PULMONARY RETENTION OF DYE FOLLOWING INHALATION OF COLOURED SMOKE

T C Marrs, Pathology and Toxicology Section, Chemical Defence Establishment, Porton Down, Salisbury, Wilts SP4 OJQ

Twenty-one female Porton mice, Wistar rats and Dunkin Hartley guinea-pigs were exposed to a smoke containing Solvent Green 3, Disperse Red 9 and Solvent Yellow 33 for 30 min at a total concentration of 0.595 g m^{-3}. Animals were sacrificed in groups of 30 at 80 min and 1, 3, 7, 10, 14 and 21 d. The lungs of rats killed at 80 min and 1 d contained Solvent Green 3. No other groups showed any evidence of dye in the lungs.

Ten animals from each species were exposed to the smoke (0.5 g m^{-3}) 1 h d^{-1} for 5 d. Two animals were sacrificed at 1 d and 2, 4, 6 and 8 weeks. Although the green dye was present in mice killed only at 1 d, the lungs of the rats still contained dye 4 weeks after the last exposure. Alveolar macrophages of the guinea-pigs still contained dye 8 weeks after exposure.

Four hundred mice and two hundred rats and guinea-pigs were divided into equal sized control, low, medium and high dose groups. The first was exposed to air only, the remainder to the smoke at the approximate concentrations of 0.1, 0.3 and 1 g m^{-3}, 1 h d^{-1}, 5 d $week^{-1}$ for up to 100 exposures. Because of the high intercurrent mortality, exposure was stopped early in the case of the guinea-pigs, a species notable for its susceptibility to aerosols (Hoar, 1976). A proportion of the mice was killed 40 weeks after the start of exposure and all the remaining survivors at 18 months. Decedents and survivors were examined post-mortem and sections of lung and 15 other organs stained with haematoxylin and eosin. The dye Solvent Green 3 was present in a large proportion of the high dose group lungs of all three species, and to a lesser extent in the middle dose groups. Particularly in the rats a vigorous foreign-body reaction was noted. There was a dose-related incidence (Snedecor and Cochran, 1971) of many pulmonary histological changes including alveolitis in the rats and mice ($P < 0.05$, both) and inflammatory changes in the mice ($P < 0.001$). Non-pulmonary changes found included a dose related incidence of fatty change in the mouse livers ($P < 0.05$) and of biliary hyperplasia in the rat livers, in one case accompanied by fibrosis ($P < 0.01$). The guinea-pigs, if they survived were remarkably free of pathological lesions, either in the lungs or elsewhere.

In none of the short term studies were the dyes Solvent Yellow 33 or Disperse Red 9 retained, nor were they in the 18 month study. By contrast Solvent Green 3 was retained in both long and short term studies, so that the latter study appeared an effective predictor of the outcome of the long term test in respect of dye retention and foreign-body response.

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LONG-TERM EFFECTS OF SINGLE INHALED DOSES OF 2-CHLOROBENZYLIDENE MALONONITRILE (CS)

H F Colgrave and T C Marrs, Pathology and Toxicology Section, Chemical Defence Establishment, Porton Down, Salisbury, Wilts SP4 0JQ

2-Chlorobenzylidene malononitrile (CS) is used as a riot control agent on account of its irritant properties. In laboratory animals, the toxicology of CS is generally low (Ballantyne, 1977; Marrs et al, 1983) especially if compared to the human IC_{50}. There is no evidence of teratogenicity or embryotoxicity (Upshall, 1973) and mutagenicity and in vivo DNA-binding experiments (von Daniken et al, 1981) do not suggest carcinogenic potential. Studies in humans (Beswick et al, 1972) showed no evidence of clinically significant changes in chest X-ray, peak flow, tidal volume, vital capacity and ECG. Nevertheless, these authors did demonstrate a significant pressor effect which lasted about 20 min.

SPF Wistar rats and golden hamsters were exposed to single doses of pyrotechnically-generated CS smoke at a concentration of 480 mg m^{-3} for 1 h or 150 mg m^{-3} for 2 h. Animals that survived 1 month were transferred to permanent quarters and observed for up to 32 months. At that time survivors were killed. All survivors as well as decedents were examined post-mortem. Sections were stained with haematoxylin and eosin, and additionally in the case of the pituitary glands with Heidenhain's azan stain (Culling, 1957). Statistical analysis of histological changes was by the χ^2 test. Where the incidence of lesions was high a 3 x 2 contingency test was applied. Where changes were infrequent, the data from the two test groups were pooled and a 2 x 2 contingency test performed against the controls.

The lifespan of test and control animals did not appreciably differ. Most individual types of tumours did not occur frequently enough to be analysed statistically. An exception was the numerous mostly acidophil pituitary tumours found in female rats. The incidence of these did not differ significantly between test and control groups. The frequent occurrence of pituitary tumours in elderly rats is often ascribed to high calorie intake (Ross et al, 1970), and our animals were fed ad libitum throughout. When all tumours were statistically analysed together regardless of type (a procedure of questionable validity, Feron, 1980), no significant relationship between exposure and tumours was found. Heterogeneity was only found in respect of pyelonephritis in the female hamsters and male rats ($P < 0.02$, both groups). This was due to a low incidence of the lesion in the 480 mg m^{-3} group. It was concluded that single doses of CS at Cts up to 28,800 mg min m^{-3} for 1 h and 18,000 mg min m^{-3} for 2 h did not produce adverse late effects.

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ENDOGENOUS GENERATION OF CYCLOOXYGENASE PRODUCTS BY HUMAN ISOLATED LUNG TISSUE

N.J. Cuthbert & P.J. Gardiner, Pharmacology Section, Miles Laboratories Ltd., Stoke Court, Stoke Poges, Slough SL2 4LY, Bucks.

The isolated guinea-pig trachea generates endogenous cyclooxygenase products which regulate its inherent tone (Orehek et al, 1973). Indomethacin 1μg/ml (a concentration known to inhibit the enzyme, cyclooxygenase) decreases this inherent tone and potentiates subsequent responses of contractant agonists (Gardiner and Collier, 1980). Although human isolated lung tissue is known to possess inherent tone, it remains uncertain whether cyclooxygenase products regulate such tone. We have attempted to clarify this situation.

Tyrodes solution (3ml) was collected from tissue baths at 15 min intervals after human isolated bronchial muscle and lung strip preparations had equilibrated. Tissue movement was monitored by Harvard isotonic transducers (with a load of 250-500mg) linked to Chessell flat bed recorders. Radioimmunoassay techniques were used to determine whether any cyclooxygenase products (TXA_2, $PGF_2\alpha$, PGE_2, PGI_2 or PGD_2) were produced by the tissues under resting conditions or in the presence of test drugs (Brennecke et al, 1982; Mitchell et al, 1982).

Both tissues were shown to possess inherent tone by the relaxant effects of isoprenaline or theophylline. Under normal resting conditions both tissues were shown to generate cyclooxygenase products with the lung strip generally producing the least, although it alone produced PGD_2 (Table 1).

Table 1 Generation of cyclooxygenase products by human isolated lung tissues

HUMAN TISSUE	n (number observations)	Cyclooxygenase Products (Mean ± s.e.mean) pg/ml bath fluid				
		TXB_2	$PGF_2\alpha$	PGE_2	6-keto $PGF_1\alpha$	PGD_2
Bronchial Muscle	10	45 ± 12	410 ± 192	351 ± 109	383 ± 217	0
Lung Strip	4	39 ± 13	50 ± 37	184 ± 84	56 ± 17	16 ± 3

TXB_2 reflects TXA_2 levels and 6-keto $PGF_1\alpha$ reflects PGI_2 levels.

Leukotriene D_4 (LTD_4) 10^{-8}M and histamine 10^{-5}M produced similar contractant responses on the bronchial muscle but little or no contraction of the lung strip. LTD_4 stimulated the production of all cyclooxygenase products except PGD_2 by both tissues. Histamine acted similarly except that it inhibited TXA_2 generation by the lung strip.

Indomethacin (1μg/ml) significantly reduced all cyclooxygenase products on three separate lung samples but no concomitant reduction of inherent tone was observed. However, on three other lung samples indomethacin potentiated cyclooxygenase, no change in inherent tone occurred. Taken together these results suggest that cyclooxygenase products do not regulate such tone in human isolated lung tissues.

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DISTRIBUTION OF SODIUM CROMOGLYCATE IN RAT KIDNEY USING MICRO-AUTORADIOGRAPHY

G.A. Buckley, A.J. Murphy[1] & M.G. Neale[1], Department of Life Sciences, Trent Polytechnic, Nottingham, NG1 4BU and [1]Safety Evaluation Group, Fisons plc, Bakewell Road, Loughborough, Leics, LE11 ORH.

Sodium cromoglycate, an organic acid, appears rapidly in bile and urine after intravenous injection. We have previously studied the distribution of (^{3}H) sodium cromoglycate in rat liver using microautoradiography (Buckley *et al* 1982) and have now extended these studies to the rat kidney.

Eight Cobs Wistar male rats (240-290 g), anaesthetised with sodium pentobarbitone were given an intravenous dose of 11 mg kg^{-1} (^{3}H) sodium cromoglycate (specific activity 40 $\mu Ci\ mg^{-1}$). One kidney was removed from a rat at 2.0, 2.5, 3.5, 4.5, 5.8, 10.5, 30.5 or 60.5 minutes after the injection. Two further rats received unlabelled sodium cromoglycate, to serve as negative controls, and a kidney was removed from the rats at either 2 or 5 minutes. Microautoradiographs were prepared according to Buckley *et al* (1982).

The cortex of the rat kidney may be divided into outer and inner zones (Sternberg *et al* 1956), glomeruli being absent from the inner cortex. Using this criterion, the concentration of silver grains/unit area was determined in distal tubules and in the proximal tubules (PT) of the outer and inner cortex.

Two methods were used for obtaining this information. The first involved the use of a Zeiss Photomicroscope in which one eye-piece contained a graticule, one small square of which represented 100 μm^2. In each tubule the number of grains in 1000 μm^2 was counted. Visual grain counting can be both tedious and inefficient therefore a second, automated system using a Quantimet 720 Image Analyser was developed by which the number of silver grains in a number of variable areas was determined. The results were converted into grains/1000 μm^2. To suppress any interference from the haemotoxylin and eosin stain, a combination of Kodak Wratten 30 and 32 filters was inserted into the light path and, under these conditions, the automated counts gave good agreement with those obtained visually.

The results showed that silver grains (indicating the presence of sodium cromoglycate) appeared at the highest concentration in the lumen of the distal tubules and in some segments of the PT. During the first six minutes the PT in the inner cortex, consisting mainly of the pars recta (or S3 segments, Maunsbach, 1966) contained five to nine times as much sodium cromoglycate as those in the outer cortex. More detailed analysis indicated that in the outer cortex most PT, consisting of pars convoluta (or S1 and S2 segments), contained low concentrations of sodium cromoglycate but approximately 15% had a higher concentration, similar to that found in the PT of the inner cortex. These latter are, probably, pars recta of superficial nephrons.

This distribution in the rat is consistent with secretion of sodium cromoglycate chiefly in the pars recta of the PT and complements existing data on acidic molecules, such as para-aminohippurate which has been shown by other techniques to be secreted in the PT.

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THE EFFECTS OF ATP, ADP, AMP, AND ADENOSINE ON THE ANTI-HAEMOLYTIC ACTION OF PROCAINE

M.L.Gonçalves & J.A.Ribeiro, Laboratory of Pharmacology, Department of Cell Biology, Gulbenkian Institute of Science. 2781-Oeiras,Portugal

It has been shown that adenosine triphosphate (ATP) and related nucleotides have anti-anaesthetic action (Kuperman, Okamoto, Beyer & Volpert, 1964; Ribeiro & Sebastião, 1983). On the other hand, it is well established that local anaesthetics have anti-hemolytic actions (e.g. Seeman, 1972). The present work was undertaken to investigate whether ATP, related nucleotides and adenosine affect the anti-hemolytic action of procaine.

Experiments were carried out on erythrocytes from rat blood, and the techniques to produce hemolysis followed the procedures described by Seeman & Weinstein (1966). The test solution used to cause 50% hemolysis contained NaCl 67 mM in 10 mM of sodium phosphate buffer, pH 7.0.

Appropriate concentrations (0.01-1 mM) of ATP, ADP and AMP antagonized the anti-hemolytic effect of procaine (10 mM) (Figure 1).

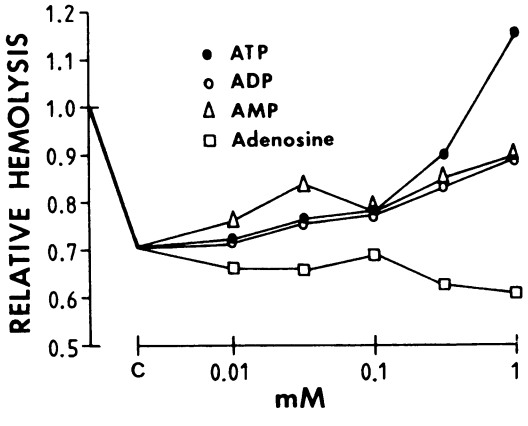

Figure 1 Effects of ATP,ADP, AMP and adenosine on the anti-hemolytic action induced by procaine 10 mM(C). A relative hemolysis of 1.0 indicates an absolute degree of hemolysis of around 50 per cent.

ATP in concentrations above 0.1 mM was more effective than ADP or AMP. ADP and AMP caused similar effects. In concentrations above 0.1 mM the nucleotides had hemolytic action and in a concentration range between 0.01 and 0.1 mM antagonized the stabilizing action of procaine without causing hemolysis. Adenosine (0.01-1 mM) exhibited some anti-hemolytic action, though smaller than that of procaine (10 mM), and in the presence of procaine accentuated slightly the anti-hemolytic action of this compound (Figure 1).

It is concluded that ATP, ADP and AMP can antagonize the anti-hemolytic action of procaine and have hemolytic properties when used in high concentrations.

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COMPARISON OF METYRAPONE AND ETHANOL AS INHIBITORS OF HEPATIC MICROSOMAL MONOOXYGENASE ACTIVITY

J R Fry, S Horner & P Paterson, Departments of Physiology & Pharmacology and Human Morphology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, UK.

Increasing awareness of the existence of multiple isozymic forms of cytochrome P450 and their mediated monooxygenations in liver microsomes has prompted the search for inhibitors capable of distinguishing these different forms in control (i.e. uninduced) rats. It is likely that a range of selective inhibitors would be most useful, and two possible candidates are metyrapone and ethanol. Metyrapone is believed to exert its inhibition by binding to the substrate-binding site of cytochrome P450 (Netter, 1980), whereas ethanol is believed to act by interfering with binding of substrate to the cytochrome without itself binding at the same site (Cinti et al, 1973). We have attempted to confirm that these two inhibitors act by different mechanisms by comparing their relative inhibitory potencies to four cytochrome P450-dependent microsomal monooxygenases.

Hepatic microsomes, isolated from uninduced male rats, were assayed for the activities of 7-ethoxycoumarin O-deethylase (7-EC), 7-methoxycoumarin O-demethylase (7-MC), biphenyl 4-hydroxylase (BP) and 4-methoxybiphenyl -demethylase (4-MBP) by published methods (Aitio, 1978; Fry, 1981), in the absence or presence of various concentrations of metyrapone (10^{-6}M-10^{-3}M) or ethanol (0.017-2.57 M). Results from previous studies, utilizing differential induction techniques, have suggested that these enzyme reactions are catalyzed by different cytochrome P450 types (Kamataki et al, 1980; Fry, 1981). The results are presented in Table 1.

Table 1. Effects of metyrapone and ethanol on four hepatic microsomal monooxygenases.

Enzyme	10^{-3}M Metyrapone (% inhibition)	Ethanol (I_{50} value; mM)
7-EC	48.2 ± 1.7	0.454 ± 0.089
7-MC	22.2 ± 4.4	0.055 ± 0.013
BP	11.0 ± 5.6	0.180 ± 0.023
4-MBP	41.7 ± 1.8	0.605 ± 0.124

Values are mean ± S.E.M., n=4.

Metyrapone was ineffective at concentrations of 10^{-6}-10^{-4}M with 7-MC, BP and 4-MBP. At a metyrapone concentration of 10^{-3}M, the rank order of susceptibility to inhibition was: 7-EC>4-MBP>7-MC>BP. From the ethanol data, I_{50} values (i.e. the concentration of ethanol required to inhibit the reaction by 50%) were calculated for each enzyme (Table 1) and these were used to compile the following rank order of susceptibility to inhibition: 7-MC>BP>7-EC>4-MBP.

It is apparent that the rank orders are different for the two inhibitors and from this we conclude that metyrapone and ethanol exert their inhibitory effects on the microsomal monooxygenase system by different mechanisms. This, in turn, suggests that these two inhibitors may be of value in distinguishing different forms of microsomal monooxygenases in uninduced liver.

The financial assistance of the Medical Research Council is gratefully acknowledged.

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NEUROMUSCULAR EFFECTS OF FENTANYL, A MORPHINE-LIKE ANALGESIC DRUG

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

Fentanyl is an opioid analgesic agent which resembles morphine in its analgesic action, but it is more potent and has a shorter duration of action than morphine (Pybus & Torda, 1982; Haberer et al., 1982).

It has been suggested that morphine-like analgesics may reduce the release of acetylcholine(ACh) from the axon terminals of the parasympathetic cholinergic neurones of Auerbach's plexus in the intestine (Simon, 1978; Snyder, 1977), and that this effect is more pronounced at low frequencies of nerve stimulation.

The object of the present experiments was to investigate the possible neuromuscular effects of fentanyl and morphine, that could be interpreted in terms of pre-and postsynaptic events at the vertebrate neuromuscular junction.

Fentanyl(1 $ug.ml^{-1}$,2.9 uM) and morphine (1.5 $ug.ml^{-1}$,2.9 uM) reduced the amplitudes of the twitch tension (produced by repetitive nerve stimulation at 0.2 Hz with 5V and 0.5 ms pulse duration) by 37±4.2% and 25±1.5% of the control responses in the rat hemidiaphragm nerve-muscle preparation ($n=6, P < 0.001$). The local anaesthetic drug lignocaine (43 $ug.ml^{-1}$,3.6 uM) completely abolished the twitch tension.

In the chick biventer cervicis(BVC) nerve-muscle preparation, ACh(0.55-11.0 mM) produced concentration-dependent contractures. A mean (±SEM) maximum tension of 4.5±0.12 g was obtained by 5.5 mM ACh in the control Krebs solution. Fentanyl (2.9 uM) and morphine (2.9 uM) reduced the ACh-induced contractures in the chick BVC muscle. The mean (±SEM) ED50s of ACh contractures in the control Krebs solution and in Krebs containing fentanyl were: 0.65±0.11 mM and 0.85±0.2 mM respectively ($n=6, P < 0.05$). Similar results were obtained by morphine (2.9 uM). The local anaesthetic drug lignocaine completely abolished the ACh-induced contractures in less than 2 min. The effects of fentanyl and morphine were reversible on washing out the drugs in Krebs solution for 10-15 min.

The present experiments show that fentanyl and morphine slightly but significantly reduce the twitch and the ACh-induced tensions at the frog and chick neuromuscular junctions.

Experiments, using the technique of incubating the preparation with radioactive choline (Rand, Story & Wong-Dusting, 1982) and intracellular recording techniques, would be useful to further elucidate the mode of action of opioid analgesic drugs at the vertebrate neuromuscular junction.

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EFFECT OF AMILORIDE ON THE CHICK BIVENTER CERVICIS MUSCLE

Sahba Fatherazi , A.Ghandiha & M.A.Khoyi[1], Department of Pharmacology, School of Pharmacy, University of Tabriz, Tabriz, Iran [1]Department of Pharmacology, Pharmaceutical Research Centre, Daroupakhsh Avenue, Karaj Road, Tehran, Iran.

Amiloride is a pyrazine derivative with a guanidine group and is used as a potassium sparing diuretic (Baer et al.,1976). In frog skin (Nagel & Dorge, 1970) , toad bladder (Bently, 1968) and human red cells (Aceves & Cereijido, 1973) amiloride is known to inhibit the entry of sodium ions into cells.

In the present work, the effect of amiloride on the chick biventer cervicis muscle was studied. The chicks(2-3 weeks old) were killed by ether. Biventer cervicis muscle was removed and transferred to a 10 ml. bath containing tyrode solution ($37^{o}C$). Isometric contractions were recorded on a Grass polygraph with the aid of a FT10C Transducer. Stimulation was carried out with an S88 Grass stimulator (0.1 Hz, 0.2 ms , 12 V).

$2.1x10^{-4}M$ - $4.3\ x10^{-4}M$ amiloride increased the twitch response to electrical stimulation. $4.3x10^{-5}M$ - $4.3x10^{-4}M$ amiloride shifted the dose response curve of acetylcholine to the left. $2.1x10^{-4}M$ amiloride reduced -log EC_{50} of acetylcholine from 3.88 ± 0.07 to 4.62 ± 0.11. In preparations incubated with physostygmine ($10^{-6}M$), amiloride had no significant effect on EC_{50} of acetylcholine (-log EC_{50} changed from 5.13 ± 0.05 to 5.43 ± 0.06). Low concentration of amiloride ($1.3x10^{-5}M$ - $2.1x10^{-4}M$) did not show any significant effect on the response to carbamylcholine (-log EC_{50} changed from 5.28 ± 0.06 to 5.58 ± 0.24). High concentration of amiloride ($4.3x10^{-4}M$ - $1.2x10^{-3}M$) decreased the maximum response to carbamylcholine. In the presence of d Tubocurarine ($4x10^{-6}M$),$2.1x10^{-4}M$ - $4.3x10^{-4}M$ amiloride didn't show any effect on contractions produced by Kcl 20 mM (2 experiments).

The results suggest that amiloride has some cholinestrase inhibiting activity on neuromuscular junction. Further experiments are needed to elucidate the mechanism of its inhibitory action on the maximum response to carbamylcholine.

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FUNCTIONAL CHARACTERISTICS OF VERAPAMIL AND NITRENDIPINE INTERACTIONS AT CALCIUM CHANNELS IN SMOOTH MUSCLE

P.P.A. Humphrey & M.J. Robertson, Department of Pharmacology, Glaxo Group Research Ltd., Ware, Hertfordshire.

Receptor binding studies suggest different classes of calcium (Ca^{++}) entry blockers interact at different sites (e.g. Murphy & Snyder, 1982). We have examined this possibility pharmacologically by measuring the inhibition of Ca^{++} induced contractions of smooth muscle produced by two different types of Ca^{++} entry blocker, verapamil (V) and nitrendipine (N), alone and in combination.

Rings of beagle left anterior descending coronary artery (0.3 - 0.8 mm ext. diam.) or strips of guinea-pig taenia coli (2-2.5 cm) were suspended in organ baths containing Ca^{++}- free, high K^+, Tyrode solution of the following composition (mmol/l): NaCl 97; KCl 40; $NaHCO_3$ 11.9; NaH_2PO_4 0.4; glucose 5.5, pH 7.1 and bubbled with 95% O_2/5% CO_2 gas at 37°C (see Spedding, 1982). An initial resting tension of 0.3g (for coronary artery) or 1g (for taenia coli) was applied and contractile responses to the cumulative addition of Ca^{++} (0.03-30 mM) determined.

Pretreatment with V or N for 30 min shifted the Ca^{++} concentration-response curve to the right in a concentration-dependent fashion. An Arunlakshana & Schild plot (1959) gave pA_2 values for V of 7.9 ± 0.1 and 8.5 ± 0.1 in taenia coli and coronary artery respectively (mean values ± s.e. mean; n = 5 for each group). Similarly, the equivalent pA_2 values for N in the two preparations were 9.4 ± 0.1 and 10.1 ± 0.1 (n = 5 for each group). In all cases the slopes of the plots were not significantly different from unity. In separate experiments the resultant shift of the Ca^{++} concentration-response curve (concentration ratio, CR) when the tissues were pretreated with V and N separately (CR_V and CR_N) or with an admixture of V and N (CR_{V+N}) is shown in Table 1:-

Table 1:

	OBSERVED				EXPECTED*	
	$CR_{(V)}$	$CR_{(N)}$	$CR_{(V+N)}$	n	Interaction Competitive	Not competitive
TAENIA COLI V 10^{-7}M N 2 x 10^{-9}M	9.3±1.8	10.7±1.6	18.8±2.6	8	19	99.5
CORONARY ARTERY V 10^{-8}M N 5 x 10^{-10}	4.0±0.7	6.5±1.8	11.1±3.9	6	9.5	26

Each value is the mean (± s.e. mean) of 6 - 8 observations.

*$CR_{(V+N)} = (CR_V + CR_N)-1$ if the antagonists are acting at the same receptor. but $CR_{(V+N)} = CR_V \times CR_N$ if the antagonists are not competing for the same receptor (see Paton & Rang, 1965).

Our results suggest that verapamil and nitrendipine compete for common functional sites on the Ca^{++} ion channel in each of the two preparations studied.

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EFFECTS OF THE CALCIUM ENTRY BLOCKER NIFEDIPINE ON CARBACHOL-INDUCED CONTRACTIONS OF GUINEA-PIG ISOLATED TRACHEAL RINGS

I. Ahmed, H.P. Rounding and R. Towart, Miles Laboratories Ltd., Stoke Court, Stoke Poges, Slough SL2 4LY, Bucks, England.

Although the contraction of tracheal smooth muscle is calcium-dependent, there are reports that calcium antagonists have little effect on acetylcholine- or carbachol- (CCh) induced contractions of this tissue (Coburn, 1979). To investigate this paradox further we have examined the effects of nifedipine on CCh-induced contractions of isolated guinea pig tracheal rings. Single rings from male Dunkin-Hartley guinea pigs (250-350g) were suspended in modified Tyrode's solution at 37°C. Isometric contractions were measured using Statham UC2 strain gauges connected to Devices or IFD bridge amplifiers (Towart, 1982). The Tyrode's solution contained 3 x 10^{-7}g/ml indomethacin to suppress the synthesis of constrictor or relaxant prostglandins (Orehek et al., 1975). After equilibration, the addition of various concentrations of CCh produced sustained contractions, and the addition of nifedipine to the established contraction caused relaxation. The results are shown in Table 1a, and clearly demonstrate that contractions induced by lower concentrations of CCh are more sensitive to inhibition by nifedipine.

To investigate the mechanism of this inhibitory effect of nifedipine, the method of Broekaert & Godfraind was used:- Addition of CCh under calcium-free conditions produces little or no contraction; marked contractions are however produced on readmission of calcium in the presence of CCh. These contractions can then be attributed to CCh-induced influx of extracellular calcium. Under these conditions nifedipine inhibited the CCh-induced calcium influx contractions (Table 1b) although the degree of inhibition by nifedipine was less dependent on the CCh concentration used.

These results show that nifedipine does affect the cholinergic response of guinea-pig tracheal smooth muscle, but that a nifedipine-resistant component may be present. This nifedipine-resistant component is at least partly due to nifedipine-resistant influx of calcium ions, and provides further evidence for the existence of more than one type of calcium entry channel.

CCh g/ml	nifedipine g/ml $3x10^{-9}$	$3x10^{-8}$	$3x10^{-6}$
$3x10^{-8}$	16.5 ± 5	68 ± 5	86 ± 9
10^{-6}	0	22 ± 5	41 ± 6
10^{-5}	0	6.5 ± 1	12 ± 3

Table 1a) % inhibition by nifedipine of established CCh-induced contractions (mean ± SEM) (4-6 observations)

CCh g/ml	nifedipine g/ml $3x10^{-9}$	$3x10^{-8}$	$3x10^{-6}$
$3x10^{-8}$	16 ± 5	40 ± 9	61 ± 10
10^{-6}	5 ± 1	30 ± 3	57 ± 5
$3x10^{-5}$	0	18 ± 5	52 ± 5

Table 1b) % inhibition by nifedipine pretreatment of the CCh-induced calcium influx contraction

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METOCLOPRAMIDE POTENTIATION OF RAT VAS DEFERENS RESPONSES TO ELECTRICAL FIELD STIMULATION

G. John & R.W. Marshall (introduced by T.L.B. Spriggs) Dept. of Pharmacology and Therapeutics, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN.

Spedding (1980) suggested that metoclopramide (MCP) 2.8-280 μM potentiates the response of the rat vas deferens to short trains of stimuli by blockade of prejunctional α-receptors. We have investigated this further by carrying out a detailed analysis of the effect of MCP (7-224 μM) on the responses of the rat vas to single and multiple pulse electrical field stimulation (EFS) using a microcomputer technique (Marshall and Sparks, 1981) to aid data collection and analysis.

Rat vasa, stripped of superficial fascia and blood vessels, were suspended between parallel platinum electrodes in 5 ml tissue baths containing Krebs solution maintained at 37°C and gassed with a 95% oxygen 5% carbon dioxide mixture. The tissues were maintained under a resting tension of 0.5 g. Periods of EFS were separated by 5 min intervals to avoid interaction between successive stimuli. Vasa used to investigate MCP action on single stimuli were never exposed to trains of stimuli (McGrath, 1978).

The early phase (1-2S) of contractile responses to 20s trains of stimuli (10 Hz, 0.3 ms pulse width) was significantly potentiated by MCP (7-56 μM). This potentiation was almost maximal at 7 μM and did not significantly increase with increasing dose. Indeed, at MCP concentrations greater than 56 μM the degree of potentiation diminished and resolved into an inhibition by 224 μM. MCP (7-224 μM) produced a dose related inhibition of the later phase (10-18s) of the responses.

The early phase (400-720 ms) of responses to single stimuli at 0.3 ms pulse width was also significantly potentiated by MCP (7-224 μM), the potentiation increasing with dose. The later phase (880-1600ms) of the response was unaffected. When the pulse width was increased to 5 ms the early phase was still potentiated although the maximum potentiation occurred at a MCP concentration of 56 μM with a decreased degree of potentiation occurring at higher concentrations. The late phase of the responses to 5 ms pulses showed a significant dose related inhibition.

We also investigated the effect of MCP on dose response curves to exogenous noradrenaline and phenylephrine. MCP (7-56 μM) did not shift the dose response curves in a parallel fashion but did increase the maximum response evoked by each agonist.

Our results confirm that MCP potentiates the responses of the rat vas to EFS. However, our finding that potentiation also occurs with single pulse EFS suggests the involvement of another mechanism besides the presynaptic α-receptor block proposed by Spedding (1980). The apparently non-specific potentiating effect of MCP on smooth muscle responsiveness cannot explain the potentiation of single pulses since the effect on single pulses is almost maximal at 7 μM whereas significant potentiation of exogenous agonists occurs only at higher concentrations.

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CALCIUM CHANNELS MEDIATING THE BARIUM CONTRACTION IN THE RAT VAS DEFERENS

D.W.P. Hay & R.M. Wadsworth, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

Barium 1mM usually produces a two-component response in the rat vas deferens: an initial small increase in baseline tone (phasic contraction) followed by larger rhythmic contractions (Hay & Wadsworth, 1983). This communication reports the effects of verapamil or nifedipine on the initial phasic contraction and on the barium-induced increase in ^{45}Ca uptake.

In tension experiments vasa deferentia were removed from Wistar rats, transversely bisected and suspended in Krebs-Henseleit solution, bubbled with 95% O_2:5% CO_2 and maintained at 37±0.5°C. Tension was recorded isometrically. Tissues were incubated for 20 min with the appropriate calcium channel inhibitor before addition of barium. Control and barium-stimulated ^{45}Ca uptake were measured by a modification of the 'lanthanum method', employing La^{3+} 50mM at 0.5°C (Hay & Wadsworth, 1982).

$BaCl_2$ 1mM or 2mM produced an initial phasic contraction of approximately 0.05-0.4g in both halves which was abolished by verapamil 2.04 µM or nifedipine 0.29µM; rhythmic activity was unaffected. (Figure 1). Rhythmic contractions are abolished by concentrations of verapamil or nifedipine 20-50X higher and are dependent on extracellular Ca^{2+} (Hay & Wadsworth, 1983). $BaCl_2$ 1mM increased ^{45}Ca uptake in the prostatic half from a basal level of 654±13.6 nmol ^{45}Ca g^{-1}, n = 30, to 854± 53.4 nmol ^{45}Ca g^{-1}, n = 18, $P < 0.001$, and in the epididymal half from a basal level of 791±27.1 nmol ^{45}Ca g^{-1}, n = 30, to 962±69.6 nmol ^{45}Ca g^{-1}, n = 16, $P < 0.05$. The stimulation of ^{45}Ca uptake in both halves was abolished by verapamil 2.04µM or nifedipine 0.29µM.

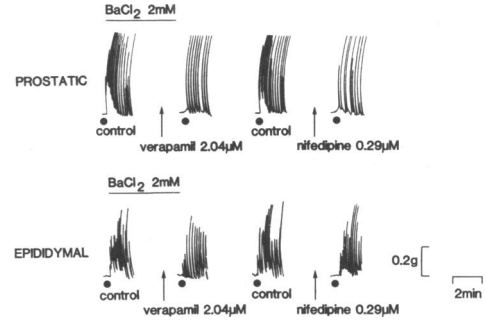

Figure 1

Effect of verapamil 2.04µM or nifedipine 0.29µM on the initial phasic contraction produced by $BaCl_2$ 2mM in the rat bisected vas deferens. ● = addition of barium.

The results suggest that the barium-stimulated increase in ^{45}Ca uptake is associated exclusively with the initial phasic contraction. Barium probably depolarises the smooth muscle initially, leading to the entry of extracellular Ca^{2+} via voltage-dependent channels and activation of the contractile proteins. The rhythmic contractions are likely to be mediated by the entry of a small amount of 'trigger' Ca^{2+}, which subsequently releases intracellular Ca^{2+} stores. The trigger Ca^{2+} enters via a separate population of membrane channels that are relatively insensitive to calcium channel inhibitors.

DWPH was supported by an MRC scholarship. We thank Bayer and Knoll for drugs.

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COMPARISON OF THE EFFECT OF OUABAIN AND AR-L 115 BS ON A MODEL OF CARDIAC AUTOMATICITY

J. Hernández, M. Romero & J.S. Serrano, Department of Pharmacology and Therapeutics; Medical School; Sevilla; Spain.

2-(2-Methoxy-4-methylsulfinyl)phenyl)-1H-imidazo(4,5-b)-pyridine(AR-L 115 BS)# is a new cardioactive drug with a greater positive inotropic effect than ouabaine (Diederen & Weisenberger, 1981). It reduces endsystolic volume without increasing heart rate or myocardial oxygen consumption (Hellige et al. 1981). Our purpose was to ascertain the effect of AR-L 115 BS, as compared with the standard inotropic drug ouabaine, upon a model of cardiac automaticity.

Spontaneous automatic activity was obtained by a local injury induced by a pair of Starling forceps on the isolated right ventricle of the rat placed in Tyrode solution maintained at 37ºC and bubbled with 95% O_2 and 5% CO_2 (Hernández & Serrano, 1982). Both drugs were tested at the following concentrations: 10^{-9}M, 10^{-8}M, 10^{-7}M 10^{-6}M, 10^{-5}M, 5 X 10^{-5}M and 10^{-4}M.

Neither ouabaine (n = 11) nor AR-L 115 BS (n = 9) at concentrations ranging from 10^{-9}M to 10^{-5}M, modified the automatic ventricular frequency. At higher concentrations (5 X 10^{-5}M and 10^{-4}M), both drugs increased the automatic activity in this preparation when compared with the control frequency (Table I). No statistical difference was found between the effect of both drugs at such concentrations.

Table I. Effect of ouabaine and AR-L 115 BS on the automatic ventricular frequency. Responses calculated as % of control value (± s.e.)

	Control	5 X 10^{-5}M	10^{-4}M
Ouabaine	100.0 ± 0.0	140.7 ± 17.2*	226.9 ± 27.9**
AR-L 115 BS	100.0 ± 0.0	150.7 ± 13.3**	179.9 ± 17.1**

*$p < 0.05$; **$p < 0.001$ when compared with control. Student t-test.

Our results indicate that, despite its higher inotropic efficacy, AR-L 115 BS does not enhance experimental cardiac automaticity more than ouabaine.

Supplied by Boehringer Ingelheim S.A.

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EFFECT OF AMINO-OXYACETIC ACID ON RAT BODY TEMPERATURE

M. Danesh-Kazemi & M.R. Zarrindast, Department of Pharmacology, Medical Faculty, University of Tehran, Tehran, IRAN .

Amino-oxyacetic acid (AOAA) is a potent competitive inhibitor of GABA transaminase. This agent is used to increase the levels of GABA in the brain (Wallach, 1961) . In the present work, the effect of AOAA on rat body temperature has been investigated.The rectal temperature was taken by the aid of an electrical thermometer. Readings were taken 0, 15, 30, 60 & 90 min after AOAA injection . The drugs were injected interaperitoneally and rats were restrained in a restainer during the experiments.
Table. 1 shows the effect of 20 mg/kg/IP of AOAA and AOAA alone or in conbination with different antagonists. AOAA decreased the rat body temperature in a dose dependent fashion .

Table 1 Effect of AOAA on rat body temperature

treatment dose mg/kg	Temperature (M±s.e.) at min after injection 0	15	30	60	90
AOAA 20	38.0±0.5	37.5±0.5	36.8±0.7	37.1±0.7	37.5±0.6
Pimozide 0.5 +AOAA 20	38.8±0.1	38.5±0.1	36.3±0.3	36.2±0.4	38.1±0.3
Bicuculline +AOAA 20	38.5±0.1	38.0±0.1	37.3±0.2	37.2±0.3	37.8±0.3
Picrotoxin +AOAA 20	37.3±0.5	36.9±0.5	36.3±0.4	36.6±0.7	37.2±0.5
Phenoxybenzamine 5 +AOAA 20	38.0±0.4	37.7±0.3	36.7±0.6	35.7±0.7	36.3±0.4
Propranolol 10 +AOAA 20	37.4±0.1	37.1±0.3	36.4±0.5	36.2±0.6	36.7±0.5
Methergoline 0.5 +AOAA 20	37.1±0.2	36.8±0.3	36.3±0.4	36.6±0.3	36.9±0.3

Pretreatment of rats with bicuculline(3mg/kg,30 min),picrotoxin (2 mg/kg , 15 min), pimozide (0.5 mg/kg , 120 min), phenoxybenzamine (5 mg/kg , 60 min) and propranolol (10 mg/kg , 60 min) did not antagonize the effect of AOAA (20 mg/kg) on body temperature. therefore the effect of AOAA on the body temperature of the rat could not be through the GABA-ergic, dopaminergic, noradrenergic mechanisms. Although methergoline (0.5 mg/kg , 120 min) slightly inhibited the fall in the body temperature of the rat induced by AOAA , for the elucidation of its mechanism further experiments showld be carried out .

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MODULATION OF VASOCONSTRICTION BY β-ADRENOCEPTORS IN ISOLATED PERFUSED RAT MESENTERIC ARTERIES

K. R. Borkowski & M. Porter, Department of Physiology, The University, Leicester LE1 7RH.

This study set out to investigate the possible modulation of vasoconstrictor responses by vascular smooth muscle β-adrenoceptors, by analysing the effects of β-adrenoceptor agonists and antagonists on pressor responses to vasoconstrictor agents in the isolated perfused rat mesenteric artery preparation.

Male normotensive Wistar rats (200-300g) were anaesthetised with ether and the mesenteric arterial bed removed as described by McGregor (1965). The preparation was kept at 37°C and perfused, via the mesenteric artery, at a rate of 4 ml per minute with Krebs solution (gassed with 95% O_2 and 5% CO_2) to which EDTA (10 $mg.dm^{-3}$) and ascorbic acid (20 $mg.dm^{-3}$) had been added. Pressor responses to bolus injections of noradrenaline (0.01 - 100μg) and methoxamine (0.1 - 300μg) in volumes of 0.1 ml were measured as increases in peak perfusion pressure. Test drugs were dissolved in the Krebs solution. Group sizes of six rats were used in all cases and data analysis performed upon responses calculated as a percentage of the maximum control response for a particular tissue. Statistical analysis was performed using the paired Student's t-test and computer assisted curve fitting.

Log dose-response curves to noradrenaline (NA) and methoxamine (ME) were reproducible over the period of the experiment. Addition of prazosin (10^{-8}M) to the Krebs abolished all responses to NA while 10^{-9}M prazosin significantly ($p<0.001$) depressed the responses. The ability of prazosin to antagonise the NA-induced increases in perfusion pressure indicates that these responses are mediated by α-adrenoceptors. Timolol (10^{-7}M) caused a significant ($p<0.001$) elevation (by ~ 20%) in the maximal response to NA. (-) isoprenaline (10^{-7}M - 10^{-5}M) caused dose-related rightward shifts of the log dose-response curves to NA and significantly ($p<0.05$) increased the slopes. 10^{-4}M (-) isoprenaline caused a 6-fold shift to the right of the NA dose-response curve with no change in the slope. 10^{-7}M and 10^{-5}M (-) isoprenaline caused a greater depression of the ME dose-response curves than the NA curves with no change in slope. The presence of 10^{-7}M timolol attenuated the depressant effects of 10^{-5}M and 10^{-4}M (-) isoprenaline on the responses of NA. The less active (+) isomer of isoprenaline (10^{-5}M) had no significant effect on the NA-induced responses. The (-) isoprenaline-induced suppression of NA-induced responses and its attenuation by timolol indicates the presence of vasodilatory β-adrenoceptor population. The increased slopes of the (-) isoprenaline-modified NA response curves may be due to the higher doses of NA displacing (-) isoprenaline from the β-adrenoceptor. The slopes of the ME-response curves are not affected by (-) isoprenaline, which indicates that ME is not capable of competing with (-) isoprenaline for the β-adrenoceptor, as is NA.

The results indicate that the mesenteric arterial bed of the rat contains an α-adrenoceptor population which mediates vasoconstriction and a smaller "physiologically antagonistic" β-adrenoceptor population which mediates vasodilatation.

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β-ADRENOCEPTOR-MEDIATED MODULATION OF STIMULATION-INDUCED VASOCONSTRICTION IN PITHED SPONTANEOUSLY HYPERTENSIVE RATS

K.R. Borkowski & P.Quinn. Department of Physiology, The University, Leicester LE1 7RH.

Since Stjärne & Brundin (1975; 1976) have shown a prejunctional β_2-adrenoceptor involvement with adrenaline-induced facilitation of ^{3}H-noradrenaline release, we have attempted to characterise the receptor involved in the adrenaline-induced restoration of stimulation-induced pressor responses in pithed, adrenal demedullated spontaneously hypertensive (SHR) rats. The effects of adrenaline infusion, before and after pretreatment with timolol, and infusions of the β_2-adrenoceptor agonists salbutamol and procaterol on stimulation (0.125 - 4Hz; 30V; 1msec; 15sec) and noradrenaline (0.03 - 10μg, i.v.) induced increases in diastolic blood pressure (DBP) are reported.

Pretreatment of pithed adrenal-demedullated SHR rats with timolol (1mg/kg, i.v., 30 min) did not significantly affect the resting blood pressure, but led to a slight increase in stimulation and noradrenaline (NA) induced increases in DBP, probably due to blockade of vasodilator β_2-adrenoceptors in the vasculature. The subsequent infusion of adrenaline (500ng/animal/min., i.v. in a volume of 5μl/min), in timolol pretreated rats, markedly enhanced NA-induced increases in DBP, while the stimulation-induced increases in DBP were significantly lower than those obtained during adrenaline-infusion alone.

Infusions of salbutamol (50-500ng/animal/min., i.v.) and procaterol (2.5ng/animal/min., i.v.) did not affect the resting blood pressure and did not affect the increases in DBP induced by bolus injections of NA. However, the stimulation-induced increases in DBP were significantly enhanced by each β_2-adrenoceptor agonist. Higher rates of infusion of salbutamol (5μg/animal/min., i.v.) and procaterol (25ng/animal/min., i.v.) attenuated the stimulation-induced increases in DBP. This is possibly due to the activation of vasodilator β_2-adrenoceptors in the vasculature, by salbutamol and procaterol in high concentrations, since the NA-induced increases in DBP were significantly depressed at these high infusion rates.

The blockade of adrenaline-induced enhancement of stimulation-induced pressor responses in pithed, adrenal demedullated rats by timolol suggests that this enhancement involves β-adrenoceptors. Furthermore, since timolol enhanced the effects of adrenaline on NA-induced increases in DBP, yet blocked adrenaline's effect on stimulation-induced responses, it would appear that adrenaline in the vasculature is capable of activating both post-junctional vasodilator β-adrenoceptors and pre-junctional β-adrenoceptors which facilitate sympathetic neurotransmitter release. Moreover, these anatomically distinct β-adrenoceptors would appear to have quite different sensitivities since procaterol and salbutamol were able to enhance stimulation-induced increases in DBP, presumably by activating pre-junctional facilitatory β-adrenoceptors, at infusion rates which had no effect on NA-induced pressor effects.

PQ is an SERC-CASE student in conjunction with ICI plc.

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AN EXAMINATION OF PRESYNAPTIC α-ADRENOCEPTORS IN THE RAT VAS DEFERENS

J.R. Docherty, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2.

Peripheral alpha-adrenoceptors were initially subclassified into $alpha_1$-postsynaptic and $alpha_2$-presynaptic adrenoceptors (Langer, 1974), but it is now known that $alpha_2$-adrenoceptors are also present on vascular smooth muscle cells (Docherty et al., 1979). Recent reports suggest that $alpha_1$-adrenoceptors may be present presynaptically in the pithed rat heart, and these, like the $alpha_2$-receptors, mediate inhibition of neurotransmitter release (Kobinger & Pichler, 1980; Docherty, 1983). The object of the present investigation is to demonstrate these presynaptic $alpha_1$-adrenoceptors under more controlled conditions in the rat isolated vas deferens.

Isometric contractions were obtained to single pulse field stimulation at 5 min intervals (supramaximal voltage, 0.5ms) of epididymal portions of the rat vas deferens. In the absence of nifedipine, the $alpha_2$-agonist xylazine produced a concentration-dependent inhibition of the isometric contraction to a single pulse, but the $alpha_1$-agonists amidephrine and cirazoline produced a concentration-dependent potentiation of the isometric contraction by action at postsynaptic $alpha_1$-adrenoceptors. The calcium entry blocker nifedipine greatly attenuates this postsynaptically-mediated potentiation by $alpha_1$-agonists of nerve-evoked contractions while leaving intact the adrenergic contraction to nerve stimulation (Docherty & McGrath, 1983). The nifedipine-treated epididymal portion is thus suitable for the investigation of the presynaptic effects of $alpha_1$-agonists in the absence of complicating postsynaptic effects.

In the presence of nifedipine (10 μM), xylazine produced a concentration-dependent inhibition of the isometric contraction to a single stimulus pulse with an IC_{50} of 7.76 ± 0.22 (mean & 95% confidence limits, -logM), and this inhibition was antagonised by the $alpha_2$-antagonist rauwolscine (100nM) with a significant shift in the IC_{50} of xylazine to 7.04 ± 0.12 ($P < 0.001$). In the presence of nifedipine (10 μM), amidephrine and cirazoline inhibited nerve-evoked contractions but the concentration-response curves had two components: at low concentrations a steep inhibitory response curve, but at higher concentrations a residual postsynaptic potentiation of nerve-mediated contractions made the upper part of the inhibitory response curve shallow. In the presence of rauwolscine (100nM) the steep inhibitory response curve to low concentrations of amidephrine and cirazoline was not significantly altered, but at higher agonist concentrations the postsynaptic potentiation of nerve-mediated contractions became more prominent.

These results demonstrate two presynaptic effects of $alpha_1$-selective agonists: at low concentrations, an $alpha_1$-mediated inhibition; at high concentrations, a rauwolscine-sensitive $alpha_2$-mediated inhibition. However, since xylazine was more potent than either $alpha_1$-agonist, it is concluded that although $alpha_1$-adrenoceptors are present presynaptically in the rat vas deferens, the predominant presynaptic receptor is $alpha_2$.

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SELECTIVE BLOCKADE OF 5-HYDROXYTRYPTAMINE NEURONAL RECEPTORS BY BENZOIC ACID ESTERS OF TROPINE

J.R. Fozard and M.W. Gittos, Centre de Recherche Merrell International, 16, rue d'Ankara 67084 Strasbourg-Cedex, France.

In an earlier report (Fozard et al.,1979) certain structural features for blockade of the responses mediated through 5-hydroxytryptamine (5-HT) receptors on the terminal sympathetic fibres of the rabbit heart were defined in a series of compounds related structurally to (-)-cocaine. In the present report we extend this work in describing selective blockade of these sites by a number of substituted benzoic acid esters of tropine. In addition, we explore the potency of these compounds as antagonists of two other responses reflecting activation of neuronal 5-HT receptors, the Von Bezold-Jarisch effect of 5-HT in the anaesthetized rat and contraction of the guinea-pig ileum incubated with methysergide.

The techniques to measure 5-HT neuronal receptor blocking activity in rabbit heart and guinea-pig ileum and to quantify antagonism of the Von Bezold-Jarisch effect *in vivo* have been described previously (Fozard et al. 1979; Fozard and Host, 1982).

Table 1. Blockade of 5-HT neuronal receptors by benzoic acid esters of tropine.

Compound	Rabbit heart (pA_2)[a]	Von Bezold-Jarisch (ED50 ; μmol/kg)	Guinea-pig ileum (pD_2')
tropine benzoate	7.19 ± 0.05 (4)	0.22 ± 0.07 (5)	5.04 ± 0.07 (4)
tropine 3-chlorobenzoate	8.63 ± 0.08 (4)	0.12 ± 0.02 (3)	5.57 ± 0.09 (4)
tropine 4-chlorobenzoate	7.00 ± 0.07 (3)	0.98 ± 0.20 (3)	4.96 ± 0.05 (4)
tropine 3,5-dichlorobenzoate	9.27 ± 0.06 (7)	0.12 ± 0.02 (4)	5.39 ± 0.15 (4)
tropine 3-methylbenzoate	8.18 ± 0.04 (3)	0.07 ± 0.01 (4)	5.42 ± 0.11 (3)
tropine 4-methylbenzoate	7.75 ± 0.08 (4)	0.13 ± 0.03 (5)	4.51 ± 0.05 (4)
tropine 3,5-dimethylbenzoate	9.02 ± 0.10 (3)	0.02 ± 0.002 (7)	5.24 ± 0.14 (4)
tropine 3,5-dimethoxybenzoate	8.35 ± 0.06 (13)	0.12 ± 0.01 (3)	4.80 ± 0.15 (3)

Mean values (± s.e.m.) are presented. [a] measured by the method of Schild (1947).

In general, substitution by chlorine, methyl or methoxy in the benzene ring leads to increased antagonist potency at the sympathetic neuronal 5-HT receptor of the rabbit heart (table 1). In each case, blockade was selective in that responses to dimethylphenylpiperazinium iodide (DMPP) were blocked only at high (> μM) concentrations.

All the compounds inhibited the Von Bezold-Jarisch effect of 5-HT at low doses and without affecting responses to submaximal electrical stimulation of the efferent vagus. In contrast, the compounds were only weak, non-selective antagonists of the indirect cholinergic response to 5-HT on the ileum (Table 1).

In conclusion, the selective blockade by a number of benzoic acid esters of tropine of the effects of 5-HT in the rabbit heart and of the Von Bezold-Jarisch effect *in vivo* suggests that the receptors mediating these responses are similar. Significantly, these sites appear to be different from the classical M-receptor of the guinea-pig ileum (Gaddum and Picarelli, 1957) since the compounds are neither potent nor selective antagonists of 5-HT in this tissue.

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THE ACTIONS OF THREE MAMMALIAN SEROTONIN RECEPTOR AGONISTS ON HELIX CENTRAL NEURONES

Anita J. Bokisch, C.R. Gardner+ & R.J. Walker, School of Biochemical and Physiological Sciences, University of Southampton, Southampton SO9 3TU and +Roussel Laboratories Ltd., Swindon, Wiltshire.

Binding studies have shown that there are two distinct serotonin receptors in the mammalian central nervous system, designated $5HT_1$ and $5HT_2$ (Peroutka & Snyder 1979). It appears that these two receptors are physiologically different, with $5HT_1$ receptors being associated with neuronal inhibition, while $5HT_2$ receptors seem to mediate rodent head twitching (Gardner & Guy, 1983) and neuronal excitation (Peroutka & Synder, 1982). In this study we have examined the actions of preferential $5HT_1$ and $5HT_2$ agonists on Helix neurones that are excited or inhibited by serotonin, in order to determine if these serotonin receptors can be classified as $5HT_1$ or $5HT_2$. RU 24969, a potent $5HT_1$ receptor agonist was tested, together with MK 212, a preferential $5HT_2$ agonist and RU 28253 which has a selectivity intermediate between the other two. We also investigated the interactions of these compounds with other putative transmitters in order to assess their specificity.

Intracellular microelectrode recordings were made from identified neurones in the isolated central nervous system of the snail, Helix aspersa. The preparation was as described by Walker (1968) and was mounted in a bath of 5ml vol. Agonists were either ionophoresed onto the cell soma from a second micropipette, or added directly over the preparation in 0.4ml of Ringer. When tested as antagonists the drugs were added to the bath in 1ml of Ringer and allowed to equilibrate for 3 to 5 mins.

On neurones that are excited by serotonin, MK 212 acted as a partial agonist, having an equipotent molar ratio (epmr $\pm$ S.E.M.) of 67$\pm$20 (n=7) and a pA_2 value against serotonin of 5.7$\pm$0.2 (n=5). In contrast, RU 24969 had no clear agonist action but it did produce a small, slow, long lasting depolarisation of the cell at $1\text{-}10\times10^{-4}$M, an effect which was also observed with RU 28253. RU 24969 and RU 28253 also antagonised the excitatory effects of serotonin (pA_2 values 4.5$\pm$0.1 , n=5 and 4.4$\pm$ 0.3, n=5 respectively). Neurones that are inhibited by serotonin were also weakly inhibited by MK 212 and RU 24969, both giving apparent epmrs of >500, although on 3/10 cells the epmr for RU 24969 was <40. Both compounds also antagonised serotonin inhibition with RU 24969 being more potent.

However, all three compounds also antagonised acetylcholine - induced excitation and acetylcholine or dopamine - induced inhibition. The antagonism of serotonin excitation by MK 212 appears to be specific as the pA_2 values for the other agonists effects were lower (4.2-4.6, n=5-6). RU 24969 and RU 28253 show some selectivity in antagonising dopamine inhibition, having pA_2 values of 5.2$\pm$0.1 (n=5) in comparison to values of 4.3-4.7 (n=5-6) for the other agonists. This is consistent with suggested similarities between dopamine and serotonin receptor requirements in Helix. The data presented here suggest that the Helix excitatory serotonin receptor is similar to the mammalian $5HT_2$ receptor with respect to the agonists but not the antagonist properties of the compounds tested.

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LACK OF EFFECT OF CHRONIC METHIOTHEPIN TREATMENT ON 5-HYDROXY-TRYPTAMINE AUTORECEPTORS

R.M. Hagan & I.E. Hughes, Department of Pharmacology, Worsley Medical and Dental Building, University of Leeds, Leeds LS2 9JT.

Chronic exposure to 5-HT receptor blocking drugs affects the number and properties of post synaptic 5-HT receptors in the CNS (Samanin et al., 1980). Since 5-HT autoreceptors might be similarly affected we have determined the effect of acute and chronic administration of the potent central 5-HT receptor antagonist methiothepin on the sensitivity of the 5-HT autoreceptors which regulate electrically evoked 5-HT release from rat hypothalamic slices.

Male Wistar rats were injected twice daily with saline or methiothepin maleate (10mg kg^{-1} per day i.p., in two doses) and were sacrificed 24h after the last injection. Hypothalamic slices (0.4mm) were incubated with 0.1μM ^{3}H-5-HT and then superfused at 0.5ml min^{-1} with modified Krebs' solution containing 5μM chlorimipramine. Each slice was electrically stimulated (50mA, 3Hz, 2ms duration) for 3 periods of 2 min (S_1,S_2,S_3) with 20 min intervals between stimulations. Drugs were added to the superfusion fluid 18min before appropriate stimulation periods. ^{3}H-5-HT accumulation, estimated as mean total tissue ^{3}H content was not significantly altered by chronic methiothepin treatment ($1.72 \pm 0.14 \times 10^5$ d.p.m., m±s.e.mean, n = 24 for saline treatment; $1.89 \pm 0.13 \times 10^5$ d.p.m., n = 33 for methiothepin treatment; $p > 0.2$). Neither acute ($p > 0.05$) nor chronic treatment with methiothepin significantly altered the mean fractional stimulation evoked overflow (s.e.o.) of ^{3}H in the control stimulation period, S_1 ($2.61 \pm 0.22 \times 10^{-2}$, m ± s.e.mean, n = 24 for chronic saline treatment; $2.30 \pm 0.10 \times 10^{-2}$, n = 33 for chronic methiothepin treatment; $p > 0.1$).

None of the pretreatments had any effect on 5-HT autoreceptor sensitivity or function as assessed by (i) the negative $\log_{10}$ of the concentration of exogenous 5-HT required to produce a 40% inhibition of the s.e.o. of ^{3}H (pIC40) and (ii) the increase in the s.e.o. of ^{3}H produced by methiothepin (3μM), expressed as the ratio of the s.e.o. in the presence of methiothepin to that in the initial (control) stimulation period, S_3/S_1.

	In vitro treatment	
In vivo pretreatment	exogenous 5-HT pIC40*	methiothepin (3μM) S_3/S_1
Acute saline (1 day)	7.09 ± 0.30 (25)	1.90 ± 0.28 (6)
Acute methiothepin (1 day)	7.04 ± 0.19 (28)	1.89 ± 0.18 (6)
Chronic saline (21 days)	7.04 ± 0.37 (40)	1.80 ± 0.28 (7)
Chronic methiothepin (21 days)	7.02 ± 0.32 (55)	1.60 ± 0.06 (10)

Values are means ± s.e.mean based on the number of observations indicated.
*Calculated by regression analysis of pooled data.

It is concluded that this long term treatment with methiothepin does not cause changes in the sensitivity or function of 5-HT autoreceptors in rat hypothalamus. Nerve terminal 5-HT autoreceptors and soma-dendritic 5-HT autoreceptors in the dorsal raphé (Blier & de Montigny, 1980) seem more resistant to pharmacological manipulations of serotoninergic transmission *in vivo* than are certain post-synaptic 5-HT sites.

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EFFECTS OF 5-HYDROXYTRYPTOPHAN ON NEUROLEPTIC-INDUCED SUPER-SENSITIVITY IN THE MOUSE

J.E. CUNDEY & J.A. DAVIES. Department of Pharmacology and Therapeutics, Welsh National School of Medicine, Cardiff. CF4 4XN.

There is now appreciable evidence for an interaction between serotonergic and dopaminergic systems in the CNS. Serotonergic agents have been shown to modulate dopamine synthesis (De Belleroche & Bradford, 1980) release (Westfall &Titterman, 1982) and dopaminergic-induced behavioural syndromes (Grabowska and Michaluk, 1974; Davies and Williams, 1983). Long-term treatment with neuroleptic drugs has been shown to induce supersensitivity in dopaminergic receptors (Seeman, et al., 1975) and also to increase 5HT synthesis and turnover (Ratogi et al., 1981). The present study examined the acute and chronic effects of 5-hydroxytryptophan (5HTP) upon spontaneous or apomorphine-induced climbing in normal mice and in mice in which supersensitivity had been induced with chronic neuroleptic treatment.

Male TO mice (WNSM strain) were housed in temperature controlled, sound-proofed cabinets maintained on a 12-hour light-dark cycle. All experimental work was performed between the 2nd and 6th hour of darkness and, where appropriate, neuroleptic-induced supersensitivity was produced with α-flupenthixol (20 mg/l α-FPT) in the drinking water for 10 days. Climbing was assessed using a 20 cm high cylindrical frame made of stainless steel mesh (holes 1 cm^2, wire 1 mm diameter) with a floor area of 100 cm^2. Apomorphine was injected s.c. in distilled water (0.05 ml/10 g body weight) 10 min prior to scoring. 5HTP was given as either a single i.p. injection with a pretreatment time of 60 min or in the drinking water (50 mg/l). The mice were observed at 6 s intervals and the following behaviours were noted:- climbing, rearing, grooming or gnawing. Spontaneous climbing activity was assessed using the same apparatus placed behind coloured film to reduce light intensity.

Apomorphine was shown to reduce spontaneous climbing at low doses (0.01 - 0.25 mg/kg, s.c.) whilst higher doses (0.5-4 mg/kg) induced climbing. Acute administration of 5HTP (10 mg/kg i.p.) attenuated the climbing responses induced by apomorphine over the higher dose range and also reduced spontaneous climbing. Similarly, animals given 5HTP in the drinking water (50 mg/l) for periods up to four days reduced apomorphine-induced climbing but had no effect on spontaneous climbing. Following α-FPT withdrawal 0.25 mg/kg apomorphine, a dose which did not induce climbing in control mice, induced marked climbing activity which reached a maximum between the second and fourth day following α-FPT withdrawal. Pretreatment with 5HTP reduced climbing induced by apomorphine over this period whether the 5HTP was given as a single dose or in the drinking water. Spontaneous climbing activity was also increased following withdrawal of α-FPT and this too was reduced by 5HTP in the drinking water.

The results show that drug-induced and spontaneous climbing behaviour in the mouse can be potentiated by neuroleptic withdrawal, and that the supersensitivity produced by such treatment can be attenuated with relatively low concentrations of 5HTP. A regulatory effect of serotonergic mechanisms on dopaminergic function is implied.

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THE ACTIONS OF QUERCETIN AND RUTIN ON CYCLO-OXYGENASE AND 15-LIPOXYGENASE

W.P. Kingston, Department of Pharmacology, Life Sciences Building, Sunderland Polytechnic, Sunderland SR1 3SD.

Arachidonic acid is a substrate for both cyclo-oxygenase and lipoxygenase enzymes. There is increasing evidence for the involvement of cyclo-oxygenase and lipoxygenase products in allergic and inflammatory conditions. Agents which modulate these two enzymes could possess considerable therapeutic potential. In this investigation the actions of a number of flavonoids on 15-lipoxygenase and cyclo-oxygenase were determined.

Lipoxygenase activity was determined polarographically with an oxygen electrode as described previously (Kingston, 1981). Incubations were performed at 37°C in 100mM phosphate buffer pH7.5 containing 240µM arachidonic acid and 80µg soybean lipoxygenase. The mixture was stirred gently and the change in oxygen saturation of the mixture was recorded for 3 min. The nature of the enzyme inhibition and the inhibitor constant (K_i) were determined as described by Tipton (1980) and Cornish-Bowden (1974). Bovine seminal vesicle microsomes were used as a source of cyclo-oxygenase. Polarographic measurements of enzyme activity were obtained as described by Vanderhoek and Lands (1973). Results represent the mean $\pm$ s.e. mean for 6 determinations performed on 2 occasions.

Quercetin was found to be an uncompetitive inhibitor of both enzymes. The K_i values for 15-lipoxygenase and cyclo-oxygenase were 17.2µM and 532µM respectively. Rutin stimulated cyclo-oxygenase and inhibited lipoxygenase. 500µM rutin produced 23.1 $\pm$ 2.9% stimulation of cyclo-oxygenase. The same concentration elicited a 15.3 $\pm$ 2.4% inhibition of lipoxygenase. A maximum lipoxygenase inhibition of 40.1 $\pm$ 3.4% was obtained with 2mM rutin. Naringenin, hesperitin and hesperidin had no effect on either enzyme.

Quercetin was the most potent of the flavonoids tested. The K_i for cyclo-oxygenase was 31 times that for 15-lipoxygenase. Quercetin compares favourably with 5,8,11- Eicosatriynoic acid which Hammarstrom (1977) reported to inhibit platelet lipoxygenase and cyclo-oxygenase. The ID_{50} of this compound for lipoxygenase is 24µM and that for cyclo-oxygenase is 340µM.

These studies suggest that Quercetin (a natural compound) may have therapeutic potential.

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EFFECT OF TWO RETINOIDS ON EXUDATE VOLUME AND LEUCOCYTE ACCUMULATION IN A PLEURAL INFLAMMATORY RESPONSE IN THE RAT

D. Bradshaw, B.B. Dodge and P.H. Franz, Department of Pharmacology, Roche Products Ltd., Welwyn Garden City, Hertfordshire AL7 3AY.

Etretinate and isotretinoin are members of a class of vitamin A derivatives, known collectively as retinoids, which are highly effective in the treatment of a variety of dermatological disorders. Retinoids have been reported to reduce leucocyte migration (Fritsch, 1981; Dubertret et al., 1982) and to have a beneficial effect in both an experimental inflammation in man (Plewig and Wagner, 1981) and psoriatic arthropathy (Stollenwerk et al., 1981). This suggests that a further indication for retinoids may be in the treatment of inflammatory/arthritic disease. In the present study we have investigated the effects of etretinate and isotretinoin in an acute inflammatory response induced by the injection of an irritant into the pleural cavity of the rat.

Etretinate was formulated either as a colloid-milled suspension in 7.5% succinylated gelatin or as a suspension in arachis oil. Isotretinoin was formulated as a suspension in arachis oil. Both compounds were administered orally, once daily, to female Alderley Park strain 1 rats (180-200g) for a period of 10 days. Control animals received the corresponding vehicle. One hour after the final dose of the compounds 0.2 ml of 1% lambda carrageenan in saline was injected into the pleural cavity of each animal. Four hours later the pleural exudate was recovered and its volume was measured. Total and differential counts were performed on the cells contained within the exudate. Animal body weights were monitored daily.

The colloid-milled formulation of etretinate, at doses of 5, 15 and 45 $mg.kg^{-1}$ day^{-1}, had no significant effect on cell accumulation within the pleural cavity. At a dose of 45 $mg.kg^{-1}day^{-1}$ exudate volume was inhibited by 29% ($P<0.05$) and at this dose the increase in body weight of the animals was also significantly reduced. As a suspension in arachis oil, etretinate produced a dose-dependent inhibition of exudate formation (34%, $P<0.05$ at 45 $mg.kg^{-1}$). At doses of 5, 15 and 45 $mg.kg^{-1}$ a significant inhibition of PMN accumulation was observed. There was no effect on MN accumulation. The increase in body weight of the animals was reduced to a greater extent with the arachis oil formulation than with the colloid-milled formulation.

Isotretinoin, at doses of 5, 15, 45 and 135 $mg.kg^{-1}day^{-1}$ for 10 days, inhibited the formation of exudate although the effect was statistically significant only for doses of 15 and 45 $mg.kg^{-1}$ (41%, $P<0.01$ at 45 $mg.kg^{-1}$). There was a dose-dependent inhibition of both PMN and MN accumulation which was statistically significant only for the highest dose (PMN, 34%, $P<0.05$; MN, 37%, $P<0.05$). Isotretinoin showed little or no effect on the increase in body weight of the animals.

The results with etretinate indicate that formulation is a major determinant of biological activity for this type of compound. When formulated in arachis oil, etretinate and isotretinoin reduced both the exudative and cellular components of a pleural inflammation. Isotretinoin differed from etretinate in being less toxic, as judged by changes in body weight, and in showing little, if any, selectivity for PMN accumulation. These activities of the two retinoids may be relevant to their effectiveness in dermatological disease and suggest further that retinoids may be of some benefit in inflammatory conditions.

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CHARACTERIZATION OF Na^+,K^+-ATPase IN DIFFERENT CELL TYPES FROM THE RAT CNS

C.K. Atterwill and R. Balazs, MRC Developmental Neurobiology Unit, 33 John's Mews, London WC1N 2NS

Brain Na^+,K^+-ATPase exhibits heterogeneity with respect to its interaction with ouabain suggesting the presence of more than one enzyme form (Marks & Seeds, 1978). Furthermore, Sweadner (1979) has demonstrated different molecular forms of the enzyme in cultured glia (α form: low ouabain affinity) and axolemma (α+form: high ouabain affinity). However, it is still uncertain whether central neurones and glia possess different forms of Na^+,K^+-ATPase because of the lack of suitable test preparations. We have, therefore, studied the ouabain sensitivity of Na^+,K^+-ATPase from both isolated and cultured cerebellar neurones and glia with a view to clarifying this problem.

Granule neurones, Purkinje cells and astrocytes were separated from the cerebellum (CBL) of 8 day old rats by unit gravity sedimentation (see Cohen et al, 1979). Enriched cultures of granule cells and astrocytes were also grown from the CBL in DMEM containing 10% foetal calf serum (see Atterwill et al, 1983). Na^+,K^+-ATPase activity was measured in membrane fractions at varying ouabain concentrations using a radiometric assay. For comparison, membrane preparations from immature and mature rat CBL, and their respective P2 fractions, as well as from rat kidney, were also examined. In agreement with previous reports (Medzihradsky et al, 1971; Kimelberg et al, 1978) the Na^+,K^+-ATPase activity in freshly-isolated astrocytes was significantly higher (3-fold) than in neuronal perikarya. In contrast, in the primary cell cultures after 14 DIV enzyme activity was 2-fold greater in neurones than in astrocytes.

The ouabain-inhibition profile of the enzyme indicated a heterogenous interaction both in whole CBL and the respective P2 fractions, and in a total cell suspension from the CBL. Computer-fitting of the data showed two components of the Na^+,K^+-ATPase activity: a 'high affinity' component ($K_i=10^{-7}$M approx, 60-75% of total) together with a smaller 'low-affinity' component ($K_i=10^{-4}$M approx). There appeared to be no difference in the inhibition of the Na^+,K^+-ATPase by various concentrations of ouabain between freshly-isolated granule, purkinje or astrocyte perikarya. However, cultured astrocyte membranes possessed predominantly a Na^+,K^+-ATPase form with low ouabain affinity (73% of total), similar to that present exclusively in rat kidney. In contrast neuronal cultures from the CBL at 14 DIV exhibited less of the low-affinity enzyme form (55% of total). Other differences in the properties of the enzyme from the various cell types were also noted. The Na^+,K^+-ATPase from cultured astrocytes was maximally activated by a lower K^+ concentration (1mM) than the enzyme from the cultured neurones (15-20mM $[K^+]$). An attempt was made to estimate Na^+,K^+-ATPase activity and its relation to ouabain in K^+-depleted 'live' cells using ^{86}Rb uptake. In these experiments the ouabain sensitivity appeared to be similar between the cultured cell types (see also Walz & Hertz, 1982).

As far as the purity of our preparations allows we conclude that although there are differences in the proportion of the two Na^+,K^+-ATPase forms in cultured CBL neurones and glia along with differences in potassium affinity, both cultured and freshly isolated cell types appear to have both forms of the enzyme present. Cultured cells present a useful model for studying Na^+,K^+-ATPase characteristics during development.

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CALCITONIN ANTINOCICEPTION AND SEROTONERGIC TRANSMISSION

R.F.L. Bates, G.A. Buckley, R.M. Eglen and C.A. McArdle, Department of Life Sciences, Trent Polytechnic, Nottingham.

Morphine antinociception in mice can be antagonised by inhibition of serotonin synthesis with p-chlorophenylalanine (pCPA) (Fennessy and Lee, 1970), and potentiated by intracerebral (i.c.) administration of serotonin (Sewell and Spencer, 1975). Much evidence of this nature exists linking opiate antinociception with central serotonergic systems. We have investigated the possible involvement of serotonin in the central antinociceptive action of salmon calcitonin (SCT) in the abdominal constriction test (Bates et al, 1981).

Groups of mice (CFLP, ♂, ♀, 25-30g) were treated with vehicle or pCPA (300 mg.Kg^{-1} S.C. in 0.9% NaCl with 0.05% polyoxyethylene-4-lauryl ether, pH 7.4) 72, 48 and 24 hours prior to test. On the day of test the mice were given SCT (0.01 - 50 IU, Kg^{-1}) or serotonin (0.5 - 10 µg/mouse) in 10µl of vehicle (50mM Tris, 100mM NaCl in 1% bovine serum albumin at pH 7.4) using the i.c. injection technique of Haley and McCormick (1957). Ten minutes later the mice received, i.p., 0.3 ml of a solution containing 1% acetic acid in 0.9% NaCl and the abdominal constriction rate was counted between the 10th and 14th minutes after injection.

The statistical validity of the results was assessed using analysis of variance and Student's 't' test. In all cases groups of mice (6-10) under test were compaired to an internal control group receiving the appropriate vehicle.

The abdominal constriction rate after treatment with vehicle (i.p. and i.c.) was 3.98 ± 0.26 ($\bar{x}$ ± s.e., n = 23; pooled controls) constrictions.min^{-1}. Pretreatment with pCPA had no significant effect on the abdominal constriction rate (4.12 ± 0.22, n = 24), whereas, SCT (0.1, 1, 2, 10, 50 IU.Kg^{-1}) produced a dose dependent reduction of the abdominal constriction rate (23.6 ± 7.6%, 38.1 ± 4.8%, 45.4 ± 8.5%, 41.3 ± 7.8%, 33.0 ± 6.7% respectively, n = 6-9, $P < 0.025$). After pretreatment with pCPA, SCT (i.c.) produced no significant reduction in abdominal constriction rate at 0.1, 1, 2 or 50 IU.Kg^{-1}, although at 10 IU.Kg^{-1} SCT, the constriction rate was reduced by 19.9 ± 6.1%, n = 7, $P < 0.05$. Intracerebral serotonin (1, 5, 10 µg/mouse) caused a dose dependent reduction in abdominal constriction rate (32.3 ± 10.0%, 49.2 ± 10.3%, 56.7 ± 10.4% respectively, n = 9-10, $P < 0.025$) which was not antagonised by pretreatment with pCPA. Furthermore, simultaneous i.c. administration of serotonin (10 µg/mouse) and SCT (2 IU.Kg^{-1}) caused a reduction in abdominal constriction rate (86.6 ± 4.0%, n = 9) which was significantly ($P < 0.02$) greater than the effect observed with either serotonin (56.7 ± 10.4, n = 10) or SCT (39.8 ± 9.5%, n = 10) alone. In addition the antagonism of SCT antinociception by pCPA was not apparent when serotonin (1 or 10 µg/mouse) was injected simultaneously with the SCT (2 IU.Kg^{-1}).

These results suggest that central serotonergic systems may be involved in the antinociceptive action of SCT.

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RELATIONSHIP BETWEEN CYCLIC AMP AND INCIDENCE OF FIBRILLATION UPON REPERFUSION AFTER VARYING PERIODS OF ISCHAEMIA

R. Crome, D.J. Hearse and A.S. Manning. The Heart Research Unit, The Rayne Institute, St. Thomas' Hospital, London, SE1, UK.

Interest in reperfusion arrhythmias which may occur following successful thrombolytic procedures, after the release of coronary artery spasm and during cardiac surgery, has led us to investigate the cellular mechanisms responsible for reperfusion arrhythmias. We have correlated the duration of ischaemia **prior** to reperfusion with the incidence of ventricular fibrillation **after** reperfusion and have also assessed the relationship between cellular cyclic AMP content and the incidence of ventricular fibrillation upon reperfusion. For this study we have used an isolated 'working' rat heart preparation with temporary coronary artery occlusion. Table 1 shows that a bell-shaped time-response curve was obtained such that short periods of coronary artery occlusion (1-3 min) did not produce ventricular fibrillation upon reperfusion but longer periods increased the incidence, so that after 15 min coronary artery occlusion over 95% hearts fibrillated. Increasing the time of coronary occlusion beyond 15 min decreased the incidence of fibrillation, so that after 30 min coronary artery occlusion only 10% hearts fibrillated.

Table 1 Relationship between duration of coronary occlusion, cellular cyclic AMP content and the incidence of fibrillation upon reperfusion (*indicates $P < 0.01$ compared to pre-occlusion values)

Time of Coronary Artery Occlusion (min)	(n)	% Incidence of fibrillation upon reperfusion	Cyclic AMP Content within Ischaemic Zone (nmol/g dry wt)
0	(8)		4.1 ± 0.3
1	(12)	0	5.5 ± 0.4*
3	(12)	0	5.0 ± 0.7
5	(12)	33	4.5 ± 0.6
15	(34)	97	2.4 ± 0.3*
25	(10)	30	-
30	(10)	10	-

Cyclic AMP content within the ischaemic zone increased dramatically ($p < 0.01$) during the first minute of coronary occlusion (when the incidence of fibrillation upon reperfusion was low) but fell significantly at the time of highest incidence of fibrillation upon reperfusion (ie 15 min occlusion). In contrast, cellular cyclic AMP levels **during** the reperfusion phase, almost doubled (2.4 ± 0.3 to 4.6 ± 0.6 nmol/g dry wt; $p < 0.01$) when hearts were reperfused at the time when incidence of fibrillation upon reperfusion was highest. When hearts were reperfused at the time of lowest incidence of fibrillation upon reperfusion (i.e. 1 min occlusion) cyclic AMP levels remained unaltered.

Thus, the incidence of reperfusion-induced fibrillation is highly dependent on the duration of the previous period of ischaemia and in this model, the changes in cellular cyclic AMP content **during reperfusion** cannot be dissociated from the incidence of fibrillation upon reperfusion.

AUTORADIOGRAPHIC LOCALISATION OF β-ADRENOCEPTORS IN GUINEA-PIG AIRWAYS

H. Carswell & S.R. Nahorski, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, Leicester. LE1 7RH. U.K.

Previous studies using the radioligands ^{125}I-(-)-cyanopindolol (^{125}I-CYP) and ^{3}H-(-)-dihydroalprenolol (^{3}H-DHA) have directly revealed a high density of beta-adrenoceptors in homogenates prepared from various areas of guinea-pig airways (Carswell & Nahorski, 1982). Most attempts at determining the precise location of beta-adrenoceptors have, in the past, relied upon an assessment of responses produced by activation of the receptor, such as relaxation of isolated airway preparations. However, the results of some of these recent studies indicate that only a proportion of the receptors identified in homogenates are located on airway smooth muscle (Carswell & Nahorski, 1982). In the present study we have utilised light-microscope autoradiographic techniques on lung sections in vitro to identify a more precise localisation for beta-adrenoceptors.

Lungs from male guinea-pigs (250-300 g) were inflated with O.C.T. (embedding fluid diluted 1:3 with buffered saline), frozen in liquid nitrogen, and cut into sections 6-12μ thick using a cryostat. Sections were then mounted onto subbed microscope slides and stored dessicated for 24 hours at 4°C. Incubations with ^{125}I-CYP were performed in 140 mM NaCl/50 mM Tris-HCl/2 mM ascorbate buffer (pH 7.8) at 37°C for 2 hours, and with ^{3}H-DHA in 50 mM Tris-HCl (pH 7.8) at 22°C for 30 minutes. Separate sections were also incubated with each ligand in the presence of 200 μM (-)-isoprenaline to determine non-specific binding which accounted for less than 2% total ^{125}I-CYP binding and 20% total ^{3}H-DHA binding. After washing, the sections were thoroughly dried before being apposed to cover-slips coated in Ilford K_5 Nuclear emulsion and exposed, dessicated at 4°C for various periods dependent on the ligand used.

The specific binding characteristics of both ^{125}I-CYP and ^{3}H-DHA to tissue sections were virtually identical to those obtained in washed homogenates with respect to dissociation constant, stereoselectivity towards the isomers of propranolol and the potency order for the beta-agonists isoprenaline, adrenaline and noradrenaline. A heterogeneous mixture of $beta_1$ and $beta_2$ adrenoceptors was also observed in tissue sections since the subtype-selective antagonists atenolol ($beta_1$) and ICI 118.551 ($beta_2$) displaced radioligand binding, generating curves that clearly deviate from mass action behaviour. Computer-assisted curve fitting revealed that the relative proportion of $beta_1$:$beta_2$ adrenoceptors was 18%:82%, which is very similar to the ratio of 15%:85% found in lung homogenates (Carswell & Nahorski, 1982).

The actual location of specific radioactive grains determined by light-microscope autoradiography, was similar to that very recently described for beta-adrenoceptors in the ferret lung by Barnes et al (1982). There appears to be a general distribution of beta-adrenoceptors throughout the lung, with most structures showing some specific labelling including airway smooth muscle and, in particular, the alveoli. There was, however, very little specific labelling of the smooth muscle in blood vessels. The same pattern of distribution was seen with both ^{125}I-CYP and ^{3}H-DHA, and although there is a slight loss of resolution with ^{125}I-CYP due to the higher energy of the radioactivity emitted, it does have a much slower dissociation rate from the receptor and the advantage that exposure times can be reduced to between 1 and 5 days, compared to the 4-10 weeks required with ^{3}H-DHA.

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CHARACTERISATION OF HUMAN PLATELET α_2-ADRENOCEPTORS BY (^{3}H)-YOHIMBINE BINDING, AGGREGATION AND ADENYLATE CYCLASE INHIBITION

J. Arroyo, J. Bergerhausen, O.-E. Brodde and N. O'Hara (introduced by S.R. Nahorski), Division of Renal & Hypertensive Diseases, Med. Klinik and Poliklinik, University of Essen, D-4300 Essen, Fed. Rep. Germany.

Human platelets containing α_2-adrenoceptors (R), which mediate aggregation (Grant & Scrutton, 1979) and inhibition of basal and PGE_1-stimulated adenylate cyclase (AC) (Jakobs et al, 1976), are suitable tissues to study α-R changes in the human being under various (patho)-physiological conditions. Recently it has been shown that platelet α_2-R can be quantitatively assessed by binding with the α_2-R antagonist ^{3}H-yohimbine (Brodde et al, 1982; Cheung et al, 1982). In the present study we have compared the efficacy and/or affinity of several α-R agonists and antagonists in these three systems to find out whether the physiological responses (aggregation, AC-inhibition) correlate with results from binding studies.

^{3}H-Yohimbine binding to platelet membranes was performed as recently described (Brodde et al, 1982). Inhibition of PGE_1 (10 µM)-stimulated AC was determined according to Jakobs et al (1976). Platelet aggregation was monitored in platelet-rich plasma at 37° in an ELVI-aggregometer. For quantification of the effects of the α-adrenergic drugs the slope of the initial linear decrease in optical density (primary aggregation) was evaluated (Lasch & Jakobs, 1979).

Adrenaline (ADR) and noradrenaline (NA) were full agonists in inducing aggregation and AC-inhibition. In both systems K_{act}-values for ADR (1-2 µM) and NA (3-6 µM) were similar and comparable with K_i-values for inhibition of ^{3}H-yohimbine binding (ADR: 0.5 µM; NA: 0.95 µM). Clonidine and guanfacine did not cause aggregation or AC-inhibition, but antagonised ADR-responses. K_i-values for clonidine and guanfacine, as well as for 5 α-R antagonists investigated, are given in Table 1.

Table 1 K_i-values (nM) of α-R drugs for inhibition of:

α-R Drugs	^{3}H-Yoh-Binding	ADR (1 µM)-induced aggregation	ADR (10 µM)-induced AC-inhibition
Clonidine	43 ± 4.1	136.0 ± 37	175.2 ± 27
Guanfacine	75 ± 8.3	592.0 ± 188	585.5 ± 77
Phentolamine	20 ± 0.2	27.3 ± 3.8	11.5 ± 2.3
Yohimbine	3 ± 0.16	98.7 ± 8.9	2.3 ± 0.5
Rauwolscine	2 ± 0.2	57.0 ± 12.6	1.8 ± 0.2
Prazosin	1500 ± 123	n.d.	n.d.
Corynanthine	990 ± 76	n.d.	n.d.

n.d. = not determinable, since effects were less than 50% at 10^{-5} M.

Since the rank order of potency for the α-R drugs (ADR ≥ NA, yohimbine, rauwolscine >> prazosin, corynanthine) was the same in all three systems, it is concluded that aggregation and/or AC-inhibition may be taken as physiological correlate to data obtained by binding studies in human platelets.

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THE RETENTION AND DISTRIBUTION OF DUAL-LABELLED LIPOSOMES INJECTED INTO ARTHRITIC JOINTS

W.C. Foong & K.L. Green, School of Pharmacy, Portsmouth Polytechnic, Portsmouth, PO1 2DT.

Liposomes are artificial phospholipid vesicles which closely resemble biomembranes and have been advocated for use as drug carriers. Dingle et al (1978) have shown that the entrapment of corticosteroids in liposomes greatly enhances their anti-inflammatory effect when injected into arthritic joints. We have investigated the possibility of entrapping cytotoxic drugs in liposomes for intra-articular injection to inhibit the proliferation of synovial tissue in inflamed joints. Methotrexate (MTX) was selected for this study because it is used clinically to treat synovitis (Wigginton et al, 1980) and because if necessary its effects can be reversed by the administration of folinic acid derivatives.

Dual-labelled multilamellar liposomes (average diameter 1 μm) containing ^{3}H-MTX were prepared from egg phosphatidylcholine, ^{14}C-cholesterol oleate and dicetyl-phosphate in a molar ratio of 5:5:1. A chronic arthritis was induced in the knee joints of rabbits using the procedure described by Consden et al (1971). 3 weeks after the induction of arthritis the joints were injected with either free or liposome-entrapped ^{3}H-MTX. Peak plasma levels of ^{3}H-MTX (6.80 ± 0.24% amount injected) occurred 1 h after the intra-articular injection of free ^{3}H-MTX, in agreement with findings in arthritic patients (Bird et al, 1977). When liposome-entrapped ^{3}H-MTX was injected, plasma levels of ^{3}H increased more rapidly than ^{14}C, suggesting that substantial amounts of ^{3}H-MTX leaked out of the liposomes within the first few minutes of injection.

The rabbits were killed 4h, 24h, 3 days or 7 days after injection and the distribution of ^{3}H and ^{14}C in the injected joints and various other tissues determined. The administration of ^{3}H-MTX in liposome-entrapped form greatly enhanced its retention in the joint (Table 1). The concentration of ^{3}H-MTX was highest in the synovial membrane, specific radioactivity of the synovium being 5 and 9 times greater than the meniscus cartilage, 24h and 7 days after injection respectively. There was no significant difference between the retention of ^{3}H and ^{14}C in the joint tissues. It seems likely that the synovial tissues incorporated the liposomal constituents mainly by endocytosis and/or fusion.

Table 1 Recovery of ^{3}H-MTX from arthritic joints expressed as a percentage of the amount injected (mean ± s.e.mean).

	Time after injection	Synovial fluid	Synovial membrane	Total recovered from joint	n
Free MTX	4h	3.18 ± 0.55	0.23 ± 0.08	3.74 ± 0.73	4
	24h	0.04 ± <0.01	0.01 ± <0.01	0.13 ± 0.02	3
Liposomal MTX	4h	46.45 ± 4.60	1.23 ± 0.30	48.66 ± 4.85	6
	24h	41.38 ± 2.93	1.18 ± 0.30	45.58 ± 2.80	4
	3 days	25.75 ± 2.43	2.18 ± 0.42	30.15 ± 3.20	4
	7 days	4.66 ± 0.90	2.73 ± 0.53	9.68 ± 1.40	4

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5-HYDROXYTRYPTAMINE RESPONSES OF MURINE NEUROBLASTOMA CELLS: IONS AND PUTATIVE ANTAGONISTS

J.A. Peters and P.N.R. Usherwood, Department of Zoology, Nottingham University, University Park, Nottingham NG7 2RD

Cells of the neuroblastoma clonal line NIE-115 (Amano, et al, 1972) respond to microperfused and ionophoretically applied 5-hydroxytryptamine (5-HT) with a membrane depolarisation and an associated conductance increase which displays rapid tachyphylaxis (Guharay & Usherwood, 1981). Differentiation of the cells by maintenance in 2% (vol/vol) dimethylsulphoxide (Khimi et al, 1976) was not a prerequisite for expression of the 5-HT response. 5-HT (10μM) microperfused using pressure ejection from a coarse (20-30 μm tip diameter) micropipette depolarised 80% (n=109) of undifferentiated cells and 73% (n=78) of cells differentiated by incubation with dimethylsulphoxide for twelve days.

The reversal value of the 5-HT induced depolarisation determined by interpolation was -8.6mV ± 2.3 (S.D.) (n=26). In chloride-free saline a similar reversal potential (E_R) of -10.0mV ± 3.5 (n=11) was obtained suggesting that chloride is not involved in the 5-HT response. Reducing the external sodium concentration $[Na]_o$ shifted the 5-HT reversal potential to more negative values whereas elevation of $[Na]_o$ produced shifts in the opposite direction. At standard $[Na]_o$ of 110mM changes in the external potassium concentration $[K]_o$ had no effect on E_R. When $[Na]_o$ was reduced to 30mM, however, increases in $[K]_o$ caused positive shifts in E_R whereas decreases in $[K]_o$ made E_R more negative. Variations of the external calcium concentration $[Ca]_o$ had no effect on E_R. These results can be quantitatively described by the Goldman-Hodgkin-Katz equation for a calculated pNa/pK ratio of 1.02. This suggests that the depolarisation of NIE-115 cells elicited by 5-HT results from a simultaneous increase in permeability to Na and K as has been observed in autonomic ganglia during application of this putative transmitter (Higashi & Nishi, 1982).

Reducing $[Ca]_o$ from standard 1.8mM to 0.18mM enhanced the 5-HT depolarization to microperfusion and ionophoresis by 60% over control. This potentiation was fully reversible and occurred in the absence of any significant changes in the passive properties of the cell membrane. Although reducing $[Ca]_o$ sometimes slightly decreased the rate of desensitization onset this effect was unpredictable. Possibly Ca blocks the channel gated by 5-HT in these cells (Kato & Narahashi, 1982).

A number of possible antagonists, competitive and non-competitive, were tested. Quipazine [2-(1-piperazinyl) quinoline maleate] and d-tubocurarine chloride at 100nM completely blocked depolarisations to ionophoretically applied 5-HT in a fully reversible manner. Atropine and metergoline also completely blocked the 5-HT response but at a higher concentration (10μM). The latter, however, elicited depolarisations similar to those produced by 5-HT itself and its possible role as an antagonist is doubtful.

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REVERSIBLE AND IRREVERSIBLE ACTIONS OF DIHYDROAVERMECTIN B1a ON GABA MEDIATED RESPONSES IN INSECT MUSCLE

I.R. Duce and R.H. Scott (introduced by P.N.R. Usherwood), Department of Zoology, Nottingham University, University Park, Nottingham NG7 2RD.

Avermectin B1a (AVM) is a polycyclic lactone with broad spectrum anthelminthic and insecticidal activities (Egerton et al, 1979; Ostlind et al, 1979). AVM irreversibly blocks inhibitory post-synaptic potentials in the stretcher muscle of the lobster walking leg (Fritz et al, 1979) and paralyses the parasitic nematode Ascaris suum without causing flaccid paralysis or hypercontraction (Kass, 1982). The action of AVM has been suggested to involve irreversible increases in Cl^- permeability (Fritz et al, 1979; Mellin et al, 1981).

GABA (10^{-3}M) induced an increase in input conductance of 17.7×10^{-7}S $\pm$ 2.03 (mean $\pm$ SE n=35) when applied to muscle bundle 33 of the extensor tibiae of the locust metathoracic leg. Experiments with Cl^- free saline confirmed the results of many previous authors that GABA induces an increase in Cl^- permeability.

Microperfusion of 22, 23, dihydroavermectin B1a (DHAVM) induced reversible dose dependent increases in input conductance at concentrations between 0.000075 µg/ml (9×10^{-11}M) and 0.0075 µg/ml. DHAVM (0.0075 µg/ml) caused an increase in input conductance of 2.6×10^{-7}S $\pm$ 0.35 (n=6) during a 2 min application, which returned to control levels after $1\frac{1}{2}$ mins in normal saline. Doses of DHAVM which induced a reversible increase in Cl^- conductance caused a dose dependent inhibition of the GABA response.

Higher doses of DHAVM (0.01 µg/ml to 1 µg/ml) induced irreversible increases in conductance. Microperfusion of 0.1 µg/ml DHAVM caused a change in input conductance of 33×10^{-7}S $\pm$ 6.91 (n=7) after 5 minutes. However, this increase continued during washing and reached 56×10^{-7}S $\pm$ 9.7 after 1 hour. Other doses of DHAVM also initiated irreversible conductance increases which continued during washing. When GABA was applied to a fibre in which DHAVM had already irreversibly increased Cl^- permeability the GABA response was enhanced. This enhancement did not change despite the continued slow increase in input conductance following DHAVM. Application of a second dose of DHAVM during the GABA response caused a decrease in input conductance. Thus DHAVM may interact with Cl^- channels reversibly activated by GABA and or Cl^- channels irreversibly activated by the initial application of DHAVM.

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SENSITIVITY TO KAINIC ACID OF LOCUST (SCHISTOCERCA GREGARIA) VENTRAL NERVE CORD

R.H. Evans & J.N.P. Kirkpatrick, Department of Pharmacology, Medical School, University Walk, Bristol, BS8 1TD

The glutamate analogue kainic acid has negligible excitant action at arthropod muscle fibres but abolishes righting in cockroaches with an ED_{50} of 43 µmole/Kg body weight suggesting the possibility of a neurotoxic action of this amino acid in insects (Castle & Evans, 1982). The present results support this suggestion.

Supramaximal electrical stimuli (0.2 m.sec pulses at 0.25 Hz) were applied to the connectives supplying the first thoracic ganglion of desheathed nerve cords. Evoked potentials and the d.c. level of nerve fibres were recorded from the abdominal connectives across a grease seal (Brookhart & Fadiga, 1960). The preparations were bathed in physiological saline (Hoyle, 1953) at room temperature (18-22^{o}C). Kainate (0.1-2mM) produced reversible, concentration-dependent depolarizing shifts in d.c. level. At the higher concentrations evoked potentials were abolished. N-Methyl-D-aspartate (NMA), quisqualate and dihydrokainate were inactive at concentrations up to 2 mM and L-glutamate was inactive at concentrations up to 20 mM. Domoic acid, a higher homologue of kainate with greater potency in the vertebrate nervous system (Biscoe et al., 1976), was found to have a mean depolarizing potency of 46.3 ± 3.2 (S.D.) times that of kainate on three preparations.

The lack of effect of NMA and quisqualate suggests that receptors for these agonists may be absent from the insect nervous system whereas the lack of effect of dihydrokainate on nerve cords suggests the presence of kainate receptors similar to those in the vertebrate (Watkins & Evans 1981) rather than the annelid (James et al., 1980) nervous system.

Previous work (Shinozaki & Shibuya, 1974) suggests that excitatory amino acid receptors at arthropod skeletal muscle fibres are of the quisqualate type. The present results suggest that a different receptor type may be present in the nervous system of arthropods.

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A RAPID IN VIVO ELECTROCHEMICAL ASSAY OF EXTRACELLULAR ASCORBIC ACID

Z.L. Kruk and J.A. Stamford, Department of Pharmacology and Therapeutics, The London Hospital Medical College, Turner Street, London E1 2AD.

Ascorbic acid has been shown to be released from nervous tissue by depolarising stimuli (Milby et al, 1981). It may modulate dopaminergic mechanisms by inhibiting dopamine binding to striatal membranes and can antagonise dopamine mediated behaviours (Heikkila et al, 1981). Autoradiographic and tissue punch analyses show that it has a heterogeneous distribution within the CNS (Hammarstrom, 1966; Milby et al, 1982) but these assays do not distinguish between intracellular and extracellular stores. Measurement of extracellular ascorbic acid concentration may be of use in establishing its neuronal functions.

High speed cyclic voltammetry (Armstrong-James et al, 1981) has been used for quantification of electroactive compounds applied by pressure ejection or iontophoresis. We report here pressure ejection of ascorbate oxidase in vivo from multibarrel carbon fibre microelectrodes as a technique for quantifying ascorbic acid.

Ascorbate oxidase (Boehringer) was dissolved in physiological saline (1000 units ml^{-1}). The enzyme catalyses the oxidation of electroactive ascorbic acid to the electrochemically inert dehydroascorbic acid. Ascorbate oxidase did not affect the electrochemical signals to dopamine, serotonin, DOPAC or 5HIAA in vitro. During use in vivo, small volumes of enzyme (estimated to be approximately 500 picolitres) were ejected by application of 35-70 kPa for 30 seconds.

Rats (280-320 g male Sprague-Dawley) were anaesthetised with chloral hydrate or urethane. Micro-application of ascorbate oxidase in the cerebral cortex or striatum caused a rapid fall in the electrochemical signal as ascorbic acid was destroyed in the extracellular fluid.

Neither saline vehicle nor heat-denatured ascorbate oxidase had an effect on the endogenous electrochemical signals. By comparing with calibration curves prepared in vitro, it was possible to calculate the extracellular ascorbic acid concentration in vivo. This was found to be 150-500 μM. The assay allows measurements to be made in vivo at narrowly separated sites.

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HPLC MEASUREMENTS OF THE RELEASE OF ENDOGENOUS AMINO ACIDS FROM RAT BRAIN IN VIVO

R.A. Webster & K. Wilson. Department of Pharmacology, University College, Gower St. London, WC1E 6BT.

High pressure liquid chromatography (HPLC) is being used increasingly to measure a number of endogenous substances and neurotransmitter candidates. Most of the existing methods of amino acid (AA) analysis by HPLC can be divided into two broad categories: a) Those measuring comparatively high AA levels (nmol ml^{-1} or $\mu mol\ g^{-1}$) in CSF and blood samples (Turnell and Cooper 1982) or in brain extracts (Chapman et al 1982). b) Those which measure the release of endogenous AAs but limit detection to 2 or 3 AAs (Van der Heyden et al 1979), or the release of AAs from in vitro preparations (Hamberger et al 1982).

The aim of our work was to design a procedure which could be used to measure the release of a range of endogenous AAs into perfusates from rat brain *in vivo* collected in cortical cups and push-pull cannulae. The HPLC system used was a Varian 5000. To optimise detection and resolution of AA peaks at pmol levels we have used pre-column derivitization of the AAs with orthophthalaldehyde and subsequent separation on a reverse-phase 'Micropak MCH-5' column followed by fluorescence detection (Varian Fluorichrom). The solvents were a modification of those used by Turnell and Cooper (1982). Solvent A contained water, sodium propionate and acetonitrile (72:20:8) at pH 6.9; solvent B contained water, acetonitrile and methanol (42:30:28) plus 1ml propionic acid per 100ml, at pH 3.8. The gradient programme was, time in minutes, (% solvent B) 0(0), 10(38), 24(38), 25(70), 30(100), 38(100), 42(0). The flow rate was 0.5ml min^{-1} with the column at 37°C. The derivatized samples were injected onto the column in a 50µl volume. It was possible to separate the following AAs: aspartate, glutamate, serine, glutamine, glycine, taurine, alanine and GABA, their fluorescence being linear over a concentration range of 2-5000 pmols. Additionally, lysine, tryptophan, phenylalanine, methionine and tyrosine have been separated.

When brain perfusates were analysed, peak separation became distorted due to contaminants in the samples which reduced the efficiency of the column and rendered it inoperable at the extremely high sensitivity required. Although we could not detect protein in the samples chemically, using the Lowry method, trace amounts of this and other substances had accumulated on the column to contribute to its loss of efficiency. Contaminants could not be removed adequately by a guard column ('Vydac SC reverse phase'10µM, Varian) or by 0.2µM filters recommended for this purpose. In contrast, filtering samples through an 'Amicon PM10' filter at 4000 rpm for 30 mins, using the holder system described by Joseph et al (1981), proved an efficient cleaning procedure. The process described now allows the accurate determination of pmol levels of AAs in perfusate samples without any rapid deterioration in the performance of the analytical column. This method appears to provide a sensitive, quick and comparitively inexpensive method of detecting endogenous AAs in brain perfusates.

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THE EFFECT OF ANGIOTENSIN II ON RAT MESENTERIC ARTERIES

K.A. Munday, Judith A. Poat and M. Rigby (Introduced by G.N. Woodruff)
Department of Physiology and Pharmacology, University of Southampton, Southampton, SO9 3TU

Angiotensin II potentiates the vasoconstrictor response to nerve stimulation in the rat isolated mesenteric artery (Malik et al, 1976). We have investigated this phenomenon by studying the mechanical response of the nerve stimulated preparation and the release of [^{3}H]-noradrenaline from preloaded mesenteric slices.

Rats (200-250 g) were sodium pentobarbitone anaesthetised or killed by cervical dislocation, the mesenteric artery and surrounding tissue was mounted in a chamber kept at 37 °C by a surrounding water jacket. The tissue was constantly perfused (3 ml per min) with Krebs' bicarbonate buffer, pH 7.4. Drugs were injected into the perfusion system by a 3 way tap arrangement. The stimulating electrode was a bipolar silver electrode, driven by a Grass SD9 stimulator, placed around the mesentery bed. Biphasic pulses of 1 msec duration and at supramaximal voltage were employed. The vasoconstriction was monitored by a transducer connected to a Servoscribe pen recorder. In the experiments designed to measure [^{3}H]-noradrenaline release small pieces of mesenteric artery were incubated with 30 nM [^{3}H]-noradrenaline (S.A. 8 Ci per mmol) for 30 min at 37 °C. At the end of this period tissue was mounted between gauze in a 4 channel superfusion chamber. The chambers were superfused at 0.5 ml per min with Krebs' bicarbonate buffer, pH 7.4, at 37 °C. Collection of samples was commenced following a 30 min washing period. Efflux was routinely stimulated by exposing the tissue to 40 mM K^+ for 10 min in the presence and absence of drugs. Additions were randomised between the chambers and one chamber always acted as a control. At completion the tissue was removed and counted, the results being expressed as the fractional free ratio of release (Smith & Pycock, 1982).

The preparation responded to nerve stimulation by vasoconstriction. The frequency response curve was linear up to a maximum of 17.5 Hz, 10 Hz being used routinely. The addition of angiotensin II (1-20 ng per ml) potentiated the effect. Thus the response was 16.5 ± 0.4 mm in the absence and 26.0 ± 0.9 ($p<0.05$) in the presence of 1 ng per ml angiotensin. This potentiation was blocked by the addition of 100 ng per ml saralasin (15.5 ± 0.5 mm). In these experiments drugs were added sequentially and each tissue acted as its own control, angiotensin was at a sub-pressor dose. Injection of noradrenaline and phenylephrine produced a dose dependent vasoconstriction (a change of 70 mm by 1 and 1.5 µg per ml respectively). Surprisingly angiotensin at 5 ng per ml also potentiated the response to noradrenaline (51.3 ± 3.0 to 70.2 ± 4.8 mm), this was also blocked by saralasin (57.8 ± 5.6, with 30 ng per ml). [^{3}H]-noradrenaline was released from preloaded mesenteric artery by the addition of K^+ in a dose dependent manner between 15-40 mM K^+. The release was Ca^{2+}-dependent suggesting a neuronal release, the addition of 17-β-oestradiol (180 µM) inhibited uptake by approximately 30%. K^+-stimulated release was potentiated by phentolamine and yohimbine, and the addition of angiotensin II significantly enhanced [^{3}H]-noradrenaline release.

In conclusion the results suggest that angiotensin can modulate noradrenaline release from rat mesenteric artery by a presynaptic receptor-mediated event. They also suggest the possibility of a postsynaptic role for the polypeptide.

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A MICROIONTOPHORETIC COMPARISON OF THE SELECTIVITY OF THE OPIATE ANTAGONISTS NALOXONE AND ICI 139462

P.B. Bradley & Anne Brookes, Department of Pharmacology, The Medical School, University of Birmingham, Birmingham B15 2TJ

There is now considerable evidence for the existence of multiple sub-types of opiate receptor (Kosterlitz and Paterson, 1980; Wood, 1982), however, further elucidation of their physiological roles would be greatly facilitated by the use of selective antagonists. Until recently naloxone has been the most selective antagonist available, having affinity for receptors in the order $\mu > \kappa > \delta$ (Hutchinson et al. 1975; Lord et al, 1976). Two new antagonists have now been produced with reputed selectivity for the δ sub-type of opiate receptor (Shaw et al. 1982). One of these, ICI 139,462, has been studied and the results are reported here.

Urethane-anaesthetised male Sprague Dawley rats were cerebellectomised and microiontophoretic application of drugs was made to single neurones in the medulla. The drugs used were as follows: μ agonist, FK 33,824 (D-Ala^2,MePhe^4,Met-O-ol), 15mM, pH 4.3; κ agonist, bremazocine hydrochloride, 40mM, pH 5; δ agonists, leucine enkephalin, 15mM, pH 4.3, BW 180C (D-Ala^2,D-Leu^5), 15mM, pH 5; μ antagonist, naloxone hydrochloride, 20mM, pH 5.3; δ antagonist, ICI 139,462 (N,N diallyl Tyr-Gly-Gly-Phe-Leu-OMe), 30mM, pH 4. The dye pontamine sky blue was present in most electrodes so that neurones could be marked at the end of the recording.

All the opioid agonists predominantly cause depression of neuronal activity in the medulla (Bradley and Brookes, 1981). Naloxone antagonised 100% of the responses produced by FK 33,824, 91% of responses to bremazocine and 86 % and 100% of responses to leucine enkephalin and BW 180C, respectively. Where more than one agonist was tested on a single neurone, naloxone antagonised the effects produced by all of them. Using this technique differential sensitivities to naloxone-antagonism of responses induced by the different classes of opioid agonist were not observed. ICI 139,462 was found to antagonise responses in 61% and 50% of neurones depressed by BW 180C and leucine enkephalin, respectively. A considerably smaller proportion of responses to FK 33,824 and bremazocine was affected, therefore it was decided to first test the effect of this antagonist on responses induced by the δ agonist BW 180C and then against the responses induced by the μ and κ agonists on the same cell. Under these conditions it was found that on occasions when ICI 139,462 had no effect on a BW 180C-induced response it likewise had no effect on an FK 33,824- or bremazocine-induced response on the same cell. However, in contrast to the observations made with naloxone, when ICI 139,462 antagonised a response to BW 180C, it only antagonised the response to FK 33,824 on the same cell in 30% of neurones studied, and in only 10% of neurones also depressed by bremazocine. A preliminary histological examination of the distribution of brain stem sites where ICI 139,462 was shown to antagonise opioid-induced responses revealed that these sites were located in a number of brain-stem nuclei in much the same pattern as the distribution of opioid-sensitive sites. Thus, the putative δ antagonist was considerably more effective in antagonising the effects induced by the δ agonist BW 180C than in antagonising the effects of the μ agonist FK 33,824 or the κ agonist bremazocine.

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LORAZEPAM IMPAIRS RATS' PERFORMANCE OF A RECOGNITION MEMORY TASK

M.W. Brown & M.H. Harries (introduced by G. Clarke) Anatomy Department, Bristol University, Bristol BS8 1TD.

Oral administration of the benzodiazepine lorazepam can produce a temporary anterograde amnesia in man (Brown et al, 1983). An unusual feature of this amnesia is that recognition memory performance may be even more impaired than recall. It was decided to test the effects of lorazepam on the performance of a visual recognition task in the rat. The rats so tested formed part of a study of hippocampal single unit activity during acquisition of the task: lorazepam was administered towards the end of training.

A rat was exposed to one of six stimulus patterns (e.g. dots or stripes) in a start area. The stimulus was then covered. After a variable interval a door was opened and the rat admitted to a choice area. In the choice area the rat faced two hinged panels, one bearing the previously exposed stimulus, the other bearing one of the other five patterns. The rat had to push open the panel bearing the previously exposed stimulus within 2 min to obtain a food pellet. The rat was first trained using a specific pair of stimuli until it achieved a criterion of 9 out of 10 trials correct. Training continued using a zero delay interval before door opening until the same criterion was attained with random pairings of the six stimuli. The delay was then increased by stages to 80 s. Sixty trials were completed at this delay. Ten rats were then tested after increasing oral doses (of between 1.0 and 10.00 mg/kg) of lorazepam in propylene glycol absorbed by food pellets. Each rat subsequently acted as its own control by ingesting propylene glycol without lorazepam.

For all 10 rats the proportion of correct to incorrect responses for trials at 80 s delay before and after the various drug doses were analysed by a generalised analysis of variance using a binomial error model: the effect of drug dose was highly significant ($P<0.001$). Further, there was a significant ($P<0.01$) rank correlation between the proportion of errors made and drug dose. Averaging across rats the mean proportion of errors before drug administration was $1.5\pm0.5\%$; after $\geq$ 5mg/kg lorazepam it was $22.6\pm5.3\%$. The proportion of errors peaked at 20-30 min after ingestion but was still considerably higher than control levels 70 min after ingestion. Post-drug performance at 0 s delay did not differ significantly from that at 80 s delay. It should be noted that at 0 s delay the rat still could not simultaneously compare the stimulus in the start box with those in the choice area since the former was covered before the start box door was opened. Performance following propylene glycol did not differ significantly from pre-drug performance. Drug administration did not significantly change the mean time for the rats to push a response panel after the start box door had opened. Thus the rats responded as fast after the drug as before it, so that lorazepam's effects would not seem to be due to gross changes in levels of arousal or motivation. It is possible that a sensory/perceptual or motor dysfunction underlies impairment in the task, although no obvious abnormalities in the rats' general behaviour were observed. A more parsimonious explanation is that the drug interferes with the input of information into memory in the rat as it does in man.

In summary, administration of lorazepam causes impaired performance in a recognition task in the rat. This finding is consistent with the drug's impairment of recognition memory performance in man.

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RAT BRAIN PERIAQUEDUCTAL GREY AND BODY TEMPERATURE: EVIDENCE FOR AN ACETYLCHOLINE-NEUROTENSIN INTERACTION

E.C.Griffiths, P.Slater & P.S.Widdowson, Department of Physiology, University of Manchester, Manchester M13 9PT

The tridecapeptide neurotensin (NT) has been identified in the periaqueductal grey (PAG) area of rat brain (Emson et al, 1982). Microinjections of NT into the PAG cause hypothermia (Widdowson et al, 1982) but the mechanisms responsible for this temperature response are unknown. Recognised PAG transmitters, other than NT, include GABA, acetylcholine (Ach) and 5-HT, and the PAG has moderate densities of muscarinic, 5-HT, $GABA_A$ and $GABA_B$ receptors (Kobayashi et al,1978; Biegon et al, 1982; Crossman et al, 1983). The role of these transmitters in the hypothermia produced by NT in the PAG has been investigated.

A permanent cannula guide was implanted above the PAG (A 0.6, H -0.5, L 0.45; König & Klippel,1963) in adult, male Sprague Dawley rats. After 10 days, compounds were injected in 1 µl volumes through a cannula inserted in the guide. Rectal temperature was measured at 10 min intervals using a thermistor probe before (20 min) and after (120 min) injection at room temperature (21°c). All injection sites were subsequently confirmed histologically.

NT (0.5 - 5 µg) in PAG caused a dose-related hypothermia. The hypothermia produced by 1 µg of NT was not affected by simultaneous injection in PAG of muscimol, (±)baclofen, bicuculline 5-HT, 5-methoxy-N,N-dimethyltryptamine, methysergide, cyproheptidine and atropine. None of these compounds alone in PAG altered body temperature. By contrast, carbachol in PAG caused hypothermia which was prevented by atropine (Table 1).

Table 1 Temperature effects of compounds in PAG

Compound-dose	Mean temperature (±S.E.) and time after injection Initial	20 min	60 min	100 min
Saline	36.7±0.2	37.2±0.2	37.2±0.3	36.9±0.3
NT 1 µg	37.2±0.1	36.6±0.1[a]	35.6±0.2[a]	37.0±0.1
Carbachol 5 µg	36.8±0.1	35.4±0.2[a]	35.2±0.4[a]	36.3±0.4[a]
Carbachol 5 µg +atropine 5 µg	36.8±0.2	36.8±0.5	37.0±0.3	37.0±0.2
NT antibodies + NT 1 µg	36.9±0.1	36.5±0.1	36.4±0.3[a]	36.9±0.2
NT antibodies + carbachol 5µg	37.3±0.1	36.8±0.3	38.0±0.3[a]	38.1±0.2[a]

[a] $P<0.01$, unpaired Student's t-test compared to saline.

Rats were pretreated for 5 days with specific NT antibodies (raised in sheep): the pretreatment prevented both NT- and carbachol-induced hypothermia. A small elevation in body temperature occurred in this latter group.
It is concluded that an NT-Ach interaction occurs in the PAG. Ach neurones involved in temperature responses may synapse on NT neurones and thus regulate NT release.

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TRITIUM-FILM AUTORADIOGRAPHY OF $GABA_A$ AND $GABA_B$ RECEPTORS IN RAT BRAIN.

A.R. Crossman[1], A. Latham, S. Patel & P. Slater, Departments of [1]Anatomy and Physiology, University of Manchester, Medical School, Manchester M13 9PT

Mammalian brain contains not only the classical GABA receptor ($GABA_A$) but also a bicuculline-insensitive [^{3}H]GABA binding site designated as the $GABA_B$ receptor (Hill & Bowery,1980). We have measured the distribution and density of these GABA receptor subtypes in rat brain.

Female Sprague Dawley rats were anaesthetized and perfused transcardially with 200 ml of 0.1% formaldehyde in pH 7.4 phosphate buffer. Cryostat-cut sections (15 μm) of frozen brain were mounted on glass slides and incubated for 45 min at 20°C in 50 mM Tris HCl buffer (pH 7.4). This was followed by 15 min incubation in Tris buffer containing 50 nM [^{3}H]GABA (65 Ci/mmol,Amersham) and 190 mM sucrose ($GABA_A$ receptors) plus 40 μM isoguvacine and 2.5 mM $CaCl_2$ ($GABA_B$ receptors) (Wilkin et al, 1981). The sections were washed twice in buffer solution,dipped in distilled water, blown dry and exposed to tritium-sensitive film (LKB) for 10 weeks. Regional density measurements were made on the autoradiograms with a fibre optic microdensitometer system (Morton et al, 1982).

$GABA_A$ binding sites were present in nearly all regions of the rat brain, with low binding to white matter. In general, the regional distribution of $GABA_A$ receptors was closely similar to the regional binding of the GABA agonist [^{3}H]muscimol (Palacios et al,1981). The greatest concentration of $GABA_A$ receptors was present in the external (I-III) layers of the cerebral cortex, parts of the thalamus (antero-ventral thalamus,nucleus parataenialis, nucleus paraventricularis) and the dentate gyrus. Moderately high concentrations were present in the striatum, superior colliculus, the medial geniculate nucleus, the periaqueductal grey, subthalamic nucleus and the substantia nigra zona reticulata.

$GABA_B$ receptors were less numerous than $GABA_A$ sites, although many brain areas had both receptor subtypes. The highest density of $GABA_B$ receptor was found in the interpeduncular nucleus (an area of low $GABA_A$ binding). The superior colliculus, parts of the amygdala - especially the lateral amygdaloid nucleus, the medial habenula, parts of the thalamus (lateral and ventral thalamic nuclei, antero-ventral thalamus) and the outer layers of cerebral cortex (laminae I-II) contained high densities of $GABA_B$ sites. There were moderate numbers of $GABA_B$ receptors in the striatum,periaqueductal grey and substantia nigra zona reticulata.

In the cerebellum, $GABA_A$ receptors were mostly in the granular layer with smaller densities in the molecular layer, whereas $GABA_B$ receptors were almost entirely confined to the molecular layer. This is in agreement with the findings of Wilkin et al (1981).

In conclusion, both GABA receptor subtypes are distributed throughout the brain. Generally, $GABA_A$ receptors are present in greater densities than $GABA_B$, although in some areas the proportion of one subtype greatly exceeds the other.

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NEUROTENSIN ANTAGONISES LOCOMOTOR STIMULANT ACTIONS OF TRH AND RX77368

E.C.Griffiths, R.Robertson & P.Slater, Department of Physiology, University of Manchester, Manchester M13 9PT

The CNS actions of neurotensin (NT), which include inhibition of locomotor activity (Jolicoeur et al,1981), are generally the opposite of those of thyrotrophin-releasing hormone (TRH). TRH injected into the nucleus accumbens (NA) of rat brain stimulates locomotor hyperactivity (Griffiths et al, 1981). The NA hyperactivity model has been used to investigate TRH and NT interactions.

Adult male Sprague Dawley rats were fitted with bilateral cannulae for microinjections (1 µl) into NA. Locomotor activity was measured in cages fitted with a pulsed infra-red beam generator and detector unit. The animals were allowed 90 min to acclimatize; recordings of activity were made 5-35 min after injection. The post-injection behaviour was monitored by an observer.

TRH in NA stimulated locomotor activity. Mean beam crossings per 30 min were: saline 17.0±3.0; 1 µg TRH 38.0±8.0; 10 µg TRH 122±24 (mean ± s.e., n=10-25). The stimulation of activity produced by TRH (10 µg) was partly prevented by prior (5 min) injection into NA of 1 µg NT (70±20 counts) and 10 µg NT (25±5 counts). By contrast, the N-terminal octapeptide [1-8 NT] did not affect TRH-induced hyperactivity.

The TRH analogue RX 77368 (pGlu-His-3,3'dimethyl ProNH_2) injected in NA also enhanced locomotor activity (0.1 µg 124±40; 0.5 µg 189±22; 10 µg 325±28). NT (10 µg) significantly reduced the stimulation produced by 0.5 µg RX 77368 (96±10), as did the NT fragment [8-13 NT] (10 µg 108±12). Once again, the [1-8 NT] fragment was inactive.

The actions of endogenous NT in NA were prevented by injecting rats once daily for 5 days in NA with 1 µl NT antisera (raised in sheep, diluted 1 in 10 with saline). The treatment enhanced the locomotor response produced by 0.1 µg RX 77368 in NA (mean counts 210±24 compared to 124±40 in normal animals). An interesting finding was that a single injection in NA of NT antisera produced a mild locomotor stimulation (saline 25±10 counts; antisera 67±13).

The ability of NT and the [8-13 NT] fragment to antagonize TRH and RX 77368 in NA may involve dopamine. TRH stimulates dopamine release in NA (Kerwin & Pycock 1979) whereas NT may inhibit dopaminergic transmission (Ervin et al, 1981; Haubrich et al,1982). NT which is present in NA (Emson et al,1982) may normally inhibit dopamine and restrain locomotor activity. This idea is supported by the present findings in which NT antisera, which inactivates endogenous NT, produced a mild locomotor stimulation and enhanced TRH-stimulated activity. The findings also provide a further example of the antagonism of TRH by NT.

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