MAST CELLS FROM GUINEA-PIG LUNG: ISOLATION AND PROPERTIES

K.E.Barrett[1], M. Ennis* & F.L. Pearce[1], [1]Department of Chemistry, University College London, London WC1E 6BT and Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 OHS

The guinea pig is generally considered to be the animal of choice for the study of immediate hypersensitivity reactions. It may be readily sensitized and responds strongly to antigenic challenge. Isolated organs or chopped tissues from the animal have thus been widely used in the investigation of the release of anaphylactic mediators. However, these experiments are per se somewhat difficult to interpret and problems of diffusion, accessibility of reagents, non-specific adsorption and masking of receptor sites may interfere with the results. For these reasons, we have now developed a method for the dispersion of guinea pig lung into its component cells.

Lungs were excised from Dunkin-Hartley guinea pigs (ca 450 g) and washed twice in HEPES-buffered Tyrode solution (Pearce & Ennis,1980). The tissue was chopped mechanically (1 mm^2) and incubated (90 min, 37°C) in a solution of collagenase (0.05%) in buffer containing bovine serum albumin (0.1%). The tissue was disrupted by expression through a syringe, the suspension filtered through gauze and the cells recovered by centrifugation (5 min, 4°C, 150 g). The cells were then washed, resuspended in buffer and finally passed through a porous plastic filter to remove remaining debris and aggregated material. Preparations were characterized as previously reported (Pearce & Ennis, 1980).

The above procedure typically yielded 5 x 10^6 mast cells per g of tissue with a recovery of histamine of ca 20%. The mast cells comprised 2% of the total nucleated cells, had a histamine content of 3 pg per cell and exhibited a low ($\leq$5%) spontaneous release of the amine.

In contrast to the rat peritoneal mast cell (Pearce & Ennis, 1980), guinea pig lung cells were unresponsive to compound 48/80 ($\leq$100 µg/ml), peptide 401 (MCD-Peptide, $\leq$100 µg/ml), dextran (12 mg/ml) and concanavalin A ($\leq$100 µg/ml). These differences cannot be attributed to artefacts induced by the isolation procedure, since treatment of the peritoneal cells in the same way did not alter their reactivity. However, the ionophores A23187 and ionomycin (0.1 - 10 µM) produced a dose-dependent release of histamine from the lung cells, with a maximum secretion of ca 50%. Cells obtained from actively sensitized animals also released histamine on challenge with the antigen ovalbumin.

The present report thus further emphasises the functional heterogeneity of mast cells from different tissues and species. The availability of dispersed tissue preparations from a range of diverse sites should facilitate continued study of this problem and may provide ideal models for the testing of anti-allergic drugs directed against specific inflammatory conditions.

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2P

THE EFFECT OF ARACHIDONIC ACID AND NON-STEROIDAL ANTI-INFLAMMATORY AGENTS ON INTRAPULMONARY AIRWAYS IN THE GUINEA-PIG

T.P. Clay and D.C. Fenske*, Department of Pharmacology, Roussel Laboratories Limited, Covingham. Swindon. Wilts.

The physiological role of exogenous arachidonic acid on airway smooth muscle remains controversial, relaxation or constriction may occur depending on the anatomical site of the airway (Mitchell and Denborough 1980). Furthermore, constriction of trachea or parenchymal strip results in either prostaglandin or thromboxane release (Gryglewski, Dembinska-Kiec, Grodzinska and Panczenko 1976).

In order to resolve the predominant effect of arachidonic acid and its metabolic products on bronchial smooth muscle, we have attempted to evaluate the role of arachidonic acid on gross airway function using a perfused guinea pig lung model.

Guinea pigs were anaesthetised and exsanguinated. The lungs were removed and the trachea cannulated. The peripheral margin of the lung was trimmed and the lungs perfused via the trachea at constant flow (5 ml/min) with oxygenated Krebs fluid at 37°C. Perfusion pressure was monitored proximal to the trachea.

Arachidonic acid, injected into the perfusing fluid produced no significant alteration of baseline pressure, even up to a concentration of 61 μM (n = 4). However, on a histamine (10-40 μM) elevated baseline (56-77 mm Hg), arachidonic acid produced a dose-related reduction of perfusion pressure, producing an ED_{50} value of 5.0×10^{-6} M (n = 10; $p < 0.001$). The absolute baseline measurement failed to influence the resultant relaxation; regression analysis yielded an r value of -0.24 which was not significant.

The non-steroidal anti-inflammatory agents, given in the perfusing fluid, inhibited the relaxation of airway smooth muscle by a supramaximal bolus dose of arachidonate (61 μM). The resultant IC_{50} values were indomethacin, 6.7×10^{-6}M; BW 755C, 2.58×10^{-5}M; phenylbutazone, 1.49×10^{-4}M.

Phenidone produced inhibition ($IC_{50} > 1.23 \times 10^{-3}$M) although airway relaxation to phenidone was observed at high concentrations, making further measurements impractical. Aspirin inhibited arachidonate relaxation ($IC_{50} > 1.1 \times 10^{-3}$M) although higher concentrations proved insoluble.

Indomethacin (8.4×10^{-6}M) and phenylbutazone (6.5×10^{-5}M) produced 31% (n = 20; $p < 0.05$) and 59% (n = 6 $p < 0.05$) constriction of a histamine elevated baseline, respectively.

In conclusion, exogenous arachidonic acid produces relaxation of contracted intrapulmonary airways, although no effect can be observed on baseline tone. All the NSAIA's tested inhibit such relaxation, and several produce baseline elevation in their own right. It is suggested that intra-pulmonary airways are dependent on prostaglandins and not thromboxanes for modulation of their reactivity.

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PARADOXICAL METABOLISM OF THROMBOXANE B_2 IN PERFUSED GUINEA-PIG LUNG

I.A. Blair, C.T. Dollery, J.R.S. Hoult[1], S.H. Peers[1], C. Robinson* & K.A. Waddell, Department of Clinical Pharmacology, Royal Postgraduate Medical School, London W12 OHS and [1] Department of Pharmacology, King's College, London WC2R 2LS.

The 13,14-dihydro-15-keto metabolite of thromboxane B_2 (TXDK) is found in pulmonary effluent of anaphylactically challenged guinea-pig lung (Boot et al, 1978), but it is uncertain whether this compound can be formed directly from TXB_2 by sequential action of prostaglandin 15-hydroxydehydrogenase (PGDH) and Δ13-reductase (Oates et al, 1980). We have found that TXB_2 is apparently transformed to TXDK in isolated perfused guinea-pig lung but paradoxically is resistant to oxidation by purified PGDH and in guinea-pig lung supernatants.

Lungs from male Dunkin-Hartley guinea-pigs were perfused at 10 ml/min for 5 min with Krebs' solution containing 1 μCi of both [^{3}H]-TXB_2 (10 ng/ml) and inulin [^{14}C]-carboxylic acid (as extracellular marker). Tissue to medium ratio measurements on solubilised homogenates showed that lungs from both normal and ovalbumin-sensitized guinea-pigs accumulated TXB_2 during the 5 min perfusion (3.10 ± 0.12 versus 2.60 ± 0.12, n = 30, $P < 0.05$) but not inulin (0.19 ± 0.01 and 0.16 ± 0.01 respectively, n = 30). Extracted centrifuged samples of effluent were chromatographed, and showed two radioactive peaks (R_F 0.3 and 0.45 in ethyl acetate : acetone : acetic acid 90:10:1), the former corresponding to TXB_2. This was the only peak found after extracting TXB_2 from Krebs' solution alone. According to this method, perfused lungs from normal and sensitised guinea-pigs converted 25.1 ± 2.3% and 32.4 ± 7.6% of TXB_2 to the second unknown product (n = 5, n.s.).

We confirmed the identity of material from the unknown peak as TXDK by capillary column GC negative ion chemical ionisation mass spectrometry (Finnegan 4000 modified for negative ion detection) of the methoxime-pentafluorobenzyl ester trimethylsilyl ether derivatives of unlabelled TXB2 infused through sensitized guinea-pig lung. Authentic and lung-derived TXDK showed the same retention times, and the characteristic ion at m/e 571 of authentic TXDK was the only feature of the mass spectrum of the lung-derived TXDK. Moreover, this ion was found exclusively at the appropriate retention time.

TXB_2 breakdown by purified human placental PGDH (4 mU/ml) or 100,000 g supernatants from lungs of normal or sensitized guinea-pigs was measured by radio-t.l.c.; after correction for reaction blanks, apparent breakdown was less than 5% in 10, 30 or 60 min incubations at substrate concentrations of 0.5, 1.0 and 10.0 μg/ml and was not altered by substituting $NADP^+$ for NAD^+.

We conclude that although TXB_2 is metabolised to TXDK in perfused guinea-pig lung, this is unlikely to be initiated as expected by oxidative attack at C-15 by PGDH.

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EFFECTS OF STREPTOZOTOCIN-INDUCED DIABETES ON THE METABOLISM OF ARACHIDONIC ACID IN RAT ISOLATED LUNG

Y. S. Bakhle, G. P. Lewis & I. S. Watts *, Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN.

Diabetes mellitus is associated with an increased incidence of intravascular platelet aggregation (Bern, 1978). Since platelet behaviour is affected by cyclo-oxygenase products (COP) of arachidonic acid (AA) synthesized by vessel walls, especially in the pulmonary circulation (Gryglewski, 1979), we have investigated the formation of a number of COP in the pulmonary circulation of perfused isolated lungs from rats made diabetic by a single injection of streptozotocin (85 mg/kg). Rats were used not less than 28 days following the injection of streptozotocin (blood glucose>400 mg/100 ml) or of saline (blood glucose 80-100 mg/100 ml). Lungs were prepared for perfusion with Krebs solution (8 ml/min) as described earlier (Alabaster & Bakhle, 1970).

Synthesis of COP was first measured by bioassay of lung effluent following bolus injections of AA (10 and 20 ug) into the pulmonary circulation, as PGE_2 equivalents on the rat stomach strip. Formation of COP in control lungs was less (14.5 ± 2.2 ng; mean$\pm$s.e.mean, n=6) than in diabetic lungs (21.9 ± 2.3 ng; $P<0.05$) for the 10 ug dose of AA. Similar results were obtained for 20 ug AA.

The COP of lung effluent were further analysed by radioimmunoassay (Jose et al, 1976) for 6-oxo-$PGF_{1\alpha}$, thromboxane B_2 (TxB_2) and PGE_2 and $PGF_2\alpha$ both before (referred to as endogenous synthesis) and after (exogenous synthesis) infusion of AA (3 min; 40 uM) in lungs from control and diabetic rats. Under all conditions, 6-oxo-$PGF_{1\alpha}$ was the major constituent of the COP present (77-58%) with TxB_2, PGE_2 and $PGF_2\alpha$ contributing roughly equally to the remainder. Endogenous synthesis of 6-oxo-$PGF_{1\alpha}$ was greater in control lungs (40 ± 6 vs 22 ± 3 ng) whereas PGE_2 formation was higher in diabetic lungs (3.3 ± 0.5 vs 5.5 ± 0.5 ng). Endogenous synthesis of TxB_2 and $PGF_2\alpha$ were the same in control and diabetic lungs.

Under conditions of exogenous synthesis, although 20 - 30 fold more COP were present in lung effluents, the only statistically significant differences between control and diabetic lungs observed were for PGE_2 (46.5 ± 17 vs 105 ± 21 ng) and $PGF_2\alpha$ (76.5 ± 8 vs 102 ± 11 ng; control vs diabetic respectively). Since these two PGs are more potent than TxA_2 or PGI_2 on the rat stomach strip, these results are compatible with those obtained by bioassay of lung effluent following injection of AA through the pulmonary circulation.

These results show that when exogenous AA is infused at a relatively high concentration (40 uM; 12.5 ug/ml) there is no difference in the production of TxB_2 and 6-oxo-$PGF_{1\alpha}$ between lungs from normal and diabetic animals; the higher PGE_2 and $PGF_2\alpha$ formation in diabetic lungs may indicate a relative excess of endoperoxide which degrades chemically to $PGF_2\alpha$ and possibly to PGE_2. However, when endogenous AA is utilized, diabetic lungs produced only half as much PGI_2 and this deficiency in a potent anti-aggregatory factor could contribute to increased intravascular platelet aggregation in diabetic animals.

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THE FATE OF LEUKOTRIENES C_4 AND D_4 IN THE PULMONARY AND CORONARY CIRCULATIONS IN VITRO

L. G. Letts, M. A. Palmer & P. J. Piper *, Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

Passage through the pulmonary vascular bed in vitro results in a loss of up to 98% of the biological activity of prostaglandins (PGs) E_2 and $F_2\alpha$ (Piper, Vane & Wyllie, 1970). The fate of leukotrienes (LT) C_4 and D_4 was investigated in the pulmonary circulation of guinea-pig and rat isolated perfused lungs and coronary circulation of guinea-pig hearts in vitro.

Lungs from guinea pigs (Dunkin Hartley, 300-450 g) or rats (Wistar, 250-300 g) were perfused via the pulmonary artery with Tyrode (5 ml min^{-1}). Coronary circulations of guinea-pig hearts were perfused at constant flow with Tyrode (5-10 ml min^{-1}) via retrograde cannulation of the aorta. Coronary perfusion pressure was recorded with an Elkomatic pressure transducer. In all experiments effluent superfused a series of strips of guinea-pig ileum smooth muscle (GPISM) blocked with mepyramine and hyoscine (3.5×10^{-7}M). Leukotriene activity in effluent was assayed against doses given directly to GPISM. Indomethacin (3×10^{-6}M) was infused into guinea-pig lungs to prevent release of cyclo-oxygenase products by LTs (Piper & Samhoun, 1981). Lungs inactivating less than 85% of infused PGE_2 were rejected.

When LTD_4, 5×10^{-10} - 5×10^{-9}M, was infused into guinea-pig or rat lungs, responses of GPISM showed 23 - 54% loss of biological activity. However, when LTC_4, 5×10^{-10} - 1×10^{-9}M was infused into either guinea-pig or rat lungs, there was a 24 - 56% increase of activity on GPISM. Infusion of either LTD_4 or LTC_4, 5×10^{-10} - 2×10^{-9}M into the coronary circulation increased coronary pressure by 50 - 125% and there was a 23 - 60% increase of biological activity of LTC_4 but a reduction of LTD_4.

In all experiments the GPISM-contracting activity in the effluents was completely blocked by FPL 55712 (1.9×10^{-6}M). The activation of LTC_4 was not blocked by δ-D-glutamyl (O-carboxy) phenylhydrazine, 2.5×10^{-4}M (inhibitor of γ-glutamyl transferase) (Griffiths & Meister, 1979) but treatment of heart or lungs with BW 755c (4×10^{-5}M) or mepacrine (4×10^{-4}M) reduced the activation of LTC_4. After intravascular administration of LTs, GPISM showed an increased sensitivity to histamine and PGE_2. These observations suggest the increase in biological activity of LTC_4 in the pulmonary or coronary circulations may result from the stimulation of phospholipase A_2 and is possibly due to the release of a lipoxygenase product.

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LEVELS OF PLASMA PROSTAGLANDIN 'RECIPROCAL COUPLING FACTOR' IN HUMAN AND EXPERIMENTAL ANIMAL DISEASE STATES

C.N. Berry, P.G. Hellewell & J.R.S. Hoult,* Department of Pharmacology, King's College, London WC2R 2LS.

Prostaglandin 'reciprocal coupling factor' (RCF) is the provisional name for the component(s) in plasma which inhibits microsomal PG synthesis and enhances cytosolic PG degradation (Moore & Hoult, 1980; Hellewell et al, 1980). We report studies on RCF activity in plasmas taken from patients with various diseases and from animals with experimentally-induced pathological states. Plasmas were assayed at 0.5 to 10.0 % v/v (a) as inhibitors of PG synthesis by seminal vesicle microsomes using bioassay, and (b) as stimulators of PG breakdown by 100,000 g supernatants of rat caecum using a radiochemical assay (Moore & Hoult, 1980).

Of eight sets of experiments, four were "cross-sectional" and four "longitudinal" (Table 1). If there was no difference between plasma groups in the PG synthesis-inhibition tests, the effect on PG breakdown was usually not tested.

Table 1 RCF activity in human and animal disease states

Condition	Change in RCF activity	
	PG synthesis-inhibition	PG breakdown-stimulation
A. Rheumatoid arthritis, human	No change	Not tested
B. Ulcerative colitis, human	No change	No change
C. Sulphinpyrazone treatment, g-pig	No change	Not tested
D. Turpentine injection, rat	Increased for 6 d	Decreased for 6 d
E. Endotoxin shock, rat	No change	Not tested
F. Alcohol challenge, human	Increased, max 10 min	Increased, max 10 min
G. Ovalbumin anaphylaxis, g-pig	Increased (not signif)	No change
H. PGE_1 infusion, human	Decreased, max 24 hr	Decreased, max 24 hr

DETAILS: A. 5 Patients with active disease compared with 5 in remission. B. 6 patients with active disease, 10 in remission, 3 control. C. Samples before 30 mg/kg given orally compared to those 6 hr later, 5 per group. D. 0.1 ml turpentine i.m., groups of 5 treated or control rats sacrificed daily for 6 d. E. 4 anaesthetised rats, samples taken before/after i.v. lipopolysaccharide W (E. coli), 20 mg/kg. F. alcohol-sensitive patient challenged with 50 ml vodka, plasmas taken before and up to 120 min after. G. 4 guinea-pigs, plasmas taken before/after anaphylactic challenge. H. patient with peripheral vascular disease, PGE_1 infusion 10 ng/kg/min for 72 hr; plasmas taken before and daily for 5 d.

The principal conclusions are: (1) "longitudinal" studies are preferable to "cross-sectional" studies (because of variation between individuals in RCF activity), (2) the synthesis-inhibiting and breakdown-stimulating components are probably different entities (results D and G), (3) RCF release is altered in some but not all disease states, with species variation.

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ACTIVATION AND INHIBITION OF PROSTAGLANDIN 15-HYDROXYDEHYDROGENASE BY PLASMA AND SULPHASALAZINE-LIKE DRUGS

C.N. Berry*, J.R.S. Hoult, J. Jeffery[1], O.T. Mak[1] and S.H. Peers. Department of Pharmacology, King's College, Strand, London, WC2R 2LS and [1]Department of Biochemistry, Marischal College University of Aberdeen, Aberdeen, AB9 1AS

Our recent studies using a number of cell-free cytosolic systems have defined the inhibition and activation of PG breakdown by sulphasalazine and mammalian plasma, respectively (Hoult & Moore, 1980; Moore & Hoult, 1980). These effects were attributed to interactions with prostaglandin 15-hydroxydehydrogenase (PGDH; EC.1.1.1.141), the enzyme responsible for the first step in the breakdown of classical PGs. We have tested sulphasalazine and plasma for the same effects using purified PGDH preparations, and sought to identify structural features needed for inhibition by sulphasalazine analogues.

Bovine lung PGDH (from BDH, 75 mU/ml) and human placental PGDH (prepared by acid precipitation pH 5.2 followed by chromatography on blue Sepharose, 200 mU/ml) were used as enzyme source. Breakdown of $PGF_{2\alpha}$ was measured as described before in 37°C incubations which generally contained 10 µg/ml substrate and 5 mM NAD^+ (Hoult & Moore, 1977). The extent of $PGF_{2\alpha}$ breakdown increased as a function of enzyme concentration, was slightly reduced at 22°C but inhibited at 0°C or if NAD^+ was omitted; double reciprocal plots of 1/V versus 1/S gave K_m values of 30.5 ± 5.3 µM and 73.1 ± 15.6 µM for the lung and placental enzymes.

Sulphasalazine dose-dependently inhibited both enzymes under all conditions tested (e.g. ID_{50} values of 23 µM and 20 µM for lung and placental PGDH respectively). Three sets of experiments to assess its mode of inhibition showed changes in both K_m and V_{max} (e.g. for placental enzyme alone values were 73.1 ± 15.6 µM and 1926 ± 223 pmoles/ml/min respectively and with 50 µM sulphasalazine were 120.4 ± 36.6 µM and 1150 ± 198 pmoles/ml/min, results expressed as mean ± s.d.). Using placental enzyme and 10 µg/ml $PGF_{2\alpha}$, breakdown increased as a function of NAD^+ concentration up to 1 mM but not thereafter both in the presence and absence of 50 µM sulphasalazine.

We tested analogues of sulphasalazine and salazine (this lacks the pyridine substituent). Optimal inhibition was obtained with the homo-derivates (carboxyl of salicylic acid replaced with $-CH_2COOH$) and ID_{50} values in µM on lung enzyme for the parent, homo- and dihomo- analogues of the two series were respectively: 23, 13 and 100 for the sulphasalazine series and 170, 15 and 600 for the salazine series.

Human plasma at 0.1-10% v/v activated $PGF_{2\alpha}$ breakdown by the lung enzyme in a dose-dependent manner. However, in only one of two series of experiments did plasma activate the placental enzyme and this activation was not dose-related.

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PLASMA INHIBITS AND SERUM STIMULATES PROSTACYCLIN (PGI_2) SYNTHESIS BY RAT AORTIC RINGS

P.J. Lewis, M.A. Orchard & J.M. Ritter*, Department of Clinical Pharmacology, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 OHS

Plasma is the physiological milieu of endothelium. It has been proposed that it contains stimulatory (Remuzzi et al, 1978) and inhibitory (Saeed et al, 1977) factors that may control endothelial PGI_2 synthesis. Elements of the coagulation process may be involved in initiation and evolution of vascular lesions and may also influence PGI_2 synthesis (Weksler et al, 1978; Coughlin et al, 1980).

Synthesis of PGI_2 by chopped rat aortic rings incubated in plasma or serum was studied by measuring 6-oxo-$PGF_{1\alpha}$ after acid hydrolysis by radioimmunoassay. In each experiment the aortas from three rats were chopped into 1 mm rings that were allotted alternately to five groups. Each group was stored in balanced salt solution (Gey's Solution, Gibco) on ice until the start of the incubation. Five parallel incubations were performed, each being initiated by adding the rings to incubation fluid in a polystyrene tube in an agitated water bath at 37°C. The fluids were: (1) Tris buffer 50 mM, pH 7.4 (Tris); (2) citrated platelet poor plasma (PPP) from patients with renal failure (U-PPP); (3) PPP from healthy age and sex matched controls (C-PPP); (4) C-PPP heated at 100°C for 5 min (H-PPP) and (5) serum prepared by adding 0.1 ml $CaCl_2$ (1M) to 5 ml citrated human platelet-rich plasma (PRP) and incubating at 37° for 3 hours (PRP-S). The pH of plasmas and serum was adjusted to 7.4-7.6 with 95% O_2 5% CO_2. Aliquots were withdrawn at 4, 8, 30 and in some experiments 60 min. These findings are summarized in Table 1.

Table 1 6-Oxo-$PGF_{1\alpha}$ production by aortic rings in buffer, plasmas and serum

Incubation fluid	V_o[a] ng $mg^{-1}min^{-1}$	Q_{30}[b] ng mg^{-1}	Q_{60}[c] ng mg^{-1}
Tris	0.28 ± 0.06	4.39 ± 0.84	-
U-PPP	0.21 ± 0.04	3.56 ± 0.80	-
C-PPP	0.20 ± 0.04	3.47 ± 0.92	3.20 ± 0.40
H-PPP	0.30 ± 0.07	5.45 ± 1.13	5.03 ± 0.79
PRP-S	0.31 ± 0.07	11.06 ± 3.01	18.26 ± 3.07

[a]Initial rate of 6-oxo-$PGF_{1\alpha}$ production, mean ± s.e. mean, n = 8
[b]6-oxo-$PGF_{1\alpha}$ produced at 30 min, mean ± s.e. mean, n = 8
[c]6-oxo-$PGF_{1\alpha}$ produced at 60 min, mean ± s.e. mean, n = 5

Approximately 1/3 less 6-oxo-$PGF_{1\alpha}$ was synthesised in C-PPP than in H-PPP ($P < 0.005$, paired t test), consistent with the presence of a heat-labile inhibitor in plasma (Saeed et al, 1977). No difference between C-PPP and U-PPP was detected (a consistent difference of > 10% would have been detected with $P < 0.005$). 6-Oxo-$PGF_{1\alpha}$ production was greater in PRP-S than in C-PPP ($P < 0.005$). This difference was most marked later in the incubation (5-6 fold increase at 60 min). The mechanisms of these effects are being studied.

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VASODILATION, THE MAJOR VASCULAR RESPONSE TO PGE_2 IN THE RAT KIDNEY

J. Haylor, Department of Pharmacology, The University, Sheffield S10 2TN.

The rat has been suggested to exhibit an important species difference in its renal vascular response to PGE_2 which produces renal vasoconstriction both in vitro (Malik and McGiff 1975) and in vivo (Baer and McGiff 1979). In the other species which have been studied, prostaglandins of the E and A series are renal vasodilators (Lee 1978). However recently, PGE_2 has been demonstrated to increase the renal clearance of p-aminohippuric acid, the effective renal plasma flow, in the pentobarbitone-anaesthetised rat (Haylor and Lote 1980). The influence of PGE_2 on renal vascular resistance therefore has been re-investigated, using an electro-magnetic flow meter and probe to monitor renal blood flow.

Male Wistar rats (350-400G) were anaesthetised with sodium pentobarbitone 60mg/kg intraperitoneally and were maintained on sodium pentobarbitone 3mg/kg every 45 minutes. Cannulas were placed in the left carotid artery, and in the right jugular vein through which 153mM sodium chloride was infused at 57µl/min. A small bore cannula (external diameter 0.61mm) was introduced into the left femoral artery and advanced up the aorta until its tip lay adjacent to the bifurcation with the left renal artery. A slow infusion of heparin saline (50 units/ml:10µl/min) was maintained through this cannula. A 2mm circumference electro-magnetic flow probe was placed on the left renal artery, which had been cleared from the surrounding tissue. It was essential to ensure that a good fit between probe and artery had been obtained and that the probe did not partially occlude flow. PGE_2 in heparin saline was infused at 10µl/min through the aortic cannula in a cumulative manner within the dose range 0.01-20µg/min. Each dose was infused for a 5 minute period.

A dose-dependent vasodilation could be seen which usually appeared at doses as low as 0.01µg/min. PGE_2 1µg/min increased renal blood flow from 8.1 $\pm$ 0.35 to 10.0 $\pm$ 0.30ml/min ($P < 0.05$, $n = 6$), the mean systemic arterial blood pressure fell slightly by about 5mm Hg, while the calculated renal vascular resistance fell from 16.0 $\pm$ 2.0 to 12.7 $\pm$ 2.1 ml/min/mmHg ($P < 0.05$). Vasodilation was still apparent at 10µg/min while at the highest dose used, vasoconstriction was clearly seen, renal blood flow being reduced to 6.1 $\pm$ 0.2 ml/min ($P < 0.05$) and vascular resistance rising to 14.4 $\pm$ 2.0 ml/min/mmHg ($P < 0.05$). Values given represent the mean $\pm$ standard error of the mean and results were compared to the pre-prostaglandin values using the paired t-test.

The results demonstrate that, contrary to previous reports, PGE_2 can vasodilate the rat kidney, although vasoconstriction is seen at high doses. If vasodilation is also produced by PGE_2 synthesised in vivo then the role suggested by Armstrong et al (1976) for altered catabolism or altered sensitivity of the kidney to renal PGE_2 in mediating some forms of hypertension in the rat may have to be re-considered.

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POTENTIATION OF TAUROCHOLATE INDUCED GASTRIC DAMAGE BY PARENTERAL ADMINISTRATION OF CYCLO-OXYGENASE INHIBITORS

P. Lidbury, G. Steel & B.J.R. Whittle*, Dept. of Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.

Parenteral administration of indomethacin greatly potentiates gastric damage induced by topical application of the bile-salt taurocholate in the anaesthetised rat (Whittle, 1977). Indomethacin inhibits prostaglandin formation and reduces mucosal blood flow, whereas topical taurocholate increases acid back-diffusion. Under conditions of reduced mucosal blood flow by indomethacin, the acid diffusing back into the mucosa may accumulate within the gastric tissue, thus leading to extensive damage. We have now correlated the ability of several parenterally-administered anti-inflammatory agents to enhance taurocholate-induced damage with their inhibitory actions on gastric mucosal prostacyclin formation in the rat.

The gastric lumen of the urethane-anaesthetised rat was perfused (0.2 ml min^{-1}) with acid-saline (100 mM HCl) or acid-saline containing sodium taurocholate (2 mM). Anti-inflammatory agents were injected subcutaneously, and the gastric damage assessed after 3 h (Whittle, 1977). The formation of prostacyclin ex vivo by strips of the gastric mucosa following a washing-vortex procedure was determined by bioassay using platelet aggregation as described previously (Whittle, 1981).

In this model, parenteral administration of low doses of indomethacin (1.25 mg kg^{-1}), naproxen (1.25 mg kg^{-1}), flurbiprofen (0.25 mg kg^{-1}) and aspirin (50 mg kg^{-1}) caused no significant damage during the 3 h perfusion of acid-saline alone. However, the erosion index following perfusion of acid-taurocholate (2 $\pm$ 1 after 3 h, n=8) was significantly ($P < 0.05$) increased by these doses of indomethacin (to 25 $\pm$ 9, n=4), naproxen (to 22 $\pm$ 6, n=4), flurbiprofen (to 35 $\pm$ 12, n=3) and aspirin (to 24 $\pm$ 9, n=4). The inhibition of prostacyclin formation ex vivo in the gastric mucosa by these doses of anti-inflammatory agents following the 3 h perfusion was 80 $\pm$ 5% of control for indomethacin, 61 $\pm$ 13% for naproxen, 97 $\pm$ 1% for flurbiprofen and 79 $\pm$ 3% for aspirin. In contrast, anti-inflammatory doses of the recently described lipoxygenase inhibitor, BW755C (100 mg kg^{-1} s.c.) failed to enhance significantly gastric damage following 3 h acid-taurocholate perfusion and likewise failed to inhibit gastric mucosal prostacyclin formation.

These studies confirm that non-steroid anti-inflammatory drugs, in parenteral doses which inhibit gastric mucosal prostacyclin formation, greatly enhance the damage induced by taurocholate and support the concept of potentiating interactions between topical irritation and cyclo-oxygenase inhibition in the gastric mucosa. The finding that BW755C did not inhibit gastric mucosal prostacyclin formation or enhance gastric damage in anti-inflammatory doses agrees with its selectivity of action on different tissue cyclo-oxygenase (Whittle et al, 1980).

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THE EFFECTS OF INDOMETHACIN AND RICINOLEIC ACID ON SMALL INTESTINAL ABSORPTION AND MYOELECTRICAL ACTIVITY

A.J. Shirley*, J. Collin[1] & I.D.A. Johnston (introduced by M.D. Rawlins), Department of Surgery, University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 4LP and [1]Nuffield Department of Surgery, John Radcliffe Hospital, Oxford.

Prostaglandins have been implicated in the causation of diarrhoea of various aetiologies. Animal studies suggest that indomethacin increases water and electrolyte absorption from the small intestine (MacGregor, 1979) and it has therefore been used to reduce the duration of illness in infantile diarrhoea (Neumann, 1980). Ricinoleic acid, the active agent of Castor oil has been shown to increase the amount of "prostaglandin-like substances" in colonic fluid (Beuber & Juan, 1979). The present study was designed to investigate the effects of indomethacin and ricinoleic acid on small intestinal water and electrolyte absorption and myoelectrical activity in the dog.

Adult female mongrel dogs were used. At operation a 75cm segment of proximal jejunum was isolated and its ends attached to the anterior abdominal wall. Unipolar recording electrodes were attached to both the proximal jejunum and to the jejunal segment. Animals were allowed two weeks to recover and were fed prior to each experiment. A Foley catheter was inserted into the proximal stoma and the isolated segment of jejunum perfused (sodium chloride 115mm/L potassium chloride 5.0mm/L glucose 70mm/L) at 3ml/min for two consecutive 60 minute periods. During the second 60 minute period either indomethacin (11mg/kg/L), sodium ricinoleate (40mg/kg/L) or indomethacin plus sodium ricinoleate (11mg/kg/L and 40mg/kg/L respectively) was added to the perfusate. The effluent was collected from the distal stoma. The first 30 minutes of each 60 minute period of perfusion was used for equilibration. During the second 30 minutes the effluent was collected, the volume measured and the fluid analysed for sodium and chloride content. The myoelectrical activity was recorded and the tracings analysed for pacesetter potential frequency and fast wave activity.

Compared to the control period indomethacin did not alter the volume or electrolyte content in the effluent. Following ricinoleic acid, however, the volume of the effluent increased significantly ($P = <0.0001$) from $36.65 \pm 2.87 \rightarrow 80.30 \pm 3.91$ mean ± SEM mls/30mins and the electrolyte content also increased to a similar extent. The effect of ricinoleic acid on both water and electrolyte content was significantly reduced by indomethacin. Neither indomethacin nor sodium ricinoleate had any effect on pacesetter potential frequency. Indomethacin, given alone, caused an increase in fast wave activity in the jejunal segment ($P = 0.001$) and sodium ricinoleate, alone, caused a significant ($P = <0.05$) reduction. This action of sodium ricinoleate was inhibited ($P = <0.0001$) by the addition of indomethacin.

We conclude that in the canine small intestine indomethacin at least partially antagonises the effects of ricinoleic acid but does not alter absorption when given alone. The effects of ricinoleic acid on the small intestine may be mediated in part by prostaglandins.

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ACTION OF THE CARBACYCLIN ZK 36374 AND OTHER PROSTANOIDS ON IRIS SPHINCTER MUSCLE

Y.J. Dong* & R.L. Jones, Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ.

ZK 36374 is a chemically stable carbacyclin analogue. It inhibits both platelet aggregation and vascular tone in vitro in the same concentration range as PGI_2 (Schrör et al, 1981). We have investigated the contractile effects of ZK 36374 and other prostanoids on bullock and dog iris sphincter muscles. Preparations were mounted in a sucrose-salt solution (Crawford et al, 1978) containing Indomethacin (10^{-6}M) and contractions were recorded isometrically.

The bullock iris sphincter is sensitive to both thromboxane- and prostaglandin E-like compounds. The thromboxane receptor antagonist EP 045 (Jones & Wilson, 1981) blocks the action of 11,9-epoxymethano PGH_2 in a competitive manner; the equilibrium dissociation constant is 1.39×10^{-7}M. The thromboxane mimic EP 011 (Jones et al, 1979) is about 40 times more active than 11,9-epoxymethano PGH_2 giving threshold responses at 10^{-10}M. PGE_2 gives a lower maximum response (80-95%) than 11,9-epoxymethano PGH_2 and its action is not blocked by EP 045 (2.6×10^{-6}M). 16,16-Dimethyl PGE_2 is 1-3 times as active as PGE_2 and also unaffected by EP 045; at high concentrations (5×10^{-6}M) it gives a maximum response similar to that of 11,9-epoxymethano PGH_2. PGD_2, PGI_2, and $PGF_{2\alpha}$ and its 16-m-trifluoromethylphenoxy analogue (ICI 81008) show weak contractile activity.

ZK 36374 appears to act as a partial agonist at the PGE-sensitive site in the bullock iris sphincter. It gives a lower maximum (30-75%) than PGE_2 and opposes the effects of PGE_2, 16,16-Dimethyl PGE_2 and $PGF_{2\alpha}$, whereas with 11,9-epoxymethano PGH_2 and carbachol there is an additive interaction. EP 045 does not block the action of ZK 36374. Analysis of the log concentration-effect relationships for PGE_2 and ZK 36374 gives an equilibrium dissociation constant of 1.00×10^{-8}M for ZK 36374.

On the dog iris sphincter muscle, which is sensitive to $PGF_{2\alpha}$ analogues, the order of potency is ICI 81008 > $PGF_{2\alpha}$ >> 11,9-epoxymethano $PGH_2 \geq PGE_2$. 16,16-Dimethyl PGE_2 shows very weak activity being 500 times less active than $PGF_{2\alpha}$. ZK 36374 also shows weak activity and its effects are additive with those of $PGF_{2\alpha}$.

In summary the PGE-sensitive system in the bullock eye is characterized by (a) the potent action of PGE_2 and 16,16-Dimethyl PGE_2 (b) the partial agonist action of ZK 36374 (c) the very low potency of ICI 81008, and (d) the inability of EP 045 to block the agonist response. Preliminary studies indicate the presence of a similar PGE-sensitive system mediating contraction in the isolated guinea pig trachea. Different structure-activity relationships are found for the relaxant effects of PGE analogues.

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A BINDING SITE FOR PENTOBARBITONE ON CHOLINERGIC MEMBRANES FROM TORPEDO CALIFORNICA

L.M. Braswell and K.W. Miller*, Departments of Anesthesia and Pharmacology, Massachusetts General Hospital and Harvard Medical School, Boston, Mass, 02146, U.S.A.

In acetylcholine receptor-rich membrane vesicles from the electric organ of Torpedo californica pentobarbitone (PB) blocks by half the fast maximal carbachol stimulated efflux of cations at 25μM. In the concentration range 0.05-5.0 mM PB also progressively and non-competitively reduces the affinity of the acetylcholine receptor for both acetylcholine and d-tubocurarine (Braswell et al, 1980). Neither effect can be mediated by lipid fluidity because the former occurs at too low a concentration, whilst the latter is additive with pressure (Sauter et al, 1979).

To test whether a barbiturate binding site is involved is difficult because of the high lipid solubility of PB. However, by preparing membranes of high acetylcholine receptor specific activity (1.5-2.0 μmoles of sites per g of protein) we have been able to demonstrate a PB binding site.

Membranes (1-10μM in acetylcholine sites) were incubated at pH 7.0 with (^{14}C)-PB in the absence and presence of 5mM unlabelled PB. The difference in PB bound in these two situations was defined as displaceable binding. Binding was assayed by centrifugation at 135,000g. for 30 min in a Beckman Airfuge, followed by washing and resuspending the pellets in SDS solution prior to counting.

Displaceable binding was 20-30% of the total binding between 0.05 and 10μM (^{14}C)-PB. Titration with unlabelled PB reduced displaceable binding by half at about 0.2-0.5 mM. Because the proportion of displaceable binding decreased with increasing PB concentration, it was difficult to establish the stoichiometry, but it is probably in the 0.5-2.0 PB sites per acetylcholine site.

A preliminary survey shows other anesthetics to vary in their interactions with the PB site. Thus, 1.2 mM ketamine failed to reduce displaceable binding, whilst both urethane (125 mM) and the local anesthetic SKF-525a (165μM) reduced displaceable binding by about one half.

Is the PB site in cholinergic membranes on the receptor protein? Pre-incubation with the esterase inhibitor, diisopropyl fluorophosphate (50μM), failed to reduce displaceable binding. Carbachol (80μM) decreased displaceable binding, but probably not by a competitive mechanism because α - bungarotoxin (5-fold excess) failed to decrease displaceable binding. Thus it is probable that the acetylcholine receptor and the PB binding site interact allosterically, occupancy of either one decreasing the affinity of the other for its ligand. That the two sites interact is also consistent with the displacement by SKF-525a for which an allosteric site has been previously identified (Krodel et al, 1979).

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THE EFFECT OF SODIUM DEOXYCHOLATE ON THE ASCENDING AND DESCENDING COLON OF THE RABBIT

K.T. Bunce*, C.F. Spraggs and R. Stables, Department of Pharmacology, Glaxo Group Research Limited, Ware, Hertfordshire. SG12 0DJ.

Di-α-hydroxy bile acids cause secretion of fluid and electrolyte in rabbit colon (Chadwick et al, 1979). Since regional differences exist in the colon of other species (Dolman & Edmonds, 1975) we have investigated the effects of the bile salt, sodium deoxycholate (NaDC), on both the ascending and descending colon of the rabbit. The effect of NaDC on the recovery of the volume marker, [^{14}C]-polyethylene glycol-4000 ([^{14}C]-PEG) has also been examined.

Male rabbits were anaesthetised with urethane, and separate segments of ascending and descending colon were cannulated by a modification of the procedure described by Grandison et al (1980). Agarose/NaCl bridges were placed in the segments for measurement of potential difference (P.D.). Each experiment consisted of four consecutive 60 min periods. During control periods the segments contained a solution consisting of 115 mM NaCl, 25 mM $NaHCO_3$, 5 mM KCl, 25 mM mannitol and [^{14}C]-PEG (5 g/l;4 μCi/l). During test periods NaCl was partially replaced with NaDC. Changes in [^{14}C]-PEG concentration were used to calculate net water fluxes; net fluxes of Na^+ and Cl^- were also measured. At the end of each period the intestinal segments were rinsed out with isotonic mannitol solution and the percentage recovery of [^{14}C]-PEG determined.

The results obtained during the fourth period only are given. NaDC, 2 mM, did not affect the recovery of [^{14}C]-PEG, whereas 5 mM decreased the percentage recovery of the volume marker in the ascending colon from 96.1 ± 3.5% to 87.5 ± 3.2% ($P > 0.05$), and from 97.9 ± 3.7% to 77.6 ± 7.3% ($P < 0.05$) in the descending colon. The effect of NaDC on water and electrolyte fluxes are shown in Table 1.

Table 1 Effects of NaDC on fluid and electrolyte fluxes in rabbit colon.

Treatment	P.D. mV	H_2O flux μl/cm/h	Na^+ flux μeq/cm/h	Cl^- flux μeq/cm/h
Ascending colon				
control (n=6)	15.2 ± 1.9	- 47.6 ± 10.9	- 22.3 ± 2.2	- 15.2 ± 2.3
2 mM NaDC (n=8)	9.5 ± 2.2	- 10.4 ± 28.4	- 23.2 ± 3.2	- 16.1 ± 3.3
5 mM NaDC (n=6)	7.7 ± 1.7*	+ 107.2 ± 33.5*	+ 5.4 ± 10.1*	+ 4.1 ± 8.8*
Descending colon				
control (n=6)	46.2 ± 2.8	- 29.5 ± 22.5	- 9.1 ± 1.5	- 4.2 ± 1.1
2 mM NaDC (n=8)	22.5 ± 5.8*	+ 28.9 ± 21.0	- 1.0 ± 2.9*	+ 2.2 ± 2.4*
5 mM NaDC (n=6)	5.8 ± 3.1*	+ 78.4 ± 29.1*	+ 7.0 ± 6.1*	+ 6.5 ± 5.3*

minus (-) sign indicates a net loss (absorption) from the lumen; positive (+) sign indicates a net gain (secretion). *$P < 0.05$ compared with control.

In the ascending colon only 5 mM NaDC affected mucosal function. In the descending colon significant changes in P.D. and fluxes of Na^+ and Cl^- ions were induced by 2 mM NaDC, and the corresponding change in H_2O flux, although not significant, was from net absorption to net secretion. Thus, in the rabbit the descending colon is more sensitive to NaDC than the ascending colon.

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ACUTE EFFECTS OF ALDOSTERONE AND ALDOSTERONE ANTAGONISTS ON ARTERIAL SMOOTH MUSCLE PERMEABILITY. IONIC FLUXES EX VIVO

Anne Marie Moura* and Manuel Worcel. Centre de Recherches Roussel-Uclaf
93230 Romainville, France

We have studied previously the effects of two aldosterone antagonists : RU 28318 (17ß-hydroxy 3.oxo 7α propyl (17α) pregn 4-ène 21 potassium carboxylate and spironolactone on blood pressure and arterial smooth muscle permeability during the mineralocorticoid dependent phase of DOCA and salt hypertension in rats. It was shown that coincidently with their antihypertensive effects, RU 28318 and spironolactone supress the DOCA and salt induced changes in membrane permeability to Na^+ and Rb^+ (Moura and Worcel, 1981 ; Jones and Hart, 1975). Both mineralocorticoid and antimineralocorticoid effects could be secondary to longterm alterations in salt metabolism, rather than the consequence of a primary effect on the arterial wall. In order to solve this problem we have studied ^{22}Na and ^{86}Rb efflux from rat tail artery (Garay, Moura et al., 1979). Corticoids appear to have a tonic physiologic action since 7 days after adrenalectomy (Sx), ^{22}Na efflux is greatly reduced, due exclusively to a decrease in the ouabaine sensitive fraction (control : 0.053 ± 0.004 min^{-1} (n=8), Sx : 0.031 min^{-1} (n=9) ($p < 0.01$)). The ouabaine insensitive (control : 0.084 ± 0.004 min^{-1} (n=8), Sx : 0.078 ± 0.004) as well as ^{86}Rb efflux (control : 0.0112 ± 0.0001 min^{-1} (n=12) ; Sx : 0.0107 ± 0.0003 min^{-1} (n=10)) are not modified. The acute administration of aldosterone(s.c. injection) is able to stimulate both the ouabaine sensitive and insensitive fractions of ^{22}Na efflux from the excised tail artery. Nonetheless a plateau effect on the pump activity is obtained faster, 2h after s.c. injection (Sx : 0.031 ± 0.003 (n=9), Sx + 10 µg/kg aldosterone : 0.056 ± 0.005 min^{-1} (n=8) ($p < 0.01$)), than on the ouabaine insensitive, 4h later (Sx : 0.078 ± 0.004 min^{-1} (n=9), Sx + 10 µg/kg 0.102 ± 0.003 min^{-1} (n=8) ($p < 0.01$)). The mineralocorticoid also stimulates ^{86}Rb efflux (Sx : 0.0107 ± 0.0003 min^{-1} (n=10) Sx + 10 µg/kg aldosterone : 0.0133 min^{-1} (n=8) ($p < 0.01$)) the plateau being attained 4h after injection. The action of the mineralocorticoid on ionic fluxes is dose-dependent, a maximal effect being obtained between 2 and 5 µg/kg. Aldosterone antagonists, administered s.c. 1h before the mineralocorticoid, block mainly its effects on the sodium pump studied 2h later. The maximal stimulation of the ouabaine sensitive ^{22}Na efflux induced by aldosterone is reduced (Sx + 5 µg/kg aldosterone : 0.054 ± 0.001 min^{-1} (n=8) ($p < 0.01$), Sx + aldosterone + 2 mg/kg spironolactone : 0.041 ± 0.001 min^{-1} (n=8)) , (Sx + 5 µg aldosterone 0.054 ± 0.001 min^{-1} (n=8), Sx + 5 µg/kg aldosterone + 2 mg/kg RU 28318 : 0.042 ± 0.001 min^{-1} (n=8) ($p < 0.01$)).

In conclusion, the present results show that mineralocorticoids as well as antimineralocorticoids may exert a primary effect on the ionic permeabilities of smooth muscle. Furthermore, aldosterone appears to have two distinct types of effect on passive and sodium pump dependent exchanges.

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ANTAGONISM BY METOCLOPRAMIDE AND QUIPAZINE OF 5-HYDROXYTRYPTAMINE -INDUCED DEPOLARISATIONS OF THE RAT ISOLATED VAGUS NERVE

S.J. Ireland*, D.W. Straughan & M.B. Tyers, Department of Pharmacology, Glaxo Group Research Ltd., Greenford Road, Greenford, Middlesex, UB6 OHE.

Quipazine antagonises 5-hydroxytryptamine (5HT)-induced depolarisations of the rabbit isolated superior cervical ganglion (SCG) (Lansdown et al, 1980), and metoclopramide is a competitive antagonist of 5HT on the rabbit isolated heart preparation (Fozard & Mabarok Ali, 1978). These effects of 5HT may be mediated via the so-called 'M' receptor (Gaddum & Picarelli, 1957). In the present study, the effects of metoclopramide and quipazine on 5-HT-induced depolarisations of the rat isolated vagus nerve (VN) have been investigated. The actions of these putative antagonists against depolarisations induced by GABA, DMPP, and muscarine have also been examined. For these latter studies the rat isolated SCG preparation was used because tachyphylaxis to GABA and DMPP develops rapidly on the VN, and muscarine is inactive on this tissue.

Male Lister Hooded rats (270-330g) were anaesthetised with chloral hydrate (300mg/kg i.p.). The SCG or VN were excised, desheathed, and mounted in 2-compartment perspex baths. The SCG preparations were positioned such that the ganglion soma lay in the first compartment, and the post-ganglionic (internal carotid) nerve projected through a greased slot into the second. The VN preparations were mounted so that about 75% of the nerve lay in the first compartment, while the remainder projected into the second. The tissues were continuously superfused with Krebs-Henseleit solution at 27°C. Drugs were applied at known concentration via the perfusion stream into the first compartment only. The D.C. potential between the two compartments was recorded using Ag/AgCl electrodes connected to the tissue via agar/filter paper bridges, and displayed on a potentiometric chart recorder.

In the VN preparation, 5HT, $1x10^{-7}$ - $3x10^{-5}$M, evoked rapid depolarisations which reached peak ampitude within 2 minutes. These depolarisations were not antagonised by methysergide, $1x10^{-4}$M, hexamethonium, $1x10^{-3}$M, picrotoxin, $1x10^{-4}$M, or bicuculline, $1x10^{-5}$M. Metoclopramide, $1x10^{-6}$ - $1x10^{-4}$M, caused a rightwards displacement of the 5HT dose-response curve, with no evidence of a reduction in slope or maximum. A plot of log [dose ratio -1] against log concentration of metoclopramide (Arunlakshana & Schild, 1959) produced a significant linear relationship ($p < 0.001$), and the best fit straight line had a gradient of 1.01 ± 0.01. The pA_2 value was 6.57. Quipazine, $1x10^{-9}$ - $1x10^{-6}$M, also caused a dose-related rightwards shift of the 5HT dose-response curve, but this was accompanied by a decline in both slope and maximum. Quipazine alone, $1x10^{-9}$ - $1x10^{-4}$M, failed to induce any depolarisation.

In the rat isolated SCG, metoclopramide, $1x10^{-4}$M, had no effect on GABA or muscarine-induced depolarisation but significantly depressed the slope of the DMPP dose-response curve. Quipazine, $1x10^{-4}$M, failed to block GABA responses, but caused a slight reduction in the dose-response curve maxima for both muscarine and DMPP induced depolarisations. Neither compound, at a concentration of $1x10^{-5}$M inhibited the effects of the non-5HT agonists. In comparison, this concentration of either metoclopramide or quipazine depressed half-maximal 5HT-induced depolarisations of the rat isolated SCG by over 80% (n=7).

The results suggest that the 5HT receptors on the rat VN may be similar to those of the rabbit SCG and heart.

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β-ADRENOCEPTOR-MEDIATED RESPONSES OF RAT SUPERIOR CERVICAL GANGLIA IN VITRO

P.M. Dunn. (introduced by D.A. Brown). Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London. WC1N 1AX

Catecholamines have previously been reported to produce an α-mediated hyperpolarization (Brown & Caulfield, 1979) and a β-mediated elevation of cyclic AMP (Lindl & Cramer, 1975; Brown, Caulfield & Kirby, 1980) in rat superior cervical ganglia *in vitro*. In this communication I describe an additional β-mediated depolarization in this tissue comparable to that initially reported in cat ganglia *in vivo* (De Groat & Volle, 1966), via receptors similar to those responsible for the raised cyclic AMP.

Extracellular DC potential changes were recorded from desheathed freshly-isolated rat superior cervical ganglia using a three-chambered bath (Brown & Marsh, 1978). The ganglion was superfused continuously with oxygenated Krebs solution at 22°C.

Addition of catecholamines to the perfusate produced small ($\leq$ 0.4 mV) reversible, concentration-dependent potential changes: adrenaline and noradrenaline (1-100 μM) produced either a hyperpolarization (as previously reported by Brown & Caulfield, 1979) or a depolarization preceded by a hyperpolarization; in contrast isoprenaline (1-100 nM; mean EC_{50} 3.4 nM) and salbutamol (0.01-1 μM; EC_{50} 50 nM) produced only depolarizations, noticeably more delayed and prolonged than the α-hyperpolarization. Isoprenaline-induced depolarizations were antagonized by (-)propranol (pA_2 8.9), ($\pm$)practolol (5.1) and ($\pm$)butoxamine (7.4), but not at all by (+)propranolol at 0.1 μM. This order of activity suggests a β_2 receptor.

Cyclic AMP changes following addition of catecholamines were measured as described by Brown *et al* (1980). Isoprenaline (0.01-1 μM; mean EC_{50} 28 nM) produced a dose-dependent elevation of cAMP of up to 30 times after 10 min incubation. This effect was antagonised by ($\pm$)practolol and ($\pm$)butoxamine, at concentrations comparable to those required to antagonize the β-depolarizations. Thus although agonist concentrations necessary to elevate cAMP exceeded those required to depolarize the neurones, antagonist activities on the two responses were similar. Other experiments to explore the relationship between these two responses are in progress.

P.M.D. is an S.R.C. CASE student.

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FUNCTIONAL ROLE OF PRESYNAPTIC α- AND β-ADRENOCEPTORS IN RAT ISOLATED SUPERIOR CERVICAL GANGLION?

D.A. Brown and I.C. Medgett*. Department of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London. WC1N 1AX

Catecholamines can both facilitate and depress ganglionic transmission; the former appears to be a β-effect, the latter an α_2-effect which in several species including the rat has been shown to be presynaptic in origin (reviewed by Brown and Caulfield, 1981). This communication describes conditions under which ganglionic transmission can be modified by activation of presynaptic α- and β-adrenoceptors.

Extracellular DC potential changes (unstimulated preparations), and compound action potentials (supramaximal preganglionic stimulation at 0.2 Hz) were recorded from desheathed rat superior cervical ganglia using a three-chambered bath (Brown and Marsh, 1978). Ganglia were superfused continuously with oxygenated Krebs solution at 22-25°C.

There was a clear difference in potency between the hyperpolarizing effect of clonidine in unstimulated preparations (postsynaptic α_2-adrenoceptor effect; mean EC_{50} ~ 2 nM) and inhibition of the compound action potential (presynaptic α_2-adrenoceptor effect; mean EC_{50} ~ 18 nM). In addition to blocking the inhibitory effects of adrenaline (10 nM-100 μM), phentolamine (1 μM) itself significantly increased the height of the compound action potential by 11% (s.e.m. ± 2%, n = 13). This latter effect, together with the observed lower potency of clonidine at pre- compared to postsynaptic α_2-adrenoceptors, suggests that, with continuous stimulation of preganglionic nerves at 0.2 Hz, an α_2-adrenoceptor-mediated trans-synaptic inhibitory feedback mechanism for release of transmitter acetylcholine operates in this tissue, activated by noradrenaline released from the dendrites of the postganglionic neurones (Martinez and Adler-Graschinsky, 1980) or from other sites (Noon et al., 1975).

(±)Propranolol (0.1 μM) did not affect the height of the compound action potential whereas the inhibitory effects of high concentrations of adrenaline (10-100 μM) were significantly increased. During an infusion of clonidine (1 μM), adrenaline (1-100 μM) and to a lesser extent noradrenaline (10-100 μM) increased the height of the compound action potential; these effects were blocked by propranolol (0.1 μM). These data suggest that facilitatory β-adrenoceptors (possibly β_2) may be present on preganglionic terminals and that activation of these receptors may facilitate transmission when the α_2-adrenoceptor-mediated inhibitory mechanism is fully operative.

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EVIDENCE FOR MUSCARINIC AUTORECEPTORS ON CHOLINERGIC NEURONES IN THE RABBIT RETINA

C. Dawson & M.J. Neal*, Department of Pharmacology, The School of Pharmacy, University of London, Brunswick Square, London, WC1N 1AX.

There is strong evidence to suggest that the rabbit retina possesses a sub-population of amacrine cells which are cholinergic (Masland & Mills, 1980). In the present study, we have examined the effects of muscarine and some cholinergic antagonists on the light evoked release of ACh from the retina.

The methods have been described previously (Neal & Massey, 1980). Briefly, rabbits were anaesthetised with urethane and an eye-cup prepared by removal of the anterior parts of the eye and the vitreous. The retina was then exposed for 30 min to [^{3}H] choline which is accumulated by some (cholinergic) amacrine cells. The release of [^{3}H]ACh from the retina was then measured at 5 min intervals. The retina was stimulated with light flashes (25% duty cycle, 330. lux) at 3Hz.

The results which are summarised in Table 1 show that muscarine reduced the light evoked release of ACh by 50%, whilst atropine, in the presence of eserine, increased the release by over 50%. Hexamethonium and pempidine were without effect.

Table 1 Effect of cholinergic drugs on light evoked release of [^{3}H]ACh

Each result is the mean ± s.e.mean of 4 experiments

Control	1.56 ± 0.1	
Muscarine (10μM)	1.28 ± 0.1	$p<0.02$
Control	2.8 ± 0.5	
Atropine (10μM) + eserine (30μM)	4.4 0.7	$p<0.01$
Control	2.1 ± 0.2	
Atropine (10μM)	2.2 ± 0.3	N.S.

The simplest explanation for these results is the presence of inhibitory muscarinic autoreceptors on the cholinergic amacrine cells.

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20P

EFFECT OF PUTATIVE DOPAMINE AUTORECEPTOR AGONISTS 3-PPP AND TL-99 ON POSTSYNAPTIC DOPAMINE-SENSITIVE ADENYLATE CYCLASE IN CARP RETINA

K. J. Watling* & M. Williams[1], The Biological Laboratories, Harvard University, Cambridge, MA 02138, USA and [1]Merck, Sharp & Dohme Research Laboratories, West Point, PA 19486, USA

Presynaptic dopamine (DA) receptors, termed autoreceptors, provide a negative feedback mechanism by which synaptically released DA can inhibit its further release from presynaptic dopaminergic terminals. A selective autoreceptor agonist, by reducing dopaminergic neurotransmission, may prove to be a novel neuroleptic agent devoid of the side effects associated with the currently used neuroleptic drugs which are believed to antagonise postsynaptic DA receptors. Two compounds, 3-PPP [3-(3-hydroxyphenyl)-N-n-propylpiperidine; Hjorth et al., 1981] and TL-99 (6,7-dihydroxy-2-dimethyl-aminotetralin; Goodale et al., 1981) have recently been proposed as selective DA autoreceptor agonists.

The carp retina possesses a well characterised population of DA receptors associated with adenylate cyclase (Watling and Dowling, 1981; Dowling and Watling, 1981). Furthermore, these DA receptors are located postsynaptically with respect to the DA-containing interplexiform cells as evidenced by the lack of any reduction in DA-stimulated adenylate cyclase activity following the destruction of DA-containing neurones using intraocularly injected 6-hydroxydopamine (20 µg/10 µl 0.9% saline/eye on each of two consecutive days). One week following the injection of 6-hydroxydopamine, histological examination of the retina confirmed the disappearance of dopaminergic nerve terminals as verified biochemically by the reduction of retinal DA levels to less than 5% of control (0.9% saline treated) eyes. The DA-induced stimulation of cyclic AMP production in the carp retina is thus a purely postsynaptic event, and as such represents an excellent system in which to evaluate the selectivity of putative presynaptic DA receptor agonists.

The effects of DA, ADTN (6,7-dihydroxy-2-aminotetralin), TL-99 and 3-PPP were examined for their ability to stimulate cyclic AMP accumulation in pieces of intact carp retina using previously described methods (Dowling and Watling, 1981). Whilst DA, ADTN and TL-99 all induced a similar dose-related increase in cyclic AMP levels with EC50 values (concentration inducing 50% of maximum response) of 3.5 µM, 3.1 µM and 3.6 µM respectively, and maximum responses occuring between 10 and 100 µM, 3-PPP was inactive at concentrations up to 100 µM. Moreover, 150 µM 3-PPP failed to elevate cyclic AMP levels in preparations of isolated horizontal cells from the enzymatically dissociated carp retina, cells which are known to receive an extensive dopaminergic innervation in this species (Ehinger and Dowling, 1978) and to possess a DA-dependent adenylate cyclase (Van Buskirk and Dowling, 1981). In contrast, 150 µM DA, ADTN or TL-99 all increased cyclic AMP production in this isolated cell preparation.

In conclusion, the inability of 3-PPP to stimulate the postsynaptic dopamine-sensitive adenylate cyclase in the carp retina supports the contention that this compound may preferentially activate presynaptic dopamine autoreceptors. In contrast, the ability of TL-99 to stimulate cyclic AMP accumulation in either intact pieces of retina or preparations of isolated horizontal cells indicates that this compound can interact with postsynaptic dopaminergic mechanisms, and as such cannot be viewed as a selective presynaptic DA receptor agonist.

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A COMPARATIVE STUDY OF THE CATECHOL-O-METHYL TRANSFERASE INHIBITORS, U-0521 AND TROPOLONE ACETAMIDE, IN RAT HEART

Lesley J. Bryan*, H. Fleig & U. Trendelenburg, Pharmakologisches Institut, Universität Würzburg, D-8700 Würzburg, Federal Republic of Germany

U-0521 has been used by various workers to inhibit catechol-O-methyl transferase (COMT) in studies on extraneuronal uptake and metabolism of catecholamines. However, it also weakly inhibits catecholamine uptake (Bönisch et al, 1974; Bryan & O'Donnell, 1979) and efflux (Bönisch et al, 1978). Tropolone analogues also inhibit COMT. In the present study, the effects of U-0521 and tropolone acetamide on the O-methylation of isoprenaline and on the tissue/medium (T/M) ratios for isoprenaline in the rat heart were compared.

Hearts were perfused with 0.01 µM [^{3}H]-isoprenaline for 15 min, in the presence of various concentrations of U-0521 or tropolone acetamide, after pre-perfusion with the inhibitor for 20 minutes. Venous samples were collected between the 11th and 15th min of perfusion, when the rate of appearance of O-methylisoprenaline (OMI) in the venous effluent is in steady state. T/M ratios were obtained by determination of isoprenaline in the heart tissue at the end of the experiment. For both U-0521 and tropolone acetamide, any reduction in the rate of appearance of OMI corresponded with an increase in the T/M ratio for isoprenaline in the heart. Fifty percent inhibition of O-methylation and 50% of the maximal increase in T/M ratio were obtained with 0.413 ± 0.070 µM (n=9) and 0.280 ± 0.050 µM (n=9), respectively, for U-0521 (from data for 0.1 to 1 µM), and 2.77 ± 0.44 µM (n=11) and 2.21 ± 0.30 µM (n=25), respectively, for tropolone acetamide. A high concentration of U-0521 (100 µM) caused a smaller increase in T/M ratio than did a lower concentration (10 µM), with ΔT/M values of 4.58 ± 0.25 and 6.65 ± 0.35, respectively. This represents the inhibition of extraneuronal uptake by U-0521. For tropolone acetamide, on the other hand, no decline of the T/M ratio was observed for concentrations as high as 1000 µM.

The effects of U-0521 and tropolone acetamide on the efflux of isoprenaline from the rat heart were also compared. Hearts were perfused with 0.3 µM [^{3}H]-isoprenaline for 10 min in the presence of 10 µM U-0521. During subsequent wash-out with amine-free solution, either 100 µM U-0521 or 1000 µM tropolone acetamide was present. The half time for efflux of isoprenaline was significantly greater ($t = 5.91$, d.f. 4, $0.01>P>0.001$) with U-0521 (geometric mean: 19.2 min; 95% confidence limits: 17.3 and 21.2 min; n=3) than with tropolone acetamide (geometric mean: 13.8 min; 95% confidence limits: 11.9 and 15.9 min; n=3).

In conclusion, the study has shown that tropolone acetamide inhibits O-methylation of isoprenaline in extraneuronal sites in the rat heart, but it is less potent than U-0521. However, tropolone acetamide has the advantage that it does not possess the weak uptake inhibitory properties that have been reported for U-0521. Furthermore, high concentrations of U-0521, but not tropolone acetamide, impaired the efflux of isoprenaline from the heart.

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β-ADRENOCEPTORS AND EXTRANEURONAL UPTAKE IN CAT TRACHEA

Cole, J.J., O'Donnell, S.R.* & Wanstall, J.C., Department of Physiology and Pharmacology, University of Queensland, Brisbane, Queensland 4067, Australia.

The β-adrenoceptor population, adrenergic innervation and extraneuronal uptake of catecholamines in cat tracheal preparations have been examined and the data compared with that previously obtained in guinea-pig trachea.

Single rings of thoracic trachea were contracted with 0.5 μM carbachol and cumulative concentration-response (relaxation) curves to β-adrenoceptor agonists obtained. Schild plots and pA_2 values were obtained, as described by O'Donnell & Wanstall (1979). For both ICI 118,551 (β_2-selective antagonist) and atenolol (β_1-selective antagonist) the Schild plots were in different locations depending on whether the agonist used was fenoterol (β_2-selective) or noradrenaline (β_1-selective). When noradrenaline was the agonist the pA_2 for ICI 118,551 was 6.96 ± 0.05, (4 animals); slope of Schild plot 0.96 ± 0.06, (12 data points) and the pA_2 for atenolol was 6.81 ± 0.02 (4); slope 0.99 ± 0.07 (12). These pA_2 values were close to pA_2 values for these two drugs on β_1-adrenoceptors. When fenoterol was the agonist the pA_2 for ICI 118,551 was 7.55 ± 0.06 (5); slope 0.86 ± 0.08 (10), i.e. higher pA_2 than on β_1-adrenoceptors, and, because the fenoterol concentration-response curve in the presence of atenolol was biphasic, two different pA_2 values were obtained for atenolol viz. 6.80 ± 0.07 (5); slope 0.85 ± 0.07 (15) and 5.94 ± 0.02 (5); slope 0.86 ± 0.11 (15). These values corresponded to values for atenolol on β_1- and β_2- adrenoceptors respectively. These results indicated that relaxation of cat tracheal preparations was mediated by both β_1- and β_2- adrenoceptors (as in guinea-pig trachea). However the β_2-adrenoceptors were too few in number to elicit a maximum relaxation response. Fenoterol and noradrenaline were approximately equipotent confirming that β_1-adrenoceptors predominated (in contrast to guinea-pig trachea in which $\beta_2 > \beta_1$).

Cat trachealis smooth muscle was more densely adrenergically innervated than guinea-pig trachealis smooth muscle (O'Donnell & Saar, 1973) and, unlike guinea-pig, there was no apparent change in density of innervation between cervical and thoracic ends. Using a fluorescence histochemical technique (Bryan & O'Donnell, 1981) the extraneuronal uptake of isoprenaline into trachealis smooth muscle was studied. The K_m for isoprenaline was 407 ± 110.4 μM (c.f. guinea-pig 266 ± 11.2 μM). The capacity of the uptake was greater than in guinea-pig. Corticosterone was a competitive inhibitor of isoprenaline uptake with a K_i of 2.64 ± 1.38 μM (c.f. guinea-pig 1.52 ± 0.53 μM). The extraneuronal uptake was functionally effective in that isoprenaline responses were potentiated by 10 and 50 μM corticosterone (2.6 and 3.6 fold respectively) and also by 50 μM metanephrine (4 fold, when corrected for the β-adrenoceptor antagonist action of metanephrine).

Thus cat trachea differs from guinea-pig trachea with respect to its β-adrenoceptor population and the density and pattern of its adrenergic innervation. However cat trachea resembles guinea-pig trachea with respect to the uptake of isoprenaline into trachealis smooth muscle cells, and the functional effectiveness of this uptake mechanism in pharmacological experiments. The predominance of β_1-adrenoceptors in cat trachea may possibly be related to the comparatively high density of adrenergic nerves, but there is no evidence to suggest that extraneuronal uptake is linked in particular to one or other β-adrenoceptor type.

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IS THERE A DIRECT LINK BETWEEN Na^+K^+-ATPase ACTIVITY AND NORADRENALINE RE-UPTAKE?

M.D. Wood* & M.G. Wyllie, Biochemical Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berks, SL6 OPH.

There is considerable circumstantial evidence linking Na^+K^+-ATPase (EC 3.6.1.3) and neurotransmitter release and re-uptake (Vizi, 1978; Gilbert and Wyllie, 1980). Generally however media of different compositions are used to measure these parameters. This is necessary because calcium ions inhibit Na^+K^+-ATPase (Schwartz et al, 1975). It is reasonable to assume therefore that in a calcium-free medium an overestimate of enzyme activity may arise. Traditionally as an alternative and to circumvent these difficulties, enzyme activity has been calculated from ouabain binding. The relationship between enzyme activity and neurotransmitter function has also been examined using drugs to selectively inhibit or stimulate either process. We describe here the assessment of Na^+K^+-ATPase activity using ^{86}Rb (2Ci g^{-1}. Radiochemical Centre, Amersham). The relationship between the activity of this enzyme and noradrenaline uptake was also examined.

Purified synaptosomes were prepared and noradrenaline uptake measured as described by Wood and Wyllie (1981). Incubations were performed at 37°C in a medium of the following composition (mm): NaCl 136; KCl 5; $MgCl_2$ 2.5; $CaCl_2$ 2.5; glucose 10; ascorbate 1 and Tris base 20 buffered to pH 7.4 with HCl. The medium was gassed with oxygen for 30 min prior to use. After pre-incubation, ^{86}Rb (1μCi ml^{-1}) and ^3H-ℓ-NA (100nM) were added to the synaptosomal suspensions. Uptake was terminated by filtration and washing on cellulose acetate 0.45μm filters. In a separate series of experiments, Na^+K^+-ATPase activity was measured as described by Gilbert and Wyllie (1980) in a medium of the following composition (mM): NaCl 150; KCl 10; $MgCl_2$ 5 and Tris-HCl pH 7.4, 50.

There is now considerable evidence showing that the ouabain sensitive uptake of ^{86}Rb is a reliable index of Na^+K^+-ATPase activity (inter alia, Meyer & Cooper, 1981). However, in the present study, enzyme activity as assessed using ^{86}Rb was only 15% of the activity measured under optimal conditions. This was undoubtedly due to the inhibitory action of calcium ions. The data also indicate that ouabain-sensitivity is independent of the method of measurement of enzyme activity. The IC_{50} for Na^+K^+-ATPase inhibition measured conventionally was 6.1 ± 0.2 (6) μM whereas using ^{86}Rb the IC_{50} was 7.4 ± 0.4 (3) μM. There was no demonstrable stimulation of ^{86}Rb uptake by noradrenaline which was in marked contrast to the effect of the catecholamine when the enzyme was measured by conventional methods in a calcium-free environment. It is also pertinent to note that there was a large discrepancy between the inhibitory potency of ouabain on noradrenaline uptake and on enzyme activity. Further, imipramine inhibited noradrenaline uptake without altering enzyme activity. The data suggest that there is no direct association between Na^+K^+-ATPase activity and noradrenaline re-uptake.

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THE EFFECTS OF NIFEDIPINE AND VERAPAMIL ON KC1-STIMULATED Ca^{2+} FLUXES IN THE RAT VAS DEFERENS

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

The organic calcium antagonists are potent inhibitors of KCl contractions in the rat vas deferens (Hay & Wadsworth, 1981) and have been shown to inhibit KCl stimulated Ca^{2+} uptake into vascular and intestinal muscle (Thorens & Haeusler, 1979; Rosenberger et al, 1979). However their effect on Ca^{2+} flux in the vas deferens is unknown. Bisected vasa deferentia from Wistar rats were incubated for 50 min at 37°C in Krebs-Henseleit solution then for 5 min at 37°C in a tris buffered solution (both containing ^{45}Ca 0.5 $\mu Ci\ ml^{-1}$) and finally for 60 min at 0.5°C in a tris buffered solution containing $LaCl_3$ 50 mM. Residual ^{45}Ca was extracted with EDTA 5 mM and measured by liquid scintillation counting. The basal ^{45}Ca uptake was greater in the epididymal half, but KCl 160 mM caused a larger accumulation of ^{45}Ca in the prostatic half (prostatic 155% increase; epididymal 38% increase). KCl stimulated uptake was abolished by nifedipine 0.29 µM in both halves. Verapamil 2 µM abolished KCl stimulated uptake in the epididymal half and substantially reduced it in the prostatic half. Basal ^{45}Ca uptake was not affected except with nifedipine in the epididymal half, where it was reduced (Table 1).

Table 1 Lanthanum resistant ^{45}Ca uptake (nmol g^{-1} wet weight) (n=6)

	Epididymal	Prostatic
Basal	746 ± 30	694 ± 37
Basal + nifedipine 0.29 µM	609 ± 21	614 ± 66
KCl 160 mM	992 ± 120	1654 ± 144
KCl 160 mM + nifedipine 0.29 µM	542 ± 82	697 ± 25
Basal	715 ± 50	663 ± 22
Basal + verapamil 2 µM	708 ± 51	638 ± 56
KCl 160 mM	1021 ± 104	1814 ± 70
KCl 160 mM + verapamil 2 µM	604 ± 42	888 ± 44

Intact vasa deferentia were loaded with ^{45}Ca 2 $\mu Ci\ ml^{-1}$ in Krebs-Henseleit solution at 37°C for 3h, then superfused at 2.7 ml min^{-1} with Krebs-Henseleit solution containing 2.5 or 0 mM $CaCl_2$ or EGTA 0.05 mM. After 90 min the superfusate was changed to an identical solution containing KCl 160 mM. The superfusate was collected every 2 min and counted by liquid scintillation. Residual ^{45}Ca was extracted and counted as above. The pattern and rate of efflux into Ca-containing and Ca-free superfusates were similar. KCl 160 mM caused an increase in ^{45}Ca efflux, which was not blocked by verapamil 2 µM or nifedipine 0.29 µM.

Since nifedipine and verapamil block ^{45}Ca uptake, we conclude that in the vas deferens the organic calcium antagonists inhibit KCl contractions by block of voltage dependent Ca^{2+} channels. However, these drugs have no effect on KCl stimulated ^{45}Ca efflux and it is possible that KCl may release Ca^{2+} from high affinity extracellular sites not directly involved in contraction. Alternatively, there may be a separate population of Ca^{2+} channels not blocked by the calcium antagonists, that permit efflux of intracellular Ca^{2+} released by KCl.

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COMPARISON OF "Ca^{++}-ANTAGONISTS" AND TRIFLUOPERAZINE IN SKINNED SMOOTH MUSCLE FIBRES

M. Spedding, Centre de Recherche Merrell International, 16, rue d'Ankara, 67084 Strasbourg, Cedex, France

Trifluoperazine and pimozide have been shown to bind to calmodulin (Levin & Weiss, 1979) and high concentrations of trifluoperazine inhibit activation of the contractile proteins by Ca^{++} in chemically-skinned smooth muscle preparations (Kerrick et al, 1980; Sparrow et al, 1981). This property may be a factor in the inhibitory effects of trifluoperazine and pimozide against Ca^{++}-induced contractions in K^{+}-depolarized smooth muscle preparations which are similar to the effects of some "Ca^{++}-antagonists" (cinnarizine, flunarizine, fendiline) but not others (verapamil, diltiazem, nifedipine) (Spedding, 1981). I have therefore compared all these drugs in taenia caeci preparations chemically skinned by the method of Sparrow et al. (1981).

Strips (10 mm x 0.5 mm) of taenia caeci from male guinea pigs were incubated in a buffer containing Triton X-100 (composition: imidazole 20 mM, ethyleneglycol bis-aminoethylether N,N-tetracetic acid (EGTA) 5 mM, KCl 50 mM, sucrose 150 mM, dithioerythritol 0.5 mM, Triton X-100 1%, pH 7.4) for 4 h at 4°C and stored for 5-10 days at -20°C in glycerol/buffer (composition: imidazole 20 mM, EGTA 4 mM, $MgCl_2$ 10 mM, adenosine 5'-triphosphate (ATP) 7.5 mM, NaN_3 1 mM, dithioerythritol 0.5 mM, glycerol 50%, pH 6.7) prior to being set up in buffer (composition: imidazole 20 mM, EGTA 4 mM, $MgCl_2$ 10 mM, ATP 7.5 mM, NaN_3 1 mM, K_2PO_4 6 mM, pH 6.7) at 20-22°C. Contractions were induced at 30-40 min intervals by adding $CaCl_2$ to give a Ca^{++} concentration of 20 µM (Sparrow et al, 1981) and were reproducible for > 3 h.

The contractions can be attributed to a direct activation of the contractile proteins because electron microscopy of the preparations showed uniform disruption of the plasma membrane. The maximum tension developed by unskinned preparations (in Tyrode solution at 35°C) was approximately 5-fold greater than that of the skinned preparations. Addition of calmodulin (0.2 µM for 3 h) increased the maximum developed tension in the skinned preparations following exposure to Ca^{++} (calmodulin-treated preparations 1.67 $\pm$ 0.19 g, n = 4; adjacent control strips 0.95 $\pm$ 0.12 g, n = 4, $p < 0.05$), suggesting some loss of soluble proteins, including calmodulin, during the skinning procedure.

A high concentration (100 µM) of trifluoperazine and pimozide inhibited Ca^{++}-induced contractions by 42 $\pm$ 11% (n = 8) and 50 $\pm$ 11% (n = 6) respectively, but a low concentration (10 µM) was without effect. Cinnarizine (100 µM, 77 $\pm$ 5% inhibition, n = 4), flunarizine (100 µM, 28 $\pm$ 12%, n = 4) and fendiline (100 µM, 34 $\pm$ 4%, n = 4) also inhibited Ca^{++}-induced contractions, but verapamil (100 µM), diltiazem (100 µM) and nifedipine (100 µM) were without effect.

Thus some, but not all, "Ca^{++}-antagonists" interact with the contractile proteins of smooth muscle cells, albeit in high concentrations. Further experiments are required to determine whether this property contributes to the inhibitory effects of these drugs in unskinned preparations and to their individual spectra of activity as "Ca^{++}-antagonists" (Spedding, 1981).

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INHIBITION OF THE BREAKDOWN OF EXOGENOUS AND ENDOGENOUS ENKEPHALINS

A.D. Corbett, H.W. Kosterlitz & A.T. McKnight*, Unit for Research on Addictive Drugs, University of Aberdeen, Marischal College, Aberdeen, AB9 1AS.

In this study we have investigated the release of enkephalins directly into the bath fluid after field stimulation of the myenteric plexus-longitudinal muscle preparation of guinea-pig small intestine (G.p.i.) when inhibitors of enkephalin degradation are included in the bathing medium. In preliminary studies we have also assessed the usefulness of other putative inhibitors by testing for increased potency of [Met]enkephalin in the in vitro assay models, G.p.i., rat vas deferens (R.v.d.) and mouse vas deferens (M.v.d.) in their presence.

For the release experiments, preparations of G.p.i. were stimulated electrically in the presence of cycloheximide and tetraethylammonium (which facilitates enkephalin loss), and the enkephalins from the tissues and in the bathing fluid were extracted and assayed (Corbett et al, 1980; Corbett et al, 1981). The effectiveness of the various inhibitors was measured by comparing the IC_{50} values for [Met] enkephalin obtained in control and treated assay preparations.

With the addition of either the exopeptidase inhibitor bacitracin (30 µM) or the mixture of dipeptides, Tyr-Tyr, Leu-Leu, Leu-Gly and Gly-Phe (all 1 mM) and Tyr-Gly (100 µM), no enkephalins could be detected in the bath fluid after field stimulation at 1 Hz, 0.5 ms, 50 mA for 30 min. When these two treatments were combined 48±13 pmol/g [Met]enkephalin and 16±3 pmol/g [Leu]enkephalin (n = 3) were found. Compared to the losses from the tissue stores these amounts correspond to "recoveries" of less than 50%, therefore more effective inhibitors were sought using the bioassay tissues.

Control IC_{50} values for [Met]enkephalin were 158.6±38.6 nM (n = 6) for G.p.i., 53.7±7.8 µM (n = 7) for R.v.d. and 18.9±1.2 nM (n = 21) for M.v.d. The combination of dipeptides produced leftward shifts of the dose-response curves for [Met] enkephalin in all three tissues; 10-fold in G.p.i., 20-fold in the R.v.d. and 5-fold in the M.v.d. In the G.p.i. and R.v.d. at least any one of the constituent dipeptides produced a shift; Leu-Leu and Leu-Gly were particularly effective and at 2 mM produced shifts equivalent to that seen with the mixture.

In the M.v.d. the enkephalinase inhibitor thiorphan 300 nM produced a 2 to 3-fold leftward shift of the dose-response curve to [Met]enkephalin without affecting the sensitivity to normorphine, β-endorphin or [D-Ala^2,D-Leu^5]enkephalin. A similar shift was observed with the aminopeptidase inhibitor bestatin and the combination of these two agents reduced the IC_{50} to 4.2±0.7 nM (n = 4). The addition of the angiotensin-converting-enzyme inhibitor captopril (10 µM) further reduced the IC_{50} to 2.6±0.2 nM (n = 4). Preliminary results with the G.p.i. and R.v.d. have shown that the combination of these three agents may be even more effective, reducing the IC_{50} to 15 nM and 2 µM, respectively.

If the combination of agents producing large increases in potency to exogenous [Met]enkephalin in the isolated tissues exerts a similar effect in protecting released, endogenous, enkephalins from breakdown it should be possible to obtain reproducibly high measures of enkephalin overflow. It may also now be possible to remove the interfering effect of metabolism in studies of receptor kinetics.

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A COMPARISON OF THE EFFECTS OF MORPHINE AND OPIOID PEPTIDES ON THE SECRETION IN VITRO OF CORTICOTROPHIN RELEASING FACTOR

Julia C. Buckingham, Academic Department of Pharmacology,
Royal Free Hospital School of Medicine, London NW3 2PF

Morphine and morphine-like drugs influence pituitary-adrenocortical activity by modifying the secretion of the hypothalamic peptide, corticotrophin releasing factor (CRF), which controls the secretion of corticotrophin (ACTH) (Buckingham & Hodges, 1980; Buckingham, 1981). In this study the effects of morphine on the secretion *in vitro* of CRF were compared with those of met-enkephalin, leu-enkephalin and β-endorphin. Hypothalami were removed from adult male Sprague-Dawley rats and incubated, as described previously (Buckingham & Hodges, 1977*a*), in the presence and absence of opioid substances. The CRF contents of the hypothalamic tissue and of the incubation media were determined by a method which depends upon the ability of the releasing hormone to stimulate segments of adenohypophysial tissue to secrete ACTH (Buckingham & Hodges, 1977*b*).

Met-enkephalin (10^{-10}-10^{-6}M) and leu-enkephalin (10^{-7}-10^{-5}M) increased the CRF content of the hypothalami and the media in which they were incubated. Morphine (10^{-10}-10^{-6}M) and β-endorphin (10^{-12}-10^{-10}M) also stimulated CRF production but in higher concentrations (10^{-5}-10^{-4}M and 10^{-10}-10^{-9}M respectively) their effects were less marked. The stimulatory effects of morphine, β-endorphin and the enkephalins were antagonized by naloxone (10^{-8}M). In complete contrast, β-endorphin, in very high concentrations (10^{-6}-10^{-4}M), inhibited the spontaneous secretion of CRF and antagonized, in a non-competitive manner, the production of the hormone which normally occurs in response to acetylcholine, 5-hydroxytryptamine or met-enkephalin. After prolonged contact with the tissue, morphine (10^{-8}-10^{-7}M) also exhibited inhibitory effects which were antagonized by naloxone (10^{-5}M).

The data explain the actions of morphine on pituitary adrenocortical activity and support the concept (Gibson, Ginsburg, Hall & Hart, 1979) that opioid peptides are concerned with the control of the secretion of corticotrophin releasing factor. They also suggest that the actions of opioid substances on the hypothalamus are mediated by more than one type of receptor.

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PHYSOSTIGMINE AS AN ANTAGONIST OF MORPHINE IN THE HUMAN SUBJECT

J.T. Davidson, I. Snir-Mor & M. Weinstock*[1], Departments of Anaesthesiology and Pharmacology[1], Hadassah Hospital Hebrew University, Jerusalem, Israel.

Some of the effects of opiate drugs may result from interference with central cholinergic transmission (Domino & Wilson, 1973). In a previous study, physostigmine was shown to reverse the respiratory depressant effect of morphine in dogs and rabbits, leaving the analgesia unimpaired (Weinstock et al., 1980). The purpose of the present study was to determine whether physostigmine could be used to effect a selective antagonism of morphine-induced respiratory depression in human subjects.

As pain itself may alter respiratory control, the first part of the study was conducted in 10 pain-free, pre-operative subjects who were given morphine as part of their anaesthesia. Nausea and peripheral cholinergic overactivity were prevented in both studies by pretreatment with droperidol (5 mg) and N-butyl hyoscine hydrobromide (Scobutan, 5-10 mg). The effect of morphine and physostigmine on respiration was assessed by the CO_2-rebreathing method of Read (1967). Changes in minute ventilation in response to increasing concentrations of CO_2 were measured before and 30 min after morphine (0.16 mg/kg i.v.), and 5-10 and 30 min after physostigmine (13-33 ug/kg i.v.), i.e. 45 and 65 min after morphine.

Morphine reduced the mean slope (± s.e.mean) of the CO_2-response curves (decreased sensitivity to CO_2) for the group from 1.96 ± 0.23 to 1.11 ± 0.13 ($P < 0.001$) and moved the CO_2-response curve significantly to the right (increased P_ACO_2 for the same minute ventilation). After physostigmine, the mean slope was increased to 1.78 ± 0.2 ($P < 0.01$, c.f. morphine), and the CO_2-response curve restored to its control position.

The second part of the study was performed in 10 post-operative subjects who required morphine for pain relief. The degree of pain was assessed both by the patient on a pain chart, and by an independent "blind" observer, on a 5 point rating scale as described by Gupta and Dundee (1974). The patients were also rated for sedation on another 5-point scale.

Morphine (0.16 mg/kg) reduced the pain score from 4 ± 0.2 to 2 ± 0.2 (severe to mild pain) 30 min after its injection. This was accompanied by an increase in sedation from 1.3 ± 0.2 to 3.3 ± 0.2. Within 5-10 min of its injection, physostigmine (12-15 ug/kg caused marked arousal, decreasing the sedation score from 3.3 ± 0.2 to 1.75 ± 0.3, while further reducing both the patient's and observer's pain scores to 1.5 ± 0.3 and 1.5 ± 0.2 respectively. The analeptic effect of physostigmine lasted 40-60 min.

It is concluded that physostigmine can antagonize the respiratory effect of morphine in human subjects by restoring the sensitivity of the respiratory centre to CO_2. It can also antagonize the somnolence associated with narcotic drugs, without reducing the analgesia.

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ANTAGONIST ACTIONS OF MORPHINE ON THE RAT VAS DEFERENS

G. Henderson*, D.S. Robinson & J.A. Sim, Department of Pharmacology, University of Cambridge, Cambridge, CB2 2QD

Morphine, unlike other opiates and opioid peptides, does not inhibit the contractile response following nerve stimulation of the rat vas deferens (Lemaire et al, 1978). Recently, Huidobro et al (1980) have shown that in the rat vas deferens morphine antagonises the inhibitory effects of β-endorphin and D-ala^2-methionine enkephalin. We have sought to characterise further this antagonist action of morphine.

Vasa deferentia from Sprague-Dawley rats were mounted in tissue baths and stimulated electrically with trains of pulses delivered at frequencies of 0.05 or 0.1 Hz. Each train consisted of 3 pulses of 0.5 ms duration at intervals of 100 ms, the voltage being sufficient to produce 80-90% of the maximal contracture.

Morphine (1-100 μM) failed to alter the amplitude of the nerve mediated contractions. However, exposure of the vasa to morphine (0.5-5 μM) produced a dose-dependent, parallel shift to the right of the dose-response curves for both D-ala^2-D-leu^5-enkephalin and D-ala^2-MePh^4-Met (O)ol^5-enkephalin (FK 33824). The equilibrium dissociation constants (K_e) for morphine against D-ala^2-D-leu^5-enkephalin and FK 33824 were 0.6 and 1.0 μM respectively. Levorphanol also acted as an antagonist in the rat vas deferens and was over 10 times more potent than its + isomer dextrorphan. Morphine (40 μM) failed to antagonise the inhibition of contractions produced by either adenosine or clonidine.

Morphine has both an agonist and a weak antagonist action on the guinea-pig ileum (Kosterlitz & Watt, 1968). It is conceivable therefore that morphine is a partial agonist and that its apparently pure antagonist action, or lack of agonist action, in the rat vas deferens results from a deficit in the number of 'spare' opiate receptors in this preparation. This view is supported by experiments using chlornaltrexamine an irreversible opiate receptor antagonist (Caruso et al, 1980). The effects of various concentrations of chlornaltrexamine on the dose-response curve for FK 33824 were studied in the rat vas deferens, mouse vas deferens and guinea-pig ileum myenteric plexus-longitudinal muscle preparations. All tissues were incubated with chlornaltrexamine for 20 min and then washed continuously for 1h (Chavakian & Goldstein, 1981). Pretreatment of the rat vas deferens with chlornaltrexamine (1-10 nM) produced a dose-dependent decrease in both the slope and maximum of the FK 33824 dose-response curve. Whereas in the guinea-pig ileum and mouse vas deferens chlornaltrexamine (3-10 nM) produced a parallel shift to the right of the FK 33824 dose-response curve; only at higher concentrations (30-100 nM) did chlornaltrexamine reduce the slope and maximum of the FK 33824 dose-response curve. The spare receptors present in the guinea-pig ileum and mouse vas deferens but not the rat vas deferens could represent either 'spare' μ receptors or receptors of a different type (δ or κ) activated by FK 33824 at higher concentrations.

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SOME CENTRAL EFFECTS OF L-HISTIDINE IN THE RAT

C.R. Calcutt*, B. Jackson & F. Roberts, Smith Kline & French Research Ltd, Welwyn Garden City, Herts, AL7 1EY.

L-histidine has been reported to decrease locomotor activity and induce a state of catalepsy following peripheral loading (Costentin et al, 1974; Maslinski et al, 1973) and to decrease neuronal firing rate when applied by microiontophoresis (Hösli & Haas, 1971). The following experiments were carried out to determine whether these effects could be attributed to the formation of histamine from histidine in the brain.

To examine the effect on neuronal firing rats (300g male Wistar from the SK&F colony) were anaesthetized with urethane (1.2 g/kg) and the activity of glutamate-stimulated somatosensory cortical cells recorded using conventional 7-barreled glass microelectrodes. Histamine dihydrochloride and L- or D-histidine monohydrochloride (0.2 M pH 4.5) were placed in the barrels. As observed by Hösli & Haas (1971) L-histidine depressed neuronal firing with a fast onset and recovery. This effect is unlikely to be the result of histamine formation because D-histidine, which is not decarboxylated to histamine, was also depressant.

Spontaneous locomotor activity was examined using a Columbus activity cage, and catalepsy using the test of Morpurgo (1962). Histidine hydrochloride or histidine methylester dihydrochloride were given i.p. in saline or i.c.v. in artificial csf, using chronically implanted cannulae, into the right lateral ventricle. Both histidine and the methylester (250-1000 mg/kg i.p.) produced a dose dependent reduction in spontaneous locomotor activity measured at 60 min. Only the highest dose of the methylester examined (1600 mg/kg i.p.) was observed to consistently produce a cataleptic effect. Histidine was not effective even at the highest dose. These effects are not thought to result from the formation of histamine because 20 mg/kg s.c. of the histamine N-methyl transferase inhibitor, amodiaquin administered 1h after the methylester (1600 mg/kg i.p.) temporarily reversed rather than potentiated the catalepsy, although it had no effect on the catalepsy produced by haloperidol (2 mg/kg i.p.). 20 µg amodiaquin i.c.v. 30 min prior to 300 mg/kg i.p. of the methylester failed to modify the effect on locomotor activity. It was also observed that i.c.v. administration of neither histidine (250-500 µg) nor the methylester (125-500 µg) had any effect on spontaneous locomotor activity or produced any significant cataleptic state.

It is therefore concluded that although some central effects of histidine may be the result of histamine formation, the depression of neuronal firing, the catalepsy and the effect on locomotor activity are not. It is suggested that the latter behavioural effects may be due to decreased endogenous brain dopamine levels. This would be consistent with the lack of effect of i.c.v. administered histidine reported here, the effect of histidine on L-DOPA uptake into brain slices (Herreros et al, 1978) and the ability of histidine to antagonize methamphetamine stereotypy but not apomorphine induced climbing behaviour in mice (Joshi et al, 1981).

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ORAL BEHAVIOUR INDUCED BY INTRANIGRAL MUSCIMOL: UNAFFECTED BY HALOPERIDOL BUT ABOLISHED BY LESIONS OF SUPERIOR COLLICULUS

P. Redgrave*, E.B. Taha & P. Dean, Department of Psychology, University of Sheffield, Sheffield, S10 2TN.

It has been suggested that GABA activity within certain parts of substantia nigra may form part of the striatal efferent pathway responsible for the expression of dopamine-related oral behaviour (Koob et al, 1978). Consistent with this suggestion are some incidental reports that bilateral injections of the GABA agonist muscimol can produce stereotyped gnawing and biting (e.g. Scheel-Kruger et al, 1977). We report here two experiments on this effect.

(1) Seven male hooded rats were prepared with bilateral guide cannulae giving access to the substantia nigra. Upon recovery from surgery each animal received each of four experimental conditions - (i) systemic vehicle, 0.5 mg/ml ascorbic acid + intranigral vehicle, 0.5 ul saline; (ii) systemic vehicle + intranigral muscimol, 1.0 nM/side; (iii) systemic haloperidol, 0.4 mg/kg + intranigral muscimol; (iv) systemic haloperidol + intranigral vehicle - in an order that was individually randomised. In a small test cage intranigral injections of muscimol produced intense, long lasting stereotyped biting and gnawing. The latency, duration and intensity of this response was unaffected by 0.4 mg/kg haloperidol; a dose which significantly attenuated the oral stereotypy induced by 8 mg/kg apomorphine or intranigral carbachol (Taha & Redgrave, 1980).

(2) Since there is some evidence that an important pathway of the substantia nigra projects to the superior colliculus, and that collicular lesions severely attenuate the oral component of apomorphine-induced stereotyped behaviour (Redgrave et al, 1980), the effects of superior colliculus lesions on oral behaviour induced by intranigral muscimol were examined. Rats were prepared with nigral cannulae and, at the same time, given a bilateral lesion in either the superior colliculus or overlying cortex. After recovery all animals received bilateral injections of muscimol (1.0 nM) into substantia nigra. The stereotyped oral behaviour which was observed in control lesioned animals was completely abolished in animals with collicular lesions, and was replaced by stereotyped locomotion and sniffing ($p<0.01$, Wilcoxon's signed rank test).

In conclusion,it appears that the oral behaviour induced by intranigral muscimol is relatively independent of dopamine transmission but is completely abolished by lesions which involve the superior colliculus. These observations are consistent with the view that the substantia nigra is a relay station between the caudate and the superior colliculus in an efferent pathway mediating dopamine-related oral behaviour.

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GENETIC VARIATION IN RESPONSE OF CENTRAL ADRENOCEPTORS TO STRESS

Campbell, I. C. & Durcan*, M. J. (introduced by E. Marley) Institute of Psychiatry, University of London.

Depressive illness has been hypothesised to arise in susceptible individuals as a result of a stress induced supersensitivity of central presynaptic autoreceptors (Cohen et al 1980), leading (by means of feedback inhibition) to abnormal neuronal firing. In this study two genetically distinct strains, the Roman High Avoidance (RHA) and the Roman Low Avoidance (RLA) were used to test this hypothesis. These animals were selectively bred on the basis of their performance in an active avoidance task (Bignami 1965). The RLA strain has been reported to be relatively more susceptible to a state of 'learned helplessness' and thus thought of as more 'depressogenic' (Seligman and Beagley, 1975).

Groups of rats (n=11) from both strains were stressed by placing them in a loose fitting 'corset' for 2hr/day for seven days. On the eighth day the animals were sacrificed, their brains removed, dissected and frozen at -80°C until analysed. Alpha adrenoceptors were assayed using ^{3}(H)-clonidine and ^{3}(H)-WB4101 and Beta adrenoceptors were assayed using ^{3}(H)-dihydroalprenolol (DHA). B_{max} values were obtained from six point Scatchard plots.

In hypothalamus, significant decreases in B-adrenoceptors were seen for both strains in response to stress, (57% in the RHA strain, P<.05, and 71% in the RLA strain P<.001). These changes are similar to those reported by Torda et al (1981). In brainstem a difference was noted for α_2-adrenoceptors, the RLA controls being significantly higher than the RHA (25.1 ± 2.4 against 16.4 ± 5.05 fmols/mg protein P<.005). In the RLA group stress significantly increased the α_2-adrenoceptors (control 25.1 ± 2.4, stress 39.3 ± 4.0 fmols/mg protein, P<.005) and B-adrenoceptors (control 25.4 ± 2.1, stress 50.0 ± 3.7 fmols/mg protein, P<.05) along with a significant fall in α_1-adrenoceptors (control 69.6, stress 52.0 ± 2.5 fmol/mg protein P<.05); no such marked changes were noted for the RHA animals. The control strain comparisons of the brainstem data, showing a significantly higher level of α_2-adrenoceptors in the RLA group perhaps indicates that the system is inherently more inhibited than that of the RHA strain. Also the stress induced changes in the RLA brainstem population of α-adrenoceptors are further in the direction of greater inhibition, and whilst similar changes occur in the RHA strain they are not so marked. The increases in B-adrenoceptors in RLA brainstem are difficult to interpret but it is interesting to note that they are in the opposite direction to those observed following chronic antidepressant drug treatment (Cohen et al 1981). In the forebrain, stress significantly reduced the B_{max} of the α_2 population in both groups. It also decreased the B-adrenoceptor number in the stressed RHA group but it increased this in the stressed RLA group (although this did not reach statistical significance). These forebrain findings preclude the idea that stress is associated with an overall development of α_2-adrenoceptor supersensitivity and is in accord with observations that stress does not result in uniform changes in catecholamines in the CNS (Kvetnansky et al 1975).

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THE EFFECT OF ACUTE AND REPEATED DESMETHYLIMIPRAMINE ADMINISTRATION ON CLONIDINE-INDUCED HYPOACTIVITY IN RATS

A.R. Green, D.J. Heal*, Sue Lister & S. Molyneux, MRC Clinical Pharmacology Unit, Radcliffe Infirmary, Oxford OX2 6HE

Injection of low doses of clonidine to rats and mice induces behavioural hypoactivity and it has been suggested that this is due to stimulation of central presynaptic α_2-adrenoceptors (Zebrowska-Lupina et al, 1977). We have previously shown that the clonidine-induced hypoactivity responses of rats are markedly attenuated 24 h after the administration of an electroconvulsive shock (ECS) once daily for 10 days, but not after a single ECS (Heal et al, 1981). Furthermore, repeated ECS also abolished the clonidine-induced decrease in rat brain MOPEG-SO_4 concentrations (Heal et al, 1981). Recently, Sugrue (1981) observed this latter effect after repeated administration of desmethylimipramine (DMI). However, this was the only antidepressant tested which caused this effect, and then only after twice daily administration for several days. In view of these findings, we have studied the effect of acute and repeated DMI injection on clonidine-induced hypoactivity in the rat.

Experiments were performed on male Sprague-Dawley derived rats (50-150 g). All drugs were injected intraperitoneally dissolved in 0.9% NaCl solution. The hypoactivity following injection of clonidine (0.5 mg/kg) was rated 0-3, according to severity, for each of 4 behavioural parameters (passivity, tactile responsiveness, posture and gait) as previously described (Drew et al, 1977; Heal et al, 1981).

When rats were pretreated with DMI (20 mg/kg) 90 min before clonidine this produced a small but highly significant reduction in the hypoactivity response ($p < 0.01$). Pretreatment with this dose of DMI 12 h earlier, however, had no effect on clonidine-induced hypoactivity. In contrast, when rats were tested with clonidine 12 h after twice daily administration of DMI (20 mg/kg) for 14 days the hypoactivity response was almost totally abolished, with all 4 behavioural parameters being significantly reduced ($p < 0.01$). Seventy-two hours after the last injection the attenuation of clonidine-induced hypoactivity was much less pronounced, although the decrease was still significant ($p < 0.01$).

GLC analysis showed that 12 h after repeated DMI injection the mean plasma DMI concentration ($\pm$ S.D.) was 675 $\pm$ 223 ng/ml ($n = 7$) and that at 72 h the concentration had decreased further so that DMI was undetectable ($<$ 20 ng/ml) in 9 out of 11 samples. Ninety min after a single DMI injection the plasma drug concentration was 252 $\pm$ 143 ng/ml ($n = 6$) and at 12 h this had fallen to 125 $\pm$ 93 ng/ml ($n = 6$).

These data show that DMI administration reduces clonidine-induced hypoactivity in rats and that this effect is markedly increased following repeated drug injection. There are, therefore, some similarities between the actions of DMI and ECS on this behavioural syndrome. However, since the maximal effects of DMI administration also coincided with the highest plasma concentrations of this drug, DMI may be acting as an α_2-adrenoceptor antagonist and its increased effectiveness following repeated administration may simply reflect the pharmacokinetics of this drug.

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HIGH-AFFINITY (^{3}H)-DESIPRAMINE BINDING LABELS A MODULATORY UNIT OF THE NEURONAL UPTAKE COMPLEX FOR NORADRENALINE

M. Briley, S.Z. Langer*, R. Raisman & M. Sette, Dept. of Biology, L.E.R.S. - Synthélabo, 58, rue de la Glacière, 75013 Paris, France.

The association of the high-affinity ^{3}H-imipramine binding site with the uptake mechanism for serotonin (Langer et al., 1980) has demonstrated that a neurotransmitter uptake complex can be labelled by a radioligand in a similar way to classical neurotransmitter receptors. We have recently reported that ^{3}H-desipramine (DMI) binds with high-affinity to membranes of the peripheral and the central nervous system (CNS) (Langer et al., 1981). We present here the further characterisation of this new high-affinity tricyclic antidepressant binding site.

^{3}H-DMI binding sites are most concentrated in regions with the highest densities of noradrenergic innervation (CNS : hypothalamus ; Bmax =156 $\pm$ 22 fmoles/mg protein, Kd = 2.6 $\pm$ 1.2 nM, n=4 : Periphery: vas deferens ; Bmax = 1015 $\pm$ 130 fmoles/mg protein, Kd = 2.6 $\pm$ 0.7 nM, n=5) suggesting a relationship with noradrenergic neurotransmission. The inhibition profile of ^{3}H-DMI binding is very similar in the CNS and the periphery. A comparison of the potencies of 9 drugs gives a highly significant correlation between the CNS and the periphery ($r=0.978$, $p<0.001$).

Only drugs which inhibit the uptake of noradrenaline (NA) are potent at inhibiting ^{3}H-DMI binding, although, interestingly, NA itself and other substrates for NA uptake such as metaraminol are inactive. ^{3}H-DMI binding is inhibited stereoselectively. In the heart the isomers of oxaprotiline show a 275 fold selectivity in favour of the (+) isomer, the isomer active at inhibiting NA uptake. When the potencies of drugs active at the ^{3}H-DMI binding site are compared with their potencies at inhibiting NA uptake a highly significant correlation is obtained ($r=0.940$, $p<0.002$, $n=8$), suggesting a close association with the NA uptake mechanism.

Thus ^{3}H-DMI binds to a specific, high-affinity site both in the peripheral and central nervous systems. These sites, which are located on noradrenergic nerve terminals (Langer et al., 1981), are probably associated with, but do not directly label, the NA uptake recognition site. We hypothesise that ^{3}H-DMI may label a modulatory unit of the neuronal NA uptake complex.

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DIFFERENTIAL EFFECTS OF CHRONIC IMIPRAMINE ON 5-HT LEVELS IN THE SUPRACHIASMATIC NUCLEUS, RAPHE, HIPPOCAMPUS AND CORTEX OF THE RAT

C. Lighton, C.A. Marsden & R. Mason*[1], Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [1] Electropharmacology Laboratory, Department of Pharmacy, University of Nottingham, Notts. NG7 2RD.

The suprachiasmatic nucleus (SCN) of the hypothalamus has been identified as a central pacemaker of many circadian rhythms (Rusak & Zucker, 1979). The SCN is rich in 5-HT terminals originating from the raphe complex. These serotonergic terminals have recently been implicated in the regulation of the intrinsic period of circadian rhythms (Wirz-Justice et al, 1982). This suggestion is based on the observation that chronic imipramine (a 5-HT uptake inhibitor) or clorgyline (a monoamine oxidase inhibitor) alter circadian rhythms when administered systemically or locally in the SCN. In view of these observations and the selectivity of SCN cells to ionophoresised 5-HT and imipramine (Groos & Mason, 1982) we have studied the effects of chronic imipramine administration on the 5-HT content of this nucleus.

Male Wistar rats (n=17) were kept on a LD 12h:12h lighting regimen (lights-on at 07.00h) and their circadian rest-activity cycle was monitored continuously by capacitance measurements over a period of 110 days. Control and experimental rats were orally intubated with distilled water, after a period of 25 days the experimental group were daily intubated with imipramine (10mg kg^{-1}). At 89 and 110 days brain 5-HT levels were estimated using HPLC with electrochemical detection (Marsden, 1981) from dissected or punched tissue samples of the raphe, SCN, hippocampus and cortex. Samples were taken during the rat's resting period (10.00-11.00h) and following onset of activity in the dark (19.00-19.30h).

Table 1 Measurements of 5-HT concentrations (pg μg^{-1} protein, ± s.e.m.) in dissected (d) and punched (p) samples.

		active control(6)	resting control(4)	resting imip.(5)
raphe complex	(p/d)	11.7 ± 2.0	20.8 ± 7.6	8.1 ± 2.1
SCN	(p)	9.3 ± 2.2	6.5 ± 1.6	19.4 ± 1.6
hippocampus	(d)	12.2 ± 2.7	10.5 ± 0.8	9.6 ± 1.6
cortex	(p)	4.2 ± 1.1	5.1 ± 1.8	3.2 ± 0.5

Levels of 5-HT in control rats peaked during the rat's active period and exhibited a reduction during their resting period in its circadian cycle. In contrast, the raphe exhibited low and high levels during the active and resting periods respectively.

Chronic treatment with imipramine resulted in significantly reduced 5-HT levels in the raphe, while elevated 5-HT levels were present in the SCN.

This demonstration of a differential effect of chronic imipramine on 5-HT levels in the SCN is consistent with the notion implicating a central circadian generator in the aetiology of manic-depressive illness (Wehr & Goodwin, 1979).

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DOES 5-HYDROXYTRYPTAMINE EXCITE CORTICAL NEURONES BY AN ACTION AT α-ADRENOCEPTORS?

C.M. Bradshaw, M.J. Stoker* & E. Szabadi, Department of Psychiatry, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT

Cerebral cortical neurones are sensitive to 5-hydroxytryptamine (5-HT): both excitatory and depressant responses to 5-HT applied by microelectrophoresis have been described (Bevan et al, 1974). On the other hand, the selective α-adrenoceptor stimulants phenylephrine and methoxamine evoke only excitatory responses (Bradshaw et al, 1981a). It was of interest to examine whether the excitatory responses to 5-HT are mediated, fully or partly, by α-adrenoceptors, since 5-HT can act at both α-adrenoceptors and specific 5-HT receptors in vascular smooth muscle (Apperley et al, 1976).

In the present experiments, we compared the effects of haloperidol and methysergide on excitatory responses of cortical neurones to 5-HT and phenylephrine. There is evidence that haloperidol is a potent antagonist at α-adrenoceptors in the periphery (Van Rossum, 1965), and, in our previous experiments, haloperidol proved to be a selective and potent antagonist of responses of cortical neurones to phenylephrine (Bradshaw et al, 1981b). Methysergide is a 5-HT antagonist (Fozard, 1975).

Spontaneously active single neurones were studied in the somatosensory cortex of the halothane-anaesthetized rat. All the drugs were applied by microelectrophoresis. Our techniques have been described elsewhere (Bevan et al, 1977).

The effect of haloperidol on responses to 5-HT and phenylephrine was compared on 13 cells. Acetylcholine was used as a control agonist. Haloperidol produced the following changes in the sizes ('total spike number'; see Bevan et al, 1977) of responses to the agonists (percentage of corresponding control; mean ± s.e. mean): 5-HT: -20.7 ± 7.0; phenylephrine: -72.6 ± 3.7; acetylcholine: +3.3 ± 3.1. Thus while responses to both amines were significantly reduced (t-test, $P<0.02$), responses to acetylcholine were not significantly affected ($P>0.1$). Furthermore, responses to phenylephrine were antagonised to a greater extent than responses to 5-HT (paired t-test, $P<0.001$).

The effect of methysergide on responses to 5-HT, phenylephrine and acetylcholine was compared on 8 cells. Methysergide produced the following changes in the sizes of responses to the agonists (percentage of corresponding control; mean ± s.e.mean): 5-HT: -81.5 ± 5.8; phenylephrine: -7.7 ± 5.1; acetylcholine: +2.1 ± 6.4. Thus while the response to 5-HT was significantly antagonised (t-test, $P<0.001$), responses to phenylephrine and acetylcholine were not significantly affected ($P>0.1$).

The results of the present experiment indicate that the excitatory responses to cortical neurones to 5-HT are mediated by a receptor which is susceptible to blockade by methysergide, and is relatively resistant to blockade by haloperidol. Thus, the excitatory 5-HT receptor is different from the excitatory α-adrenoceptor which is susceptible to blockade by haloperidol, but is unaffected by methysergide.

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CHRONIC INTRA-ACCUMBENS DOPAMINE: BEHAVIOURAL CONSEQUENCES OF CONTINUOUS INFUSION OR SINGLE DAILY INJECTIONS

Brenda Costall, Annette M. Domeney* & R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP.

We have shown the continuous infusion of dopamine (DA) into the nucleus accumbens (ACB) of rat to cause profound motor behavioural change at doses far smaller than those generally employed to cause a motor response from acute challenge with a single intracerebral injection (Costall et al, 1981). In the present study we attempt a detailed comparison of the motor behavioural effects of continuous infusion or single daily injections of DA.

Male Sprague-Dawley rats, selected as low responders to 0.05 mg/kg s.c. (-)N-n-propylnorapomorphine ((-)NPA) (see Costall et al, 1980) were subject to standard stereotaxic surgery for the implantation of chronically indwelling guides for subsequent bilateral intra-ACB injection. 2 weeks after this implantation animals were separated into two groups; one group was re-anaesthetised for the sub-cutaneous (back neck region) implantation of two Alzet osmotic minipumps which were filled and primed with DA (4.16, 2.08 and 1.04 µg/µℓ) or vehicle, and fixed via injection units in the guides to deliver continuously (0.5 µℓ/h) to the centre of the ACB for 13 days. During this period the second group of rats received single daily intra-ACB injections of 50, 25 or 12.5 µg DA, delivered over 60 s. The development of hyperactivity (photocell measure) or stereotyped movements (scored) both to the DA treatments and to challenge with 0.05 mg/kg s.c. (-)NPA were measured.

Animals subject to DA infusion developed a hyperactivity by day 3, and maxima were recorded on days 3-4 and again on days 10-11; the intensity of response increased with dose. After discontinuation of infusion, the DA treated animals exhibited the same spontaneous responding as the animals receiving vehicle. However, the DA treated rats were distinguished from control by their increased responsiveness to (-)NPA developing 1-3 weeks after infusion ceased; the duration of this enhanced response correlated with dose (9 and 6 weeks at 50 and 25 µg, absent at 12.5 µg). When DA was given in single daily injections (12.5 µg-50 µg) a spontaneous hyper-activity response peaked on the 5th day. Further, a repetitive biting/chewing response, never observed when DA was delivered by infusion, developed to all doses from day 2. After discontinuing the 50 µg injection after 13 days rats exhibited an enhanced spontaneous locomotion which persisted for 3 weeks; this was not observed using lower doses. After discontinuing daily injections of both 50 and 25 µg (but not 12.5 µg) DA, the normal motor responses to (-)NPA were enhanced, dependent on dose. All behavioural responses were haloperidol (0.5 mg/kg i.p.) sensitive.

Thus, the hyperactivity response of rats to intra-ACB DA can be enhanced both by repeated daily injection or by infusion of the DA, although the time courses of changes vary between treatments, and only daily injections allow the development of repetitive biting. Enhanced motor responses to (-)NPA are apparent for several weeks after DA withdrawal. Such data has important implications for the repeated use of animals, and to our understanding of changed motor responses resulting from DA over-stimulation - such changes clearly depend on whether the increased stimulation is slow and continuous, or in intense 'pulses'.

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DOPAMINE RECEPTOR BLOCKING PROPERTIES OF N-CHLOROETHYLAPORPHINE DERIVATIVES

A.A. Mustafa*, R.P. Sharma and G.N. Woodruff. Dept. Physiology & Pharmacology, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU.

The apomorphine analogues N-(chloroethyl) norapomorphine (NCA) and N-(hydroxyethyl) norapomorphine (NHA) have been reported to block central dopamine receptors whereas the N-(chloroethyl)-10 methoxynorapomrophine derivative (NCC) was inactive in both behavioural and biochemical tests (Costall, Fortune, Granchelli, Law, Naylor, Neumeyer and Nohria, 1980). As previously reported, the injection of ADTN (2-amino-6,7,-dihydroxy-1,2,3,4,-tetrahydronaphthalene) into the nucleus accumbens of conscious rats causes a strong and long-lasting stimulation of locomotor activity (Elkhawad and Woodruff, 1975). We have investigated the effect of intra-accumbens injections of N-chloroethylaporphine derivatives on the hyperactivity caused by ADTN and two other dopamine agonists, ergometrine and pergolide.

Male Wistar rats (200 g) were anaesthetised with sodium pentobarbitone (60 mg/kg i.p.), placed in a stereotaxic frame and guide cannulae were implanted bilaterally above the nucleus accumbens as previously described (Woodruff, Watling, Andrews, Poat and McDermed, 1977). Drugs were injected in a volume of 1 µl using a Hamilton 5 µl microsyringe. Locomotor activity was measured using conventional activity cages. Groups of 6-18 rats were used for each individual experiment.

The aporphine derivatives were injected intracerebrally, 4 h before agonist administration. The intra-accumbens injections of ADTN (85 nmol), pergolide (90 nmol) and ergometrine (45 nmol) caused a strong locomotor stimulation which lasted for 18, 16 and 7 h respectively. The hyperactivity caused by ADTN (85 nmol) or pergolide (90 nmol), following injection into the nucleus accumbens, was partially blocked by NCA (57 nmol). The hyperactivity during the first 6 h to either agonist was not inhibited by NCA but the rest of the hyperactivity was effectively blocked. The locomotor stimulation produced by ergometrine was not affected by NCA (57 nmol). Up to 6 days after injection of NCA, pergolide or ADTN locomotion was still inhibited after the initial 6 h period which was insensitive to NCA.

The hydroxy derivative (NHA) in a dose of 57 nmol similarly inhibited the hyperactivity caused by ADTN (85 nmol), but it was considerably less active. The methoxy derivative (NCC) was completely inactive and it did not inhibit any part of the hyperactivity caused by the bilateral injection of ADTN.

Sulpiride, the potent dopamine receptor antagonist, in a dose of 15 nmol, injected into the nucleus accumbens protected against NCA inhibition. ADTN (85 nmol) injected two days after sulpiride and NCA (57 nmol) pretreatment caused a full hyperactivity response which lasted for 18 h.

The present results confirm the ability of NCA to inhibit central dopamine receptors. The inability of NCA to block the hyperactivity caused by ergometrine or part of that induced by either ADTN or pergolide suggest that the locomotor stimulant drugs act by more than a single mechanism.

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IMPORTANCE OF SULPHYDRYL GROUPS IN THE BINDING OF DOPAMINERGIC AGONISTS AND ANTAGONISTS

S.B. Freedman*, Wilma W. Templeton & G.N. Woodruff. Dept. Physiology and Pharmacology, University of Southampton, Bassett Crescent East, Southampton SO9 3TU.

The use of radioactive ligands has led to suggestions of multiple binding sites for dopamine in the CNS (Seeman, 1980). Thus agonists and antagonists may bind to sites with different pharmacological profiles, while it has been proposed that antagonists can bind to different sites, cis-flupenthixol labelling D_1 receptors and sulpiride labelling D_2 receptors (Kebabian & Calne, 1979).

Recently we have shown that an SH-group is associated with the (^{3}H)-sulpiride binding site. This study examines the effect of the thiol-alkylating agent N-ethylmaleimide (NEM) and the disulphide bond reducing agent dithiothreitol (DTT) on (^{3}H)-cis-flupenthixol and (^{3}H)-ADTN binding sites and compares this with (^{3}H)-sulpiride binding site sensitivity.

Binding studies of the ligands (^{3}H)-sulpiride (15 nM), (^{3}H)-ADTN (5 nM) and (^{3}H)-cis-flupenthixol (4 nM), specific binding defined by 1 μM S-(-)-sulpiride, 1 μM (±)-ADTN and 1 μM (+)-butaclamol, respectively, to partially purified rat synaptic membranes were as previously described, with the omission of ascorbic acid from the incubation buffer. Preincubation with protein modifying agent was for 30 min at 37°C. For protection studies, the membranes were thoroughly washed to remove the dopaminergic drugs before the binding assay (Freedman, Poat & Woodruff, 1981).

Preincubation of the membranes with DTT had relatively little effect on the binding of (^{3}H)-sulpiride and (^{3}H)-cis-flupenthixol. In contrast, DTT completely abolished (^{3}H)-ADTN binding ($I.C._{50}$ 1.3 mM). NEM produced a dose dependent inhibition of binding of all three ligands. The concentrations of NEM required to inhibit binding by 50% were 2.5 mM for (^{3}H)-cis-flupenthixol and 1.8 mM for (^{3}H)-sulpiride. (^{3}H)-ADTN binding was much more sensitive, 1 μM causing 60% inhibition of binding.

The ability of unlabelled sulpiride and cis-flupenthixol to protect (^{3}H)-sulpiride binding sites from NEM inactivation was tested. With no other additions, 3 mM NEM inhibited (^{3}H)-sulpiride binding by 74%. Addition of sulpiride 15 min before addition of NEM protected the site in a dose related manner, 1 nM, 10 nM and 100 nM sulpiride causing a recovery of binding to 41%, 64% and 74% of control, respectively. Similarly, 1 nM cis-flupenthixol caused recovery to 48% of control. Higher concentrations of cis-flupenthixol could not be satisfactorily removed by the washing procedure, and hence interfered in the binding assay, in contrast to sulpiride, which is less lipid soluble and hence more easily removed.

This study demonstrates that the binding sites for all three ligands are sensitive to NEM, although (^{3}H)-ADTN binding is much more sensitive. Similarly, DTT distinguishes between the sites as (^{3}H)-ADTN binding is sensitive to this agent while (^{3}H)-sulpiride and (^{3}H)-cis-flupenthixol are not. Binding sites for the two antagonists, at least with respect to sulphydryl modifying agents, appear to be similar.

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COMPARISON OF IN VIVO BINDING OF (^{3}H)-ET 495, (^{3}H)-S 3608 AND (^{3}H)-N,n-PROPYLNORAPOMORPHINE IN THE RAT

M.D.Hall*, P.Jenner & C.D.Marsden, University Department of Neurology, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5 8AF, UK

ET 495 (piribedil; 4-(piperonyl)1-(2-pyrimidyl)piperazine) and S 3608 (coumaranyl 5-methyl)-4(thiazolyl-2)1-piperazine) act as dopamine agonists (Corrodi et al, 197; Poignant et al,1975), but do not structurally resemble dopamine or other known direct dopamine agonists. Specific in vitro binding of ^{3}H-ET 495 to rat striatal preparations cannot be demonstrated (unpublished observations) and that of ^{3}H-S 3608 does not parallel the known distribution of dopamine or other neurotransmitter receptors in the rat (Kato, unpublished observations). We now compare the specific in vivo binding of ^{3}H-ET 495 and ^{3}H-S 3608 with that of a more conventional dopamine agonist ^{3}H-N,n-propylnorapomorphine (NPA) using unlabelled ET 495, S 3608, (+)-butaclamol or apomorphine to define specific binding.

^{3}H-ET 495 (50 uCi; 17 Ci/mmole, CEA; 20 min prior to death), ^{3}H-S 3608 (50 uCi; 9 Ci/mmole, CEA: 20 min prior to death) or ^{3}H-NPA (25 uCi; 50-70 Ci/mmole, NEN; 10 min prior to death) in 0.25 ml 0.9% saline were administered via the tail vein to female Wistar rats (120-150 g). In displacement experiments animals received also unlabelled ET 495 (40 mg/kg i.p., 20 min prior to death), S 3608 (40 mg/kg i.p., 20 min prior to death), (+)-butaclamol (5 mg/kg i.p., 30 min prior to death) or apomorphine (0.5 mg/kg s.c., 15 min prior to death). The timing and doses utilised were determined by maximal behavioural effect in the 6-hydroxydopamine turning rat model.

^{3}H-ET 495 was displaced by ET 495 (40 mg/kg) from the substantia nigra, nucleus accumbens, cervical spinal cord and the pons-medulla. No specific binding was observed in the striatum, tuberculum olfactorium, frontal cortex, hippocampus, olfactory lobes or cerebellum. Unlabelled S 3608 (40 mg/kg) displaced ^{3}H-ET 495 from the nucleus accumbens and cervical spinal cord only. In addition, (+)-butaclamol (5 mg/kg) and apomorphine (0.5 mg/kg) displaced bound ^{3}H-ET 495 from the nucleus accumbens and substantia nigra only. Again no displacement was observed in the striatum and the ligand did not accumulate in that area.

^{3}H-S3608 was displaced by S 3608 (40 mg/kg) from the substantia nigra, nucleus accumbens, cervical spinal cord, pons-medulla and, in addition, to the tuberculum olfactorium and frontal cortex, but from no other areas including the striatum. Unlabelled ET 495 (40 mg/kg) caused displacement in the substantia nigra, nucleus accumbens, frontal cortex and cervical spinal cord, but not the tuberculum olfactorium or pons-medulla.

(+)-Butaclamol (5 mg/kg i.p.) caused displacement of ^{3}H-NPA from the substantia nigra, nucleus accumbens, striatum, tuberculum olfactorium, and also from the olfactory lobes and hypothalamus. The failure of ET 495 and S 3608 to label dopamine receptors in the striatum was confirmed by their inability to displace ^{3}H-NPA from that area, although ET 495 did cause displacement of this ligand from the substantia nigra, nucleus accumbens and cervical spinal cord and S 3608 (40 mg/kg) from the substantia nigra, tuberculum olfactorium and nucleus accumbens.

^{3}H-ET 495 and ^{3}H-S 3608 appear to label different sites from those identified by ^{3}H-NPA. In particular, the former compounds selectively label sites in the substantia nigra, but not in the striatum.

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