1-purinoceptors, reducing neuronal release of noradrenaline (Stone, 1981; Willemot & Paton, 1981). However, a recent study (Satchell, 1986) suggested that AMP actually blocks the twitch response by acting as an antagonist of post-junctional P_2-purinoceptors, inhibiting the transmitter action of neuronally released ATP. To test whether AMP could be useful as a selective antagonist of P_2-purinoceptors, we have studied its effect on excitatory junction potentials (e.j.p.s) in guinea-pig vas deferens, since we have previously demonstrated that e.j.p. magnitude in this preparation can be reduced either by P_1-purinoceptor agonists acting prejunctionally (Sneddon et al., 1984) or by antagonism or desensitization of post-junctional P_2-purinoceptors (Sneddon et al., 1982; Sneddon & Burnstock, 1984). Intracellular microelectrodes were used to record membrane potential and e.j.p.s evoked by field stimulation at 0.5 Hz, 0.5 ms pulse width. Addition of AMP, at concentrations from 5×10^{-6} to 10^{-3}M, did not produce any effect on the control resting membrane potential (70.6 ± 1.6 mV, n=18), indicating that it had no P_2-agonist action, but the same concentrations of AMP produced a dose dependent reduction in the magnitude of e.j.p.s. Subsequent addition of the specific P_1-purinoceptor antagonist 8-phenyltheophylline (8-PT) at 10^{-5}M completely reversed the effect of 10^{-5}M AMP, and partially reversed the effect of 10^{-4}M AMP. (See figure 1). The effects of both drugs were rapidly reversed by washout.

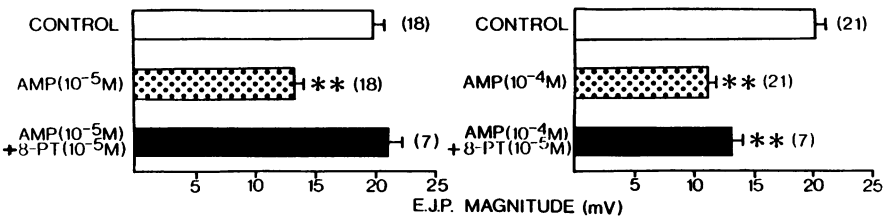

Figure 1 Effect of AMP and 8-PT on e.j.p. magnitude in guinea-pig vas deferens. All drug effects on e.j.p.s are compared with controls obtained in the same cell. * * = Significant difference at 1% level using Students t-test for paired data.

From these results we conclude that AMP cannot be used as a selective P_2-purinoceptor antagonist to investigate the putative role of ATP as a cotransmitter in guinea-pig vas deferens, since its primary action in this tissue is to reduce responses to sympathetic nerve stimulation by acting prejunctionally as a P_1-purinoceptor agonist.

Satchell, D. (1986) European J. Pharmacol. 132 305-308
Sneddon, P. & Burnstock, G. (1984) European J. Pharmacol. 100 85-92
Sneddon, P., Meldrum, L. & Burnstock, G. (1984) European J. Pharmacol. 105 293-299
Sneddon, P., Westfall, D.P. & Fedan, J.S. (1982) Science 218 693-694
Stone, T.W. (1981) European J. Pharmacol. 75 93-102
Willemot, J. & Paton, D.M. (1981) Naunyn-Schmiedeb. Arch. Pharmacol. 317 110-114

FOCAL APPLICATION OF TTX TO SYMPATHETIC NERVE TERMINALS USING INTERNALLY PERFUSED SUCTION ELECTRODES

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It has always been difficult to obtain qualitative information about the sequence of events involved in depolarization-secretion coupling in sympathetic nerve terminals and in particular about the precise site of action of drugs. Recently, we reported a method of extracellular recording using a suction electrode which allows simultaneous measurement of both the nerve action potential and transmitter release from postganglionic sympathetic nerve terminals and have demonstrated that intermittent transmitter release is not due to failure of the action potential to invade the terminals (Brock & Cunnane, 1987).

To determine the characteristic features of action potential propagation in sympathetic nerve terminals and the mode of action of drugs known to modify the release process, the method has been modified to allow internal perfusion of the suction electrode. Thus we are now able to study the effects of applying drugs locally at the site of recording. It was of particular interest to apply tetrodotoxin (TTX) to determine whether the action potential actively invades the varicose terminals. TTX (10^{-7} M) blocked the inward current of the nerve terminal action potential and abolished evoked transmitter release; spontaneous transmitter release was unaffected. The residual electrotonic depolarization of the terminals was not sufficient to activate the transmitter release mechanism (Figure 1). These effects of TTX were readily reversed by wash. Release from varicosities located outside the electrode was unaffected by TTX applied within the electrode.

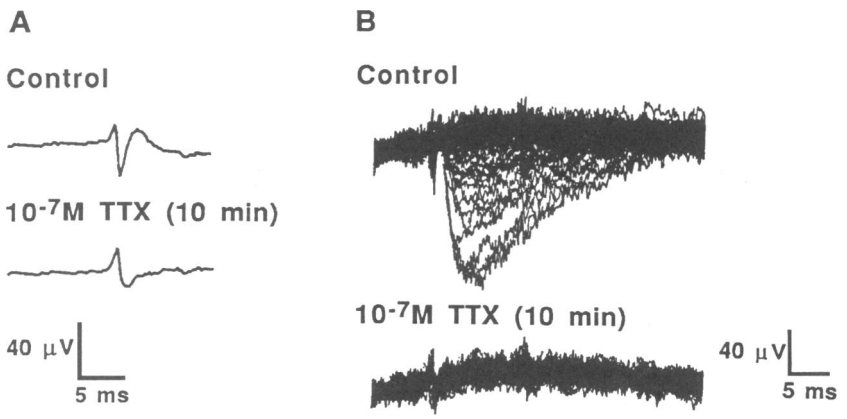

Figure 1. Effects of local application of TTX on evoked electrical activity recorded extracellularly from the guinea-pig vas deferens. (A) Averages of 80 action potentials recorded at 1 Hz. (B) The nerve terminal action potential and evoked release (100 pulses, 1 Hz) .

In conclusion (1) sympathetic nerve terminals are actively invaded by sodium-dependent nerve action potentials and (2) active invasion of the terminals is normally required for transmitter release to occur.

Brock, J.A. & Cunnane, T.C. (1987) *Nature* **326**, 605-607
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On a range of isolated tissues, distinct patterns of agonist potency have been suggested to reflect the existence of multiple classes of tachykinin receptors (Iversen 1985). Substance P (SP) and physalaemin have greater affinity for NK1 receptors and kassinin, eledoisin, and substance P (6–11) are more active at NK2 receptors. Recent work has revealed the existence of two novel tachykinins in mammalian systems, neurokinin A (NKA) and neurokinin B (NKB).

Previous evidence has suggested that the non-cholinergic, non-adrenergic response produced by electrical stimulation of the iris sphincter muscle may be mediated by a tachykinin like peptide released from the peripheral terminals of primary afferent fibres (Ueda et al, 1982; Jamieson and Russell 1986). However, in order to investigate the transmitter responsible for this response we have used the tachykinin antagonist ($DPro^4$, $DTrp^{7,9,10}$)-S.P (4–11) against a range of tachykinins and against the slow response to electrical field-stimulation.

Cumulative dose responses were constructed to application of SP, physalaemin, substance P (6–11), NKA, NKB, eledoisin, and kassinin.

Application of the tachykinin antagonist ($DPro^4$, $DTrp^{7,9,10}$)-S.P (4–11) (10 μM, 15 mins) caused a parallel rightward shift in the dose responses to NKA (Dose Ratio = 5.2) substance P (6–11) (dr = 7.3) eledoisin (dr = 5.7) and kassinin (dr = 5) whilst responses to SP (dr = 1.2) physalaemin (dr = 1.8) and NKB (dr = 0.8) were unaffected (n = 6).

Electrical stimulation produced an initial rapid cholinergic contraction followed by a slow peptidergic response. In the presence of the tachykinin antagonist the fast cholinergic response was unaffected (–12% ± 7%, mean ± s.e.m., n=6) whilst the slow peptidergic contraction was significantly attenuated (–71% ± 9%, mean ± s.e.m., n=6).

Thus under these conditions the tachykinin antagonist ($DPro^4$, $DTrp^{7,9,10}$)-S.P (4–11) attenuated responses to agonists acting at NK2 type receptors (substance P (6–11), eledoisin, and kassinin) whilst those induced by agonists acting at NK1 type receptors (SP, and physalaemin) were unaffected. The response to NKA was attenuated and therefore probably mediated by NK2 type receptors whilst that to NKB was unaffected. The slow response to electrical stimulation was also attenuated by the tachykinin antagonist.

We conclude therefore that the endogenously released tachykinin exerts its action on NK2 type receptors and the probable transmitter candidate for this response is a NKA-like tachykinin.

Iversen, L. (1985). In: Tachykinin Antagonists (Eds : R. Hakanson and F. Sundler) Elsevier, Amsterdam.

Ueda, N. et al (1982). Life Sci. 31, 369–375.

Jamieson, A. and Russell, N.J.W. (1986). Brit. J. Pharmac., London Inst. Education, C52, 17–19th December.

VAGAL NERVE FIBRES INFLUENCE RESPONSES OF RAT STOMACH TO NEUROKININ A

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Substance P (SP) and neurokinin A (NKA) have been shown to contract the rat stomach both *in vitro* and *in vivo*, NKA being 10 times more potent than SP. Contrary to observations *in vitro*, the dose-response curve for SP *in vivo* was flatter than that for NKA (Holzer-Petsche et al., 1987). This study aimed at investigating whether reflexes elicited by i.a. infusion of tachykinins *in vivo* were responsible for either increasing the response to NKA or decreasing the response to SP.

Rats were anaesthetized with urethane and laparotomized. The abdominal aorta was cannulated for drug infusion into the coeliac artery. The stomach was cannulated *via* the duodenum and filled with 3 ml Tyrode solution. Gastric motility was recorded by monitoring changes in intragastric pressure. Possible reflex loops were eliminated by surgical removal of the coeliac-superior mesenteric ganglion complex (ganglioniectomy) or by subdiaphragmatic vagotomy. Either of these operations was performed immediately before the experiment.

The contractile responses of the stomach to infusion of NKA (0.02 - 2 nmol min^{-1} for 3 min) increased markedly after vagotomy. Ganglionectomy had only a small stimulating effect on gastric contractions due to NKA.

In rats pretreated with guanethidine (20 mg kg^{-1} s.c.) and atropine (1 mg kg^{-1} i.v.) electrical stimulation of the distal end of one cut vagus nerve (10V, 20 Hz, 5 ms) caused relaxation of the stomach. These relaxations were completely blocked by application of a cotton wool swab soaked in a 10 mg ml^{-1} solution of capsaicin (in 10% Tween 80, 90 % paraffin oil) to the nerve. Relaxations recovered within 60 min after removal of the capsaicin swab from the vagus.

The results are compatible with the proposition that NKA might be able to stimulate a vagal inhibitory reflex, the effect of which is masked by the direct contractile activity of NKA on gastric smooth muscle.

U. Holzer-Petsche, F. Lembeck & H. Seitz, *Br. J. Pharmac.* (1987), 90, 273-279.

EFFECT OF ATRACURIUM ON TONE, CONTRACTILITY AND THE CONTRACTIONS PRODUCED BY PERIARTERIAL STIMULATION IN THE RAT ISOLATED ILEUM

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Atracurium is a new non-depolarizing neuromuscular blocking agent (Stenlake,1979), with a rapid onset of action and of intermediate duration. Unlike its effect at the skeletal muscle,i.e producing relaxation, atracurium and other muscle relaxants produce a contraction in the intestinal smooth muscle (Wali,1984; Wali,Suer, McAteer,Hayter,Tugwell and Makinde,1987). This latter effect of atracurium may,in part,be due to histamine release and in part to a direct effect of atracurium, e.g on muscle intracellular calcium ions.

In the present investigation,we have studied the effect of atracurium on tone, contractility and on the contractions produced by periarterial nerve stimulation in rat ileum to see if atracurium modified neurotransmitter release and cholinergic and non-cholinergic responses in the rat ileum.

Atracurium (1-100 μM) had no significant effect on the spontaneous contractions of rat ileum (control 0.5±0.1 g tension).However,atracurium produced concentration-dependent contractions in the muscle. A mean maximum contraction of 1.2±0.1 g was obtained with 100 μM of atracurium (mean±s.e.,n=6). In the presence of mepyramine (0.6 μM) and methysergide (1 μM),to block histamine and serotonin-induced contractions,the contraction produced by atracurium (10 μM) was reduced by 32±4.1% and 11±2.3%, respectively(means±s.e.,n=6,$P < 0.05$, P ; N.S., with respect to their control values). In the presence of these drugs and calcium-free Krebs-Henseleit solution, atracurium (10 μM) still produced small contractions in the ileum (0.3± 0.1 g),suggesting that the drug may affect intracellular calcium stores in the muscle.

Repetitive nerve stimulation,of the autonomic nerve supply to the gut, at 1-100 Hz, with 10-40 V (maximal) and 0.2-0.5 ms pulse duration, produced frequency-dependent contractions, or relaxations in the rat ileum. At low rates of stimulation, e.g 0.2-2 Hz, only contractions were produced (0.6±0.2 g) . At intermediate rates of stimulation,e.g 5-25 Hz, a biphasic response was produced; i.e an initial relaxations (0.2-0.7 g) followed by a prolonged and low amplitude contraction (0.5-0.8 g tension). At high rates of stimulation (e.g 50 Hz), only relaxations were produced. Atracurium, reduced these contractions,i.e produced by periarterial nerve stimulation, in the rat ileum. In the presence of guanethidine (10 μM) and propranolol (1 μM),the contractions produced by atracurium remained unchanged,indicating a non-adrenergic dependency of atracurium action.

It is not clear how atracurium reduced the contractions produced by periarterial nerve stimulation,but this may be due to interference with neurotransmitter action at the rat intestinal smooth muscle. In conclusion, we have demonstrated that atracurium (a) had little effect on spontaneous contractions, (b) modified the contractions produced by periarterial nerve stimulation,and (c) produced a marked contraction,by itself, in the rat intestinal muscle.

Stenlake,J.B. (1979). In: Advances in Pharmacology & Therapeutics,ed., J.C.Stocklet , Pergamon Press, New York, p. 303

Wali,F.A. (1984). Br.J.Anaesth. 56 , 421-422

Wali,F.A., Suer,A.H., McAteer,E.,Hayter,A.,Tugwell,A.C.,& Makinde,V.(1987). Agents and Actions, In Press.

EFFECTS OF ATROPINE AND GLYCOPYRROLATE, ON TWITCH, TETANIC AND POST-TETANIC TWITCH RESPONSES IN THE RAT DIAPHRAGM PREPARATION

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Atropine (a tertiary amine) and glycopyrrolate (a quaternary ammonium) are anti-muscarinic drugs used, in a mixture with neostigmine, to reverse the residual neuromuscular blockade produced by non-depolarizing muscle relaxants (Mirakhur, Dundee & Clarke, 1977). Moreover,it has been suggested that glycopyrrolate may be superior to atropine, in stabilizing the heart rate and antagonizing cholinergic responses in man (Ostheimer, 1977; Cozanitis,Dundee,Merrett,Jones & Mirakhur,1980).

In the present investigation, we have studied and compared neuromuscular effects of atropine and glycopyrrolate, by analysing their effects on indirectly-elicited twitch,tetanic and post-tetanic twitch response and potentiation in the rat isolated preparation.

The phrenic nerve was stimulated, at 0.2-100 Hz, with 2-5 V (maximal) and 0.1-0.2 ms pulse duration. The post-tetanic twitch response(0.2 Hz) was elicited 5s after a tetanic stimulation,e.g at 50 Hz for 1s duration. Mechanical responses of the rat diaphragm, were recorded isometrically, in the absence and presence of atropine (0.001-10 μM) or glycopyrrolate (0.01-100 μM) . The results are shown in Table 1. Atropine (1.4 μM), which produced near maximum increase in twitch tension, increased all neuromuscular responses,i.e the twitch (T),tetanus (TT),post-tetanic twitch response (PTT) and the post-tetanic twitch potentiation (PTP).In contrast, glycopyrrolate (6.25 μM),which is large enough to produce any effect, had little effect on the amplitude of the twitch tension,but it significantly reduced the tetanic and post-tetanic twitch responses (Table 1).

The mechanism of action of muscarinic antagonists at the neuromuscular junction is not clear, but it is clear that the action of atropine may be different from that of glycopyrrolate. Atropine facilitated (Abbs & Joseph,1981) whereas glycopyrrolate inhibited neuromuscular responses of the rat diaphragm preparation.

Table 1. Effects of atropine (1.4 μM) and glycopyrrolate (6.25 μM) on indirectly-elicited twitch,tetanic,and post-tetanic twitch responses and potentiation in the rat diaphragm preparation (means±s.e., n=6).

	T E N S I O N (g)			(PTT-T)/T X 100
	Twitch(T)	Tetanus(TT)	PTT	PTP (%)
Control (C)	0.9±0.1	3.9±0.7	1.2±0.1	33±2.3
Atropine (A)	1.1±0.2	4.5±0.3	1.6±0.1	+45±2.4
% Change (A of C)	22 ±2.1	15 ±1.0	33±3.1	+36±2.9
P (A & C)	0.02	0.05	0.01	0.01
Glycopyrrolate (G)	0.8±0.1	2.4±0.3	1.0±0.1	-25±1.6
% Change (G of C)	11 ±2.0	38 ±3.5	17 ±1.2	-24±3.1
P (G & C)	N.S.	0.01	0.05	0.02

Abbs,E.T. & Joseph,D.N. (1981). Br.J.Pharmac. 73, 481-483
Cozanitis,D.A.,Dundee,J.W.,Merrett,J.D.,Jones,C.J.& Mirakhur,R.K.(1980). Br.J.Anaesth. 52, 85-89
Mirakhur,R.K.,Dundee,J.W.& Clarke,R.S.J.(1977). Br.J.Anaesth. 49, 825-829
Ostheimer,G.W.(1977). Anaesth.Analg. 56, 182-186

SEVERE IDIOPATHIC CONSTIPATION IS ASSOCIATED WITH REDUCED ACTIVITY OF COLONIC CHOLINERGIC NERVES

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There is evidence to suggest an abnormality of the colonic myenteric plexus in severe chronic constipation (Preston & Lennard-Jones, 1985; Krishnamurthy et al., 1985). The present study investigates whether this abnormality involves functional changes in the cholinergic innervation of human colon. Human taenia coli muscle strips (taenia), previously incubated with [^{3}H] choline to radiolabel neuronal stores of acetylcholine, were subjected to electrical field stimulation (1 ms, 1 Hz or 10 Hz, 480 pulses at 200 mA). The stimulation evoked release of tritiated material, shown previously to accurately represent neural [^{3}H]-acetylcholine release (Burleigh & Trout, 1986) was significantly depressed in tissue resected for constipation compared to control tissue, i.e. that resected for carcinoma. Evoked release of tritiated material was significantly reduced by overnight storage of taenia at 4°C or by increasing the frequency of stimulation. However release was significantly increased by a 30 minute period of stimulation during incubation with [^{3}H]-choline.

TABLE 1. Stimulation evoked release of tritiated material, expressed as % radioactivity remaining released (%RRR), from human taenia coli muscle strips. Data expressed as medians with inter-quartile ranges, n = number of muscle strips, with number of specimens in parenthesis. * P < 0.05, **P < 0.01 compared to control.

CONTROL (1 Hz stimulation frequency)	CONSTIPATED	OVERNIGHT STORAGE (4°C)	STIMULATION DURING INCUBATION (10 Hz)	STIMULATION FREQUENCY OF 10 Hz
0.95	0.74*	0.45**	1.39**	0.71**
(0.85-1.15)	(0.64-0.88)	(0.34-0.69)	(0.84-1.85)	(0.61-0.89)
n = 43 (9)	n = 30 (6)	n = 30 (6)	n = 30 (6)	n = 20 (4)

Regional differences in transmitter release were not observed in colon resected for constipation. Thus in 4 specimens taenia cut from the ascending colon gave a %RRR value of 0.82 (0.61-1.03, 8 muscle strips), while taenia cut from the sigmoid region gave a %RRR value of 0.78 (0.70-0.98, 12 muscle strips, P > 0.5).

The results indicate reduced activity of cholinergic nerves may occur within the bowel wall of colon removed for severe chronic constipation. Further experiments are required to indicate whether cholinergic nerves projecting to the muscle layers were affected, whether the abnormality was restricted to neurones within the myenteric plexus, or whether activity of the extrinsic nerve supply is reduced.

BURLEIGH, D.E. & TROUT, S.J. (1986). Br. J. Pharmac. 88: 307-313.
KRISHNAMURTHY, A., SCHUFFLER, M.D., ROHRMAN, C.A. & POPE, C.E. (1985). Gastroenterology 88: 26-34.
PRESTON, D.M. & LENNARD-JONES, J.E. (1985). Dig. Dis. Sci. 30: 289-294.

EVIDENCE FOR DISSIMILARITY BETWEEN 5-HT_1 LIKE RECEPTORS IN THE GUINEA-PIG ILEUM AND RAT FUNDIC STRIP

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5-HT has been shown to cause relaxation of the guinea-pig ileum by depolarization of enteric inhibitory neurones via 5-HT_3 receptors (Gunning & Humphrey, 1987 and by a direct effect on smooth muscle 5-HT_1 receptors (Feniuk et al 1983). The receptor sub-type mediating contraction of the rat fundic strip has also been tentatively designated as 5-HT_1 like (Bradley et al 1986). However, clear differences in the affinity of methysergide for these receptors has been demonstrated (pA_2 values of 7.37 and 8.5 in guinea-pig ileum and rat fundic strip, see Trevethick et al 1984) suggesting that the current receptor classification is in need of clarification. We have evaluated the effects of the novel 5-HT antagonist, ICI 169369 (Blackburn et al 1987a), methysergide and ketanserin against 5-HT and the selective 5-HT_1 agonist 8-OHDPAT in the guinea-pig ileum and rat fundic strip.

Rat fundic strip was set up as previously described (Blackburn et al 1987b) and guinea-pig ileum as described by Feniuk et al (1983). The effects of the antagonists were evaluated against contractions to 5-HT and 8-OHDPAT in the rat fundic strip and against relaxations to both agonists in histamine (1μM) pre-contracted guinea-pig ileum. In the rat fundic strip 5-HT was 1000x as potent as 8-OHDPAT (EC_{50} 0.03±0.02 and 30.0±18.2μM respectively) whereas in the guinea-pig ileum this difference in potency was approximately 7 fold (EC_{50} for 5-HT and 8-OHDPAT 2.4±0.13 and 16.1±1.6μM respectively). Moreover, in the rat fundic strip similar maximal responses were obtained to both agonists, whereas in the guinea-pig ileum 5-HT produced relaxations 46±5% of the maximal obtainable effect (i.e. a relaxation equivalent to that necessary to reduce a histamine-induced contraction to resting basal tone) compared with 97±2% obtained with 8-OHDPAT.

ICI 169369 (1μM) produced no significant antagonism of either 5-HT or 8-OHDPAT in the guinea-pig ileum whereas in the rat fundic strip marked non-competitive antagonism of both agonists was observed at 0.1μM and above. Methysergide (0.1μM) produced only weak antagonism of 5-HT (dose ratio = 4.98±0.8) and no significant antagonism of 8-OHDPAT in guinea-pig ileum, whereas at 0.01μM in the rat fundic strip marked non-competitive antagonism of both agonists was observed. Ketanserin (0.1μM) produced non-specific effects in the guinea-pig ileum, responses to histamine being significantly attenuated. In the rat fundic strip no significant antagonism of either 5-HT or 8-OHDPAT was observed.

These results suggest that the receptor sub-types mediating relaxation in the guinea-pig ileum and contraction in the rat fundic strip are not identical and that further evaluation is necessary before a definite classification can be made.

Blackburn T P et al (1987a) Br J Pharmac 90, 256P
Blackburn T P et al (1987b) Ibid 90, 277P
Bradley P B et al (1986) Neuropharmacology 25, 563-576
Feniuk W et al (1983) Eur J Pharmac 96, 71-78
Gunning S J and Humphrey P P A (1987) Br J Pharmac in press
Trevethick M A et al (1984) Life Sci 35, 1521-1528

BUSPIRONE CONTRACTS THE ISOLATED RAT ILEUM BY AN INTERACTION WITH 5-HT_2 RECEPTORS

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Buspirone and isapirone (TVXQ 7821) have been shown to be potent and selective inhibitors of 5-HT_{1A} binding sites in bovine brain membranes being inactive at 5-HT_{1B} sites while displaying moderate affinity for 5-HT_2 sites (Peroutka, 1985). The same study revealed that the affinity of buspirone for 5-HT_2 binding sites was five fold greater than that of isapirone. In this report we demonstrate that buspirone, but not isapirone, has a significant spasmogenic effect on the isolated rat ileum preparation and, by use of the 5-HT_2 antagonist ketanserin (Van Nueten et al, 1983) as well as the 5-HT_3 antagonist ICS 205-930 (Richardson et al, 1985), provide evidence that this effect is mediated by 5-HT_2 receptors.

Rat ileum sections were incubated at 35°C in Tyrode's solution and dose response curves constructed for 5-HT, buspirone and isapirone in the presence and absence of ICS 205-930 (0.1 - 100nM) and ketanserin (10 - 100nM). In each case, the tissues were allowed to equilibrate with the antagonist for 30 min prior to retesting the effects of 5-HT and buspirone and ED_{50} values (mean $\pm$ S.E.; n = 5 - 9) determined.

ICS 205-930 (1nM) significantly reduced the response to 5-HT with the ED_{50} value being increased from $1.3 \pm 0.2 \times 10^{-6}$M to $5.2 \pm 0.3 \times 10^{-6}$M ($P<0.01$) and the maximum response being reduced by 37%. In the presence of 10nM ICS 205-930, the maximum response to 5-HT was reduced by 72%. The effect of ketanserin (10nM) on the response to 5-HT was similar to that of 1nM ICS 205-930 with the ED_{50} value being increased from $2 \pm 0.3 \times 10^{-6}$M to $7 \pm 0.6 \times 10^{-6}$M ($P<0.01$) and the maximum response being reduced by 38%. Higher concentrations of ketanserin did not reduce further the response to 5-HT.

Isapirone (0.1 - 10μM) caused weak dose independent contractions of the isolated ileum. By comparison, buspirone (0.1 - 1μM) induced significant contraction of the tissue with a maximum response equivalent to 54% of the 5-HT maximum. The spasmogenic effect of buspirone was unaffected by ICS 205-930 (1 - 100nM) but was reduced significantly by ketanserin (10nM) with the ED_{50} value being increased from $6 \pm 0.4 \times 10^{-7}$M to $2 \pm 0.3 \times 10^{-6}$M ($P<0.01$) and the maximum response being reduced by 48%. In the presence of 100nM ketanserin, the maximum response of buspirone was reduced by 79%.

The data reported above suggests that, while 5-HT induced contraction can be mediated by 5-HT_2 and 5-HT_3 receptors, the spasmogenic effect of buspirone is brought about by an interaction with 5-HT_2 receptors because 5-HT_1 ligands, such as 8-OH-DPAT and RU 24969, have no spasmogenic effects on the rat ileum (Freeman et al, 1986). Furthermore, the lack of contractile effect of isapirone confirms the earlier (Peroutka, 1985) observation about the relative potency of buspirone and isapirone at 5-HT_2 receptors.

Freeman, E., Osborne, R.H. & Williams, C. (1986) Br. J. Pharmacol 88, 368P
Peroutka, S.J. (1985) Biol Psychiatry 20, 971
Richardson, B.P., Engel, G., Donatsch, P. & Stadler, P.A. (1985) Nature 316, 126
Van Nueten, J.M., Leyson, J.E., Schuurkes, J.A.J. & Vanhoutte, P.M. (1983) Lancet Feb: 297

COMPARISON OF THE ACTIVITIES OF MUSCARINIC AGONISTS ON RABBIT THORACIC AORTA PREPARATIONS

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Muscarinic agonists can have two effects on rabbit isolated thoracic aorta preparations: stimulation of receptors in the presence of the endothelium produces a relaxation whereas stimulation of receptors on the smooth muscle produces a contraction (Furchgott & Zawadzki, 1986). Activities of agonists in the absence and presence of antagonists could therefore be complicated by activity at receptors associated with two functionally opposing effects. Aorta preparations without ('rubbed') and with ('unrubbed') the endothelium attached were therefore prepared as described by Furchgott and Zawadzki (1980) and set up in 5ml tissue baths. A Krebs-Henseleit type bathing medium, containing $6x10^{-5}$M EDTA and $3x10^{-6}$M indomethacin, bubbled with 95% oxygen and 5% carbon dioxide, and maintained at 37°C, was used. Cumulative concentration-response curves were obtained to muscarine until these were reproducible. An alternative agonist was then examined and its pD_2 and maximum effect relative to that of muscarine estimated.

As shown in Table 1, all the agonists were appreciably less potent on the rubbed than on the unrubbed preparations. On the rubbed preparations, all produced maximum effects lower than that of muscarine. This is not thought to be due to desensitisation because muscarine's concentration effect curve was found to be reproducible. It is noted though that the pD_2 of 5-methylfurmethide on the rubbed preparation may be underestimated because the concentration-effect curve was biphasic and so the maximum effect corresponding to the muscarinic receptor of interest was difficult to define. Compounds such as McN-A-343 and pilocarpine behaved as antagonists on both preparations, with pK_B values of 4.78±0.06(8), 4.82±0.01(6) and 6.04±0.08(8), 5.56±0.14(5) respectively on the unrubbed and rubbed preparations (pK_B defined as log (dose ratio - 1)/[antagonist concentration]).

Table 1 pD_2 values for muscarinic agonists on 'unrubbed' and 'rubbed' rabbit thoracic aorta preparations. Mean values are shown ± sem, (n) being the number of tissues * = maximum effect less than that of muscarine

	'UNRUBBED"	'RUBBED'
Muscarine	6.34±0.58(18)	4.93±0.05(29)
Carbachol	6.30±0.06 (4)	4.45±0.06 (2)*
Arecoline	5.80±0.03 (6)	4.40±0.01 (4)*
Oxotremorine	6.47±0.06 (6)	5.16±0.19 (4)*
5-methylfurmethide	6.45±0.08 (6)	4.50±0.17 (6)*

In conclusion, the pD_2 values reflecting the activity of muscarinic agonists on the rubbed preparation are significantly lower than those reflecting the activity on the unrubbed preparation. This and the similar antagonist activity of pilocarpine and McN-A-343 on the two preparations are consistent with the receptors involved being similar but being associated with different receptor reserves. Under these circumstances the activity of muscarinic agonists in the unrubbed preparation is unlikely to be complicated by activity at an opposing receptor system.

Furchgott, R.F. & Zawadzki, J.V. (1980) Nature, 288, 373-376.

THE EFFECTS OF IDAZOXAN ON CHEMICALLY-INDUCED ILEUS IN THE RAT

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Nociceptive stimulation of the peritoneum has been shown to reduce gastrointestinal motility in the rat by a mechanism which is believed to involve noradrenergic nervous reflexes (Sjoqvist et al., 1985; Holzer et al., 1986). This phenomenon resembles postoperative or adynamic ileus which has been observed in man and experimental animals following surgery. Previous reports investigated the effects of phentolamine and yohimbine in chemically-induced ileus; the present study was designed to determine the effectiveness of idazoxan, a potent and selective α_2-adrenoceptor antagonist (Doxey et al., 1983) in antagonising the development of ileus.

Chemical ileus was induced in groups (n=8-10) of male rats (Sprague-Dawley, 150-180 g) by i.p. administration (10 ml/kg) of acetic acid 0.6% w/v. In order to determine the degree of ileus the gastrointestinal transit of a charcoal meal was measured by the method of Boura and Fitzgerald (1966) 20 min after the administration of acetic acid. The rats were given 1 ml of a charcoal meal and 15 min later they were sacrificed and the distance of the meal front from the pylorus was determined. Drugs or saline vehicle were given s.c. 15 min prior to the administration of acetic acid. Control rats received injections of saline by s.c. and i.p. routes.

Administration of acetic acid (60 mg/kg i.p.) reduced significantly ($p<0.01$; Kruskal Wallis test) the transit of a charcoal meal to 18% of that in control animals. The administration of the α_1-adrenoceptor antagonist prazosin (1 mg/kg s.c.) and the ß-adrenoceptor antagonist propranolol (1 mg/kg s.c.) prior to acetic acid did not affect the degree of ileus observed. Rats which were pretreated with idazoxan (3 mg/kg s.c.) prior to acetic acid exhibited meal transit which was significantly greater than in rats receiving saline and acetic acid; however, the idazoxan-treated rats still possessed reduced transit which was approximately 70% of that of normal, untreated animals. Normal rats treated with idazoxan (3 mg/kg s.c.) showed an increase in transit of the charcoal meal by approximately 35% ($p<0.01$). The selective α_2-adrenoceptor agonist clonidine (0.3 mg/kg, i.p.) inhibited transit of the charcoal meal and this was fully antagonised by idazoxan (3 mg/kg, s.c.).

Thus, we have demonstrated that i.p. administration of acetic acid induces a state of ileus which may be antagonised, although not completely, by the selective α_2-adrenoceptor antagonist idazoxan. This supports the view that chemically-induced ileus is largely a result of an activation of α_2-adrenoceptors of the gastrointestinal tract. The activity of idazoxan in normal rats would suggest the presence of some degree of basal noradrenergic tone influencing gastrointestinal motility.

Boura, A.L.A. and Fitzgerald, A.F. (1966). Br. J. Pharmac., 26, 307.
Doxey, J.C. et al. (1983). Br. J. Pharmac., 78, 489.
Holzer, P. et al. (1986). Gastroenterol., 91, 360.
Sjoqvist, A. et al. (1985). Dig. Dis. Sci., 30, 749.

METABOLISM AND POTENCY OF TETRAHYDROCANNABINOL HOMOLOGUES

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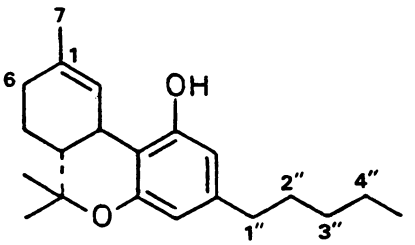
The psychoactivity of drugs obtained from Cannabis sativa L. is associated, with cannabinoids and, particularly, with delta-1-tetrahydrocannabinol (delta-1-THC, I).



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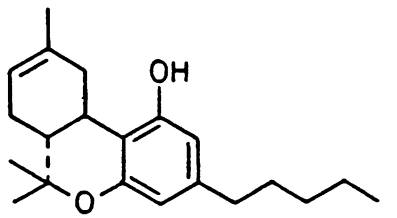

II

Major cannabinoids contain a pentyl side-chain but lower homologues also occur naturally, propyl and methyl being the most common (Turner et al., 1980). Little work has been reported on their metabolism or pharmacological activity.

Homologues (methyl to hexyl) of delta-1- (I) and the related, psychoactive, delta-6-THC (II) were synthesised by condensation of 1,3-dihydroxy-5-alkyl benzenes with 1S-(-)-verbenol. The heptyl homologue of delta-1-THC was a gift of Dr E.W. Gill. Metabolism was studied in vivo in male Charles River CD-1 mice (23-25g) after IP administration. The livers were removed after 1 hr and the metabolites were extracted with ethyl acetate. These were isolated by chromatography on Sephadex LH-20 and converted into TMS, [$^2H_9$]-TMS, methyl ester TMS and methyl oxime-TMS derivatives for examination by GC/MS (Harvey et al., 1977).

Major metabolic routes for all compounds involved hydroxylation at C-7 followed by oxidation to the corresponding carboxylic acid. Other hydroxylations were observed at C-6 and, in the higher homologues, at positions 2", 3" and 4" of the side-chain. Polysubstituted metabolites were common. A decreasing concentration of the 7-oic acid was found with ascending alkyl side-chain length. The ratio of the 7-hydroxy to 7-oic acid metabolite was found to be linearly correlated with both the side-chain length and the octanol-water partition coefficient (estimated by a gas chromatographic method). This is thought to reflect the increasing lipophilicity of the higher homologues which would make them more accessible to the membrane-bound cytochrome P-450 monooxygenase system resulting in increased hydroxylation. Conversely they would be less available to the cytosolic alcohol dehydrogenases responsible for oxidation of the 7-OH group to a carboxylic acid.

Cannabinoid potencies were estimated on the electrically stimulated guinea pig ileum by the method described by Rosell et al. (1976). Although the higher homologues were found to be the most potent, a correlation between activity and chain-length was not found as dose-response curves were not parallel. It would appear that the higher homologues were so lipophilic that they were effectively inactivated by being lipid bound, restricting access to the possible active site.

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Harvey, D.J., Martin, B.R. & Paton, W.D.M. (1977) J. Pharm. Pharmac. 29, 482.
Rosell, S., Bjorkroth, U., Agurell, S., Leander, K., Ohlsson, A., Martin, B. & Mechoulam, R. (1976) in 'Marihuana: Biological Effects': Nahas, G.G & Paton, W.D.M. (eds.), pp 63-70, Oxford, Pergamon.
Turner, C.E., ElSohly, M.A. & Boeren, E.G. (1980) J. Nat. Prod. 43, 169.

LABORATORY ANAESTHETICS AND PLASMA RENIN ACTIVITY IN THE RAT

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We have previously reported that urethane anaesthesia, in comparison to pentobarbitone and 'Hypnorm/Hypnovel' anaesthesia, results in an alteration in the pharmacokinetics of p-aminohippuric acid (PAH) (Gumbleton et al, 1987), an alteration which is likely mediated via renal haemodynamic changes. We have also observed that urethane anaesthesia results in intra-abdominal toxicity following intraperitoneal (ip) injection. This present study in male Wistar rats (245±25g) has investigated the extent to which some laboratory anaesthetics can alter plasma renin activity (PRA), with assessments in mean arterial pressure (MAP). The anaesthetic regimens studied were :- fentanyl + fluanisone (0.26+8.3 mg/kg ip; 'Hypnorm') given in combination with midazolam (4.16 mg/kg ip; 'Hypnovel') (H); urethane (1.75 g/kg ip) (U) and pentobarbitone (67 mg/kg ip) (P). Plasma renin activity was assessed by radioimmunoassay at experimental times 30, 60 and 90 min, with terminal blood collections obtained by decapitation. PRA assessment was performed in surgically-intact and surgically-prepared rats. MAP was monitored via a carotid catheter in surgically-prepared rats.

Table one. Influence of anaesthetics on plasma renin activity (ng Angiotensin 1/ml/hr in surgically-intact rats.

	Experimental time (min)		
	30 *	60 *	90 *
urethane ip	15.43 ±1.12	14.42 ±2.38	15.42 ±0.72
pentobarbitone ip	3.78 ±0.49	3.39 ±0.56	3.09 ±0.76
'Hypnorm/Hypnovel' ip	8.31* ±3.27	8.49* ±2.11	8.17* ±0.92
saline ip	2.52 ±0.48	2.04 ±0.69	2.09 ±0.73

*Significantly different ($P<0.01$) to all other treatments. Results mean s.d (n=5) Two-way analysis of variance and Duncan's test.

The MAP in Uip animals was significantly lower ($P<0.05$) (79±9 mmHg) compared to the MAP in Pip (120±8) and Hip (104±5) animals.
The effect of surgery (carotid and jugular cannulation) and blood sampling (12 x 100 µl over 90 min, with saline replacement) on PRA resulted in a 320% elevation in Pip animals (3.39 to 10.85), a 61% elevation in Hip animals (8.49 to 13.73) and a 62% elevation in Uip animals (14.42 to 23.23). The PRA in the Uip animals being significantly ($P<0.01$) elevated compared to both Pip and Hip animals. The results demonstrate that different anaesthetics will influence PRA to varying extents and that the elevated PRA observed in Uip animals may be responsible for the reported alterations in the pharmacokinetic handling of some renally eliminated compounds when disposition studies are conducted in Uip anaesthetised animals (Woolfrey et al, 1985; Pipkin and Stella 1982).

Gumbleton et al, (1987) Int J Pharmaceutics 38, 261-263.
Pipkin and Stella (1982) J Pharm Sci 71, 2, 169-172.
Woolfrey et al (1985) Int J Pharmaceutics 26, 35-43.

INHIBITION OF HUMAN PLACENTAL AROMATASE BY SUBSTITUTED AZOLES

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Many imidazole antifungal agents inhibit mammalian Cytochrome P450 enzymes including the aromatase which converts androgens into oestrogens (Mason et al. 1985). Back & Tjia (1985) reported that the ability of substituted imidazoles to inhibit tolbutamide metabolism in the rat depended on the position of the substitution; compounds bearing substitutions at positions 1, 4 or 5 were potent inhibitors, while those bearing substitutions on position 2 were ineffective. In order to determine whether the same structure-activity relationship applies to aromatase, a series of azole compounds were tested for their ability to inhibit the aromatisation of testosterone to oestradiol by human placental microsomes.

Aromatase activity was assayed (Graves and Salhanick, 1979) by measuring the release of tritiated water from [1,2-^{3}H]-testosterone (1.5μM) in the presence or absence of azoles (20μM). The compounds tested were (positions of substitutions in brackets): 1-methylimidazole (1), 2-methylimidazole (2), 1,2-dimethylimidazole (1,2), miconazole (1), clotrimazole (1), econazole (1), metronidazole (1,2,5), ketoconazole (1), itraconazole (1), cimetidine (4,5) and midazolam maleate (1,2,5).

Of the compounds bearing a substituent at position 1, econazole, clotrimazole and miconazole were the most effective inhibitors (>80% inhibition compared to controls), followed by ketoconazole (ca. 35%). In separate experiments, the concentrations of these compounds producing 50% inhibition were found to be approximately 0.06μM for econazole and clotrimazole, 0.16μM for miconazole, and 36μM for ketoconazole. Metronidazole and 1-methylimidazole were ineffective as inhibitors. Apart from midazolam, which had a weak (20%) inhibitory effect, the 2-substituted compounds were inactive. Cimetidine and itraconazole (a triazole compound) both showed less than 10% inhibition.

These preliminary results suggest that the molecular features required for aromatase inhibition may be different from those required for inhibition of tolbutamide metabolism. Substitution at position 1 of the imdazole ring is not sufficient per se to cause inhibition since the smallest compound, 1-methylimidazole, was ineffective. This compound does inhibit tolbutamide metabolism (Back & Tjia, 1985). The most effective inhibitors (clotrimazole, econazole, miconazole and ketoconazole) all have relatively large aromatic side-chains; itraconazole's lack of effect, however, may be due to steric hindrance resulting from its bulky side-chain.

In conclusion, the ability of azole compounds to inhibit aromatisation depends to a large extent on the position and nature of their substituent groups. Further study of these structure-activity relationships could provide valuable insights into the nature of the enzyme's active site.

Back, D.J., Tjia, J.F. (1985) Br. J. Pharmac. 85: 121-126
Graves, P.E., Salhanick, H.A. (1979) Endocrinology 105: 52-57
Mason, J.I. et al. (1985) Biochem. Pharmac. 34: 1087-1092

FURTHER EVIDENCE FOR THE INDUCING ACTIVITY OF AMINOGLUTETHIMIDE IN THE RAT AND MOUSE

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Previously it has been shown that aminoglutethimide (AG) induces hepatic mixed function oxidation in rats and mice (Damanhouri and Nicholls, 1986). The present work further investigates this activity in relation to different substrates for oxidation and other enzymes in female rats (120g) and mice (20g) following an oral dosing schedule of AG 60mg/kg daily for 3 days.

In rats (n=6), AG significantly ($P<0.05$) lowered plasma levels of phenytoin at 1 and 4h after dosing (15mg/kg i.p.) from 2.50±0.10 and 0.46±0.05µg/ml (control) to 1.13±0.18 and 0.30±0.01µg/ml (AG) respectively. There was a significant ($P<0.05$) reduction in the blood glucose lowering effect of tolbutamide 4h after dosing with this hypoglycaemic agent (50mg/kg i.p.) in AG treated rats (86.1±3.5mg glucose/100ml blood, n=6) compared to controls (62.5±4.2mg glucose/100ml, n=6). The ultra short-acting anaesthetic propandid is metabolised by hydrolysis in the liver (Nousiainen, 1985). In mice (n=10) the propandid-induced (750mg/kg i.p.) sleeping time was significantly ($P<0.05$) shortened by AG pretreatment (3.2±1.1 min) compared with controls (6.3±0.9 min). The activity of UDP-glucuronyl- and glutathione-S- transferases in the microsomal fraction of rat liver was determined by methods described by Gibson and Skett (1986) using 2-aminophenol and 1-chloro-2,4-dinitrobenzene respectively as substrates. For glucuronyl transferase, AG pretreatment significantly ($P<0.05$) increased the activity (nmole 2-aminophenol glucuronide formed/min/g.liver) from 3.3±0.3 (control) to 5.2±0.1 (AG). In the case of glutathione transferase, AG pretreatment significantly ($P<0.005$) increased the activity (µmole S-2,4-dinitrophenyl glutathione formed/min/mg microsomal protein) from 0.40 ±0.03 (control) to 0.55±0.02 (AG). Together with the data previously obtained (Damanhouri and Nicholls, 1986), these results indicate that AG has the characteristics of an inducing agent of the phenobarbitone type. This work re-emphasises that, in the treatment of hormone-dependent breast cancer with AG, the possibility of drug interactions based on hepatic enzyme induction must always be considered.

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Damanhouri, Z. and Nicholls, P.J. (1986) Br. J. Pharmac. 88, 430P.
Gibson, G.G. and Skett, P. (1986) Introduction to Drug Metabolism. pp.259-263. London: Chapman and Hall.
Nousiainen, U. (1985) Gen. Pharmac. 16, 49-53.

ALTERED PHARMACOKINETICS OF AMINOGLUTETHIMIDE IN MICE RECEIVING THE DRUG CHRONICALLY

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When the aromatase inhibitor aminoglutethimide (AG) is employed in patients with advanced breast cancer, various CNS-depressant side-effects are commonly observed. However, these effects are self-limiting and subside within 2-6 weeks of commencing therapy (Santen et al. 1981). In the mouse, chronic dosing with AG induces a marked degree of tolerance to the ataxic and anticonvulsant actions of the drug (Abusrewill et al. 1986). To provide additional information about this phenomenon, the pharmacokinetic profile of the drug has been examined in such animals. Either AG (50mg/kg suspended in 0.75% w/v carboxymethylcellulose) or vehicle (0.1ml/10g body weight) was administered orally to male albino mice (20-22g) daily for 14 days. On day 15, all mice received AG (50mg/kg) orally and blood samples were collected by cardiac puncture under ether anaesthesia at either 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 9 or 12h. At each time, 16 vehicle-pretreated and 16 AG-pretreated mice were employed. In each group AG was determined in pooled plasma samples from 4 animals. After extraction into dichloromethane, AG was assayed by hplc using a reverse phase system with a 10μ Bondapak C_{18} column. A further group of mice received either vehicle or AG (100mg/kg, orally) daily for 14 days. On day 15, they were given intravenous AG phosphate in a dose equimolar to AG (50mg/kg). Blood was collected as described above at 5, 10, 20 and 40 min and 1, 2, 3, 4, 6 and 8h after dosing, for the assay of AG. The plasma level data were analysed by computer programme (Johnston et al. 1983) assuming a one-compartment model (preliminary examination showed that the data could be best fitted to this type of model). The results are presented in the Table.

Table. Pharmacokinetic parameters of AG in mice receiving AG chronically

Pretreatment	Vehicle[1]	AG[1] (50mg/kg)	AG[2] (100mg/kg)
$t_{0.5}$ (h)	3.7 ± 0.2	2.2 ± 0.1*	2.1 ± 0.1*
V_d (ml)	50.3 ± 0.9	56.7 ± 3.8	58.0 ± 4.1
Total Clearance (ml/min)	0.16± 0.01	0.31± 0.01*	0.34± 0.01*

Values are means ± sem, n=4 (pooled samples)
[1]=AG challenge 50mg/kg p.o. [2]=AG phosphate challenge i.v. (equivalent to AG 50mg/kg). *P<0.01.

It may be observed that pretreatment with both doses of AG did not alter the apparent volume of distribution (V_d). However, in comparison with vehicle-pretreated controls, AG chronic pretreatment caused a significant (P<0.01) reduction in its plasma $t_{0.5}$ and increase in total clearance. The magnitude of effects of 50 and 100mg/kg pretreatment were similar. In vehicle-pretreated mice, the plasma AUC_{∞}s for oral and i.v. dosing with AG (154±7 and 161±11μg.h/ml, respectively) were not significantly different indicating virtual complete bioavailability of the oral dose. In addition, the AUCs in the AG-pretreated mice, though significantly lower than the corresponding values for the vehicle-treated animals, were not significantly different for the oral and i.v. routes (82±5 and 78±7μg.h/ml, respectively). This latter finding suggests that a first pass metabolism is unlikely to have been induced by AG-pretreatment. The results indicate that a dispositional component is most likely implicated in the tolerance to the CNS-depressant effects of AG in the mouse.

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Abusrewill, M. et al. (1986) Br. J. Pharmac. 89, 832P.
Johnston, A. et al. (1983) J. Pharmac. Methods 9, 193.
Santen, R.J. et al. (1981) New Engl. J. Med. 305, 545.

TRIIODOTHYRONINE-INDUCED CHANGES IN UDP-GLUCURONYOSYL TRANSFERASE ACTIVITY TOWARDS PHENOLPHTHALEIN IN THE RAT

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Oxazepam clearance is increased in hyperthyroid patients (Scott et al, 1984) which suggests an increase in the activity of UDP-glucuronosyl transferase (UDP-GT). However, male rats treated with, 3,3',5-triiodothyronine (T_3-20μg) showed no increase in p-nitrophenol glucuronidation (Marselos et al, 1975). The aim of this study was to investigate the effect of different doses of T_3 on UDP-GT activity in male and female Sprague-Dawley rats using phenophthalein as substrate.

Male (6) and female (4) rats (200-250g) were injected with T_3, 5μg-200μg intraperitoneally daily for seven days. Male rats were obtained from Charles River UK Ltd whereas female rats were in-house bred from Charles River stock. Blood samples were taken from the caudal vein before treatment and on day six for the measurement of thyroxine (T_4) and free T_3 concentrations. Liver microsomes were prepared and UDP-GT activity measured using phenolphthalein as substrate, by the method of Morrison et al (1984) with the modification of 0.025% $^{v}/_{v}$ Triton X-100 for activation and 2mM UDPGA. Phenolphthalein glucuronide was quantified using hplc. Statistical analysis was by one way analysis of variance with significance assigned using Dunnett's test.

Activated and 'native' UDPGT activity (nmoles glucuronide formed/mg protein/minute) in male and female rats following pretreatment with T_3 are shown below (mean ± s.e. mean)

T_3 dose (μg)	0	5	20	40	200
Male					
Activated	1.35 ± 0.11	2.06 ± 0.12*	2.63 ± 0.29*	1.10 ± 0.20	1.28 ± 0.09
'Native'	1.02 ± 0.06	1.24 ± 0.06	1.56 ± 0.31	0.80 ± 0.03	1.13 ± 0.13
Female					
Activated	2.67 ± 0.20	2.46 ± 0.35	3.60 ± 0.40	2.91 ± 0.13	2.96 ± 0.15
'Native'	1.21 ± 0.08	1.02 ± 0.04	2.29 ± 0.25*	1.17 ± 0.05	1.03 ± 0.03

* $p < 0.05$ when compared with control values

UDPGT activity reached a maximal level in both male and female rats at a dose of 20μg T_3 ($p < 0.05$) At higher doses of T_3, the activity was not significantly different from control values.

Serum T_3 concentrations showed a non-linear increase over the dose range studied, whereas serum T_4 levels were suppressed below the limit of detection (30 nmol/l) in all treated animals. Administration of T_3 results in a dose-dependent, but sex-independent effect on UDPGT activity. Studied are in progress to determine the substrate specificity of the increase in UDPGT seen after T_3 administration.

Scott A K et al (1984) Br J clin Pharmacol, 17, 49-53
Marselos M et al (1976) Acta pharmacol et toxicol, 38, 273-280
Morrison MH and Hawksworth GM (1984) Biochem Pharmacol, 33, 3833-3838

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CARDIOVASCULAR AND GENERAL TOXIC EFFECTS OF DOXORUBICIN (ADRIAMYCIN) AND 4-DEMETHOXY-DAUNORUBICIN (IDARUBICIN) IN RATS

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Doxorubicin (Dox) and Daunorubicin are well known anthracyclines highly effective in the clinical oncology. Their therapeutic application is limited by their acute and late toxic effects mainly myelodepression and cardiotoxicity (Blum & Carter, 1974) and by their i.v. route of administration.

4-demethoxy-daunorubicin (Idarubicin^R, Ida) is a new anthracycline-derivative with a cytostatic potency about 5 times that of Dox in animal models. Further it is therapeutically active when given orally to patients (Casazza *et al*, 1980, Martoni *et al*, 1985).

The purpose of the present study was to compare the general toxic and cardiovascular effects of Ida with that of Dox. Groups of 7 rats were given a single i.v. injection of Dox 5 mg/Kg, Ida 1 mg/Kg and Saline 4 ml/Kg and the influence on behaviour, growth-rate, hematological and renal parameters, arterial BT, heart rate and ECG was followed for a period of 40 days. Prolongation of the QRS- and QαT-intervals of the ECG was taken as an early sign of cardiotoxicity (Villani *et al*, 1985).

Dox had a significant toxic effect evidenced by a marked decrease in body weight gain, depression of bone marrow function, severe proteinurea and changes in renal morphology.

In the Ida group one rat died at day 7 for unknown reasons. Otherwise the only adverse effect caused by Ida was the same qualitative change in renal function and -histology as induced by Dox but less marked.

With regard to hemodynamic parameters the systolic blood pressure was not changed at any time by either drugs whereas the heart rate was significant increased in the Dox treated rats both compared with the Ida and the Saline treated group ($p < 0.05$). The QRS-interval of the ECG was significantly prolonged in the Dox group in a reversible way the greatest increase being 12% vs the control group at day 17. Dox also caused a prolongation of the QαT-interval which increased progressively from day 17, the increase being 35% vs the control group and 20% vs the Ida treated group at day 36. The Ida treatment did not influence the QRS-interval and caused only a prolongation of the QαT-interval at day 36 (12% vs control).

From the findings in this study it is concluded that Ida has a significantly less general- and cardiac toxicity than Dox at the tested doses. This observation speaks in favour of Ida on the assumption that the tested doses of the two anthracyclines are equipotent with regard to their cytotoxic effect.

Blum , R.H. & Carter S.K. (1974) Ann. Intern. Med. 80, pp 249-254.
Casazza, A.M., Pratesi, G., Giulini, F. & Di Marco, A. (1980) Tumori 66, pp 549-564.
Martoni, A., Pacciarini, M.A., & Pannuti, F. (1985) Europ. J. Cancer Clin. Oncol. 21. pp 803-806.
Villani, F., Monti, E., Piccinini, F., Favalli, L., Lanza, E., Dionigi, A.R. & Poggi, P. (1986) Tumori 72, pp 323-329.

GUINEA-PIG C4 RESEMBLES HUMAN C4A IN ITS REACTIVITY TOWARDS NITROGEN AND OXYGEN NEUTROPHILES

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Activation of the classical pathway of complement (C1, C4 & C2) is important in handling immune complexes and individuals with deficiencies of C1, C2 or C4 are at increased risk of SLE, characterised by tissue deposition of immune complexes. During activation of the classical pathway, C4 becomes covalently bound to the activating surface (Porter, 1985). Binding of C4 then leads to binding of C3, the major complement component by a similar transacylation reaction. C3 binding promotes solubilisation of immune complexes and leads to their ingestion via cells bearing receptors (CR1, CR3) for bound C3 (Fearon & Wong, 1983) and inhibition of these processes are likely to result in tissue deposition of immune complexes (Schifferli & Peters, 1983). In humans, C4 is encoded in the MHC at two polymorphic loci, C4A and C4B. The two protein products differ in reactivity. Nitrogen nucleophiles, including hydralazine (Sim & Law, 1985), are more potent inhibitors of the covalent binding reaction of C4A than of C4B. In contrast, C4B is more reactive with oxygen nucleophiles. In idiopathic SLE there is a correlation between disease and inheritance of non-functional allele at the C4A locus (Fielder et al., 1983): C4A appears more important than C4B in immune complex handling.

There is no satisfactory animal model of drug-induced SLE and so we have investigated whether C4 from animals shows C4A or C4B reactivity. Animal sera were used on the day of collection and were incubated with particulate inulin to deplete C3 by activation of the alternative pathway of complement, without affecting C4. Then the effect of hydrazine, a nitrogen nucleophile, and acetohydroxamate, an oxygen nucleophile, were compared as inhibitors of C4 binding in a C4-dependent haemolytic assay. Sheep erythrocytes were coated with rabbit antibody and guinea pig C1 (EAC1 cells). The EAC1 cells were then incubated with inulin-activated serum as a source of C4 or pure C4 in the presence or absence of potential inhibitor. The EAC14 cells were washed and incubated with C4-deficient guinea pig serum and haemolysis was measured. The ratios of the concentrations of hydrazine to acetohydroxamate required to give 50% inhibition were calculated and for guinea pig C4 the ratio was found to be 0.06 compared with 0.05 for pure human C4A and 6.2 for human C4B. Guinea pig C4, which is encoded at a single locus, is more like C4A than C4B in its reactivity. The guinea pig is, thus, a potentially useful animal for investigation of hydralazine-induced SLE.

Fearon, D.T. & Wong, W.W. (1983) Ann. Rev. Immunol. 1, 243-271.

Fielder, A.H.L., Walport, M.J., Batchelor, J.R., Rynes, R.I., Black, C.M., Docli, I.A. & Hughes, G.R.V. (1983) Brit. Med. J. 286, 425-428.

Porter, R.R. (1985) Immunol. Rev. 87, 8-17.

Schifferli, J.A. & Peters, D.K. (1983) Lancet 2, 957-959.

Sim, E., Gill, E.W. & Sim, R.B. (1984) Lancet 2, 422-424.

Sim, E. & Law, S.K.A. (1985) FEBS Letts 184, 323-327.

TISSUE DISTRIBUTION AND TOXICITY OF 2-t-BUTYL-4-METHOXYPHENOL (BHA) IN THE RAT

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BHA, a widely used antioxidant food additive, exhibits a very low toxicity in mammals when given orally. In rodents, however, its toxicity is greatly enhanced after i.p. administration resulting in gut paralysis (Della Corte & Sgaragli, 1984). The low oral toxicity of BHA may be partially explained by the finding that rat intestine is capable of transforming in vivo this compound into the less toxic peroxidation product di-BHA (Guarna et al., 1983).

The present investigation was aimed to look further into the mechanisms responsible for the different toxic behaviour shown by the oral and i.p. administration of BHA. Male Sprague Dawley rats, 180-200 g body weight, were used throughout. Plasma and tissue concentrations of BHA and di-BHA, from 1-72 h following i.p. administration of BHA LD_{50} (32 $mg.kg.^{-1}$ body weight), were determined using a GC/MS method (Guarna et al., 1983). The kinetic behaviour of BHA in the intestine was also studied in relation to the functional impairment by measuring the contraction induced by ACh (7.10^{-8} M) in preparations obtained from BHA-treated animals.

High BHA levels were found in the intestine and liver, where values of the area under experimental concentration curve (AUC_{0-24}) were 285 and 49 times higher, respectively, than those observed in plasma (945 $ng.h.ml^{-1}$). AUC_{0-24} values in kidney, spleen, RBC and brain were 2-7 times higher, whereas values below those found in plasma were observed in lung and muscle. The metabolite di-BHA could be detected in the intestine, kidney and spleen, amounting to 5-8% of BHA. In the intestine and liver BHA levels remained high up to 24 h (5.5-20.7 and 1.8-3.3 $\mu g.g^{-1}$ wet weight, respectively). Intestine ACh-induced contraction was unchanged up to 24 h, whereas showed 60% inhibition at 48 h, when BHA levels were 2% of those observed at 24 h (20.7 ± 11.8 $\mu g.g^{-1}$). At 72 h, when BHA levels were almost undetectable, the ACh-induced contractility was completely abolished. These data suggest that the intestinal toxicity observed after i.p. LD_{50} administration of BHA, might not be related to the concentrations of this compound as such. However, the peroxidative transformation of BHA into di-BHA, previously shown after oral administration, also occurs after i.p. administration. The possibility that the formation of intermediate free-radicals occurring during this metabolic transformation (Sgaragli et al., 1980) may play a role in the early toxic events induced by i.p. BHA, is under investigation.

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Della Corte, L. and Sgaragli, G.P. (1984) Pharmac. Res. Comm. 16, 1041-1047.
Guarna, A., Della Corte, L., Giovannini, M.G., De Sarlo, F. and Sgaragli, G.P. (1983) Drug Metab. Dispos. 11, 581-584.
Sgaragli, G.P., Della Corte, L., Puliti, R., Francalanci, R., Guarna, A., Dolara, P. and Komarynsky, M. (1980) Biochem. Pharmac. 29, 763-769.

CHARACTERISTICS OF BINDING OF [^{3}H]-IDAZOXAN TO HUMAN PLATELET MEMBRANES

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Alpha-2-adrenoceptors have been shown to be important in the mediation of platelet aggregation. The receptors display similar characteristics to those located in the central nervous system (Garcia-Sevilla et al, 1981). Idazoxan (RX 781094) is a potent α_2 antagonist in both systems (Kerry & Scrutton, 1982). The present study was designed to investigate the binding characteristics of [^{3}H]-idazoxan to human platelet membranes.

Expired human platelets were obtained as an enriched preparation and platelet membranes were prepared according to the method of Hoffman et al (1980), and stored at -20°C. For kinetic binding studies, thawed membrane preparations (200-300 mg platelet protein) were incubated for various times, in duplicate, at 22°C in tris-HCl buffer (50 mM; pH 7.2) containing EDTA (0.5 mM), in the presence of [^{3}H]-idazoxan (2 nM). Equilibrium studies were carried out in triplicate for each experiment. Platelet membranes were incubated with buffer containing [^{3}H]-idazoxan (0.25-8.0 nM) for 20 min. Non-specific binding was defined as that displaced by unlabelled idazoxan (10 µM).

Binding of [^{3}H]-idazoxan (2 nM) was rapid ($t_{½}$ = 1.2 min) and reversible ($t_{½}$ = 8.5 min; n = 5). Equilibrium was reached at 10-20 min with a k_{ob} of 0.16 min^{-1} (n = 5). The dissociation reaction followed first order kinetics with a k_2 = 0.09 min^{-1} (n = 5). A second order rate constant (k_1) of 0.34 min^{-1} nM^{-1} was calculated. The equilibrium dissociation constant ($K_D = k_2/k_1$ was determined as 2.6 nM from the kinetic studies.

The specific binding of [^{3}H]-idazoxan was saturable and to a single population of binding sites. Scatchard analysis showed a K_D = 1.43 ± 0.53 nM, and a B_{max} = 240 ± 63.9 fmol/mg platelet protein (n = 5). Hill analysis of [^{3}H]-idazoxan binding gave a value for the dissociation constant (K'_D) of 1.12 ± 0.4 nM, with n_H = 0.93, indicating a lack of co-operativity.

These findings suggest that idazoxan can be used as a ligand for labelling the α_2-adrenoceptor binding sites in human platelet membrane preparations.

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Garcia-Sevilla, J.A. et al (1981) Eur. J. Pharmac. 74, 329-341
Hoffman, B.B. et al (1980) Proc. Natl. Acad. Sci. 77, 4569-4573
Kerry, R. & Scrutton, M.C. (1982) Br. J. Pharmac. 81, 91-102

CHARACTERISATION OF [^{125}I]-IODOCYANOPINDOLOL BINDING TO CULTURED HUMAN LYMPHOBLAST CELLS

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Human lymphocytes possess β_2- type adrenoceptors which are positively linked to adenylate cyclase. These receptors have been characterised by radioligand binding techniques using a number of ligands including [^{125}I]-iodocyanopindolol ([^{125}I]-CYP) (Brodde *et al*, 1981). Attempts to utilize the lymphocyte as a model cell with which to monitor human β-adrenoceptor function have been handicapped by the sensitivity of the receptor to modulation by plasma constituents, particularly β-adrenoceptor agonists (Aarons *et al*, 1983; Brodde, 1983). Such problems are not encountered in cell culture, where the extracellular medium is closely controlled. Normal lymphocytes do not replicate *in vitro* therefore are of limited use. Transformation by Epstein-Barr virus, however, converts such cells into replicating lymphoblasts which are readily maintained in culture. This study describes the characterisation of [^{125}I]-CYP binding in such transformed B-cell lymphoblasts.

Stable human lymphoblastoid cell lines were maintained in RPMI 1640 medium supplemented with 10% foetal calf serum. After harvesting, cells were disrupted by homogenisation and sonication in hypotonic medium and nuclear material removed by low speed centrifugation. The resulting membrane preparation was washed twice and finally resuspended in incubation medium (50 mM Tris, 10 mM $MgCl_2$, pH 7.4). Incubation of the tissue with [^{125}I]-CYP (range 5-100 pM) was maintained for 90m min at 37°C and terminated by filtration through Whatman GF-C filters. Specific binding was defined by 1 uM CGP 12177 and constituted 70% total binding at 20 pM ligand concentration.

Specific binding of [^{125}I]-CYP to lymphoblast membranes was saturable with an association half-time of 23 min. Displacement of specific [^{125}I]-CYP binding by β-adrenoceptor antagonists demonstrated an order of potency ICI 118551 > CGP 12177 = l-Propranolol > Atenolol > CGP 20712, typical of a β_2-adrenoceptor profile. Stereoisomers of propranolol and isoprenaline showed 50-100 fold differences in inhibitory potency. Displacement of [^{125}I]-CYP binding by β-adrenoceptor antagonists occurred with Hill coefficients close to unity whereas for agonists this value was significantly less than unity, suggesting coupling of the receptor to nucleotide binding proteins associated with adenylate cyclase. In four subjects blood samples were taken and lymphocytes isolated to assess [^{125}I]-CYP binding directly and as sources of lymphoblastoid cell lines. Binding capacity of [^{125}I]-CYP in the transformed cells was significantly lower ($p < 0.001$, Student's paired t-test) than that in the circulating cells when assessed both in terms of sites per cell (77 ± 11 and 483 ± 55 respectively) and per unit protein (11.3 ± 1.9 fmol/mg protein and 92.8 ± 15.0 fmol/mg protein respectively).

In conclusion, human B-cell lymphoblasts possess β_2-adrenoceptors which appear qualitatively similar to those of the parent lymphocyte cells although the density of receptors is substantially lower in the transformed cells than in the circulating cells.

Aarons *et al* (1983) J. Pharm. Exp. Ther. 224, 1-6.
Brodde (1983) J. Receptor Res. 3, 151-162.
Brodde *et al* (1981) Life Sci. 29, 2189-2198.

INFLUENCE OF EXTRACELLULAR Ca^{2+} ON AGGREGATION AND Ca^{2+} TRANSIENTS IN QUIN 2- AND FURA 2-LOADED HUMAN PLATELETS

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Whilst extracellular Ca^{2+} is essential for platelet aggregation it is not known whether a transmembranal influx of Ca^{2+} is a prerequisite for this event. Development of the fluorescent Ca^{2+} indicators Quin 2 (Tsien et al., 1982) and more recently Fura 2 (Grynkiewicz et al., 1985) has enabled direct and continuous measurement of platelet cytosolic Ca^{2+} ($[Ca^{2+}]i$) to be made. Quin 2 and Fura 2 are analogues of EGTA and effectively report $[Ca^{2+}]i$ by chelation of intraplatelet Ca^{2+}. Therefore, before these indicators can be used to investigate the relationship between agonist-induced rises in $[Ca^{2+}]i$ and functional end-points such as platelet aggregation, it is essential to determine whether function per se is affected when platelets are loaded with concentrations of these indicators necessary to monitor $[Ca^{2+}]i$. To this end we have evaluated aggregation of Quin 2- and Fura 2-loaded human platelets induced by ADP and collagen under two different extracellular Ca^{2+} conditions frequently studied experimentally, namely 1 mM Ca^{2+} and nominally Ca^{2+}-free (Ca^{2+} F) (approximately 50 μM Ca^{2+}).

Platelets in citrated PRP were exposed to the acetoxymethyl esters of either Quin 2 (20 μM), Fura 2 (4 μM) or DMSO vehicle (30 min; 37°C) then gel-filtered (GFP) using a nominally Ca^{2+} F buffer. Aggregation concentration-response curves were constructed at 37°C using GFP (+ fibrinogen 200 μg/ml) to ADP (0.3-30 μM) and collagen (0.04-4 μg/ml) using a platelet counting (UF 100) (Lumley and Humphrey, 1981) technique. In 1 mM Ca^{2+} both agonists produced concentration-related aggregation with equal sensitivity in both Quin 2-, Fura 2- and DMSO-treated platelets. However, compared with DMSO-treated platelets aggregation of Quin 2-loaded platelets was reduced to both agonists in nominally Ca^{2+} F buffer. Thus the mean ADP and collagen EC_{50} values in DMSO-treated platelets were 0.9 μM and 0.34 μg/ml and in Quin 2-loaded platelets were 17.5 μM and 3.1 μg/ml representing an approximate 20-fold and 10-fold reduction in sensitivity to ADP and collagen respectively. In marked contrast, the sensitivity of Fura 2-loaded platelets to ADP and collagen was indistinguishable from the DMSO-treated samples (e.g. mean ADP EC_{50} values of 1.4 and 0.9 μM respectively). Thus in this low Ca^{2+} medium platelet function of Quin 2- but not Fura 2-loaded platelets was markedly affected. Interestingly, the reduction in platelet aggregation seen in Quin 2-loaded platelets was also mirrored by the changes in $[Ca^{2+}]i$. Thus compared with 1 mM Ca^{2+}, the peak rise in $[Ca^{2+}]i$ induced by both agonists under nominally Ca^{2+}F conditions was reduced by up to 90% in Quin 2-loaded platelets. In contrast, peak rises in $[Ca^{2+}]i$ in Fura 2-loaded platelets were not significantly different under the two Ca^{2+} conditions.

In conclusion, loading platelets with Quin 2 or Fura 2 appears to have no obvious effect on aggregation at physiological Ca^{2+} concentrations. However, when extracellular Ca^{2+} is lowered the sensitivity of Quin 2-, but not Fura 2-loaded platelets to aggregatory agonists is reduced. These results suggest that Quin 2, which is present in the platelet cytosol at some 20-30 times higher concentration than Fura 2 (1-1.5 mM compared with 0.05-0.07 mM, Pollock and Rink, 1986) renders the platelet more dependent upon an influx of extracellular Ca^{2+} for aggregation to occur. The question of whether an influx of Ca^{2+} is a pre-requisite for this process to occur will therefore need to be addressed using indicators such as Fura 2, which do not themselves appear to modify Ca^{2+} homeostasis within the platelet.

Grynkiewicz, G, Poenie, M and Tsien, RY (1985). J. Biol. Chem., 260, 3440-3450.
Lumley, P and Humphrey, PPA (1981). J. Pharmacol. Methods, 6, 153-166.
Pollock, WK and Rink, TJ (1986). Biochem. Biophys. Res. Commun., 139, 308-314.
Tsien, RY, Pozzan, T and Rink, TJ (1982). J. Cell Biol., 94, 325-334.

ADENOSINE ANALOGUES AS INHIBITORS OF HUMAN PLATELET AGGREGATION

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Adenosine receptors have been proposed to exist as two subtypes based on the rank orders of potency of N-ethylcarboxamido-adenosine (NECA), R-phenylisopropyl adenosine (R-PIA) and adenosine as agonists in a range of tissues (Londos et al., 1980; Van Calker et al., 1979). Human platelets contain receptors for adenosine and its mimetics which mediate inhibition of platelet aggregation. On the basis of stimulatory effects on intra-platelet levels of cAMP (Haslam and Lynham, 1972) human platelet adenosine receptors were classified as an A_2 subtype. We have attempted to classify the human platelet adenosine receptor on the basis of the functional response to a range of adenosine analogues. The adenosine analogues, NECA, R- and S-phenylisopropyl adenosine (R-PIA, S-PIA), cyclohexyladenosine (CHA) and cyclopentyl-adenosine (CPA) were tested as inhibitors of U46619-induced aggregation (Born, 1962) in human platelet-rich plasma (PRP) and gel-filtered platelets (GFP).

Platelet rich plasma was prepared by centrifugation of citrated human blood and platelets were separated from plasma by gel-filtration on columns of sepharose CL-2B equilibrated with a modified Hepes-buffered Tyrodes solution. Concentration-inhibition curves to NECA, R-PIA, S-PIA, CHA and CPA were constructed following incubation with PRP or GFP (5 minutes, 37°C), prior to aggregation with U46619. Concentration-ratios were calculated by comparison of IC_{50} values (IC_{50} test/IC_{50} NECA).

In PRP, concentration-ratios were NECA ($IC_{50}=2.1x10^{-7}M$ 95% confidence limits $1.4-3.0x10^{-7}M$; n=14) > R-PIA > CPA > CHA > S-PIA: (1:8.5: 22: 26: 145). In GFP, the potency of R-PIA appeared to increase relative to NECA and the concentration ratios were NECA ($IC_{50}=5.38x10^{-8}M$ 95% confidence limits $3.4-8.4x10^{-8}M$; n=19) > R-PIA > CPA > CHA > S-PIA (1: 2.7: 14: 17: 51). In parallel experiments using the same PRP, GFP was prepared and then re-mixed with autologous plasma. Concentration-inhibition curves were found to be similar to those obtained prior to filtration suggesting that filtration alone does not account for the change in R-PIA potency. Finally, concentration-inhibition curves for NECA, R-PIA and S-PIA were again established in GFP: NECA ($IC_{50}=2.94x10^{-8}M$ 95% confidence limits $1.1-8.0x10^{-8}M$; n=4) > R-PIA > S-PIA (1:2.8:38). The GFP was then mixed with plasma and the rank orders of potency redetermined in reconstituted PRP: NECA ($IC_{50}=1.33x10^{-7}M$ 95% confidence limits $0.6-3.1x10^{-7}M$; n=4) > R-PIA > S-PIA (1:12:174). These results suggest that plasma may selectively bind R-PIA or that plasma is required for normal responses to stimulation by R-PIA.

Adenosine A_1 receptor subtypes are generally classified on the basis of approximately equal potency for NECA and R-PIA. At adenosine A_2 subtypes, NECA is at least ten times more potent than R-PIA. On this basis, we are unable to classify the platelet receptor as belonging clearly to A_1 or A_2 subtypes as proposed (Londos et al., 1980).

Born G.V.R. 1962, Nature, 194, 927-929
Haslam R.J. & Lynham J.A. 1972, Life Sci., 2, 1143-1154
Londos C. & Wolff J. 1977, Proc. Natl. Acad. Sci. U.S.A., 74 5482-5486
Van Calker, D., Muller, M. & Hamprecht B., 1979, T. Neurochem., 33, 999-1005

[^{3}H]-NORADRENALINE UPTAKE AND RELEASE BY ANOCOCCYGEUS MUSCLES FROM CONTROL, DIABETIC AND STATIL TREATED DIABETIC RATS

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The accummulation of sorbitol and the depletion of myo-inositol and Na^+K^+ATPase activity are interelated metabolic changes which occur during poorly controlled diabetes mellitus in nervous and vascular tissues which are susceptible to chronic diabetic damage. These changes, which may contribute to such damage, can be prevented by aldose reductase inhibition (Gillon et al, 1983, Green, 1986). We have investigated the effects of experimental diabetes with or without an aldose reductase inhibitor, Statil, on ^{3}H-noradrenaline (^{3}H-NA) uptake and release by isolated rat anococcygeus muscles.

Anococcygeus muscles were isolated from 6 control and 12 six-week streptozotocin diabetic rats. Six of the latter had received Statil (25 mg day^{-1}orally) from day 3 after streptozotocin. After 1h incubation at 37°C in the presence of 1μCi ^{3}H-NA ml^{-1} (total NA concentration: 0.614μM) tissues were superfused by 1ml Krebs min^{-1} at 37°C under 0.5g tension between 2 platinum wire electrodes. After 1h 300 pulses, 2ms duration, 5Hz, supramaximal voltage were applied. This stimulation was repeated 3 times. (S_1, S_2 and S_3) at subsequent 30 minute intervals with 2, 4 minute superfusate samples being collected each time, one before, and one from the start of stimulation. 25 min before S_2 0.2μM desipramine was added to the superfusate. 25 min before S_3 the superfusate was further modified by the addition of 1μM yohimbine. The results are shown in the table. ^{3}H overflow was calculated as equivalent p.mol. ^{3}H-NA.min^{-1}.g tissue^{-1}. Pre-stimulation sample values were taken as basal overflow,stimulated ^{3}H overflow being estimated by subtracting these values from those obtained from the second (with stimulation) samples. Statistical analysis was by analysis of variance, followed, where $p<.05$ by Tukey's multiple range tests.

Table 1	Controls	Diabetic	Diabetic Statil
Blood glucose conc. (mM)	4.34±0.75[e]	28.36±2.34	27.05±2.45
Stimulated ^{3}H overflow: (See text)			
S_1 (no drugs)	30.8 ±4.2	31.1 ±6.0	35.8 ±6.5
S_2 (+ desipramine)	39.1 ±5.9	31.6 ±5.9	49.7 ±8.0[e]
% increase	26.6 ±4.7[a]	2.8 ±4.0	40.1 ±7.2[e]
S_3 (+ desipramine & yohimbine)	58.4 ±9.5	44.9 ±7.4	69.4 ±10.4
% increase	50.8 ±8.4	47.3 ±10.5	40.5 ±10.6

results are mean ± sem, all groups n = 6, significant differences from the untreated diabetic group values are indicated by a, $p<.05$, b, $p<.001$.

No significant differences between the three groups in basal ^{3}H release or in stimulated ^{3}H release in the absence of drugs (S_1) were observed. Desipramine, however, produced a markedly smaller increase in stimulated ^{3}H overflow in the untreated diabetic group than in either the control or treated diabetic group. Further increases in stimulated ^{3}H overflow produced by 10^{-6} yohimbine were similar in each group. The data indicate a reduction, by diabetes, in reuptake of electrically stimulated ^{3}H-noradrenaline in this tissue, an effect which was prevented by Statil.

Gillon, K.W.R. et al (1983) Diabetologia 25, 365-371
Green, D.A. (1986) Metabolism 35 Suppl. 1, 6)-65

BLOOD PLATELETS: A NOVEL SYSTEM TO EXAMINE CENTRAL GABA-TRANSAMINASE INHIBITION?

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The inhibition of the degradative enzyme GABA-T is an effective mechanism for augmenting GABAergic systems by increasing presynaptic GABA (Jung, 1982), but since enzyme inhibition in the CNS is difficult to monitor, a peripheral indicator is desirable. GABA-T is present in human blood platelets (White, 1979) with a specific activity 1000-fold less than in brain tissue. To examine the correlation between central (CNS) and peripheral (blood platelet) enzyme inhibition, the time course of inhibition in each tissue was compared, following in vivo treatment with the suicide enzyme inhibitor, vigabatrin.

Male rats (200g-350g) were dosed with vigabatrin (1500mg/kg, ip in aqueous solution). Control animals were dosed with saline (1ml per 100g). At various times after drug administration (see Fig.1) 5 animals from each group were sacrificed. GABA-T activity and vigabatrin concentration were measured in plasma, platelets and brain tissue.

Peak vigabatrin concentration occurred in plasma at 2 hours and in brain at between 2 and 4 hours and was undetectable at 72 hours. GABA-T activity in platelets declined rapidly to a minimum of 2% of control levels at 4 hours (Fig. 1). Activity recovered slowly, there being no significant difference between control and treated levels at 336 hours post administration. The inhibition of GABA-T in brain was slower and smaller in magnitude. A minimum activity of 30% of control levels occurred at 24 hours, recovering slowly, but remaining significantly different from controls at 336 hours.

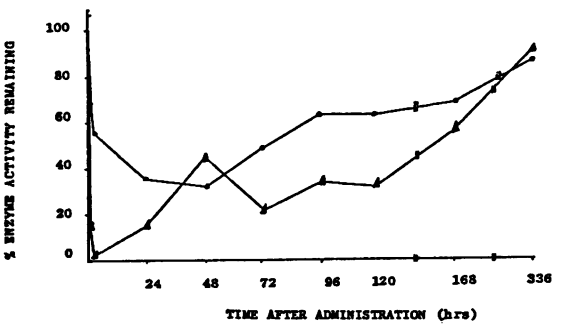

Fig 1 Comparison of the time course of inhibition of GABA-T in rat brain (•) and platelet (▲) following a single i.p. dose of vigabatrin (1500mg/kg).

These results suggest the existence of different rates of uptake of vigabatrin into rat platelets and brain synaptosomes, the latter being the major site of GABA-T activity in brain and, quantitatively, the most important pool of enzyme activity contributing to the anticonvulsant activity of GABA-T inhibitors (Wood et al, 1980). However, the similarity in time course of inhibition between the two tissues suggests that platelet enzyme inhibition correlates well with central inhibition and that platelet GABA-T monitoring may be useful in determining the efficacy of GABA-T inhibiting compounds.

Jung, M.J. (1982) In:Neurotransmitter Interaction and Compartmentation Ed. Bradford, H.G. pp 329-344 Plenum N.Y.
White, H.L. (1979) Science 205 696-698
Wood, J.D. et al (1980) J. Neurochem 35 125

DRUG DISPLACEMENT OF RADIOLABELLED ALFENTANIL FROM PROTEIN BINDING SITES IN HUMAN PLASMA

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The short acting opioid analgesic alfentanil is primarily bound to α_1-acid glycoprotein in plasma (Meuldermans et al., 1982) and a number of basic drugs have been shown to bind to this protein (Piafsky and Borga, 1977). We have shown that marked displacements can be achieved by di-isopropylfluorophosphate (Green and Kitchen, 1985) and the possibility therefore exists for drug interaction problems in the use of this opioid. We report here the effect of several drugs which might be used concurrently with alfentanil as an anaesthesic/analgesic upon the protein binding in human plasma in vitro.

Blood was collected from three healthy volunteers (2 male and 1 female) into citrated tubes. Plasma was prepared by centrifugation (2000g. for 10 mins) and stored at -20°C in 2 ml aliquots. Plasma protein binding was determined using an ultracentrifugation technique as previously described (Green and Kitchen, 1985). All drugs studied were dissolved in 0.9% saline and final concentrations in the incubations were equivalent to the reported therapeutic plasma levels in man. Each incubation contained 250 µl of plasma, 25 µl of ^{3}H alfentanil (containing 41nCi of tritium label at a final concentration of 300 ng ml^{-1}) and 25 µl of the displacing drug under investigation.

There was a high fractional binding of alfentanil to plasma proteins in all three subjects (93.2 ± 0.3%; each subject determined from 8 estimations).

Table 1. Effect of various drugs on the binding of alfentanil to human plasma in vitro

Drug and concentration ($\mu g ml^{-1}$)		% Free Alfentanil		
		Subject		
		HD	IK	MY
Saline	-	6.2 ± 0.2	7.1 ± 0.3	7.1 ± 0.2
Thiopentone	(42)	6.2 ± 0.4	7.1 ± 0.2	7.2 ± 0.2
Benzylpenicillin	(10)	6.6 ± 0.6	7.5 ± 0.9	7.7 ± 0.2
Suxamethonium	(10)	5.9 ± 0.6	5.8 ± 0.3	5.6 ± 0.2
Gallamine	(30)	6.6 ± 0.2	7.2 ± 0.5	6.2 ± 0.2
Diazepam	(0.4)	6.8 ± 0.9	7.0 ± 0.1	7.5 ± 0.3
Omnopon	(0.5)	6.7 ± 0.2	6.5 ± 0.3	8.3 ± 0.9
Omnopon-scopolamine	(0.5)	6.7 ± 0.1	6.6 ± 0.3	7.8 ± 0.6
Propranolol	(0.1)	5.4 ± 0.2	6.3 ± 0.3	7.1 ± 0.2

Each value represents the mean ± s.e.mean of 4-8 determinations.

There were no significant changes in free alfentanil in the presence of any of the drugs studied. It therefore seems unlikely that displacement from protein binding sites will cause a problem in its therapeutic use.

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Green, P.G. & Kitchen, I. (1985) Br. J. Pharmac. 86, 558P

Meuldermans, W.E.G., Hurkmans, R.M.A. & Heykant, J.J.P. (1982) Arch. Int. Pharmacodyn. 257, 4-19.

Piafsky, K.M. & Borga, O. (1977) Clin. Pharmacol. Ther. 22, 545-549.