POSTER COMMUNICATIONS

Different changes in dopamine metabolism in the striatum and olfactory tubercle of the rat after HA-966

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Baclofen (β(p-chlorophenyl)GABA), GBL (γ-butyrolactone) and HA-966 (1-hydroxy-3-amino-pyrrolidone-2) depress the firing rate of nigrostriatal and mesolimbic dopaminergic neurones (Roth, Walters & Aghajanian, 1973; Olpe, Koella, Wolfe & Haas, 1977; Nowycky & Roth, 1977), resulting in decreased dopamine (DA) release. Concomitantly an increase in DA synthesis occurs, which is probably related to a decrease in stimulation of autoreceptors on dopaminergic nerve endings (Roth, Walters, Murrin & Morgenroth, 1975).

Because of the current interest in differences between nigrostriatal and mesolimbic/mesocortical systems (Scatton, Glowinski & Julou, 1976; Mos & Van Valkenburg, 1979), we compared changes in DA turnover after HA-966 in the striatum and olfactory tubercle (OT) of the rat.

Male Wistar rats were given α-methyl-*p*-tyrosine (αMpT, 250 mg/kg) and 30 min later HA-966 (100 mg/kg i.p.); the DA decrease was taken as a measure of impulse-dependent DA release. HA-966 proved to block the DA decrease in OT for about 3 h as it did in the striatum (Broxterman, Noach & Van Valkenburg, 1979). HA-966 (without αMpT) caused an increase in DA of similar magnitude in both structures. DA control levels were reached again after 3 h. However, while in striatum a rapid rise in 3,4-dihydroxyphenylacetic acid (DOPAC) (max. 260% at t = 2 h) and homovanillic acid (HVA) (max. 115% at t = 3 h) was seen, in OT DOPAC and HVA were always below control levels until t = 3 h. These changes in DOPAC and HVA were not due to interference of HA-966 with the disappearance of these DA metabolites, since HA-966 did not significantly change the fractional rate constants of DOPAC and HVA decline after monoamine oxidase inhibition with pargyline in either OT or striatum.

In another series of experiments rats were injected once a day during seven days with HA-966 (100 mg/kg), GBL (750 mg/kg) or baclofen (40 mg/kg) ('subacute treatment'). No tolerance was observed in either structure for the HA-966 induced inhibition of DA decrease after αMpT. However, DA accumulation in striatum caused by these drugs was significantly lower after subacute than after acute treatment. Moreover, significant cross-tolerance between these drugs was seen. In OT tolerance to DA accumulation was much less than in striatum, mostly not reaching significance.

In conclusion, inhibition of the nerve impulse-flow by HA-966 had different effects on DA metabolism in striatum and OT. This could point to differences in regulation of DA turnover in various central dopaminergic systems. Furthermore, repeated administration of HA-966 leads to marked tolerance to DA accumulation in striatum, while tolerance in OT was only limited. Possibly regional differences in sensitivity of (pre-)synaptic DA receptors or in feedback regulation of DA synthesis are involved.

References

BROXTERMAN, H.J., NOACH, E.L. & VAN VALKENBURG, C.F.M. (1979). Differential effects of acute and subacute HA-966 treatment on storage and release of striatal dopamine. *Eur. J. Pharmac.*, **60**, 153–161.

MOS, J. & VAN VALKENBURG, C.F.M. (1979). Specific effect of social stress and aggression on regional drug metabolism in rat brain. *Neurosc. Letters*, **15**, 315–327.

NOWYCKY, M. & ROTH, R.H. (1977). Reversible lesion of central dopaminergic neurones with 1-hydroxy-3-amino-pyrrolidone-2. 8th Annual Meeting of the American Soc. for Neurochemistry, Abstract 72.

OLPE, H.R., KOELLA, W.P., WOLFE, P. & HAAS, H.L. (1977). The action of baclofen on neurones of the substantia nigra and of the ventral tegmental area. *Brain Res.*, **134**, 577–580.

ROTH, R.H., WALTERS, J.R. & AGHAJANIAN, G.K. (1973). Effect of impulse flow on the release and synthesis of dopamine in the rat striatum. In: Frontiers in Catecholamine Research; ed. by E. Usdin & S.H. Snyder, pp. 567–574, Pergamon Press, New York.

ROTH, R.H., WALTERS, J.R., MURRIN, L.C. & MORGENROTH, V.M. (1975). Dopamine neurones: Role of impulse flow and presynaptic receptors in the regulation of tyrosine hydroxylase. In: Pre- and Postsynaptic receptors; ed. by E. Usdin & W.E. Bunney, pp. 5–48, Marcel Dekker, New York.

SCATTON, B., GLOWINSKI, J. & JULOU, L. (1976). Dopamine metabolism in the mesolimbic and mesocortical dopaminergic systems after single or repeated administration of neuroleptics. *Brain Res.*, **109**, 184–192.

Simultaneous measurement of endogenous release of dopamine, noradrenaline, 5-hydroxytryptamine and thyrotrophin releasing hormone (TRH) from rat brain slices *in vitro*

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Current interest in the interactions between neuropeptides and monoamines in the CNS is hampered by a lack of suitable techniques for appropriate rapid tissue preparation and estimation of picomole levels of peptide and amine released from brain tissue. The method described allows routine simultaneous measurement of endogenous monoamines and TRH released *in vitro* from regions of rat brain weighing between 5–30 mg.

Tissue was sliced from freshly dissected and weighed brain regions (hypothalamus, striatum, nuc. accumbens, brain stem and hippocampus) in two dimensions (0.5 × 0.5 mm), using a novel 'egg slicer' method. Slices prepared in this rapid and convenient way showed comparable rates of transmitter release and tissue respiration to those prepared with a McIlwain tissue chopper. The slices were washed in gassed Ca^{2+} free buffer prior to resuspension and then incubation for 20 min in Krebs bicarbonate buffer pH 7.4, containing glucose (10 mM), pargyline (5×10^{-5} M), and bacitracin (2×10^{-5} M), at tissue concentrations of 5–15 mg/0.5 ml or 15–30 mg/ml. KCl (55 mM), (+)-amphetamine (10^{-4} M) or TRH (10^{-5} & 10^{-4} M) were added for the last 15 min of incubation to single bilateral tissue regions using the contralateral tissue as control. After incubation the samples were centrifuged and catecholamines, 5-hydroxytryptamine (5HT) and TRH assayed in separate aliquots of the supernatant.

Monoamines were assayed by high performance liquid chromatography with electrochemical detection using a 50 × 2.1 cm strong cation exchange column to separate dopamine and noradrenaline (Plotsky, Wightman, Chey & Adams, 1977) and a similar 25 × 2.1 cm column for 5-HT (Lake & Marsden, 1980). The potential of the working electrode in both cases was +0.65 V and 20 or 100 µl samples of deproteinised supernatant (0.1 M perchloric acid) were injected onto the column. TRH was assayed by radioimmunoassay (Bennett, Edwardson, Holland, Jeffcoate & White, 1975) following ethanol extraction at 0°C (Jackson & Reichlin, 1979).

There was brain regional variation in basal monoamine release with the greatest release of dopamine from the striatum and nuc. accumbens while noradrenaline release was most marked from the hypothalamus and absent from the striatum. 5-HT release was observed in all regions studied. TRH release was greatest in the hypothalamus and least in the brain stem and hippocampus. Both monoamine and TRH release were stimulated by KCl (55 mM) from all regions with the effects being most apparent on dopamine release from the striatum and nuc. accumbens; d-Amphetamine (10^{-4} M) increased dopamine and noradrenaline release from regions where basal release was observed. Preliminary results indicate that TRH (10^{-4} but not 10^{-5} M) stimulates dopamine release from the striatum and nuc. accumbens and noradrenaline from the hypothalamus.

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References

BENNETT, G.W., EDWARDSON, J.A., HOLLAND, D., JEFFCOATE, S.L. & WHITE, N. (1975). Release of immunoreactive luteinising hormone-releasing hormone and thyrotrophin releasing hormone from hypothalamic synaptosomes. *Nature*, **257**, 323–325.

JACKSON, I.M.D. & REICHLIN, S. (1979). TRH in the blood of the frog, *Rana pipiens*: its nature and possible derivation from regional locations in the skin. *Endocrinology*, **104**, 1814–1821.

LAKE, D.M. & MARSDEN, C.A. (1980). Electrochemical detection of 5-hydroxytryptamine and tryptamine. *Br. J. Pharmacol.* (in press).

PLOTSKY, P.M., WIGHTMAN, R.M., CHEY, W. & ADAMS, R.N. (1977). Liquid chromatographic analysis of endogenous catecholamine released from brain slices. *Science*, **197**, 904–906.

Prevention of L-tryptophan induced hypoactivity by 5-HT antagonists and by the peripheral aromatic amino acid decarboxylase inhibitor RO4-4602

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As tryptophan administration increases brain 5-HT synthesis this has usually been thought responsible for the behavioural effects of the amino acid. However, it may also alter behaviour through other mechanisms (Patkina & Lapin, 1976; Marsden & Curzon, 1979). We have now investigated this possibility with respect to the reduced motor activity in an open field of rats given L-tryptophan at moderate dosage (20 mg/kg i.p.) (Taylor, 1976; Tricklebank, Smart, Bloxam & Curzon, 1978).

Male Sprague-Dawley rats (250–300 g) were injected i.p. during the first 4 h of darkness with either saline or L-tryptophan (20 mg/kg) and immediately placed in an open field. Behaviour was observed remotely for 5 min, commencing 15 min after placement as previous work (Tricklebank *et al.*, 1975) showed that tryptophan did not significantly alter behaviour during the first 5 min and decreased ambulation through a period from 15 to 90 min after placement.

Tryptophan was without significant effect on behaviour when the open field was illuminated by dim red light but in bright white light, rearing, headlifting and ambulation were all considerably and significantly reduced. These changes were no longer significantly apparent in rats pretreated with the 5-HT antagonists metergoline (2.5 mg/kg), methysergide (1 mg/kg) or cyproheptadine (10 mg/kg) 25 min before giving tryptophan.

Although results indicate that 5-HT receptors are involved in the behavioural effects of tryptophan, brain 5-HT was not significantly altered and 5HIAA increased only slightly in rats killed shortly after observation in the open field. furthermore, the behavioural effects of tryptophan were completely prevented by RO4-4602 at a dose sufficient to inhibit peripheral but not central L-aromatic amino acid decarboxylase (25 mg/kg, 25 min before testing). Findings suggest that the reduced motor activity is caused by a tryptophan metabolite formed outside the blood brain barrier. This could either be 5-HT itself or another substance, the synthesis of which is inhibited by R04-4602 and which can influence behaviour involving 5-HT receptors.

References

MARSDEN, C.A. & CURZON, G. (1979). The role of tryptamine in the behavioural effects of trancylcypromine + L-tryptophan. *Neuropharmacology*, **18**, 159–164.

PATKINA, N.A. & LAPIN, I.P. (1976). Effect of serotonergic drugs on positive and negative reinforcing systems in cats. *Pharmac. Biochem. Behav.*, **5**, 241–245.

TAYLOR, M. (1976). Effects of L-tryptophan and L-methionine on activity in the rat. *Br. J. Pharmac.*, **58**, 117–119.

TRICKLEBANK, M.D., SMART, J.L., BLOXAM, D.L. & CURZON, G. (1978). Effects of chronic experimental liver dysfunction and L-tryptophan on behaviour in the rat. *Pharmac. Biochem. Behav.*, **9**, 181–189.

Behavioural and voltammetric evidence for involvement of 5-hydroxytryptamine in tail pinch induced gnawing

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Tail pinch causes increased motor activity with intense gnawing and licking at cage bars, food pellets and tail clip. This response appears to depend on a nigrostriatal dopaminergic pathway as it was reduced in rats with nigral 6-OHDA lesions (Antelman, Szechtman, Chin & Fisher, 1975). However, using conventional methods, these authors were unable to demonstrate that tail pinch increased DA turnover. Using linear sweep voltammetry *in vivo*, we have previously obtained evidence for increased DA release in the caudate nucleus following tail pinch (Curzon, Hutson & Knott, 1979). We now report experiments suggesting that 5-HT also has a role in the behavioural response to tail pinch.

Carbon microelectrodes were stereotactically implanted in the caudate nuclei of male Sprague–Dawley rats (Curzon *et al.*, 1979). Oxidation currents generated at 0.35 V by the application of an 0–1 sweep to the electrodes were measured as an indication of extraneuronal DA essentially as described by

Kissinger, Hart & Adams (1973). Recordings were made at 4 min intervals. After approximately 30 min a bulldog clip was applied to the tail for 10 min and oxidation current measurements continued. The total time spent gnawing at the tail clip or food pellet during 5 min of tail pinch was determined in separate experiments.

The 5-HT releasers *p*-chloroamphetamine (PCA 0.5–2.0 mg/kg i.p.) and fenfluramine (1–15 mg/kg i.p.) given 30 min before pinch caused dose-related decreases of the time spent gnawing. This decrease did not occur when PCA was given after pretreatment with the 5-HT synthesis inhibitor *p*-chlorophenylalanine (150 mg/kg × 3 i.p.) so that 5-HT was presumably no longer available for release. As previously described, tail pinch caused an increase of oxidation current at +0.35 V. This no longer occurred when tail pinch was applied 30 min after PCA (2.0 mg/kg). The same dose of PCA did not prevent the increase of oxidation current on tail pinch after pretreatment with *p*-chlorophenylalanine.

These results suggest that release of 5-HT provoked by PCA and fenfluramine prevents both the release of caudate DA and associated behavioural change following tail pinch.

References

ANTELMAN, S.M., SZECHTMAN, H., CHIN, P. & FISHER, A.E. (1975). Tail pinch induced eating, gnawing and licking behaviour in rats: dependence on the nigrostriatal dopamine system. *Brain Res.*, **99**, 319–337.

CURZON, G., HUTSON, P.H. & KNOTT, P.J. (1979). Voltammetry *in vivo*: effects of stressful manipulations and drugs on the caudate nucleus of the rat. *Br. J. Pharmac.*, **66**, 127P–128P.

KISSINGER, P.T., HART, J.B. & ADAMS, R.N. (1973). Voltammetry in brain tissue—a new neurophysiological measurement. *Brain Res.*, **55**, 209–213.

HPLC analysis of tryptophan, 5HIAA and HVA using fluorescence and electrochemical detection; the effect of probenecid studied in primate ventricular CSF

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In vivo studies on the turnover of brain monoamines in man, and in animals, in relation to pathological and drug-induced changes would be very much facilitated by a simple and sensitive method for measuring the principal monoamine metabolites in CSF. Chronic studies, especially in non-human primates also require a technique for repeated sampling.

We describe here an HPLC technique for the estimation, in untreated CSF, of the major metabolites, and the amino acid precursors, of the three principal brain monoamines. Its use is exemplified by observing the effect of probenecid on tryptophan, 5HIAA and HVA in repeated CSF samples obtained from the 4th ventricle of primates using a chronically implanted sampling device.

The device consisted of a subcutaneously implanted modified Ommaya hydrocephalus reservoir from which 0.5 ml. CSF samples were obtained at 2-hourly intervals as previously described (Baker & Ridley, 1979). Samples were frozen without preservative at −40°C in plastic tubes pending analysis. Prior to injection into the HPLC system, CSF was ultrafiltered through an Amicon PM10 membrane (NMW cut off 10,000). 25 µl, of the ultrafiltrate was then used to make the injection into the HPLC system.

The running buffer was 0.1 M sodium phosphate pH 4.0 containing 15% methanol and the HPLC system consisted of an Altex 110A pump, a Rheodyne syringe loading injection valve (20 µl loop) and a 15 cm. Hypersil ODS 5 column. Detection and quantification was achieved using an Aminco fluoromonitor (254, 320 nm) in series with a Bioanalytical Systems electrochemical detector. (Oil based carbon paste electrode, V = 1.0 relative to Ag/AgCl reference). Retention times were: tyrosine, 2.2 min; tryptophan, 5.6; 5HIAA, 7.6; HVA, 10.1.

In two male cynomologous monkeys (3.3 and 4.0 kg) i.m. administration of probenecid (100 mg/kg at time 0, and 50 mg/kg at 2, 4 and 6 h later) resulted in a marked and progressive rise in HVA (peaking at 2–3 fold) and in 5HIAA (4 to 5 fold) and a more modest rise in tryptophan (1–2 fold). All these changes were maximal at 8–10 h and were still evident at 24 h. In a female rhesus monkey (6.0 kg) definite but small increases in HVA and 5HIAA were seen, but no rise in tryptophan was apparent. Baseline levels of HVA were about 500 ng/ml, in agreement with a previous report (Gordon *et al.*, 1975). Levels of trypto-

phan and 5HIAA have not been reported for non-human primates, but the values found here (about 500 and 100 ng/ml respectively) agree with those quoted for human ventricular CSF (Bridges *et al.*, 1976).

The implants have remained patent for more than two years in these animals, and probenecid treatments were repeated 12–15 months later. Two series of CSF samples were analysed both by HPLC and by a GLC technique for HVA (Watson, Travis & Wilk, 1974). These two methods showed good agreement ($r = 0.90$, $n = 14$). In addition, the results of probenecid administration were similar on the two occasions for each monkey ($r = 0.98$ and 0.92, for HVA, 0.91 and 0.81 for 5HIAA).

MHPG was not quantified in these samples because of its low level in ventricular fluid. However, in lumbar CSF it can be quantified by running the samples in 10% (in place of 15%) methanol, and operating the electrochemical detector at V = 0.7 (in place of 1.0 V). In summary, this would appear to be a method of wide applicability to the study of amine metabolism via the CSF.

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References

BAKER, H.F. & RIDLEY, R.M. (1979). Increased HVA levels in primate ventricular CSF following amphetamine administration. *Brain Res.*, **167**, 206–209.

BRIDGES, P.K., BARTLETT, J.R., SEPPING, P., KANTAMANENI, B.D. & CURZON, G. (1976) Precursors and metabolites of 5-HT and dopamine in the ventricular CSF of psychiatric patients. *Psychol. Med.*, **6**, 399–405.

GORDON, E., PERLOW, M., OLIVER, J., EBERT, M. & KOPIN, I. (1975). Origin of catecholamine metabolites in monkey CSF. *J. Neurochem.*, **25**, 347–349.

WATSON, E., TRAVIS, B. & WILK, S. (1974). Simultaneous determination of 3,4-dihydroxyphenylactic acid and homovanillic acid in milligram amounts of rat striatal tissue by gas-liquid chromatography. *Life Sciences*, **15**, 2167–2178.

The effect of thiopentone and methohexitone on [^{14}C]-GABA and [^{3}H]-D-aspartate release from rat thalamic slices

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Despite intense investigation, the site and mechanism of action of barbiturate anaesthetics in the production of anaesthesia remain unknown. Angel & Unwin (1970) have shown that transmission through the ventrobasal thalamus is more susceptible to anaesthetic agents than other synapses in the dorsal column-lemniscothalamic sensory system, and it has been suggested that the thalamus may be a critical site of action of anaesthetic agents in the production of anaesthesia (Angel, 1977). This investigation therefore examines some of the characteristics of putative transmitter release in the thalamus. In particular, the effects of two barbiturate anaesthetics on the K^+ stimulated efflux of exogenous γ-amino butyric acid ([^{14}C]-GABA) and [^{3}H]-D-Aspartate ([^{3}H]-D-Asp) from rat thalamic slices are described. D-Aspartate was used in preference to L-Aspartate since unlike the latter it is not metabolised by brain slices although it is transported by the same carrier system as L-Aspartate (Davies & Johnston, 1976).

The thalamus from three adult Wistar rats was removed for each experiment (mean wt. 215 ± 5.3 mg s.e. mean $n = 27$) and chopped into small slices (0.1 × 0.1 × 2.0 mm approx). The slices were incubated with 22 nM [^{3}H]-DAsp and 0.45 μM [^{14}C]-GABA for 15 mins at 37°C and collected by filtration. Slices were then perfused with warm oxygenated Krebs Phosphate solution containing 10 μM amino-oxyacetic acid to prevent metabolism of [^{14}C]-GABA. Exposing the slices to 40 mM K^+ for 9 min resulted in a 4.9 ± 0.23 s.e. mean, $n = 66$) fold increase in the fractional efflux rate constant (FERC) for [^{14}C]-GABA and a 2.6 ± 0.11 (s.e. mean, $n = 63$) fold increase for [^{3}H]-D-Asp. In the absence of Ca^{2+} the increase in the FERC was reduced to 77 ± 4% (s.e. mean $n = 3$) & 63 ± 6% (s.e. mean $n = 3$) of control for [^{3}H]-D-Asp and [^{14}C]-GABA respectively. Exposure of the slices to Protoveratrine A (10 μM) for 9 min elevated the FERC for [^{3}H]-D-Asp by a factor of 2.2 ± 0.10 (s.e. mean, $n = 3$) and for [^{14}C]-GABA by a factor of 2.3 ± 0.06 (s.e. mean, $n = 3$). In the absence of Ca^{2+} in the superfusing medium [^{3}H]-D-Asp and [^{14}C]-GABA efflux was enhanced to 122 ± 3% (s.e. mean, $n = 3$) and 143 ± 6% (s.e. mean, $n = 3$) of control respectively. Thiopentone added to the superfusing medium significantly reduced release

of both transmitters, maximum inhibition of release being approached at a concentration of 300 μM when the FERC was reduced to 71 ± 6% (s.e. mean, $n = 4$, $P < 0.025$) of control for [^{3}H]-D-Asp and to 65 ± 8.1% (s.e. mean, $n = 5$, $P < 0.025$) of control for [^{14}C]-GABA. Corresponding values for methohexitone (300 μM) were 78 ± 3% (s.e. mean $n = 3$, $P < 0.05$) and 65 ± 5.3% (s.e. mean $n = 3$, $P < 0.01$) of control. However, thiopentone (1 μM) significantly increased the FERC to 148 ± 12% (s.e. mean, $n = 7$, $P < 0.005$) of control for [^{14}C]-GABA and to 130.8 ± 10% (s.e. mean, $n = 6$, $P < 0.025$) for [^{3}H]-D-Asp. Results with methohexitone indicate that a similar phenomenon occurs at low concentrations.

These results demonstrate a complex biphasic interaction of thiopentone and methohexitone on transmitter release in the thalamus, and the fact that both barbiturates had similar effects upon [^{14}C]-GABA and [^{3}H]-D-Asp release make it unlikely that such agents act through a single transmitter system. However, the consistently greater enhancement of [^{14}C]-GABA efflux over [^{3}H]-D-Asp release at low barbiturate concentration may be of importance in the anaesthetic action of these substances.

References

ANGEL, A. (1977). Processing of Sensory Information. *Progress in Neurobiology*, **9**, 1–122.

ANGEL, A. & UNWIN, J. (1970). Effect of Urethane on transmission along dorsal column sensory pathway in rat. *J. Physiol. (Lond.)*, **208**, 32P.

DAVIES, L. P. & JOHNSTON, G.A.R. (1976). Uptake and release of D- and L-Aspartate by rat brain slices. *J. Neurochem.*, **26**, 1007–1014.

The effects of the stereoisomers of the GABA uptake inhibitor 2,4-diaminobutyric acid on GABA metabolism *in vivo*

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The significance of the neuronal and glial GABA uptake systems in terminating the action of synaptically-released GABA is still open to question. L-2,4-diaminobutyric acid (L-DABA) and nipecotic acid, which inhibit neuronal uptake, have been shown to potentiate iontophoretically applied GABA in spinal cord (Lodge, Curtis & Johnston, 1978). Anticonvulsant activity in mice has been shown for L-DABA against picrotoxin and 3-mercaptopropionate (Taberner & Roberts, 1978), and for nipecotic acid against pentylenetetrazol (Frey, Popp & Loscher, 1977) and audiogenic seizures (Horton, Collins, Anlezark & Meldrum, 1979). D- and L-DABA are both weak inhibitors of GABA aminotransferase although D-DABA does not appear to be an anticonvulsant (Taberner & Roberts, 1978).

The effects of D- and L-DABA on the metabolism of GABA *in vivo* were therefore determined by measuring the relative incorporation of ^{14}C from L-8[U-^{14}C] glutamate or D-[U-^{14}C] glucose into brain amino acids in mice pretreated with either isomer. The methods used were exactly as described previously (Adcock & Taberner, 1978). Adult LACG mice were given L-DABA, D-DABA or δ-aminovaleric acid at 4 mmoles/kg, i.p. 15 min prior to labelled glucose or glutamate. After 5 min the mice were killed and the relative incorporation of ^{14}C into glutamate, aspartate and GABA determined in the neostriatum.

Following D-DABA or δ-aminovalerate the incorporation of label from [U-^{14}C] glutamate into aspartate, GABA and the neutral fraction (consisting of glutamine, serine and alanine) and the activity remaining in glutamate were unaltered from the values obtained in saline-treated control animals. The values, expressed as the percentage of total soluble supernatant activity recovered were: glutamate, 49.1–51.9; aspartate, 7.18–8.39; GABA, 1.07–1.78; and the neutral fraction, 39.4–41.3. In L-DABA treated mice incorporation into GABA was reduced by 60% from control and glutamate reduced by 24% whilst the incorporation into the neutral fraction increased by 41%. This reflects an alteration in the balance of glutamate metabolism suggesting a reduction in GABA synthesis and an increased formation of glutamine from glutamate.

The results obtained with [U-^{14}C]-glucose as precursor were less clear cut, but incorporation into GABA was slightly increased by L-DABA and δ-aminovaleric acid relative to controls, and unchanged by D-DABA. Incorporation of ^{14}C into glutamate, aspartate or the neutral fraction was unchanged by any of the drug treatments.

The apparent reduction in GABA synthesis from exogenous glutamate observed after L-DABA is unexpected in view of its known ability to block neuronal

GABA uptake. However, recent evidence has indicated that L-DABA is readily taken up into GABAergic nerve terminals, possibly displacing endogenous GABA (Weitsch-Dick, Jessell & Kelly, 1978), and it is likely that any released GABA would be taken up into glia and rapidly metabolised. The present results do not preclude an increased rate of GABA breakdown rather than a diminished rate of synthesis; they do, however, provide one possible explanation for the convulsive activity observed with L-DABA in the longer term (Horton *et al.*, 1979).

References

ADCOCK, C. & TABERNER, P.V. (1978). Measuring changes in cerebral glutamate and GABA metabolism prior to convulsions induced by 3-mercaptopropionate. *Biochem. Pharmac.*, **27**, 246–248.

FREY, H.-H., POPP, C. & LOSCHER, W. (1979). Influence of inhibitors of the high affinity GABA uptake on seizure thresholds in mice. *Neuropharmacology*, **18**, 581–590.

HORTON, R.W., COLLINS, J.F., ANLEZARK, G.M. & MELDRUM, B.S. (1979). Convulsant and anticonvulsant actions in DBA/2 mice of compounds blocking the reuptake of GABA. *Eur. J. Pharmac.*, **59**, 75–83.

LODGE, D., CURTIS, D.R. & JOHNSTON, G.A.R. (1978). Does uptake limit the action of GABA agonists *in vivo*? Experiments with muscimol, isoguvacine and THIP in cant spinal cord. *J. Neurochem.*, **31**, 1525–1528.

TABERNER, P.V. & ROBERTS, F. (1978). The anticonvulsant action of L-2,4-diaminobutyric acid. *Eur. J. Pharmac.*, **52**, 281–286.

WEITSCH-DICK, F., JESSELL, T.M. & KELLY, J.S. (1978). The selective neuronal uptake and release of [^{3}H]-DL-2,4-diaminobutyric acid by rat cerebral cortex. *J. Neurochem.*, **30**, 799–806.

The action of adenosine on [^{3}H]-neurotransmitter release from rat striatal slices *in vitro*

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Although the role of adenosine in transmission in the central nervous system (CNS) is unclear, it has been suggested that this purine may inhibit the release of several transmitters, including noradrenaline (Harms, Wardeh & Mulder, 1978), dopamine (DA) and 5-hydroxytryptamine (5-HT; Harms, Wardeh & Mulder, 1979). In an attempt to define adenosine's mechanism of action more precisely in this respect, we have looked at the effect of adenosine on the calcium (Ca^{2+}) stimulated release [^{3}H]-DA, [^{3}H]-5HT and [^{3}H]-γ-aminobutyric acid ([^{3}H]-GABA) from superfused rat striatal slices. Voltage dependent secretory mechanisms were studied by stimulating transmitter release with Ca^{2+} (1 mM) in the presence of potassium chloride (25 mM, KCl; Haycock, Cotman & White, 1976): voltage independent mechanisms were studied by stimulating transmitter release with Ca^{2+} (1 mM) following preincubation with calcium ionophore A23187 (300 μM) (Göthert, 1979). Calcium free Krebs bicarbonate buffer, pH 7.4, was used in all experiments and contained 0.1% Ascorbic acid and Pargyline (50 μM) or amino-oxyacetic acid (10 μM) to inhibit metabolism of ^{3}H-DA and ^{3}H-GABA respectively.

Release of all three [^{3}H]-transmitters was stimulated by Ca^{2+} (1 mM) in the presence of KCl (25 mM) or after preincubation with A23187. Adenosine (100 and 500 μM) produced a modest inhibition of the release of [^{3}H]-5-HT and [^{3}H]-DA stimulated by $CaCl_2$ (1 mM) in the presence of KCl (25 mM) ($P < 0.05$ in both cases) but had no effect on the release of these amines stimulated by $CaCl_2$ (1 mM) following preincubation with A23187. In neither case was the release of [^{3}H]-GABA affected by adenosine (100 and 500 μM). Adenosine was without effect on spontaneous efflux both in the presence of KCl (25 mM) and in the presence of physiological KCl (4 mM). Adenosine was also without effect on spontaneous efflux following preincubation with A23187.

Since adenosine was unable to influence calcium dependent release in the absence of depolarizations (i.e. following preincubation with A23187) but inhibited release in the presence of depolarization (i.e. with 25 mM KCl) it is suggested that the modulatory effect of adenosine is at potential/calcium dependent mechanisms rather than directly influencing the release process itself.

RWK is an MRC student.

References

GÖTHERT, M. (1979). Ca^{2+}-induced noradrenaline release from central noradrenergic neurons promoted by high K^+ concentration or ionophore A23187. *N.S. Arch. Pharmacol.*, **307**, 29–37.

HARMS, H.H., WARDEH, G. & MULDER, A.H. (1978). Adenosine modulates depolarization induced release of [^{3}H]-noradrenaline from slices of rat brain neocortex. *Eur. J. Pharmacol.*, **49**, 305–308.

HARMS, H.H., WARDEH, G. & MULDER, A.H. (1979). Effects of adenosine on depolarization induced release of various radiolabelled neurotransmitters from slices of rat corpus striatum. *Neuropharmacology*, **18**, 577–580.

HAYCOCK, J.W., COTMAN, C.W. & WHITE, W.F. (1976). Stimulus-secretion coupling processes in brain. Analysis of noradrenaline and γ-aminobutyric acid release. *J. Physiol.*, **254**, 475–505.

Release of glutamate from cortico-striatal terminals is regulated by activation of dopamine receptors

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There is now convincing evidence that glutamate is the excitatory transmitter released by the terminals of the cortico-striatal pathway in the rat (Divac, Fonnum & Storm-Mathisen, 1977; Kim, Hassler, Haug & Paik, 1977; McGeer, McGeer, Scherer & Singh, 1977; Reubi & Cuénod, 1979; Rowlands & Roberts, 1980). Recently, two groups (Schwarcz, Creese, Coyle & Snyder, 1978; Garau, Govoni, Stefanini, Trabucchi & Spano, 1978) indicated the possibility that a separate group of dopamine receptors, not associated with adenylate cyclase, may be present on the terminals of the cortico-striatal pathway—[^{3}H]-haloperidol and [^{3}H]-spiperone binding were decreased in the striatum of rats following unilateral lesions of the frontal cortex, whereas dopamine-sensitive adenylate cyclase activity was unaltered.

In this study we have investigated the effect of dopamine on the release of endogenous glutamate from rat striatal slices. The slices (0.5 mm thick) were superfused at a constant rate of 1 ml/min by means of a Watson-Marlow peristaltic pump. The perfusing medium (Krebs-bicarbonate buffer) could readily be exchanged for one containing an elevated potassium concentration (usually 50 mM) and/or dopamine. Five millilitre fractions were extracted in di-(2-ethyl)-hexylphosphoric acid (0.1 M) in chloroform, followed by desalting by passage through Amberlite cation exchange columns. Released endogenous glutamate was assayed by the method of Graham & Aprison (1966) with modifications.

Dopamine (100 μM) had no effect on the basal, unstimulated release of glutamate. It did however, exert a strong inhibitory effect on the potassium-stimulated, Ca^{2+}-dependent release of glutamate: 100 μM dopamine decreased the release of glutamate by 40%, from 0.531 ± 0.061 nmol/mg tissue in controls to 0.307 ± 0.030 nmol/mg tissue. Ascorbic acid (0.1 mg/ml) and nialamide (1 μM) were present in both control and dopamine media. The effect of dopamine appeared to have a threshold at approximately 1 μM, and increased in a concentration-dependent manner, although it was not practicable to examine concentrations of dopamine above 250 μM.

The effect of dopamine (100 μM) was increased to a 60% inhibition, when the experiment was performed in the presence of the potent dopamine uptake inhibitor, benztropine (1 μM). Apomorphine (100 μM) and ADTN (100 μM), both dopamine agonists, also decreased the potassium-stimulated release of glutamate by 55% and 40% respectively. Noradrenaline and catechol (both at 100 μM) were devoid of any action on the release of glutamate.

These initial experiments indicate that dopamine exerts a specific inhibitory effect on the calcium-dependent, potassium-evoked release of L-glutamate from rat striatal slices. It remains to be determined whether this action is direct, as receptor-binding studies would suggest, or whether it is mediated through striatal interneurons.

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References

DIVAC, I., FONNUM, F. & STORM-MATHISEN, J. (1977). High-affinity uptake of glutamate in terminals of cortico-striatal axons. *Nature, Lond.*, **266**, 377–378.

GARAU, L., GOVONI, S., STEFANINI, E., TRABUCCHI, M. & SPANO, P.F. (1978). Dopamine receptors: pharmacological and anatomical evidences indicate that two distinct dopamine receptor populations are present in rat striatum. *Life Sci.*, **23**, 1745–1750.

GRAHAM, L.T. JR. & APRISON, M.H. (1966). Fluorimetric determination of aspartate, glutamate and gamma-aminobutyrate in nervous tissue using enzymic methods. *Analyt. Biochem.*, **15**, 487–497.

KIM, J.-S., HASSLER, R., HAUG, P. & PAIK, K.S. (1977). Effect of frontal cortex lesion on striatal glutamic acid levels in rat. *Brain Res.*, **132**, 370–374.

MCGEER, P.L., MCGEER, E.G., SCHERER, U. & SINGH, K. (1977). A glutamatergic corticostriatal path? *Brain Res.*, **128**, 369–373.

REUBI, J.C. & CUÉNOD, M. (1979). Glutamate release *in vitro* from corticostriatal terminals. *Brain Res.*, **176**, 185–188.

ROWLANDS, G.J., & ROBERTS, P.J. (1980). Specific calcium-dependent release of endogenous glutamate from rat striatum is reduced by destruction of the corticostriatal tract. *Exptl. Brain Res.* in press.

SCHWARCZ, R., CREESE, I., COYLE, J.T. & SNYDER, S.H. (1978). Dopamine receptors localized on cerebral cortical afferents to rat corpus striatum. *Nature*, **271**, 766–768.

Is there a common receptor mediating the inhibitory actions of 5-hydroxytryptamine (5-HT) and dopamine on leech Retzius cells?

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There is evidence for the occurrence of 5-HT and dopamine in the leech nervous system and so both compounds may be putative transmitters (Rude, Coggeshall & Van Orden, 1969; McAdoo & Coggeshall, 1976). Both amines hyperpolarise leech Retzius cells and both events are mediated by increased chloride permeability (Walker & Smith, 1973; Sunderland, Leake & Walker, 1979). Evidence is presented which suggests that on leech Retzius cells these amines act on a single common receptor.

Intracellular recordings were made from Retzius cells in isolated segmental ganglia of the leeches *Hirudo medicinalis* and *Haemopis sanguisuga* using glass microelectrodes filled with molar potassium acetate, resistance 10–40 MΩ. Potentials were amplified using conventional electrophysiological techniques and permanent records obtained using a Hewlett-Packard pen recorder. The Ringer used had the following composition: NaCl 115 mM; KCl 4 mM; $CaCl_2$ 2 mM; glucose 10 mM; Tris/chloride buffer 10 mM, pH 7.4. All compounds were made up in this Ringer and 1 ml aliquots added to the bath (10 ml) containing the preparation. Each experiment was performed on at least five different preparations.

Dose-response curves for 5-HT and dopamine were constructed for Retzius cells in both leech species. Both amines produced the same maximum hyperpolarisation in *Haemopis* but in *Hirudo* the dopamine response was approximately 20% lower. The dose-response curves were parallel for the two amines in both species except for the reduced maximum for dopamine in *Hirudo*. Dopamine was 4.1 and 20 times respectively less potent than 5-HT on *Haemopis* and *Hirudo* Retzius cells. The time to reach 50% of the maximum response respectively for *Hirudo* and *Haemopis* for 5-HT was 4.0 ± 0.6 and 3.3 ± 0.7 s and for dopamine, 10.0 ± 1.7 and 3.2 ± 0.3 seconds. Desensitisation experiments showed that for both species both amines caused cross desensitisation.

Compounds were tested for their ability to block the action of either dopamine or 5-HT differentially on Retzius cells. Atropine and morphine, previously shown to block 5-HT at this site (Smith & Walker, 1975) blocked both amines at concentrations up to 10^{-4} M. The dopamine antagonists fluphenazine, metoclopramide and ergometrine (up to 10^{-4} M) also failed selectively to block these amines. Phentolamine and propranolol had no antagonist action on either amine. Strychnine and gallecron (up to 10^{-4} M) blocked both 5-HT and dopamine. It is suggested that both dopamine and 5-HT act at the same receptor.

Structure-activity studies using 20 tyramine analogues revealed that some of the requirements for potent agonist activity were: an hydroxyl and ring C_4; an hydroxyl on ring C_3 (*Haemopis* only); no substitution on the side-chain terminal nitrogen; no hydroxyl substitution on the side-chain β-carbon; no substitution on the side-chain α-carbon (*Hirudo* only). A comparison was made between these results and the tryptamine analogues structure-activity results of Smith & Walker (1974) and models for joint 5-HT/dopamine receptors proposed.

References

MCADOO, D.J. & COGGESHALL, R.E. (1976). Gas chromatographic-mass spectrometric analysis of biogenic amines in identified neurones and tissues of *Hirudo medicinalis*. *J. Neurochem.*, **26**, 163–167.

RUDE, S., COGGESHALL, R.E. & VAN ORDEN, L.S. (1969). Chemical and ultrastructural identification of 5-hydroxytryptamine in an identified neurone. *J. Cell Biol.*, **41**, 832–854.

SMITH, P.A. & WALKER, R.J. (1974). The action of 5-hydroxytryptamine and related compounds on the activity of Retzius cells of the leech *Hirudo medicinalis*. *Br. J. Pharmac.* **51**, 21–27.

SMITH, P.A. & WALKER, R.J. (1975). Further studies on the action of various 5-hydroxytryptamine agonists and antagonists on the receptors of neurones from the leeches, *Hirudo medicinalis* and *Haemopis sanguisuga*. *Comp. Biochem. Physiol.*, **51C**, 195–203.

SUNDERLAND, A.J., LEAKE, L.D. & WALKER, R.J. (1979). The ionic mechanism of the dopamine response in Retzius cells of two leech species (*Hirudo medicinalis* and *Haemopis sanguisuga*:. *Comp. Biochem. Physiol.*, **63C**, 129–133.

WALKER, R.J. & SMITH, P.A. (1973). The ionic mechanism for 5-hydroxytryptamine inhibition on the Retzius cell of the leech *Hirudo medicinalis*. *Comp. Biochem. Physiol.*, **45**, 979–993.

The voltage sensitivity of GABA antagonists at the bag region of Ascaris muscle

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The voltage sensitive blocking action of a number of cholinoceptor antagonists has been described (e.g. Ascher, Large & Rang, 1979). This report describes some observations on the voltage sensitivity of the GABA block produced by bicuculline, benzylpenicillin and picrotoxin.

Recordings were made from the bag region of *Ascaris suum* muscle using a 2 microelectrode voltage clamp technique. The voltage recording pipette was filled with 4 M K acetate (adjusted to pH 7.6), while the current injection pipette was filled with 0.5 M K_2SO_4. GABA was applied iontophoretically to the bag with a third pipette placed over the bag between the other two pipettes. The preparation was perfused constantly with cool (25°C) Ringer (NaCl, 135 mM; KCl, 3.0 mM; $CaCl_2$, 3.0 mM; $MgCl_2$, 15.7 mM; glucose 3 mM; buffered to pH 7.6 with 5 mM trismaleate). This abolished spontaneous depolarizing potentials.

Iontophoresis of GABA produced an outward flow of current when the bag was clamped near its resting potential (−30 mV). The current peaks slowly about 4 s after the GABA pulse. The amplitude of this current declines and will reverse in direction on hyperpolarization.

The effects of bath-applied antagonists on the amplitude of the peak of the GABA currents was observed. The degree of block can be defined as Λ-1 where Λ is the ratio of the GABA current before and during the application of antagonists (Ascher, Large and Rang, 1979). The voltage sensitivity of the block produced by each antagonist was expressed as $(\Lambda_h - 1)/(\Lambda_L - 1)$ where $\Lambda_L - 1$ is the block produced at a low membrane potential (−30 to −40 mV) and $\Lambda_h - 1$ is the block produced on hyperpolarization of the membrane potential by 30 mV (−60 to −70 mV). The values obtained for bicuculline (20 μM), picrotoxin (0.5 mM) and benzylpenicillin (2 mM) were respectively 1.48 ± 0.15 ($n = 7$), 0.80 ± 0.11 ($n = 4$), and 0.54 ± 0.11 ($n = 6$), means ± s.e. mean. Thus the degree of block produced by bicuculline increased slightly on hyperpolarization, while the results obtained with picrotoxin suggest little voltage sensitivity. The degree of block produced by benzylpenicillin decreased on hyperpolarization.

It is easy to correlate the direction of the voltage sensitivity of the antagonists with their ionic charges but there may be several reasons, including channel block, why antagonists show voltage sensitivity (Colquhoun, Dreyer & Sheridan, 1979).

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References

ASCHER, P., LARGE, W.A. & RANG, H.P. (1979). Studies on the mechanism of action of acetylcholine antagonists on rat parasympathetic ganglion cells. *J. Physiol.*, **295**, 139–170.

COLQUHOUN, D., DREYER, F. & SHERIDAN, R.E. (1979). The actions of tubocurarine at the frog neuromuscular junction. *J. Physiol.*, **293**, 247–284.

Effects of fluphenazine and sulpiride at peripheral pre- and postsynaptic dopamine receptors

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The blocking potencies of fluphenazine and sulpiride at presynaptic dopamine receptors in the hindlimb (Buylaert, Willems & Bogaert, 1977) and at postsynaptic dopamine receptors in the mesentery (Crumley, Pinder, Hinshaw & Goldberg, 1976) have been measured simultaneously in anaesthetised dogs.

Graded doses of apomorphine or N,N-di-n-propyldopamine were injected directly into the femoral and mesenteric arteries before, and 15 min after, intravenous injection of cumulatively increasing doses of fluphenazine or sulpiride. Very low doses of both antagonists caused parallel displacements of the vasodilator response curves to each agonist; after higher antagonist doses, the agonist dose-response curves became flatter as the vasoconstrictor effects of the agonists predominated. The vasodilator responses to large submaximal doses of both agonists were reduced 50% (ED_{50}) by fluphenazine (5–13 μg/kg) and sulpiride (20–120 μg/kg) in the hind-limb. Much higher antagonist doses were required in the mesentery. The ED_{50} for fluphenazine against either agonist was 2.5–10 mg/kg, but sulpiride blocked only the response to N,N-di-n-propyldopamine; its ED_{50} was 2–8.6 mg/kg. Neither antagonist had much effect on resting blood pressure.

The antagonists' blocking potencies in the mesentery were also obtained under more controlled conditions using dogs pretreated with phenoxybenzamine (5 mg/kg i.a.) and propranolol (1 mg/kg i.v.). Under these conditions, sulpiride and fluphenazine caused dose-dependent, parallel displacement of the vasodilator responses to intra-arterially injected dopamine and N,N-di-n-propyldopamine, but did not reduce the maximum responses; only fluphenazine antagonised apomorphine. The dose of each antagonist required to cause a 10-fold shift (DR_{10}) in the dose-response curve to each agonist is shown in Table 1.

The results show that fluphenazine and sulpiride are much more potent antagonists at pre- than at postsynaptic dopamine receptors. In the mesentery both drugs exert specific competitive antagonism; apomorphine may act at a different site from dopamine and N,N-di-n-propyldopamine in this vascular bed.

References

BUYLAERT, W.A., WILLEMS, J.L. & BOGAERT, M.G. (1977). Vasodilatation produced by apomorphine in the hindleg of the dog. *J. Pharmac. exp. Ther.*, **201**, 738–746.

CRUMLEY, H.J., PINDER, R.M., HINSHAW, W.B. & GOLDBERG, L.I. (1976). Dopamine-like renal and mesenteric vasodilation caused by apomorphine, 6-propylnorapomorphine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthaline. *Nature*, **259**, 584–587.

Table 1 Postsynaptic dopamine receptor blocking potencies of fluphenazine and sulpiride in anaesthetised dogs

	Fluphenazine		*Sulpiride*	
	DR_{10} *(mg/kg)*	*Slope**	DR_{10} *(mg/kg)*	*Slope**
Dopamine	5.8 (4.3–7.5)	1.17 (1.1–1.3)	11.9 (6–23)	1.07 (1.03–1.11)
N,N-di-n-propyldopamine	7.2 (3.8–10.5)	1.2 (1.1–1.3)	7.7 (4.5–12.8)	1.2 (0.95–1.4)
Apomorphine	9.4 (7.8–12.0)	1.08 (0.9–1.16)	> 30	—
Acetylcholine, Histamine	Not antagonised		Not antagonised	

Results expressed as geometric mean (and range) of 3–4 experiments.
* Slope of the plot of agonist dose-ratio—1 versus log dose of antagonist.

Spatial delayed alteration behaviour in the monkey (*Macaca mulatta*): Comparative effects of thieno- and benzo-diazepines

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In previous studies we have been concerned with the effect of benzodiazepines in the monkey (Nicholson & Wright, 1973; Curry, Whelpton, Nicholson & Wright, 1977), and it would appear that these drugs may have differential effects on behaviour. To explore this possibility further we have extended our studies to some benzodiazepines which have activity not shown by the whole group. We have studied the effects of the 1,5-benzodiazepine, clobazam, and the triazolothieno-1,4-diazepine, brotizolam, together with the 1,4-benzodiazepines, nordiazepam and temazepam, on spatial delayed alternation behaviour.

Five male monkeys (*Macaca mulatta*) were tested 1 h after an intraperitoneal injection of drug or drug vehicle (placebo control). The doses were 0.5, 1.0, 2.0, 2.5 & 3.0 mg/kg. Analysis of performance was concerned with the number of correct responses, the number of repeat errors and the total response time, each compared with placebo. There were no effects with temazepam and clobazam. Nordiazepam (1.5 mg/kg and above) reduced the number of correct responses without an increase in total response time, and brotizolam (1.5 mg/kg and above) reduced the number of correct responses and increased total response time. A further experiment was carried out with temazepam and clobazam using doses of 5.0, 10.0, 15.0, 20.0 and 25.0 mg/kg. There were no effects of clobazam. Temazepam decreased the number of correct responses with 15.0 mg/kg and above, and increased total response time with 20.0 mg/kg.

Clobazam would appear to have little or no effect on delayed alternation, and this would be consistent with other studies on motor function in animals (Fielding & Hoffman, 1979), while nordiazepam may impair behaviour without an increase in total response time. Temazepam and the triazolothieno-diazepine, brotizolam, impair behaviour with increased response time though the effect of brotizolam is much more marked than that of temazepam. These observations suggest that differential effects of thieno- and benzo-diazepines may exist within restricted dose ranges, though it is difficult to be certain to what extent these observations in the monkey relate to higher nervous function in man. Nevertheless, subtle effects on behaviour with some benzodiazepines may arise, and such effects may be more likely with the triazolo derivatives of the diazepines. It may be particularly important when using triazolo compounds in man either as anxiolytics or as hypnotics, to define carefully the dose range as they may be much more likely to lead to behavioural effects than the usual 1,4- and 1,5-benzodiazepines.

References

CURRY, S.H., NICHOLSON, A.N., WHELPTON, R. & WRIGHT, C.M. (1977). Behavioural and pharmacokinetic studies in the monkey (*Macaca mulatta*) with diazepam, nordiazepam and related 1,4-benzodiazepines. *Br. J. Pharmac.*, **61**, 325–330.

NICHOLSON, A.N. & WRIGHT, C.M. (1973). Inhibitory and disinhibitory effects of nitrazepam, diazepam and flurazepam hydrochloride on delayed matching behaviour in monkeys (*Macaca mulatta*). *Neuropharmacology*, **13**, 919–926.

FIELDING, S. & HOFFMAN, I. (1979). Pharmacology of anti-anxiety drugs with special reference to clobazam. *Br. J. clin. Pharmac.*, **7**, 7–15s.

A novel procedure for comparing the transport numbers of unlabelled drugs released from micropipettes by microelectrophoresis

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When comparing the potencies of drugs applied by microelectrophoresis, it is important to establish the transport numbers of the drugs whose effects are being studied, since drugs having different transport numbers are released from micropipettes at different rates by identical ejecting currents, and may thus achieve different concentrations at receptor sites in the tissue (see Curtis, 1964). Transport numbers may be determined by measuring the release of radiolabelled drugs from micropipettes *in vitro*. However the applicability of this method is limited by the commercial availability of the appropriate labelled compounds. We report here a method whereby the relative transport numbers of unlabelled compounds may

be estimated. The method is based on the principle that the introduction of a 'foreign' ion into an electrolyte results in a reduction in the transport number of the 'host' ions, the magnitude of this reduction being a function of the absolute mobility and concentration of the 'foreign' ion. In the present experiments we have utilised this method to estimate the relative transport numbers of methoxamine and phenylephrine.

Two series of experiments were conducted, each using twelve six-barrelled micropipettes prepared as described previously (Bradshaw, Roberts & Szabadi, 1973). In the first series, three barrels of each micropipette (1–3) contained [^{14}C]-noradrenaline bitartrate (0.05 M, 1.0 mCi/mmol), and the remaining three (4–6) a mixture of [^{14}C]-noradrenaline bitartrate (0.05 M, 1.0 mCi/mmol) and methoxamine hydrochloride (0.05 M). In the second series, barrels 1–3 contained a mixture of [^{14}C]-noradrenaline bitartrate (0.05 M, 1.0 mCi/mmol) and methoxamine hydrochloride (0.05 M), and barrels 4–6 a mixture of [^{14}C]-noradrenaline bitartrate (0.05 M, 1.0 mCi/mmol) and phenylephrine hydrochloride (0.05 M). In each micropipette the rate of efflux of ^{14}C was measured during a series of 10-min periods. First, the rate of release was determined during the passage of +25, +50, +75 and +100 nA through barrels 1–3 (two samples at each current value), then the procedure was repeated using barrels 4–6. Transport numbers of noradrenaline were calculated for each sample after subtraction of the spontaneous efflux.

The transport number of noradrenaline, released from the noradrenaline bitartrate solution was 0.333 ± 0.017 (s.e.). In the presence of phenylephrine hydrochloride the transport number of noradrenaline was 0.125 ± 0.004. In the presence of methoxamine hydrochloride the transport number of noradrenaline was 0.122 ± 0.003 (first series) and 0.115 ± 0.004 (second series). The reduction in transport number brought about by methoxamine hydrochloride was not significantly different from that produced by phenylephrine hydrochloride (t test, $P > 0.1$), indicating that methoxamine and phenylephrine have similar mobilities. Thus the greater apparent potency of phenylephrine than methoxamine in exciting cortical neurones (Bradshaw, Pun, Slater & Szabadi, 1980) is not due to a higher transport number of phenylephrine, but presumably reflects a genuine biological potency difference.

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References

BRADSHAW, C.M., PUN, R.Y.K., SLATER, N.T. & SZABADI, E. (1980). The effect of methoxamine on single cortical neurones. *Br. J. Pharmac.*, in press.

BRADSHAW, C.M., ROBERTS, M.H.T. & SZABADI, E. (1973). Kinetics of the release of noradrenaline from micropipettes: interaction between ejecting and retaining currents. *Br. J. Pharmac.*, **49**, 667–677.

CURTIS, D.R. (1964). Microelectrophoresis. In *Physical Techniques in Biological Research*, Vol. Va., ed. Nastuk, W.L., pp. 144–190. New York: Academic Press.

Evaluation of antinociceptive activity in the conscious marmoset (*Callithrix jacchus*) by using stimulation of the tooth pulp

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Evaluation of the dependence liability of new analgesic drugs is best carried out in non-human primates. However, species variations in the actions and metabolism of drugs are such that the data obtained in these studies may be irrelevant unless it has been demonstrated that the test drug possesses antinociceptive activity in the same primate species. This paper describes a method in the conscious adult marmoset (*Callithrix jacchus*, male, 250–350 g) for evaluating the antinociceptive activities of drugs against responses to electrical stimulation of the tooth pulp.

Marmosets were trained to sit quietly in specially constructed restraining chairs; this training period took about three weeks. The animals were anaesthetised with Althesin (Glaxo) and a triamel-coated, stainless steel stimulation electrode (Medwire Corp. 0.002 in O.D. single; 0.008 in coated) which was bared for 2 mm at the end was implanted in the dentine of an upper canine tooth using an operative procedure similar to that described previously for dogs (Skingle & Tyers, 1979). The electrode wire was passed subcutaneously, using a suture needle to an exit point at the back of the neck. A reference electrode comprising a small (4 mm^2) silver disc crimped onto triamel-coated wire was sited subcutaneously in the upper lumbar region and the wire passed rostrally for about 3 cm to an exit point. Electrode wires were cut and bared at

the ends so that about 1.5 cm protruded. Marmosets were housed individually and were ready for testing 10 days post-operatively; they were then tested twice each week. Nociceptive thresholds were determined using trains of square wave pulses of 5 ms pulse width, 10 Hz frequency, 5 s duration and gradually increasing voltage using a Devices (type 2533) isolated stimulator. The resistance of the tooth pulp electrodes (5–20 kOhm) were monitored on an oscilloscope such that failure and deteriorations of the stimulation circuit could be detected. Nociceptive responses were characteristic for each marmoset but usually comprised a jaw-open response sometimes accompanied by licking. Nociceptive thresholds (0.25–2.0 v) remained quite constant for each marmoset.

For determinations of antinociceptive activity thresholds were determined prior to and then at 20 min intervals after drug administration up to 3 hours. Drug effects were expressed as mean percentage increase in threshold compared with pre-drug values. All testing was carried out blind.

Statistically significant ($P < 0.05$, $n = 5$), dose-dependent increases in nociceptive thresholds were obtained with the μ-opiate receptor agonists morphine, 1–4 mg/kg orally (4–17%) and 0.5–2 mg/kg i.p. (6–19%) and codeine, 2–8 mg/kg orally with peak effects occurring at 60 min after dosing. However, the k-opiate receptor agonists ethylketazocine 0.01–0.03 mg/kg i.p., pentazocine 0.15–0.45 mg/kg i.p. and ketazocine 0.01–0.03 mg/kg i.p. caused inconsistent increases in thresholds to electrical stimulation of the tooth pulp which were not significantly different from placebo.

It is concluded that while the marmoset has been found to be a useful model for evaluating the antinociceptive activities of μ-opiate receptor agonists it has no value for the evaluation of the activities of the k-agonists. In this respect tooth pulp stimulation in the marmoset differs from that in the dog in which clear dose-dependent increases in nociceptive thresholds may be obtained with low doses of k-agonists (Skingle & Tyers, 1980).

References

SKINGLE, M. & TYERS, M.B. (1979). Evaluation of antinociceptive activity using electrical stimulation of the tooth pulp in the conscious dog. *J. Pharmacol. Methods*, **2**, 71–80.

SKINGLE, M. & TYERS, M.B. (1980). Further studies in opiate receptors that mediate antinociception: Tooth pulp stimulation in the dog. *Brit. J. Pharmacol.* (in press).

Differences between receptors for 5-hydroxytryptamine on autonomic neurones

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5-Hydroxytryptamine (5-HT) stimulates transmitter release from both the postganglionic cholinergic nerves of the guinea-pig ileum (Gaddum & Picarelli, 1957) and the terminal sympathetic fibres of the rabbit heart (Fozard & Mwaluko, 1976) by activation of specific receptor sites (Fozard & Mobarok Ali, 1978). In terms of the comparative potencies of a number of selective agonists, the receptor sites in each tissue can be considered similar, although a major anomaly is that 5-methoxytryptamine is a potent stimulant of the nerves of the ileum yet essentially devoid of activity on the heart (Fozard & Mobarok Ali, 1978). Recently nor-(−)-cocaine was shown to be a selective antagonist of responses to 5-HT mediated through the receptors present on the sympathetic neurones of the rabbit heart (Fozard, Mobarok Ali Newgrosh, 1979). I now report that nor-(−)-cocaine is a significantly more potent antagonist of neuronal stimulant responses to 5-HT in the heart than in the ileum.

Rabbit hearts were perfused by the Langendorf technique at constant pressure with Tyrode solution containing atropine (1.7 μM) at 37°C. Right atrial and ventricular tensions and cardiac rate were recorded as previously described (Fozard & Muscholl, 1971). Segments of ileum were set up in Tyrode solution containing methysergide (2.8 μM).

Cardiac rate responses to bolus injections of 5-HT (2.8 to 182 nmol) were antagonized concentration-dependently by perfusion of hearts with nor-(−)-cocaine. At concentrations of 16, 31 and 124 nM the curves were shifted in parallel to the right; at 248 nM there was a further shift to the right but the maximum response was depressed. Analysis of the data obtained with the three lowest concentrations of nor-(−)-cocaine by the method of Arunlakshana & Schild (1959) gave a significant straight line relationship between log 5-HT (dr-1) and negative log [nor-(−)-cocaine] (molar) ($r = 0.90$, $P < 0.001$) with a slope of -1.07 ± 0.16. The mean pA_2 value was 7.79 ± 0.11, $n = 13$. A similar pA_2 value, 7.98 ± 0.02, $n = 4$, was

obtained when methysergide (2.8 μM) was included in the perfusion fluid.

On the guinea-pig ileum, incubated with methysergide, nor-(−)-cocaine was also a selective and surmountable antagonist of 5-HT although the concentrations needed (0.31–9.9 μM) were higher than those shown to be effective in the heart. Schild analysis revealed a significant straight line relationship between log 5-HT(dr-1) and −log [nor-(−)-cocaine] (molar) ($r = 0.94$, $P < 0.001$) with a slope of -1.28 ± 0.12. The mean pA_2 value was 6.49 ± 0.07, $n = 16$ which is significantly lower ($P < 0.001$) than the value obtained on the heart.

These results suggest that the receptor sites for 5-HT on the cholinergic nerves of the ileum and the sympathetic fibres of the heart are different. They therefore lend support to the similar tentative conclusion of Fozard & Mobarok Ali (1978) based on the observation that 5-methoxytryptamine was a potent agonist on the ileum yet inactive in the heart.

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References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

FOZARD, J.R. & MOBAROK ALI, A.T.M. (1978). Receptors for 5-hydroxytryptamine on the sympathetic nerves of the rabbit heart. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **301**, 223–235.

FOZARD, J.R., MOBAROK ALI, A.T.M. & NEWGROSH, G. (1979). Blockade of serotonin receptors on autonomic neurones by (−)-cocaine and some related compounds. *Eur. J. Pharmacol.*, **59**, 195–210.

FOZARD, J.R. & MUSCHOLL, E. (1971). A useful muscarinic parameter and the differential recording or atrial and ventricular tension in the perfused rabbit heart. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **270**, 319–325.

FOZARD, J.R. & MWALUKO, G.M.P. (1976). Mechanism of the indirect sympathomimetic effect of 5-hydroxytryptamine on the isolated heart of the rabbit. *Br. J. Pharmac.*, **57**, 115–125.

GADDUM, J.H. & PICARELLI, Z.P. (1957). Two kinds of tryptamine receptor. *Br. J. Pharmac. Chemother.*, **12**, 323–328.

Direct excitant action of convulsant barbiturates

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The convulsant barbiturate 5-(2-cyclohexylidene ethyl)-5-ethyl barbituric acid (CHEB) directly excites dorsal root ganglion cells in the cat (Downes, 1970). We have applied a series of depressant and convulsant barbiturates to isolated dorsal root fibres of immature (3–9 day old) rats. 5-Ethyl-5-(3-methylbut-2-enyl) barbituric acid (3M2B) and CHEB but not pentobarbitone or amylobarbitone depolarized these fibres by a bicuculline (100 μM) insensitive mechanism. The threshold concentration for depolarizations evoked by CHEB was 2.5 μM compared to a threshold of 1 μM for depolarizations induced by kainic acid. The threshold concentration for 3M2B-induced depolarizations was 12.5 μM and the non-barbiturate convulsants bemegride (500 μM) and 4,6,6-trimethyl-3-caprolactum (500 μM) had no depolarizing action on these preparations. Rat vagus nerve (threshold 25 μM) and superior cervical ganglion (threshold 200 μM) were also depolarized by CHEB, but not kainate and rat phrenic nerve was unaffected by CHEB (500 μM).

CHEB or 3M2B, but not kainate, induced depolarizations of immature rat dorsal roots were abolished by omission of Ca^{2+} from, or addition of 2 μM ruthenium red (Goddard & Robinson, 1976) to, the bathing medium.

Depressant barbiturates potentiate the effects of GABA on these preparations (Evans, 1979). In the present experiments 3M2B (100 μM) potentiated GABA-induced depolarizations when applied in the presence of ruthenium red (2 μM).

These observations show that convulsant barbiturates directly depolarize unmyelinated nerve fibres by a Ca^{2+} dependent mechanism.

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References

DOWNES, H. (1970). Effects of a convulsant barbiturate on dorsal root ganglion cells and other components of the spinal reflex arc. *Ph.D. Thesis, Department of Pharmacology, University of Utah, U.S.A.*

EVANS, R.H. (1979) Potentiation of the effects of GABA by pentobarbitone. *Brain Res.*, **171**, 113–120.

GODDARD, G.A. & ROBINSON, J.D. (1976). Uptake and release of calcium by rat brain synaptosomes. *Brain Res.*, **110**, 331–350.

Behavioural effects of microinjections of picrotoxin into rat superior colliculus, and their modulation by intranigral 6-OHDA

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Recent evidence has indicated that in rats striato-nigro-tectal pathways are important in the mediation of specific dopamine (DA)-related behaviours of striatal origin. For example, electrolytic lesions of the superior colliculus selectively antagonise the oral stereotyped behaviour induced by systemic injections of apomorphine (Dean *et al.*, 1979). Available biochemical evidence relating to these pathways (Kim *et al.*, 1971, Vincent *et al.*, 1978) suggests that increased DA transmission within the striatum may cause a decrease in the release of GABA from the nigro-tectal pathway. If so, it should be possible to mimic some of the behavioural effects of DA-agonists by local administration of a GABA antagonist into the superior colliculus. This was tested by observing the behavioural effects of microinjections of picrotoxin systematically administered to different sites within the tectum.

Male hooded rats were prepared with bilateral guide cannulae aimed at different regions of the superior colliculus (AP −4.3 to −5.7; L 1.0 to 2.0; HV −2.0) according to Pelligrino & Cushman (1967). Injector needles of varying length were used to administer a range of doses of picrotoxin (12.5–50 ng/0.5 µl saline) at different depths within the tectum and underlying tegmentum.

The principle behaviours induced by intra-tectal injections of picrotoxin, but not by control injections of 0.9% saline, were as follows: (1) 5–25 min (max 10 min) after injection at most collicular sites avoidance reactions were observed which ranged from flinching to violent jumping and running (explosive motor behaviour, EMB). The focus for these avoidance reactions was the intermediate layers of the colliculus, at which sites maximal incidence of the reactions was observed at the low dose of 12.5 ng. (2) 10–35 min (max 25 min) after injection gnawing and biting were observed after injections into the intermediate and deep layers only. These were more pronounced at the higher dose of 25 ng. They occurred when appropriate objects were placed close to or in contact with the animal's snout, and were often accompanied by mild seizure activity. (3) 20–40 min (max 30 min) rearing and grooming were observed at all sites.

Rats given collicular injections of picrotoxin during the week following bilateral injections of 6-hydroxydopamine (8 µg/2 µl) into substantia nigra (AP −3.0; L 1.5; HV −7.8) exhibited EMB and gnawing only when doses were increased to 50–100 ng. Surprisingly, the sensory neglect shown by the animals with 6-OHDA injections was not reversed by intratectal picrotoxin.

It appears that, among other behavioural effects, injections of picrotoxin into colliculus induce oral behaviours that may be related to the DA stereotypies: and that nigrostriatal damage shifts the dose-response curve for intratectal picrotoxin to the right. These findings are consistent with the view that the nigrotectal pathway is concerned with the expression of DA-induced oral stereotypies.

References

DEAN, P., REDGRAVE, P. & POPE, S.G. (1979). Effects of superior-colliculus lesions on apomorphine-induced activity and stereotypy in rats. *Soc. Neurosci. Abstr.* **5**, No. 1097.

KIM, J.S., BAK, I.J., HASSLER, R. & OKADA, Y. (1971). Role of γ-aminobutyric acid (GABA) in the extra-pyramidal motor system. 2. Some evidence for the existence of a type of GABA-rich striato-nigral neurones. *Exp. Brain Res.*, **14**, 95–104.

PELLIGRINO, L.J. & CUSHMAN, A.J. (1967). A stereotaxic atlas of the rat brain. Appleton-Century-Crofts, New York.

VINCENT, S.R., HATTORI, T. & MCGEER, E.G. (1978). The nigrotectal projection: a biochemical and ultrastructural characterization. Brain Res., **151**, 159–164.

Characteristics of L-[^{3}H]-aspartate binding to cerebellar synaptic membranes

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The cerebellum is an area of the brain in which amino acids are likely to be important transmitters. Current evidence strongly favours glutamate as the transmitter of the excitatory granule cells (Young, Oster-Granite, Herndon & Snyder, 1974; Hudson, Valcana, Bean & Timiras, 1976; Nadi, McBride & Aprison, 1977; Sandoval & Cotman, 1978) and we have recently demonstrated the binding of [^{3}H]-glutamate to presumed receptors on postsynaptic cerebellar membranes (Foster & Roberts, 1978). The afferent mossy and climbing fibres release unknown excitatory transmitters, which could possibly be glutamate or aspartate and, since aspartate exhibits a different relative distribution from glutamate in this tissue (Nadi, McBride & Aprison, 1977), we have investigated whether there are binding sites for aspartate, distinct from those for glutamate.

Freshly-prepared, sonicated and extensively-washed cerebellar synaptic membranes (Sharif & Roberts, 1980) were resuspended in Tris-citrate buffer (pH 7.1) and 0.5 ml aliquots were incubated at 37°C for 10 min with L-[^{3}H]-aspartate (15 Ci/mmol), usually at a final concentration of .25 nM, in the absence or presence of unlabelled aspartate, or with related substances. Specific aspartate binding was determined by subtraction from the total binding of that component which persisted in the presence of aspartate (10^{-5}–10^{-3} M).

Specific L-[^{3}H]-aspartate binding was optimal under physiological conditions of temperature and pH, attained equilibrium within 10 min, and exhibited saturability. Scatchard analysis revealed homogeneity of sites with a K_d = 874 nM and a B_{max} = 44 pmol/mg protein, and which exhibited no cooperativity (Hill coefficient = 1.2). A preliminary investigation of the pharmacological specificity of the aspartate binding site, has revealed a number of differences from the glutamate system (Foster & Roberts, 1978). L-glutamate was marginally less active than L-aspartate as a displacer, while 4-fluoroglutamate, quisqualate and 2-amino-4-phosphonobutyrate, which were good displacers of glutamate binding, were only weakly active in the system described here. Binding was displaced by DL-α-aminoadipate and DL-α-amino-suberate and HA-966, but not by the presumed 'aspartate-preferring' analogue, N-methyl-D-aspartate. In common with the glutamate receptor, kainic acid exhibited negligible affinity for the aspartate binding site.

As we have reported for the glutamate system (Sharif & Roberts, 1980), L-[^{3}H]-aspartate binding was almost abolished following freezing of membranes. Lyophilisation has been found to preserve binding, although this process results in differential effects on the two binding sites.

These findings indicate that aspartate interacts with a single population of binding sites, distinct from those for glutamate, and which exhibit some, but not all of the pharmacological characteristics predicted for an aspartate receptor.

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References

FOSTER, A.C. & ROBERTS, P.J. (1978). High-affinity L-[^{3}H]-glutamate binding to postsynaptic receptor sites on rat cerebellar membranes. *J. Neurochem.*, **31**, 1467–1477.

HUDSON, D.B., VALCANA, T., BEAN, G. & TIMIRAS, P.S. (1976). Glutamic acid: a strong candidate as the neurotransmitter of the cerebellar granule cell. *Neurochem. Res.*, **1**, 83–92.

NADI, N.S., MCBRIDE, W.J. & APRISON, M.H. (1977). Distribution of several amino acids in regions of the cerebellum in the rat. *J. Neurochem.*, **28**, 453–455.

SANDOVAL, M.E. & COTMAN, C.W. (1978). Evaluation of glutamate as a transmitter of the cerebellar parallel fibres. *Neuroscience*, **3**, 199–206.

SHARIF, N.A. & ROBERTS, P.J. (1980). Problems associated with the binding of L-glutamic acid to synaptic membranes: methodological aspects. *J. Neurochem.*, in press.

YOUNG, A.B., OSTER-GRANITE, M.L., HERNDON, R.M. & SNYDER, S.H. (1974). Glutamic acid: selective depletion by viral-induced granule cell loss in hamster cerebellum. *Brain Res.*, **73**, 1–13.

Responses of neurones in medial caudal medulla to iontophoretically applied opioids

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Neurones in the dorsal column nuclei (DCN) and the underlying reticular formation (NRV) are readily depressed by morphine and by met-enkephalin (Hill, Pepper & Mitchell, 1976). As the density of opiate receptors (Atweh & Kuhar, 1977) and enkephalin immunoreactivity (Uhl, Goodman, Kuhar, Childers & Snyder, 1979) is low in these regions of the rat caudal medulla, there was a possibility that these responses were not being operated via the classical opiate receptor. Experiments were accordingly performed to examine this.

Halothane or urethane anaesthetized rats were prepared as previously described (Hill *et al.*, 1976). Conventional six barrelled micropipettes were used for extracellular action potential recording and for the microiontophoretic application of drugs. Drug barrels contained combinations of sodium glutamate (0.5 M: pH 8.5), morphine sulphate (50 mM), met-enkephalin (8 mM), BW180C (D-ALA^2, D-LEU^5 enkephalin) (16 mM), BW195C (D-ALA^2, LEU^5, THR^6 enkephalin) (16 mM), BW227C (D-TYR^1, D-ALA^2, LEU^5 enkephalin) (16 mM), BW96C (D-PHE^4, LEU^5 enkephalin) (16 mM), levorphanol (50 mM), dextrorphan (50 mM) and naloxone (50 mM).

The stereoisomeric pair, levorphanol and dextrorphan, produced similar depression of the firing of 27 out of 32 caudal medulla neurones tested, with levorphanol being more effective as a depressant on the remaining five. The enantiomers were similar to morphine in that they frequently depressed action potential amplitude and increased duration. It thus seemed likely that a proportion of these depressant responses were not produced by activation of a stereospecific opiate receptor.

The enkephalins readily depressed the firing of caudal medulla neurones without distorting action potential shape although on occasion the amplitude was increased. Metabolically stable enkephalins (e.g. BW180C) sometimes produced a very long duration depression of firing (i.e. $\simeq$30 min following the end of drug application). The potency ranking of the enkephalin analogues used, in terms of iontophoretic current, was in the order BW180C, BW195C, met-enkephalin, > BW227C and BW96C and this parallels their potency as opiate receptor binding ligands (Miller, Clay, Cuatracasas, Wilkinson, Lowe, Beddell & Follenfant, 1978), thus suggesting that the depressant actions are exerted via an opiate receptor.

This apparent contradiction was resolved when it was found that iontophoretic naloxone would partially reverse the depressant effects of morphine or the enkephalins in approximately 50% of trials (25 of 57 neurones tested). In many of the unsuccessful trials naloxone depressed neuronal firing rate and distorted action potential shape in a similar way to morphine.

In conclusion NRV and DCN neurones were found to be particularly sensitive to nonspecific membrane effects of morphine and other opiates but did show differential sensitivity to peptides with differing receptor binding affinity.

BW peptides were supplied by Dr. S. Wilkinson, WRL, Beckenham.

References

ATWEH, S.F. & KUHAR, M.J. (1977). Autoradiographic localization of opiate receptors in rat brain. I. Spinal cord and lower medulla. *Brain Res.*, **124**, 53–67.

HILL, R.G., PEPPER, C.M. & MITCHELL, J.F. (1976). The depressant action of iontophoretically applied met-enkephalin on single neurones in rat brain. In *Opiates and Endogenous Opioid Peptides*, ed. Kosterlitz, H. North Holland. pp. 225–230.

MILLER, R.J., CHANG, K.-J., CUATRACASAS, P., WILKINSON, S., LOWE, L., BEDDELL, V. & FOLLENFANT, T. (1978). Distribution and pharmacology of the enkephalins and related opiate peptides. In *Centrally Acting Peptides*, ed. Hughes, J. Macmillan Press, pp. 195–214.

UHL, G.R., GOODMAN, R.R., KUHAR, M.J., CHILDERS, S.R. & SNYDER, S.H. (1979). Immunohistochemical mapping of enkephalin containing cell bodies, fibers and nerve terminals in the brain stem of the rat. *Brain Res.*, **166**, 75–94.

Dopamine receptors mediating different motor responses may have differential topography in the guinea-pig striatum

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An increase in striatal dopamine function can enhance locomotor activity, induce stereotyped motor behaviour and cause dyskinesias. Evidence that receptor populations mediating these different motor responses may have different topographical distributions within the striatal complex has been derived from studies in the cat (Cools, 1973). The present studies attempt to establish whether similar topographical differences are to be found in the rodent brain.

Male Dunkin-Hartley guinea pigs (500–600 g) were stereotaxically implanted at a total of nine different anterior-lateral coordinates, Ant. 6.0, 8.0 and 10.0 each at the lateral placements of ±2.5, ±3.5 and ±4.5 (coordinates with respect to the zero of the Kopf stereotaxic instrument with the incisor bar raised 5.0 mm). 1 µl 2-(N,N-dipropyl)-amino-5,6-dihydroxytetralin (12.5 µg) or vehicle was injected over a 1 min period at a location 1.0 mm below the guide tips on the first occasion of use, 2.0 mm on the second occasion, 3.0 mm on the third occasion and so forth, with intervening recovery periods of 7–10 days. Immediately after intracerebral injection animals were placed in Perspex boxes equipped with photocells to record hyperactivity (counts/5 min); dyskinetic biting was visually assessed as present or absent. In order to facilitate a comparative analysis of effects in many brain areas an expression for biting ('biting index') was derived from

$$\frac{\text{'Intensity'}\ (\%\ \text{animals responding}) \times \text{Duration (min)}}{\text{Onset (min)}}$$

whilst hyperactivity was determined as the 'area under the hyperactivity curve from the time of onset to completion or a limit of 120 min'.

Four important distinctions could be made between the loci of tetralin action to induce biting and hyperactivity, (i) marked hyperactivity could be obtained in the absence of consistent biting at the most lateral (±4.5) locations in the entire rostral-caudal plane, (ii) within these lateral locations the dorsal placements were more effective, (iii) marked biting behaviour was induced from the ventromedial locations (±2.5, ±3.5) where the hyperactivity response was variable, and i.v.) marked hyperactivity, but only modest/weak biting, was apparent at all rostral locations. Intrastriatal fluphenazine (12.5–50 µg, 15 min pretreatment) reduced or abolished hyperactivity whilst tiapride (25–100 µg, 15 min pretreatment) was a more effective antagonist of the biting response. Intrastriatal piperoxan, propranolol, atropine (50 µg) or methysergide (10 µg) failed to modify either hyperactivity or biting.

In a more limited study dopamine (25–100 µg) and apomorphine (5–50 µg) were injected at Ant. 10.0, Lat. ±2.5, Vert. −4.0 mm (with respect to guide tips), Ant. 8.0, Lat. ±3.5, Vert. −6.0 mm and Ant. 6.0, Lat. ±3.5, Vert. −4.0 mm following pretreatment of guinea-pigs with 75 mg/kg i.p. nialamide (animals used on one occasion only). Apomorphine was ineffective at all three locations whilst dopamine caused hyperactivity, hyperactivity + biting, biting (and weak hyperactivity) at the rostral, medial and caudal locations respectively.

The data indicates a differential distribution of striatal sites capable of mediating hyperactivity and biting; the specific but differential blockade of these responses by fluphenazine and tiapride may indicate that the dopamine mechanisms have different characteristics.

Reference

COOLS, A.R. (1973). *The Caudate Nucleus and Neurochemical Control of Behaviour.* Nijmegen: Drukkerij Brakkenstein.

Comparison of core temperature changes induced by intrahypothalamic injection of 5-hydroxytryptamine and tryptamine in the rat

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Although some workers have postulated that tryptamine and 5-hydroxytryptamine (5-HT) act on different receptors (Clineschmidt & Lotti, 1974; Frankhijzen & Bonta, 1974), others have suggested that they act on the same receptor population (Dooley & Quock, 1976). Since we have previously shown that 5-HT produces a fall in core temperature by acting on specific receptors in the hypothalamus (Cox & Lee, 1979), it was of interest to examine the effect of tryptamine at this site.

Male Sprague-Dawley rats were used at an ambient temperature of 22°C. Drugs were injected into the preoptic anterior hypothalamus through previously implanted guide cannulae in a dose volume of 1 μl. Core temperature was measured by means of a rectal probe.

Intrahypothalamic injection of 5-HT caused a dose-related fall in core temperature (40 μg causing a mean maximum response of -1.2 ± 0.2°C). In contrast, intrahypothalamic injection of tryptamine caused hyperthermia 5 μg causing a mean maximum response of $+0.98 \pm 0.2$°C). Intrahypothalamic pretreatment with a series of putative indoleamine antagonists for 15 min inhibited the core temperature changes induced by either 5-HT or tryptamine in a dose-related manner. The ID_{50} values of these antagonists, calculated from a linear regression analysis, are shown in Table 1. Methergoline appeared to be more selective for the antagonism of tryptamine, whereas cyproheptadine was more selective for the antagonism of 5-HT. Propranolol and haloperidol were ineffective against either substance.

Thus the fact that tryptamine and 5-HT produce qualitatively different responses when injected into the rat hypothalamus and that they show differing susceptibilities to antagonist drugs, raises the possibility that there are different indoleamine receptors present in this region.

References

CLINESCHMIDT, B.V. & LOTTI, V.J. (1974). Indoleamine antagonists: relative potencies as inhibitors of tryptamine- and 5-hydroxytryptamine-evoked responses. *Br. J. Pharmac.*, **50**, 311–313.

COX, B. & LEE, T.F. (1979). Possible involvement of 5-hydroxytryptamine in dopamine-receptor-mediated hypothermia in the rat. *J. Pharm. Pharmac.*, **31**, 352–354.

DOOLEY, D.J. & QUOCK, R.M. (1976). Tryptamine and 5-hydroxytryptamine induced hypothermia in mice. *J. Pharm. Pharmac.*, **28**, 775–776.

FRANKHIJZEN, A.L. & BONTA, I.L. (1974). Receptors involved in the action of 5-HT and tryptamine on the isolated rat stomach fundus preparation. *Eur. J. Pharmac.*, **26**, 220–230.

Table 1 ID_{50} values (nmole) for the antagonism of 5-HT- and tryptamine-induced core temperature changes in the rat

Drugs	*Tryptamine Hyperthermia*	*5-HT Hypothermia*	*Trypt./5-HT Ratio*
Cyproheptadine	9.90	6.44	1.54
Methiothepin	0.36	0.25	1.44
Cinanserin	7.97	7.73	1.03
Methysergide	6.58	7.10	0.93
Methergoline	0.93	5.20	0.18
Haloperidol	No antagonism up to 13.3 nmole		
(−)Propranolol	No antagonism up to 38.6 nmole		

Effect of intrapallidal injection of GABA drugs on head-turn induced by electrical stimulation of the striatum in rats—*in vivo* evidence for different GABA receptors in the CNS?

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Bicuculline sensitive and insensitive GABA receptors have been identified in peripheral nervous system (Bowery & Brown, 1974; Bowery & Hudson, 1979). The bicuculline insensitive receptor modulates transmitter release and differs in its chemical specificity from that producing neuronal depolarisation. Although baclofen appears to be an agonist at this receptor it is insensitive to other more classical GABA agonists e.g. 3-amino-propane sulphonic acid (3-APS) (Bowery *et al.*, 1979). GABA and baclofen but not 3-APS have also been shown to inhibit the K^+ evoked release of ^{3}H-neurotransmitters from rat brain

Table 1 Effect of a range of putative GABA agonist drugs on striatally mediated head-turning

*Drug & Dose** (μg)	*n*	*Mean pre-drug head-turn time* (*s*)	*Mean post-drug head-turn time* (*s*)	*P***
Saline 0.5 μl	20	3.3 ± 0.9	3.1 ± 0.6	NS
GABA				
25	4	2.7 ± 0.3	5.2 ± 0.7	<0.05
50	5	3.3 ± 0.2	10.5 ± 0.6	<0.005
75	5	3.1 ± 0.4	16.1 ± 0.9	<0.001
GABA 50 + Bicuculline 4	3	3.1 ± 0.2	10.0 ± 0.9	<0.02
Muscimol				
10 ng	6	3.2 ± 0.2	7.0 ± 0.4	<0.001
25 ng	6	3.4 ± 0.2	15.3 ± 0.5	<0.001
50 ng	6	2.7 ± 0.3	20.2 ± 0.8	<0.001
Muscimol 25 ng + Bicuculline 4	4	3.4 ± 0.2	14.2 ± 0.8	<0.001
N-Me GABA				
5	5	3.1 ± 0.5	6.1 ± 0.8	<0.005
10	5	3.0 ± 0.4	7.2 ± 0.7	<0.01
20	6	2.9 ± 0.3	11.5 ± 0.9	<0.001
N-Me GABA 10 + Bicuculline 4	3	3.3 ± 0.2	7.9 ± 0.9	<0.05
DL Baclofen				
2.5	4	3.5 ± 0.2	6.6 ± 0.5	<0.01
5	6	3.0 ± 0.2	9.4 ± 0.6	<0.001
10	4	2.8 ± 0.2	14.3 ± 1.6	<0.01
DL Baclofen 5 + Bicuculline 4	3	3.5 ± 0.2	9.4 ± 0.4	<0.01
3-APS				
10	6	3.6 ± 0.2	6.5 ± 0.9	<0.02
20	4	3.9 ± 0.2	8.3 ± 1.4	NS
50	6	3.2 ± 0.3	6.8 ± 0.7	<0.005
Isoguvacine				
10	6	3.1 ± 0.3	4.2 ± 0.5	<0.01
20	6	3.3 ± 0.2	5.5 ± 0.3	<0.001
50	4	3.5 ± 0.4	8.5 ± 0.4	<0.001
THIP				
5	5	3.2 ± 0.5	6.6 ± 0.6	<0.005
10	5	3.1 ± 0.3	7.3 ± 0.6	<0.005
25	5	3.3 ± 0.3	9.1 0.5	<0.001
Bicuculline 4	6	2.9 ± 0.3	4.0 ± 0.3	<0.01

* All drugs were administered intrapallidally in a volume of 0.5 μl.
The figures represent mean ± s.e. mean.
** Determined by a paired '*t*-test'.

slices, a bicuculline insensitive effect (Bowery *et al.*, 1980).

The head-turning model of Crossman, Lee & Slater (1977a, b) which is a useful *in vivo* method for the evaluation of GABA drugs has been used to investigate the effect of putative GABA agonist drugs on central GABA receptors.

Electrical stimulation of the caudate nucleus elicits a contralateral head-turn which can be modified by GABA drugs injected into the ipsilateral globus pallidus. The mean time taken for a 90° head-turn was determined for 10 periods of stimulation at 2 min intervals (pre and post drug or saline injection) for individual rats and was used to calculate group means. The initial head-turn latency of every animal was between 2 and 6 s and was unaffected by saline injections (Table 1).

The influence of a range of putative GABA agonists are also shown in Table 1. Muscimol, GABA, N-methyl GABA and Baclofen all increased the head-turn time and produced steep dose reponse curves, the L-isomer of baclofen was twenty times more potent than the D-isomer. Bicuculline alone produced a slight increase in head-turn time but did not affect the responses to the above agonists. In contrast 3-APS, tetrahydroisoxazolopyridin-3-ol (THIP) and isoguvacine all produced shallow dose response curves and were much less potent than would be predicted from *in vitro* binding studies (Karobath *et al.*, 1979; Stone & Topham, 1980).

Thus the head-turn model, which is believed to be a measure of GABA-ergic activity in the basal ganglia, appears to have properties which more closely resemble those of the peripheral neuroterminal receptor than the ganglionic depolarising receptor.

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References

BOWERY, N.G. & BROWN, D.A. (1974). Depolarising actions of γ-aminobutyric acid and related compounds on rat superior cervical ganglia *in vitro*. *Br. J. Pharmac.*, **50**, 205–218.

BOWERY, N.G. & HUDSON, A.L. (1979). γ-Aminobutyric acid reduces the evoked release of [^{3}H]-noradrenaline from sympathetic nerve terminals. *Br. J. Pharmac.*, **66**, 108P.

BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., SHAW, J.S. & TURNBULL, M.J. (1979). Baclofen: a selective agonist for a novel type of GABA receptor. *Br. J. Pharmac.*, **67**, 444P.

BOWERY, N.G., HILL, D.R., HUDSON, A.L., DOBLE, A., MIDDLEMISS, D.N., SHAW, J.S. & TURNBULL, M.J. (1980). (−)Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature* (*Lond.*), **283**, 92–94.

CROSSMAN, A.R., LEE, L.A. & SLATER, P. (1977a). Effects of manipulating pallidal and nigral GABA on striatally-mediated head-turning in the rat. *Br. J. Pharmac.*, **61**, 483P.

CROSSMAN, A.R., LEE, L.A. & SLATER, P. (1977b). A method for investigating neurotransmission in the basal ganglia using combined stimulation and intracerebral drug injection. *Br. J. Pharmac.*, **61**, 488P.

KAROBATH, M., PLACHETA, P., LIPPITSCH, M., KROGSGAARD LARSEN, P. (1979). Is stimulation of benzoidiazepine receptor binding mediated by a novel GABA receptor? *Nature* (*Lond.*), **278**, 748–749.

STONE, M.A. & TOPHAM, L.D. (1980). Does binding to the high and low affinity GABA binding sites represent binding to benzodiazepine receptor linked and non-benzodiazepine receptor linked GABA receptors? *Br. J. Pharmac.* (in press). Proceedings of Pharmacological Society Meeting, December, 1979.

The effects of centrally administered dopamine and 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) on water and food intake in the rat

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A dopaminergic mechanism has been implicated in the control of thirst, since intracranial injections of dopamine stimulate drinking in rats (Fitzsimons & Setler, 1975). However, the role of dopamine in food intake is not clear, and noradrenaline appears to be more important in feeding (Van der Gugten, De Kloet, Versteeg & Slangen, 1977). To further examine the involvement of dopamine in drinking, it was decided to investigate the effects of the powerful dopamine agonist 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) (Woodruff, 1978) and dopamine itself upon water and food intake after their intracranial administration.

Chronic cannulae were implanted into either the lateral cerebroventricles (A 6.1 L 1.5 H +1.7), the lateral hypothalamus (A 6.3 L 3.8 H −2.2, angled at 16°), or bilaterally into the nucleus accumbens (A 9.4 L 1.5 H −0.6, angled at 13°), of male Wistar rats (200 g) under pentobarbitone anaesthesia. Stereotaxic

coordinates used were according to Konig & Klippel (1963). Following a recovery period of seven days with free access to food and water, animals were injected intracranially with either ADTN, dopamine or 0.9% saline vehicle in a total volume of 0.5 to 2.0 μl, and food and water intake were measured gravimetrically over a 2 h period. Neuroleptics or alpha adrenoceptor antagonists were injected down the cannula 15 min prior to dopamine or ADTN.

Dopamine, injected intracerebroventricularly (i.v.t.) in doses ranging from 260 to 1040 nmol was a weak dipsogen, with 3.3 ± 0.6 mls drunk/2 h (n = 8) after 780 nmol. The response was magnified by pargyline pretreatment (620 μmol/kg i.p.), and control injections of 0.9% saline did not stimulate significant water intake, with rats drinking 0.67 ± 0.2 mls/s h (n = 16).

However, i.v.t. injections of ADTN (9 to 230 nmol) stimulated food intake, but surprisingly there was no drinking. Food intake reached a maximum at 138 nmol ADTN, where rats eat 3.2 ± 0.42 g/2 h (n = 23), compared with 0.1 ± 0.03 g/2 h (n = 14) after 2 μl 0.9% saline.

The ADTN eating response was not significantly altered by i.v.t. injected haloperidol (39 nmol), cis-flupenthixol (39 nmol) or (±)-propranolol (34 nmol), but was completely abolished by i.v.t. administered phentolamine (35 nmol) and reduced by pretreating the animals with reserpine (9.6 μmol/kg, subcutaneously).

Injections of ADTN into the lateral hypothalamus (9 to 230 nmol) or into the nucleus accumbens (10 to 20 nmol) again resulted in eating, but there was no water intake. Phentolamine (35 nmol) extinguished the eating response, whilst haloperidol (39 nmol) was without effect.

The results confirm that dopamine is involved in the control of water intake, since dopamine itself is dipsogenic when administered intracranially. However, at the doses used, ADTN does not act as a dopamine like agonist, but rather like an alpha adrenergic agonist since it stimulates food intake. This effect is possibly due to release of noradrenaline, since the eating response is reduced in reserpinised animals.

References

FITZSIMONS, J.T. & SETLER, P.E. (1975). The relative importance of central nervous catecholaminergic and cholinergic mechanisms in drinking in response to angiotensin and other thirst stimuli. *J. Physiol.*, **250**, 613–631.

KONIG, J.F.R. & KLIPPEL, R.A. (1963). A stereotaxic atlas of the rat brain. Williams and Wilkins Company, Baltimore.

VAN DER GUGTEN, J., DE KLOET, E.R., VERSTEEG, D.H.G. & SLANGEN, J.L. (1977). Regional hypothalamic catecholamine metabolism and food intake regulation in the rat. *Brain Res.*, **135**, 325–337.

WOODRUFF, G.N. (1978). Biochemical and pharmacological studies on dopamine receptors. *Advanc. Biochem. Psychopharmac.*, **19**, 89–107.

[^{3}H]-(±)-Sulpiride specific binding to rat striatal preparations is sodium dependent

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Sulpiride is a cerebral dopamine antagonist believed to act selectively at post-synaptic dopamine receptors not linked to dopamine stimulated adenylate cyclase (Kebabian & Calne, 1979). [^{3}H]-(±)-sulpiride binds specifically to rat striatal preparations (Theodorou, Crockett, Jenner & Marsden, 1979) and we now report the dependence of specific binding on the cation composition of the incubation buffer in comparison to the specific binding of [^{3}H]-spiperone.

Using fresh or frozen striata from female Wistar rats (150 ± 10 g) tissue homogenates were prepared (Leysen, Gommeren & Laduron, 1978) such that the final homogenate was suspended in tris-HCl buffer (50 mM, pH 7.4) containing 0.1% ascorbic acid and pargyline hydrochloride (10 μM) in the presence or absence of varying concentrations of NaCl (25–200 mM), KCl (1–100 mM), $MgCl_2$ (1–10 mM) or $CaCl_2$ (1–100 mM). Binding experiments were carried out at 37°C for 10 min, specific binding was defined using (+)-butaclamol (5×10^{-6} M) for [^{3}H]-spiperone (0.5 nM) and (−)-sulpiride (5×10^{-6} M) for [^{3}H]-sulpiride (15 nM). In the presence of standard cation concentrations (NaCl 120 mM, KCl 5 mM, $MgCl_2$ 1 mM and $CaCl_2$ 2 mM) specific binding by [^{3}H]-spiperone in a typical experiment utilising fresh tissue was 13.1 ± 1.5 pmoles/g wet weight of tissue and [^{3}H]-sulpiride 5.0 ± 0.8 pmoles/g wet weight of tissue. Omission of the cation content of the final incubation buffer reduced specific [^{3}H]-sulpiride binding by 93% but only decreased [^{3}H]-spiperone binding by 27%. Incorporation of NaCl (25–200 mM) into such incubates restored specific [^{3}H]-sulpiride and [^{3}H]-spiperone binding to the levels observed in the presence of all

the cations or above. In contrast the individual incorporation of KCl (1–100 mM), $CaCl_2$ (1–100 mM) and $MgCl_2$ (0.5–10 mM) in general did not enhance specific [^{3}H]-sulpiride or [^{3}H]-spiperone binding. In addition the inclusion of KCl (1–10 mM), $CaCl_2$ (1–10 mM) or $MgCl_2$ (1–10 mM) in the presence of NaCl (120 mM) either had no effect or at the highest concentration decreased specific [^{3}H]-sulpiride binding.

To investigate the involvement of active processes in the specific binding of [^{3}H]-sulpiride or [^{3}H]-spiperone striatal tissue was frozen at −20°C for between 24–72 hours. Freezing did not markedly alter total specific binding of either [^{3}H]-sulpiride or [^{3}H]-spiperone in the presence of standard concentrations of all cations. Removal of the cations from the incubation media again abolished specific [^{3}H]-sulpiride binding an effect also reversed by the incorporation of NaCl (25–200 mM) ($P < 0.05$). In contrast [^{3}H]-spiperone binding was no longer reduced by removal of the cations and the subsequent inclusion of NaCl (25–200 nM) had no effect.

In conclusion, the specific binding of [^{3}H]-sulpiride can be distinguished from that of [^{3}H]-spiperone on the basis of its dependence on sodium ions. The binding site for [^{3}H]-sulpiride does not appear to involve active processes.

References

KABABIAN, J.W. & CALNE, D.B. (1979). Multiple receptors for dopamine. *Nature*, **277**, 93–96.

LEYSEN, J.E., GOMMEREN, W. & LADURON, P.M. (1978). Spiperone: A ligand of choice for neuroleptic receptors. 1. Kinetics and characteristics of in vitro binding. *Biochem. Pharmac.*, **27**, 307–316.

THEODOROU, A., CROCKETT, M., JENNER, P. & MARSDEN, C.D. (1979). Specific binding of ^{3}H-sulpiride to rat striatal preparations. *J. Pharm. Pharmac.*, **31**, 424–426.

Opiate receptors in rat spinal cord

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The spinal cord is believed to be an important site in the analgesic action of opiates. For example morphine has been shown to be a potent anti-nociceptive agent when administered to rats intrathecally (Yaksh & Rudy, 1977). Autoradiographic studies have shown that a large proportion of opiate receptors in spinal cord are destroyed on dorsal root transection in the rat (Atweh & Kuhar, 1977) and immunohistochemical data support the presence of enkephalins in the substantia gelatinosa of the cord (Snyder, Uhl & Kuhar, 1978). This evidence has been used to suggest that the opioid peptide-opiate receptor system at the spinal level acts presynaptically to inhibit the transmission of nociceptive information (Jessel & Iverson, 1977). Della Bella, Casacci & Sassi (1978) have demonstrated binding sites for dihydromorphine and methionine enkephalin in the spinal cord.

We have investigated the binding of [^{3}H]-etorphine, [^{3}H]-dihydromorphine and [^{3}H]-Tyr D-Ala Gly PheD-leu (BW 180C), a peptide with selective affinity for the δ-receptor postulated by Waterfield *et al.* (1978) (Chang *et al.*, 1979) to homogenates of rat spinal cord.

Whole spinal cord tissue of female Sprague–Dawley rats (250 *g*) was homogenised in 15 ml 0.32 M sucrose containing 0.05 M Tris-HCl (pH 7.4) and centrifuged at 10,000 *g* for 10 min. The resultant pellet was resuspended in sucrose-tris (in a volume 50 × the original weight of tissue). Incubations consisted of 960 µl of the above homogenate, 1 nM [^{3}H]-ligand and various concentrations of competing ligand, in a total volume of 1 ml. Reactions were allowed to proceed to equilibrium at 30° for 60 min and then terminated by centrifugation. The radioactivity in the pellets was determined. In some experiments the crude homogenate was further purified to give a synaptosomal fraction free of mitochondria and myelin (Grey & Whittaker, 1962).

The binding of all three radiolabelled ligands to spinal cord homogenates was stereospecific, as determined by the difference between the displacement of bound label by the potent antagonist (−)2-(3-furylmethyl)5,9-diethyl-2′-hydroxy-6,7-benzomorphan (MR 2266) and its inactive (+) isomer (MR 2267) (Waterfield & Kosterlitz, 1975). Specific binding was approximately 40% of total binding.

Bound etorphine was displaced in the order etorphine (IC_{50}, 1.7 nM) = diprenorphine > buprenorphine = MR 2266 > morphine. However morphine appeared only to displace approximately two-thirds of the [^{3}H]-etorphine binding inhibited by MR 2266. This suggests that etorphine may be binding to more than one class of sites, as observed with brain tissue (Chang and Cuatrecasas, 1979). [^{3}H]-Dihydromorphine binding was readily displaced in the order levorphanol > MR 2266 > morphine > Tyr D-Ala-

glyMePheNHCH$_2$CH$_2$NMe$_2$ (RX783016). RX783016 has been shown to be selective for μ receptors (Bower *et al.*, 1980). By comparison leucine enkephalin which shows δ-selectivity did not displace [^{3}H]-dihydromorphine (9% displacement at 2 μM).

The total number of binding sites for the ligands was lower than that found in whole brain (less cerebellum). Etorphine, as expected, bound to most sites (3.3 pmoles/g wet tissue), DHM (0.6 pmol./g) and BW180C the least (0.3 pmol./g wet tissue).

The results confirm the presence of opiate receptors in rat spinal cord and suggest the presence of more than one class of receptor site, including a proportion which appear similar in character to the μ receptors described in whole brain (Waterfield *et al.*, 1975).

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References

ATWEH, S.F. & KUHAR, M.J. (1977). Autoradiographic localisation of opiate receptors in rat brain. I Spinal cord and lower medulla. *Brain Res.*, **124**, 53–67.

BOWER, J., HANDA, B.K., LANE, A.C., LORD, J.A.H., METCALFE, G., MORGAN, B.A., RANCE, M.J., RICHARDS, P.M. & SMITH, C.F.C. (1980). Structure function studies in peptides related to the βLPH$_{61-64}$ sequence. In *Endogenous and Exogenous Opiate Agonists and Antagonists*, ed. Way, E.L., Pergamon, New York, pp. 29–32.

CHANG, K.-J., COOPER, B.R., HAZUM, E. & CUATRECASAS, P. (1979). Multiple opiate receptors: different regional distribution in the brain and differential binding of opiates and opiod peptides. *Mol. Pharmacol.*, **16**, 91–104.

CHANG, K.-J. & CUATRECASAS, P. (1979). Multiple opiate receptors. *J. Biol. Chem.*, **254**, 2610–2618.

DELLA BELLA, D., CASACCI, F. & SASSI, A. (1978). Opiate receptors: different ligand affinity in various brain regions. *Adv. Biochem. Psychopharmacol.*, **18**, 271–277.

GREY, E.G. & WHITTAKER, V.P. (1962). The isolation of nerve endings from brain: an electron microscopic study of cell fragments derived by homogenisation and centrifugation. *J. Anat.*, **96**, 79–88.

JESSELL, T.M. & IVERSON, L.L. (1977). Opiate analgesics inhibit substance release from rat trigeminal nucleus. *Nature*, **268**, 549–551.

SNYDER, S.H., UHL, G.R. & KUHAR, M.J. (1978). Comparative features of enkephalin and neurotensins in the mammalian central nervous system. In *Centrally Acting Peptides*, ed. Hughes, J., pp. 85–97. Baltimore University Park Press.

WATERFIELD, A.A. & KOSTERLITZ, H.W. (1975). Stereoselective increase by narcotic antagonists of evoked acetylcholine output in guinea-pig ileum. *Life Sci.*, **16**, 1787–1792.

WATERFIELD, A.A., LORD, J.A., HUGHES, J. & KOSTERLITZ, H.W. (1978). Differences in the inhibitory effects of normorphine and opiod peptides on the responses of vas deferentia of two strains of mice. *Eur. J. Pharmacol.*, **47**, 249–250.

YAKSH, T.L. & RUDY, T.A. (1977). Studies on the direct spiral action of narcotics in the production of analgesia in the rat. *J. Pharmac. exp. therap.*, **202**, 411–428.

Thermoregulatory effects of guanosine 3′,5′-monophosphate in the cat

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Putative neurotransmitters thought to be involved in central thermoregulatory pathways during active thermoregulation include noradrenaline, 5-hydroxytryptamine, dopamine and acetylcholine (see review by Milton, 1978). The intraneuronal events occurring when these substances alter body temperature are poorly understood but may involve the cyclic nucleotides, adenosine 3′,5′-monophosphate and/or guanosine 3′,5′-monophosphate (cyclic GMP). Several workers have reported that N^6-2′-O-dibutyryl adenosine 3′,5′-monophosphate alters deep body temperature in the cat (Varagić & Beleslin, 1973; Clark, Cumby & Davis, 1974; Dascombe & Milton, 1975). We have investigated the thermoregulatory effects of cyclic GMP in this species.

Cyclic GMP, its N^2-2′-O-dibutyryl derivative (Db-cGMP) and guanosine 5′-monophosphate (GMP) (sodium salts, Sigma) have been injected into the third cerebral ventricle, at the level of the preoptic and the anterior hypothalamic nuclei, in conscious female cats and the effects of these compounds on rectal temperature recorded. Temperature responses were assessed as a thermal response index (Dascombe & Milton, 1976). In addition, the behavioural and autonomic effects following the injection of these substances have been observed.

Cyclic GMP and Db-cGMP (10, 50, 250 and 1250 nmol) were without effect ($P > 0.05$) on body temperature in cats at an ambient temperature of 22 ± 2°C over a period of 5 h, although both compounds induced ear skin vasodilation and sweating

from the foot pads. This autonomic heat loss activity had its onset 4–10 min after drug administration and lasted 40–60 min, being most pronounced after Db-cGMP (1250 nmol), which produced polypnoea and panting also in some cats. At an ambient temperature of 10 ± 1°C, injection of cyclic GMP (1250 nmol) or Db-cGMP (1250 nmol) produced a fall in rectal temperature ($P < 0.05$) beginning within 10 min after injection of the nucleotide and lasting about 2.5 hours. The development of hypothermia induced by cyclic GMP ($TRI_{2.5}$ − 1.24 ± s.e. mean 0.25°C.h, $n = 5$) and by Db-cGMP ($TRI_{2.5}$ − 1.26 ± s.e. mean 0.18°C.h, $n = 5$) was associated with cessation of cold-induced shivering, piloerection and cutaneous vasoconstriction and the onset of cutaneous vasodilation and, with Db-cGMP, polypnoea. The return of body temperature to control values was associated with the redevelopment of shivering, piloerection and vasoconstriction. No other effect on body temperature ($P > 0.05$) or thermoregulatory activity was apparent in nucleotide-treated cats up to 5 h after injection when compared with responses to 50 µl 0.9% saline. GMP (1250 nmol) and sodium *n*-butyrate (2500 nmol) did not have a hypothermic effect in cold (10°C) exposed cats.

These results indicate that exogenous cyclic GMP and Db-cGMP may produce hypothermia in cats by inhibiting central events mediating heat production and conservation, and by activating those governing heat dissipation.

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References

CLARK, W.G., CUMBY, H.R. & DAVIS, H.E. (1974). The hyperthermic effect of intracerebroventricular cholera enterotoxin in the unanaesthetized cat. *J. Physiol.*, **240**, 493–504.

DASCOMBE, M.J. & MILTON, A.S. (1975). The effects of cyclic adenosine 3′,5′-monophosphate and other adenine nucleotides on body temperature. *J. Physiol.*, **250**, 143–160.

DASCOMBE, M.J. & MILTON, A.S. (1976). Cyclic adenosine 3′,5′-monophosphate in cerebrospinal fluid during thermoregulation and fever. *J. Physiol.*, **263**, 441–463.

MILTON, A.S. (1978). The hypothalamus and the pharmacology of thermoregulation. In *Pharmacology of the Hypothalamus* ed. Cox, B., Morris, I.D. & Weston, A.H. pp. 105–134. London: Macmillan.

VARAGIĆ, V.M. & BELESLIN, D.B. (1973). The effect of cyclic N-2-O-dibutyryl-adenosine-3′,5′-monophosphate, adenosine triphosphate and butyrate on the body temperature of conscious cats. *Brain Res.*, **57**, 252–254.

The mechanism of veratridine evoked GABA release from brain slices

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The use of veratridine to evoke the release of GABA from brain tissues may have an advantage over potassium chloride in that it appears to be more effective in causing release from neurones rather than glia (Neal & Bowery, 1979). However, we have recently found that the veratridine-evoked release of [^{3}H]-GABA from brain slices was strikingly potentiated in Ca-free medium, although the release of noradrenaline and ACh was Ca-dependent (Neal, 1979).

In the present study, we have examined the mechanisms involved in the veratridine-evoked release of labelled GABA from brain slices.

Slices of rat cerebral cortex were incubated at 25°C with [^{3}H]-GABA (10^{-8} M) in the presence of aminooxyacetic acid (AOAA) (10 µM) and then superfused at room temperature in a small chamber at a rate of 1.2 ml.min^{-1}. The radioactivity in 2.4 ml samples was measured by liquid scintillation counting. The tissue was exposed to veratridine (10 µM) or KCl (25 mM) for periods of 4 minutes. This caused large increases in [^{3}H]-GABA release. However, whilst the K-evoked release was reduced by 60% in the absence of Ca, the veratridine-evoked release was increased to almost three times that seen in controls. A similar striking potentiation of the veratridine evoked release of [^{3}H]-GABA was obtained with slices of substantia nigra but not cerebellum. This effect was not due to the presence of the GABA-transaminase inhibitor, (AOAA), since a similar effect was seen with [^{14}C]-GABA in the absence of AOAA.

The veratridine evoked release of [^{3}H]-GABA was abolished by sodium free medium and tetrodotoxin but not by Cl-free medium. The evoked release was not due to a reversal of the GABA transport system caused by an increase in $[Na]_i$ because it still occurred in Li medium and after loading the tissue with L-DABA. The veratridine evoked release of [^{3}H]-GABA was potentiated in calcium free medium

even when the slices were depolarised with 120 mM KCl.

Ruthenium red, a specific inhibitor of mitochondrial Ca uptake, potentiated the veratridine evoked release of GABA in both normal and Ca-free medium. It is suggested that the increase in $[Na]_i$ produced by veratridine causes Ca release from intraterminal mitochondria, which in turn, triggers GABA release.

The increase in the veratridine evoked release of GABA seen in Ca-free medium may be due to the absence of an ion antagonistic to the action of veratridine.

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References

NEAL, M.J. & BOWERY, N.G. (1979). Differential effects of veratridine and potassium depolarization on neuronal and glial GABA release. *Brain Res.*, **167**, 337–343.

NEAL, M.J. (1979). Potentiation of veratridine-evoked GABA release from brain slices by the absence of calcium ions. *J. Physiol. (Lond.)*, **292**, 47P.

The radioautographical localization in the vertebrate retina of [^{3}H]-(±)-cis-aminocyclohexane carboxylic acid (ACHC) a selective inhibitor of neuronal GABA transport

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ACHC is a conformationally restricted analogue of GABA which appears to be a relatively selective inhibitor of neuronal GABA transport (Bowery, Jones & Neal, 1976). Thus, the concentrations of ACHC required to inhibit [^{3}H]-GABA uptake by 50% (IC_{50}) in small slices of rat cerebral cortex and frog retinae, tissues in which GABA uptake is mainly neuronal, were 62 μM and 960 μM respectively. In neural tissues in which GABA uptake is mainly glial (rat retina, spinal and sympathetic ganglia) the IC_{50} of ACHC was >50 mM.

ACHC is a competitive inhibitor of GABA uptake in cortical slices and frog retina and is itself accumulated by these tissues (Neal & Bowery, 1978; Neal, Cunningham & Marshall, 1979). In the frog retina, radioautographical studies indicated that both [^{3}H]-ACHC and [^{3}H]-GABA were localised in horizontal cells, a result consistent with the suggestion that ACHC is transported by the GABA carrier. In the present study, we have extended these observations by using radioautography to localise the sites of [^{3}H]-ACHC uptake in the retina of the rat, guinea-pig and rabbit. The methods have been described previously (Neal, Cunningham & Marshall, 1979).

The results clearly indicated that in the rat, guinea-pig and rabbit retina, [^{3}H]-ACHC was accumulated predominantly by the glial Müller fibres. The intensity of the glial labelling may have obscured uptake by amacrine cells but no evidence of uptake by horizontal cells was observed.

A similar disagreement between inhibitor and radioautographical studies was found with L-2,4-diaminobutyric acid (DABA) in the frog retina. Thus, although high (mM) concentrations of DABA inhibited the uptake of [^{3}H]-ACHC at low concentrations (μM), implying that DABA was acting on the GABA transport system of horizontal cells, it was found that DABA itself was accumulated exclusively by glial cells and cones. Even at high concentrations, [^{3}H]-DABA was accumulated mainly by glial cells and no evidence of uptake by horizontal cells was observed.

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References

BOWERY, N.G., JONES, G.P. & NEAL, M.J. (1976). Cis-aminocyclohexane carboxylic acid: A selective neuronal uptake inhibitor and release stimulant of GABA. *Nature*, **264**, 281–284.

NEAL, M.J. & BOWERY, N.G. (1977). Cis-3-aminocyclohexane-carboxylic acid: a substrate for the neuronal GABA transport system. *Brain Res.*, **138**, 169–174.

NEAL, M.J., CUNNINGHAM, J.R. & MARSHALL, J. (1979). The uptake and radioautographical localization in the frog retina of [^{3}H]-(±)-aminocyclohexane carboxylic acid, a selective inhibitor of neuronal GABA transport. *Brain Res.*, **176**, 285–296.

Effect of a triazolo-thienodiazepine (brotizolam) on sleep and on performance in man

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Many modifications of the benzodiazepine molecule have been made to provide hypnotics with improved profiles. An example is the use of heterocyclic ring structures across the 1,2-positions, and one such compound is the triazolothienodiazepine, brotizolam (2-bromo-4-(2-chlorphenyl)-9-methyl-6*H*-thieno-[3,2-*f*]-1,2,4-triazolo-[4.3-a]-1,4 diazepine). Animal studies and early human investigations indicate that it is a potent drug with the advantage of low toxicity, and little propensity for coma in overdosage. The mean elimination half life in man is estimated at 4.4 h, and it is uncomplicated by a long-acting metabolite (Boehringer Ingelheim—Internal Report). Brotizolam is likely to be useful in the management of insomnia when residual sequelae must be avoided, and so we have investigated its effects on sleep, and its immediate and residual effects on performance.

The sleep of six healthy male volunteers aged between 18 and 27 years was studied using electroencephalography. Two adaptation nights with ingestion of placebo, separated by one week, preceded the study. Afterwards each subject ingested brotizolam (0.2, 0.4 and 0.6 mg), diazepam (10 mg) as an active control, and on two further occasions, placebo. A week separated each assessment. All medication was identical in appearance, and the experimental stage of the study was double blind with treatments presented in a random order. Details of recording and analysis are given elsewhere (Nicholson & Stone, 1979). Over the dose range 0.2–0.6 mg, brotizolam increased total sleep time, reduced stage 1 sleep, and improved the sleep efficiency index ($P < 0.05$), while 0.4 mg and 0.6 mg also reduced awake activity ($P < 0.05$). There was some evidence that brotizolam delayed the first REM period, but only after the highest dose was the total duration of REM sleep reduced ($P < 0.05$). There were no changes in slow wave sleep.

In the performance study six healthy female volunteers each took brotizolam (0.2, 0.4 and 0.6 mg) at night with placebo in the morning, placebo at night followed by 0.4 mg in the morning, and placebo on both occasions. A test of visuo-motor coordination (Borland & Nicholson, 1974) was used to assess performance which was measured 9.5, 10.5, 12.5, 14.5 and 17.0 h after overnight ingestion and 0.5, 1.5, 3.5, 5.5 and 8.0 h after morning ingestion. There were no residual sequelae after the overnight ingestion of 0.2 mg brotizolam. After the morning ingestion of 0.4 mg performance was impaired for up to 5.5 h, and there was a residual effect at 9.5 h after overnight ingestion. There were decrements in performance for up to 14.5 h with 0.6 mg taken at night.

Brotizolam would appear to be a useful hypnotic with a dose range free of adverse effects on sleep and residual effects on performance. The usual dose is likely to be around 0.2 mg. However with 0.4 mg the hypnotic effect is marked with only minimal residual effects, and so this dose may prove to be more appropriate for the severe insomniac than currently available drugs, such as nitrazepam and flurazepam hydrochloride, which have effects on performance persisting well into the next working day.

References

BORLAND, R.G. & NICHOLSON, A.N. (1974). Human performance after a barbiturate (heptabarbitone). *Br. J. clin. Pharmac.*, **1**, 209–215.

NICHOLSON, A.N. & STONE, B.M. (1979). L-tryptophan and sleep in healthy man. *Electroencephalogr. clin. Neurophysiol.*, **47**, 539–545.

Cardiovascular effects in man of impromidine, a novel and specific histamine H_2-receptor agonist

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Impromidine is a novel, potent and specific histamine H_2-receptor agonist (Durant *et al.*, 1978). We have carried out two separate cardiovascular studies in healthy volunteers using rapid intravenous injections of this compound.

In the first study, impromidine was given before and after placebo or cimetidine (200 mg i.v.) to six subjects on 2 days separated by 1 week. The treatments were randomised and the study was single-blind. BP and heart rate were measured by automatic sphygmomanometer (Arteriosonde) and heart rate meter, respectively, at half minute intervals for 1 min before and 8 min after each impromidine dose. The presence and distribution of flushing of the skin was recorded.

Impromidine caused dose-dependent falls in diastolic BP, increases in heart rate and flushing. Systolic BP was essentially unchanged. There were no significant differences between the three control log-dose response curves for impromidine (dose range 0.05–0.4 mg) for changes in BP and heart rate. Cimetidine antagonised the responses to impromidine permitting doses of 0.3–2.4 mg impromidine to be given to re-establish the dose-response curves which were displaced to the right. The analysed log-dose data after placebo and cimetidine were linear ($P < 0.05$) and parallel.

In the second study, six subjects were given impromidine before (dose range 0.1–0.3 mg) and after (dose range 0.6–1.8 mg) cimetidine (200 mg i.v.) on the same day. BP was measured at 1 min intervals as before. Heart rate, stroke volume, cardiac output and systolic time intervals were derived and averaged for 25 beats from the impedance cardiogram (dz/dt) and electrocardiogram and continuously monitored on-line by purpose-built minicomputer. BP and cardiac output values were used to calculate peripheral resistance.

Impromidine caused dose-dependent decreases in diastolic pressure, peripheral resistance, pre-ejection period (onset of Q wave of electrocardiogram to onset of dz/dt wave form) and RZ interval (peak of R wave of electrocardiogram to peak of differentiated impedance cardiogram) and increases in heart rate, cardiac output and dZ/dt/RZ index (Balasubramanian *et al.*, 1978). Changes in stroke volume were variable. Dose-response curves to impromidine were displaced to the right in the presence of cimetidine.

The responses to impromidine were well tolerated and reproducible. The results are consistent with the presence of histamine H_2-receptors in human peripheral blood vessels (causing vasodilatation) and heart (causing positive chronotropic and inotropic responses). Indirect cardiac effects, e.g. secondary to BP changes, cannot be excluded.

References

DURANT, G.J., DUNCAN, W.A., GANELLIN, C.R., PARSONS, M.E., BLACKEMORE, R.C. & RASMUSSEN, A.C. (1978). Impromidine (SK&F 92676) is a very potent and specific agonist for histamine H_2 receptors. *Nature*, **276**, 403–405.

BALASUBRAMANIAN, V., MATHEW, O.P., ARUN BEHL, TEWARI, S.C. & HOON, R.S. (1978). Electrical impedance cardiogram in derivation of systolic time intervals. *British Heart Journal*, **40**, 3, 268–275.

Salicylate frusemide interactions

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Diflunisal has been shown to produce higher plasma levels of hydrochlorothiazide (Tempero *et al.*, 1977), which suggests that it may interfere with the renal tubular excretion of diuretics, but its interaction with frusemide has not been studied. Aspirin has been shown to inhibit the diuretic and natriuretic action of frusemide in dogs (Berg & Loew, 1977), but not in man; a study by Berg (1977) showed that pretreatment with aspirin (2.0 g) did not affect the diuresis and natriuresis produced by frusemide (40 mg).

Eighteen healthy subjects of both sexes, aged 20–23 years, took part in a double-blind, double dummy, controlled study of the interactions of each of these analgesics with frusemide. They were allotted to four regimes at weekly intervals. Each regime consisted of two oral treatments given 60 min apart: diflunisal (250 mg) plus frusemide (20 mg), aspirin (300 mg) plus frusemide (20 mg), placebo plus placebo and placebo plus frusemide (20 mg). After the second treatment, urine output was measured hourly for four hours and the sodium and potassium content were measured.

Urine output (Fig. 1) following placebo plus placebo was significantly less than for each of the other three regimes after 2 and 3 hours. Urine output following aspirin plus frusemide was significantly less after 2 h than with either diflunisal plus frusemide or placebo plus frusemide. Sodium excretion followed a similar pattern to water excretion on all four regimes. Potassium excretion showed a reduction after aspirin plus frusemide and also after diflunisal plus frusemide when compared with placebo plus frusemide. This study demonstrates that aspirin inhibits the diuretic and natriuretic action of frusemide. The mechanism deserves clarification, and may be related to competition for tubular excretion or to inhibition of synthesis of intrarenal prostaglandins.

References

BERG, K.J. (1977). Acute effects of acetylsalicylic acid on renal function in normal man. *Eur. J. clin. Pharmac.*, **11**, 117–123.

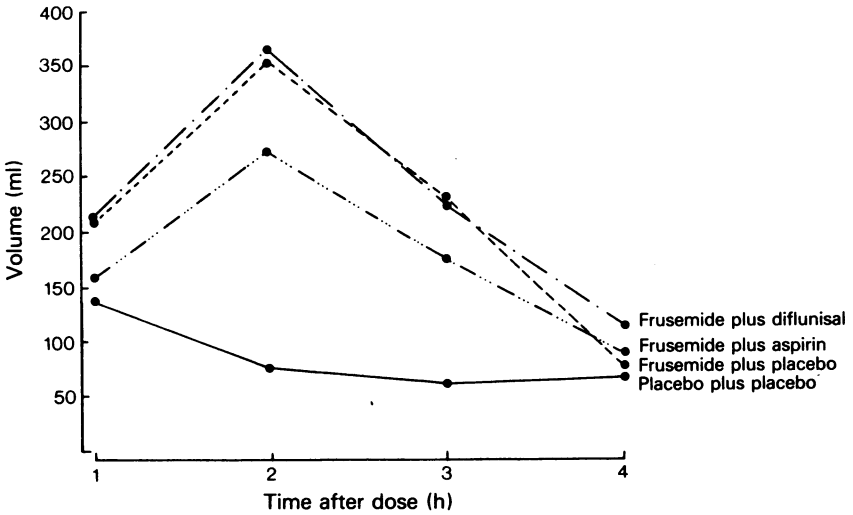

Figure 1 Hourly urine output for 4 h after oral doses of frusemide (20 mg) and aspirin (300 mg) or frusemide (20 mg) and diflunisal (250 mg) or frusemide and placebo or placebo alone.

BERG, K.J. & LOEW, D. (1977). Inhibition of furosemide induced natriuresis by acetylsalicylic acid in dogs. *Scand. J. clin. lab. Invest.*, **37**, 125–131.

TEMPERO, K.F., CIRILLO, V.J. & STEELMAN, S.L. (1977). Diflunisal: a review of pharmacokinetic and pharmacodynamic properties, drug interactions, and special tolerability studies in humans. *Br. J. clin. Pharmac.*, **4**, 31S–36S.

β-Endorphin and parturition

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We are attempting to study the role of circulating β-endorphin during labour, in order to examine the hypothesis that this endogenous opioid acts as a natural analgesic.

Initially, 16 patients were studied, from whom venous blood samples were taken at the following times, before labour, during early and late first stage, second stage and postpartum. Additionally, a cord blood sample was taken. At the time of blood sampling patients were asked to indicate the intensity of their pain on an analogue pain scale.

β-Endorphin-like-immunoreactivity (β-ELI) was extracted from plasma (Höllt *et al.*, 1979) and assayed using a commercially available radioimmunoassay kit (NEN).

The concentrations of plasma β-ELI at various stages during labour are shown in Table 1. Our results are in agreement with those reported by two other groups (Csontos *et al.*, 1979; Akil *et al.*, 1979).

When the β-ELI concentrations for each patient are expressed as a percentage of their control value, the majority of patients show a small increase at second stage, which is maintained postpartum. However, a few patients exhibited a greater rise at second stage, which then dropped slightly post partum. No other differences were evident between these two groups, however, further work may bring to light other distinguishing factors.

Although only a few patients were studied, there is some evidence that during the first stage of labour there is a positive correlation between pain score and β-ELI concentration. The situation post partum does, however, appear to be reversed with higher concentrations of β-ELI, corresponding to lower pain scores. One may therefore be seeing an increasing release of β-ELI during labour in response to pain and that at some point in late labour or after delivery β-ELI concentration is such that pain relief ensues.

There appears to be an inverse relationship

Table 1 Concentration of plasma β-ELI during labour

Stage of labour	Mean Concentration of plasma β-ELI (fmole/ml plasma) (±s.e. mean)	Number of patients
Prelabour	11.5 ± 2.8	9
Early first	13.3 ± 3.0	9
Late first	13.0 ± 3.9	8
Second	43.8 ± 29.2	3
Post partum	58.3 ± 30.3	9
Cord	40.9 ± 6.8	8

between birthweight and the concentration of β-ELI in the cord blood. It may be that high circulating levels of β-endorphin, while fulfilling a role in utero, are not required by the baby after birth and therefore increasing foetal size and maturity triggers a fall in the concentration of β-ELI in foetal blood.

References

AKIL, H., WATSON, S.J., BARCHAS, J.D. & LI, C.H. (1979). β-endorphin immunoreactivity in rat and human blood: Radioimmunoassay, comparative levels and physiological alterations. *Life Sci.*, **24**, 1659–1666.

CSONTOS, K., RUST, M., HÖLLT, V., MAHR, W., KROMER, W. & TESCHEMACHER, H.J. (1979). Elevated plasma β-endorphin levels in pregnant women and their neonates. *Life Sci.*, **25**, 835–844.

HÖLLT, V., PRZEWLOCKI, R. & HERZ, A. (1978). Radioimmunoassay of β-endorphin—Basal and stimulated levels in extracted rat plasma. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **33**, 171–174.

The evaluation of a new electronic counting technique for measurement of platelet aggregation in human whole blood *in vitro*

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The most commonly used method for studying platelet aggregation *in vitro* involves the photometric measurement of aggregation in platelet rich plasma (Born, 1962). This method has been invaluable in platelet function studies but has its limitations (Born & Hume, 1967). Other methods have also been described (Gordon, 1973; Silversten, 1976; Cardinal & Flower, 1979) but these too involve either indirect measures of platelet aggregation or the separation of platelets from other blood cell types before counting. We present here details of a new electronic counting method for studying platelet aggregation in whole blood *in vitro*.

The technique involves the use of the Ultra-Flo 100 whole blood platelet counter (Clay Adams) which counts individual platelets and is a modification of an original method described by Adam, Schulz & Kratt (1976). Blood was collected by venepuncture from the antecubital vein of healthy human volunteers (who had received no medication for at least 2 weeks) and mixed in the proportion 9 to 1 with 0.129 mol/l trisodium citrate. Aliquots (0.5 ml) of whole blood were incubated for 30 min in stoppered plastic vials with aspirin (2.0×10^{-3} mol/l) at 37°C in a water bath with shaking (Hearson, 90 shakes/min). Prior to sealing the vials a stream of 5% carbon dioxide in air was directed into them and this procedure was always repeated upon re-sealing. Throughout the period of the experiments the blood pH was stable at 7.32 ± 0.01 (mean ± s.e. mean from 20 experiments).

After the initial equilibration period the platelet count in each aliquot was determined (mean ± s.e. mean, from 20 donors, was $207 \pm 10 \times 10^3/\mu l$) and a known concentration of an aggregating agent added, the vial re-sealed and returned to the water bath. Platelet numbers were then re-determined at various times and the count expressed as a percentage of the control count. By adding a different concentration of an aggregating agent to each aliquot of blood, concentration-effect curves were constructed (Humphrey & Lumley, 1980). Experiments normally lasted 2–3 h during which time the viability of the platelets was good as shown by the highly reproducible concentration-effect curves obtained with various aggregating agents.

There was good agreement between the numbers of single platelets (in the presence and absence of different concentrations of U-46619) obtained using the Ultra-Flo 100 and those counted manually using phase-contrast microscopy (Dacie & Lewis, 1975). Furthermore the reduction in platelet count produced by a variety of aggregating agents was abolished by ethylenediaminetetra-acetic acid (Humphrey & Lumley, 1980). These findings suggest that the fall in platelet numbers obtained by our method is a measure of the degree of platelet aggregation. The method is simple and reproducible and could easily be used for the rapid assessment of platelet reactivity in man *ex vivo*.

We are grateful to Susan Roberts for skilled technical assistance.

References

ADAM, W., SCHULZ, J. & KRATT, E. (1976). The electronic counting of thrombocytes from whole blood. *Blut*, **32**, 347–352.

BORN, G.V.R. (1962). Quantitative investigations into the aggregation of blood platelets. *J. Physiol.*, **162**, 67–68P.

BORN, G.V.R. & HUME, M. (1967). Effects on the numbers and sizes of platelet aggregates on the optical density of plasma. *Nature, Lond.*, **215**, 1027–1029.

CARDINAL, D.C. & FLOWER, R.J. (1979). The study of platelet aggregation in whole blood. *Br. J. Pharmac.*, **66**, 94–95P.

DACIE, J.V. & LEWIS, S.M. (1975). Basic haematological techniques. In: *Practical Haematology*, 5th edition, pp. 21–67, London: Churchill Livingstone.

GORDON, J.L. (1973). Evaluation of a semi-micro method for measuring platelet aggregation in whole blood samples. *Thrombos. Diathes. haemorrh.* (Stuttg.), **30**, 160–172.

HUMPHREY, P.P.A. & LUMLEY, P. (1980). A method for the analysis of drug-receptor interactions on platelets in human whole blood *in vitro*. *Br. J. Pharmac.* **70**, 125–126P.

Effects of β-adrenoceptor blockers on platelet function and serotonin concentrations

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The improved prognosis in patients treated for hypertension can be attributed to a reduction in strokes, cardiac and renal failure but myocardial infarction remains unchecked. Regular consumption of drugs which inhibit platelet aggregation may be associated with a decreased risk from coronary thrombosis (Boston Collaborative Drug Surveillance Group, 1974). β-adrenoceptor blockers are the treatment of choice in hypertension and propranolol may diminish the sensitivity of platelets to aggregating agents (Frishman, Webster, Christodoulu, Smithen & Killip, 1974), possibly by non-specific membrane effects which also influence serotonin turnover (Nathan, Dvilansky, Sage & Korczyn, 1977). We have performed *in vitro* and *in vivo* tests of platelet aggregation and platelet serotonin concentrations in 11 hypertensive patients receiving placebo for 4 weeks and then during treatment for more than one month with optimal antihypertensive doses of oxprenolol and acebutolol given in randomized double blind cross-over fashion.

In vitro platelet aggregation tests were performed on citrated platelet rich plasma samples diluted to a platelet concentration of 2.5×10^9/l. Aggregation was induced by adding incremental concentrations of adenosine diphosphate (ADP; 1–10 μM/l and adrenaline (0.15–3 μM/l). The sensitivity of the platelets was expressed as the threshold concentration of aggregating agent required to produce a biphasic response.

In vivo platelet function was assessed by platelet aggregation ratio (PAR; Wu & Hoak, 1974) and β-thromboglobulin (BTG) estimations, by radioimmunoassay (Radiochemical Centre, Amersham). Platelet serotonin concentrations were estimated by fluorometric o-phthaldialdehyde assay (Drummond & Gordon, 1974).

Blood pressure and exercise induced heart rate were significantly lower after active treatment than after placebo (paired t-test $P < 0.001$). However, apart from an increased sensitivity to adrenaline in oxprenolol treated subjects (Rank test, $P < 0.05$) there was no change in platelet aggregation tests or platelet serotonin concentrations (Table 1). Therapeutic doses of oxprenolol and acebutolol do not inhibit platelet function in hypertensive patients.

References

BOSTON COLLABORATIVE DRUG SURVEILLANCE GROUP (1974). Regular aspirin intake and myocardial infarction. *Br. med. J.*, **1**, 440–443.

DRUMMOND, A.H. & GORDON, J.L. (1974). Rapid, sensitive

Table 1 Effect of β-blockers on platelet function tests (median or mean ± s.d.) B.P. and exercise tachycardia

	ADP Threshold ($\mu M/l$)	*Adrenaline Threshold* ($\mu M/l$)	*P.A.R.*	*B.T.G.* (*ng/ml*)	*Serotonin* ($ng/10^9$)	*B.P.* (*mm Hg*)	*% reduction Exercise tachycardia*
Placebo	3.0	0.75	0.94 ± 0.19	62 ± 44	596 ± 196	160/105 ± 23/8	0
Oxprenolol	2.5	0.3*	0.92 ± 0.27	66 ± 48	463 ± 153	144/94 ± 23/7	12.4 ± 7.5
Acebutolol	2.0	0.5	0.96 ± 0.30	39 ± 41	442 ± 206	142/91 ± 24/6	18.8 ± 7.5

* Lower than placebo only $P < 0.05$.

microassay for platelet 5HT. *Thromb. Diath. Haemorrh.*, **31**, 366–367.

FRISHMAN, W.H., WEBSTER, B., CHRISTODOULU, J., SMITHEN, C. & KILLIP, R. (1974). Reversal of abnormal platelet aggregability and change in exercise tolerance in patients with angina pectoris following oral propranolol. *Circulation*, **50**, 887–896.

NATHAN, I., DVILANSKY, A., SAGE, J. & KORCZYN, A. (1977). Effects of propranolol and pindolol on platelet aggregation and serotonin release. *Life Sci.*, **20**, 407–412.

WU, K.K. & HOAK, J.C. (1974). A new method for the quantitative detection of platelet aggregation in patients with arterial insufficiency. *Lancet*, **ii**, 924–926.

Inhibitors of platelet aggregation have different activity in blood than in platelet-rich plasma

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The method most commonly used to assess platelet aggregation and its inhibition *in vitro* is that described by Born (1962). This method measures light transmission through platelet-rich plasma (PRP) and hence necessitates removal of erythrocytes. The purpose of the present communication is to illustrate that the presence of erythrocytes can appreciably alter the relative potencies of anti-aggregant compounds *in vitro*.

Blood (20 ml) drawn from volunteers who denied taking aspirin within the previous seven days was mixed with 0.1 vol 129 mM trisodium citrate. Some samples were centrifuged at 150 *g* for 12 min to prepare PRP. Collagen (Hormon-Chemie, Munich) or 154 mM NaCl (control) was added to 0.5 ml aliquots of blood or PRP stirred at 1100 rev/min in an aggregometer at 37° after 3 min preincubation with compound or appropriate solvent. Exactly 4 min later, 0.5 vol. 4.5% formaldehyde was added to fix platelet aggregates, which were then sedimented with erythrocytes in a Thrombofuge (Coulter Electronics Ltd.). Single platelets remaining in the supernatant were counted electronically. Responses, expressed as per cent fall in platelet count, were obtained to a range of collagen concentrations. The concentration producing a 50% maximal effect (EC_{50}) was estimated from concentration-response curves. Dose-ratios were calculated from the ratio of EC_{50}'s in the presence and absence of compound under test. Where PRP was used, responses to collagen were also quantified as changes in light transmission (mm) and dose-ratios calculated as described above.

Results on three compounds, VK 774, aspirin and dipyridamole are summarized in Table 1. The comparison in PRP showed that assessing the activity of compounds by the method based on platelet counts gave essentially the same results as the photometric method. VK 774 was four times more active in PRP

Table 1 Comparison of the anti-aggregant potencies of three compounds in human platelet-rich plasma and blood

		Dose-ratio		
		In platelet-rich plasma		*In Blood*
Compound	*Concentration* (μM)	*Photometric method*	*Platelet count method*	*Platelet count method*
VK 774	2.5	3.1 ± 0.7 (3)	3.6 ± 0.4 (3)	—
	10	11.6 ± 2.4 (3)	16.5 ± 4.9 (3)	3.8 ± 1.8* (3)
Dipyridamole	400	2.8 ± 0.5 (3)	2.8 ± 0.6 (3)	5.5 ± 1.0* (3)
	200	1.4 ± 0.2 (3)	1.4 ± 0.3 (3)	3.7 ± 1.1* (4)
Aspirin	200	—	—	2.4 ± 0.3 (3)
	100	2.9 ± 0.7 (3)	3.0 ± 0.5 (3)	1.8 ± 0.4 (5)
	50	1.4 ± 0.3 (3)	1.1 ± 0.3 (3)	1.4 ± 0.3 (3)

Values are mean ± s.e. mean (*n*).
* $P < 0.05$, Mann Whitney 'U' test (1947).

than in blood whereas dipyridamole was at least twice as active in blood than in PRP. Aspirin had similar activity in blood and PRP. These results show that studies in PRP alone may give misleading information, especially when calculating drug potency ratios.

J.P.N.W. is a sandwich student from Liverpool Polytechnic.

References

BORN, G.V.R. (1962). Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature (Lond.)*, **194**, 927–929.

MANN, H.B. & WHITNEY, D.R. (1947). On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Statist.*, **18**, 52–54.

The effect of magnesium ions on the responses of arterial smooth muscle to noradrenaline, histamine and adenosine 5′ triphosphate (ATP)

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Alterations in extracellular magnesium ion concentration have been reported to affect the magnitude of contractile responses in isolated blood vessels and to be involved in the regulation of vascular tone (Altura & Altura, 1971, 1978). Withdrawal of magnesium ions produces differential effects on vascular responses to various agonists but the direction and magnitude of the reported changes are inconsistent (Somlyo, Woo & Somlyo, 1966; Altura & Altura, 1971; Jurevics & Carrier, 1973; Fujiwara, Kitagawa & Kurahashi, 1978). These changes are generally considered to be due to altered permeability, binding and/or distribution of calcium in arterial muscle.

In the present study the effects of changing the extracellular magnesium ion concentration on the responses of an arterial preparation to noradrenaline, histamine and ATP at normal and increased calcium ion concentrations were examined.

Segments of the central ear artery of female New Zealand white rabbits were perfused at a constant rate of 8 ml/min with Krebs bicarbonate solution at 37°C. An equilibration period of one hour was used; only one modification of ionic content was made and one agonist administered to each segment ($n = 6$–8 for each procedure). Osmotic changes were prevented by adjusting the sodium chloride content of the solution or by adding sucrose.

Withdrawal of magnesium ions from the perfusate caused no significant change in responses to noradrenaline or to adrenaline (1 to 160 ng) with the exception of the response to the highest dose of adrenaline used which was significantly potentiated ($P < 0.02$, correlated 't' test). However, responses to histamine (0.01 to 1.2 μg) and ATP (0.001 to 8.0 mg) were significantly potentiated at all dose levels ($P < 0.05$ to $P < 0.001$, correlated 't' test). Doubling the extracellular magnesium ion concentration caused little change in responses to noradrenaline, histamine or ATP except at the highest doses used where a small reduction occurred. When the magnesium content was increased fourfold the responses to high doses of all three agonists were significantly reduced to a similar extent ($P < 0.05$ to $P < 0.001$, correlated 't' test).

Doubling the extracellular calcium ion concentration caused no significant change in the responses to noradrenaline but caused significant increases in responses to histamine and ATP ($P < 0.05$ to $P < 0.001$, correlated 't' test). Withdrawal of magnesium ions from the double calcium solution caused little change in responses to noradrenaline or histamine but significantly potentiated responses to ATP at the lower dose levels ($P < 0.05$ to $P < 0.001$, students 't' test). These results show that magnesium withdrawal causes differential effects on responses to the three agonists which are similar to those observed in increased calcium solution. However, increases in magnesium ion concentration up to four times normal, caused relatively small reductions in responses to the higher doses of all three agonists and no differential effects were observed.

References

ALTURA, B.M. & ALTURA, B.T. (1971). Influence of magnesium on drug induced conctractions and ion content in rabbit aorta. *Am. J. Physiol.*, **220**, 938.

ALTURA, B.M. & ALTURA, B.T. (1978). Magnesium and vascular tone and reactivity. *Blood Vessels*, **15**, 5.

FUJIWARA, M., KITAGAWA, H. & KURAHASHI, K. (1978). Effects of magnesium on contractile responses induced by electrical transmural stimulation and noradrenaline in rabbit thoracic aorta. *Br. J. Pharmac.*, **63**, 51–56.

JUREVICS, H.A. & CARRIER, O. JR. (1973). Effect of magnesium on responses of aortas from normal and reserpine-treated rabbits. *Am. J. Physiol.*, **225**, 1479–1485.

SOMLYO, A.V., WOO, C. & SOMLYO, A.P. (1966). Effect of magnesium on posterior pituitary hormone action on vascular smooth muscle. *Am. J. Physiol.*, **210**, 705–714.

Comparison of the 5-hydroxytryptamine-induced contraction of human basilar arterial and rat aortic strips *in vitro*

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There are conflicting reports regarding the functional significance of 5-Hydroxytryptamine (5-HT) in the aetiology of cerebral arterial spasm (CAS). Allen, Gold, Chou & French (1974) provide evidence to implicate 5-HT in CAS where as Boullin, Du Boulay & Rogers (1978) suggest that 5-HT does not play a role. The major argument stems from the observation of Boullin *et al.* (1978) that BW-501C67 a reputedly selective 5-HT antagonist (Mawson & Whittington, 1970) does not prevent CAS in baboons induced by injection of blood intracisternally, but it did antagonise CAS produced by 5-HT. The human basilar artery has been shown to be 100 times more sensitive to 5-HT compared to the rat aorta (Forster, Whalley, Mohan & Dutton, 1980). This study investigates the effect of several 5-HT antagonists (methysergide, cyproheptadine, methergoline and BW-501C67) on responses of the human basilar artery and rat aorta to 5-HT and noradrenaline (NA).

Human basilar arteries were obtained at post-mortem from patients showing no neurological symptoms and were dissected into spiral strips. Male Sprague–Dawley rats (300–400 g) were killed and the thoracic aortae was removed and similarly dissected into spiral strips. The strips were bathed in bubbled (95% O_2:5% CO_2) Krebs Hensleit solution and maintained at 37°C. The tissue was allowed to equilibrate for at least 2 h before full cumulative concentration-effect curves to either 5-HT or NA were constructed.

Ninety min following the first concentration effect curve, a concentration of antagonist was added to the tissue baths and left in contact for 30 minutes. One tissue was kept as a control and no antagonist was added. The concentration-effect to 5-HT in the presence of the antagonist was repeated. The same method was utilised for 2–3 further concentrations of the same antagonist. Only one antagonist was used on one tissue.

Methysergide (10^{-7} to 10^{-5} M), cyproheptadine (10^{-8} to 10^{-6} M) and methergoline (10^{-9} to 10^{-6} M) acted as competitive antagonists against 5-HT on the rat aorta, whereas NA was unaffected. BW-501C67 (10^{-10} to 10^{-8} M) acted as a non-competitive antagonist against both 5-HT and NA on rat aorta.

On the human basilar artery all antagonists used (except methysergide which had no effect on NA) acted as non-competitive antagonists against both 5-HT and NA. Methysergide was a partial agonist on the human basilar artery.

These results demonstrate that, on the human basilar artery, 5-HT is antagonised in a non-competitive fashion by the antagonists used in this study. This also appears to be the case (using methysergide and cyproheptadine) with cat and dog intracranial vessels (Hardebo, Edvinsson, Owman & Svengaard, 1978; Saxena, 1974). It is possible that the receptor mediating 5-HT induced contraction of the human basilar artery is different from that in rat aorta and may be similar to that described recently in dog saphenous vein by Apperley, Feniuk, Humphrey & Levy (1980).

References

ALLEN, G.S., GOLD, L.H.A., CHOU, S.N. & FRENCH, L.A. (1974). Cerebral arterial Spasm. Part 3: *in vivo* intracisternal production of spasm by serotonin and blood and its reversal by phenoxybenzamine. *J. Neurosurg.*, **40**, 451–458.

APPERLEY, E., FENIUK, W., HUMPHREY, P.P.A. & LEVY, G.P. (1980). Evidence for two different types of receptor for 5-hydroxytryptamine in dog isolated vasculature. *Br. J. Pharmac.*, In Press.

BOULLIN, D.J., DU BOULAY, G.H. & ROGERS, A.T. (1978). Aetiology of cerebral arterial spasm following subarachnoid haemorrhage: evidence against a major involvement of 5-hydroxytryptamine in the production of acute spasm. *Br. J. Clin. Pharmac.*, **6**, 203–216.

FORSTER, C., WHALLEY, E.T., MOHAN, J. & DUTTON, J. (1980). Vascular smooth muscle response to fibrinogen degradation products and 5-Hydroxytryptamine: possible role in cerebral vasospasm in man. *Br. J. Clin. Pharmac.*, In Press.

HARDEBO, J.E., EDVINSSON, L., OWMAN, CH. & SVENGAARD, N.AC. (1978). Potentiation and antagonism of serotonin effects on intracranial and extracranial vessels. *Neurology*, **28**, 64–70.

MAWSON, C. & WHITTINGTON, H. (1970). Evaluation of the peripheral and central antagonistic activities against 5-hydroxytryptamine of some new agents. *Br. J. Pharmac.*, **39**, 323P.

SAXENA, P.R. (1974). Selective vasoconstriction in the carotid vascular bed by methysergide: Possible relevance to its antimigraine effect. *Eur. J. Pharmac.*, **27**, 99–105.

Effects of a benzotriazinium salt on guinea-pig atria and in the pithed rat

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Agents which increase transmembrane calcium ion influx have been demonstrated to restore excitability to guinea-pig isolated atria which are partially depolarised by immersion in potassium (22 mM). Ringer solution (Pappano, 1970; Thyrum, 1974; Schrader, Rubio & Berne, 1975). Such agents include catecholamines, methylxanthines and calcium itself. The indirectly acting amine tyramine also restores excitability to depolarised atria, its action possibly being related to the release of endogenous catecholamines from sympathetic fibres.

In five experiments both noradrenaline (2×10^{-6} M) and tyramine (3.2×10^{-5} M) restored contractions in electrically stimulated left atria depolarised by high potassium solution (22 mM). These restorations were inhibited by propranolol (5×10^{-7} M) but were unaffected by phentolamine (5×10^{-6} M), indicating involvement of the atrial β-adrenergic receptor. In atria from animals pretreated 18–24 h previously with reserpine (5 mg/kg), tyramine did not restore contractions while noradrenaline was still capable of doing so.

The benzotriazinium compound TnPBI (2-n-propyl-4-p-tolylamino-1,2,3-benzotriazinium iodide) which has been shown to possess a variety of effects on cardiac tissue (French & Scott, 1977, 1979), did not cause restoration of contractions to depolarised atria, although it has been shown to increase the maximum rate of depolarisation of 'calcium action potentials' and to increase the height of the plateau phase, both an index of increased calcium influx (French, 1979).

However, in the presence of TnPBI (1×10^{-5} M), the actions of tyramine (3.2×10^{-5} M) were markedly potentiated, while the restorations due to noradrenaline were not significantly altered. As expected, the uptake$_1$ blocking agent desipramine potentiated the effects of noradrenaline and antagonised those of tyramine in this preparation. However, in the presence of desipramine (5×10^{-6} M), TnPBI showed a small but significant restorative effect.

In the pithed rat preparation TnPBI (1 mg/kg) caused small, transient increases in blood pressure and a reduction in heart rate. The pressor responses to TnPBI were potentiated by desipramine (100 μg/kg) and partially antagonised by phentolamine (100–200 μg/kg), but neither drug altered the negative chronotropic effect. In reserpinised rats the pressor effects of tyramine were greatly reduced, while those of TnPBI were similar to those observed in normal animals.

The effects on the isolated, depolarised atria suggest that TnPBI facilitates the tyramine-induced release of noradrenaline. The restoration of excitability caused by TnPBI in the presence of desipramine may be caused by the release of small amounts of noradrenaline which would be potentiated due to inhibition of uptake$_1$. Such noradrenaline-releasing activity of TnPBI thus appears to differ from that of tyramine in that TnPBI does not depend on the uptake$_1$ mechanism. The results from the pithed rat suggests that noradrenaline release by TnPBI is not the only mechanism responsible for the pressor responses, since the drug is still active in reserpinised animals. It is possible that there is also a direct effect on the blood vessels involving calcium mobilisation. Such a mechanism has already been demonstrated on frog rectus abdominis muscle (Muir & Scott, 1977).

References

FRENCH, A.M. (1979). *In vitro* and *vivo* effects of a benzotriazinium salt on cardiac tissue. *Ph.D. Thesis*, Heriot-Watt University.

FRENCH, A.M. & SCOTT, N.C. (1977). A benzotriazinium salt as a potential antiarrhythmic agent. *Br. J. Pharmac.*, **61**, 131–132P.

FRENCH, A.M. & SCOTT, N.C. (1979). Comparison of the effects of a benzotriazinium iodide and quinidine on guinea-pig heart. *Br. J. Pharmac.*, **66**, 19–24.

MUIR, C.K. & SCOTT, N.C. (1977). Comparison of the effects of caffeine and a 2-alkyl-1,2,3-benzotriazinium iodide on frog rectus abdominis. *Br. J. Pharmac.*, **60**, 375–378.

PAPPANO, A.J. (1970). Calcium-dependent action potentials produced by catecholamines in guinea-pig atrial muscle fibres depolarised by potassium. *Circulation Res.*, **27**, 379–390.

SCHRADER, J., RUBIO, R. & BERNE, R.M. (1975). Inhibition of slow action potentials of guinea-pig atrial muscle: a possible effect on Ca^{2+} influx. *J. mol. cell. Cardiol.*, **7**, 427–433.

THYRUM, P.T. (1974). Inotropic stimuli and systolic transmembrane calcium flow in depolarised guinea-pig atria. *J. Pharmac. exp. Ther.*, **188**, 166–179.

'In vivo' and *'in vitro'* measurement of changes in sympathetic nervous control of vascular and non-vascular tissues in rats chronically treated with Cd^{2+}

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Chronic treatment of rats with Cd^{2+} causes hypertension (Schroeder & Vinton, 1962) and i.v. injection of Cd^{2+} increases blood pressure (Perry, Erlanger, Yunice, Schoepfle & Perry, 1970) however Cd^{2+} inhibits the responses of non-vascular (Fadloun & Leach, 1979) and vascular (Fadloun & Leach, 1980) smooth muscle preparations to noradrenaline (NA), perimural stimulation and potassium ions (K^+). The present studies were, therefore, designed to further analyse the nature of possible differences between the *in vivo* and *in vitro* effects of Cd^{2+}.

Male Sprague Dawley rats (200–300 g) received Cd^{2+} (5, 12.5 or 25 ppm) added to their drinking water for 4 weeks (Cd^{2+} concentrations up to 25 ppm did not affect daily fluid intake or body weight). The animals were then anaesthetised (pentobarbitone sodium, 60 mg/kg i.p.) and blood pressure was measured via the carotid artery. The rats were then pithed using the method of Gillespie & Muir (1967) and the effects of lower sympathetic outflow stimulation were tested at 20 v, 0.3 ms, 1–25 Hz, 10 s duration, and responses to noradrenaline (NA) (25–500 ng/kg) and tyramine (2.5, 25 μg/kg) were assessed after injection via the femoral vein. Portal vein and vas deferens preparations were then removed and superfused with Kreb's solution (37°C) at a rate of 2 ml/min. The effects of perimural stimulation (20 v, 0.3 ms, 1–25 Hz, 30 s) (Fadloun & Leach, 1978), NA and K^+ were assessed and compared with similar preparations obtained from untreated animals.

The resting blood pressures of rats treated with Cd^{2+} (12.5 and 25 ppm) were significantly higher than those receiving tap water alone (e.g. 173/130 mmHg at 25 ppm Cd^{2+}, 130/110 mmHg control). The increase in systolic pressure was greater than that seen in the diastolic (42–20 mmHg at 25 ppm Cd^{2+}). Heart rate was increased with 12.5 and 25 ppm Cd^{2+}. Blood pressure changes to sympathetic outflow stimulation were enhanced at all stimulation frequencies (1–25 Hz) in rats receiving 5 ppm Cd^{2+}, but the responses of these given Cd^{2+} (12.5 and 25 ppm) were only increased at 12 and 25 Hz. Treatment with Cd^{2+} (5 and 12.5 ppm) tended to reduce NA increases in blood pressure ($P > 0.05$), whilst all Cd^{2+} treatments reduced tyramine responses ($P < 0.05$). Cd^{2+} pretreatment (5, 12.5, 25 ppm) altered the responses of portal vein preparations to perimural stimulation. The nature of the alteration being dependent on the frequency used; responses to 1 and 3 Hz were potentiated whilst responses to 12 and 25 Hz were reduced. Cd^{2+} (12.5 and 25 ppm) also enhanced contractions to K^+ and NA. Furthermore, Cd^{2+} generally enhanced (although dependent on Cd^{2+} concentration) the responses of the vas deferens to electrical perimural stimulation, NA and K^+.

In conclusion, it is suggested that there may be a relationship between Cd-induced hypertension and changes in sympathetic nervous function which appears coincident with differential modification of the sympathetic function of vascular and non-vascular smooth muscle.

References

FADLOUN, Z. & LEACH, G.D.H. (1978). Measurements of noradrenaline overflow and dopamine-β-hydroxylase activity in superfused preparations. *Br. J. Pharmac.*, **64**, 467p.

FADLOUN, Z. & LEACH, G.D.H. (1979). The effects of Cd^{2+} on the responsiveness of the rat anococcygeus muscle and vas deferens to electrical stimulation, noradrenaline, tyramine and K^+. *Br. J. Pharmac.*, **66**, 495p.

FADLOUN, Z. & LEACH, G.D.H. (1980). The effects of Cd^{2+} on the myogenic activity and the responsiveness of the rat portal vein to the perimural stimulation, noradrenaline and potassium ions. *Br. J. Pharmac.*, (in press).

GILLESPIE, J.S. & MUIR, T.C. (1967). A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. *Br. J. Pharmac.*, **30**, 78–87.

PERRY, H.M. JR., ERLANGER, M., YUNICE, A., SCHOEPFLE, E., PERRY, E.F. (1970). Hypertension and the tissue metal levels following intravenous cadmium, mercury and zinc. *Am. J. Physiol.*, **219**, 755–761.

SCHROEDER, H.A., VINTON, W.H. JR. (1962). Hypertension induced in rats by small doses of cadmium. *Am. J. Physiol.*, **202**, 515–518.

Abnormal electrocardiographic activity revealed by rat isolated heart preparations at various times after experimental myocardial infarction

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Detailed long-term studies of certain experimental models of myocardial infarction have concentrated on the structural changes (eg, Rona *et al.*, 1959; Ferrans *et al.*, 1964; Rona & Kahn, 1967) and knowledge of the functional consequences has been inferred rather than defined directly. In the present study a modified version of the Langendorff isolated perfused heart preparation (Sahyoun & Hicks, 1978) has been used to provide evidence whereby changes in the electrocardiogram may be related to the state of lesions in infarct bearing rat hearts after various times.

Isoprenaline (160 mg/kg) was used to provoke experimental myocardial infarction in rats '*in vivo*'. Control animals received saline. Hearts were removed for functional and morphological studies 2, 7, 14, 28 and 42 days after treatment. These were immediately attached to a Langendorff column and perfused with Krebs-Henseleit saline equilibrated with 95% O_2/5% CO_2 at 37°C. As described elsewhere (Sahyoun & Hicks, 1978), two leads were used to record the electrocardiogram from the isolated heart, one from a stainless steel perfusion cannula inserted into the aorta and the other from the perfusion fluid collected in the lower Langendorff chamber, in which only the apical tip of the heart was immersed. Electrocardiograms were displayed and photographed using a storage oscilloscope. After perfusion for a short period, the hearts were prepared for microscopic examination. Myocardial infarct lesions were graded using the system devised by Rona *et al.* (1959).

In hearts from rats killed 2 days after isoprenaline treatment, ECG abnormalities were observed, such as deeper and wider Q waves, reduced shorter S waves and depressed T waves, typical of acute ischaemia. These were associated with widespread myocardial degeneration and necrosis. At 2 weeks the extent and severity of the lesions had subsided, leaving a residue of necrotic tissue at the apex and lower ventricular walls; some collagen deposition was apparent. At this time R waves were also depressed, QRSR̀ waves had occurred and inverted T waves were common.

At 4 and 6 weeks necrotic tissue was replaced by collagen fibrosis. This decline and repair of the lesions was associated with the normalisation of the R, S and T wave aberrations. However, deeper and wider Q waves persisted and some arrhythmias were displayed. These were attributable to the scar tissue.

Such an investigation provides the means for defining some aspects of cardiac malfunction in association with an investigation of experimental infarcts. The system is free from complications of nervous and cardiovascular connections and therefore revealed the consequences of the lesions alone. Such information could be of use in studying the influence of drugs on the course of myocardial damage.

References

FERRANS, V.J., HIBBS, R.G., BLACK, W.C. & WEILBUECHER, D.G. (1964). Isoprenaline induced myocardial necrosis: a histochemical and electron microscopic study. *American Heart Journal*, **68**, 71–90.

RONA, G., CHAPPEL, C.I., BALAZA, T. & GAUDRY, R. (1959). An infarct-like myocardial lesion and other toxic manifestations produced by isoprenaline in the rat. *Archives of Pathology*, **67**, 443–455.

RONA, G. & KAHN, D.S. (1967). The healing of cardiac necrosis as reflected by experimental studies. In *Methods and Achievements in Experimental Pathology*, **3**, ed. Bajusz, E. & Jasmin, G., pp. 300–249. Karger, Basel.

SAHYOUN, H.A. & HICKS, R. (1978). Electrocardiographic recording of normal and infact bearing rat hearts in a perfused isolated preparation. *Journal of Pharmacological Methods*, **1**, 351–360.

Some agents that stimulate presynaptic α_2-adrenoceptors produce bradycardia in pentobarbitone anaesthetised rats by an action on peripheral cardiac sympathetic nerves

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Clonidine can reduce heart rate in pentobarbitone-anaesthetised dogs by decreasing central efferent sympathetic nerve activity and by increasing vagal activity. Additionally clonidine can peripherally inhibit adrenergic neurotransmitter release through stimulation of α_2-adrenoceptors (Langer, 1977) situated on cardiac sympathetic nerve endings (Cavero & Roach, 1980).

We have now studied the mechanism by which para-aminoclonidine (PAC) and 3,4-dihydroxyphenyl-aminoimidazoline (DPI) lower heart rate in pentobarbitone anaesthetised rats. Furthermore, this study was extended to oxymetazoline since this agent was reported by Kobinger & Pichler (1975) not to produce a negative chronotropic effect in vagotomised rats anaesthetised with urethane due to its poor penetration to medullary cardiovascular centers.

Normotensive male rats (Charles River, Sprague–Dawley) weighing 250–300 g were used. In animals anaesthetised with sodium pentobarbitone (55 mg/kg, i.p.) intravenous injections of PAC (0.3–5.0 μg) produced bradycardia the magnitude and duration of which were proportional to the dose administered. Three minutes after PAC (1.0 μg i.v.) the heart rate was reduced by 80.6 ± 7.1 bts/min (mean ± s.e. mean, $n = 5$; $T_{1/2} = 28$ min). This effect was not altered by vagotomy plus ligation of carotid arteries. In rats pretreated with syrosingopine (5 mg/kg, s.c., 18 h previously) to deplete peripheral amine stores, PAC (1.0 μg, i.v.) did not decrease heart rate even when the lower heart rate of this preparation was elevated by an infusion of isoprenaline (0.008 mg kg^{-1} min^{-1}). However, PCA, like clonidine, markedly lowered (by 56.7 ± 3.1 bts/min) the tachycardia (of approximately 100 bts/min) produced by continuous electrical stimulation of the spinal cord of pithed rats (Cavero, Depoortere & Lefèvre-Borg, 1980). In rats pretreated with intravenous phentolamine (1.0 mg/kg) the bradycardia produced by PAC was reduced by 85%.

Findings with DPI were similar to those obtained with PAC although the former agent was of shorter duration of action ($T_{1/2} = 5$ min). In anaesthetised rats an intravenous injection of DPI (1.0 μg) was followed by bradycardia (67 ± 6 bts/min after 3 min) which was markedly less (by 75%) after i.v. phentolamine pretreatment.

Oxymetazoline (1.0 μg, i.v.) also produced a lowering of heart rate in anaesthetised rats and as occurred with DPI, the bradycardia was considerably reduced in animals given phentolamine.

Thus, PAC, DPI and oxymetazoline produced a negative chronotropic effect in intact pentobarbitone-anaesthetised rats. This action is compatible with a peripheral site of action mediated by stimulation of α_2-adrenoceptors on the cardiac sympathetic nerves since these compounds in the dose used do not appear to reach central cardiovascular sites perhaps due to their physico-chemical properties. This hypothesis is supported by our observations that intracerebroventricular injection PAC and DPI produce bradycardia of longer duration (e.g.: $T_{1/2} = 78$ min after PAC i.c.v.) than that found after i.v. administration. The half-lives obtained with clonidine were similar for the two routes of administration.

References

CAVERO, I. & ROACH, A.C. (1980). Effects of clonidine on canine cardiac neuroeffector structures controlling heart rate. *Br. J. Pharmac.* In press.

CAVERO, I., DEPOORTERE, H. & LEFÈVRE-BORG, F. (1980). Pharmacological studies on para-aminoclonidine. *Br. J. Pharmac.* In press.

KOBINGER, W. & PICHLER, T. (1975). Investigation into some imidazoline compounds with respect to peripheral α-adrenoceptor stimulation and depression of cardiovascular centers. *Naunyn-Schmiedebergs Arch. Pharmacol.* **291**, 175–191.

LANGER, S.Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmac.*, **60**, 481–497.

An analysis of 'adrenaline reversal' and of other depressor responses in the pithed rat

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In current pharmacological terms, 'adrenaline reversal' (Dale, 1906) involves blockade of the pressor, α-adrenoceptor mediated action of adrenaline to unveil the depressor, β_2-adrenoceptor mediated action (Grundy & Grundy, 1975). A further condition for reversal, implied by Dale (who used 'brain pithed' cats) and later amplified by Nickerson (1949), is that some vasoconstrictor tone of nervous or humoral origin must be present in order to see vasodepression.

We have now found, in routine class experiments, that 'adrenaline reversal', involving α_1- and β_2-adrenoceptors, can be demonstrated in the fully pithed rat where no neurogenic or exogenously induced vasoconstriction is present.

Male Wistar rats (250–300 g) were pithed under halothane anaesthesia and respired with O_2 (Clanachan & McGrath, 1976). Diastolic arterial blood pressure (DBP) was used as an index of vascular response since this is little affected by cardiac responses in pithed animals (McGrath & Mackenzie, 1977). Drugs were administered intravenously.

Adrenaline (1 μg/kg) produced a pressor response, which in the presence of phentolamine (0.3–10 mg/kg) was changed to a depressor response. The size of the fall depended on the initial resting level of DBP and on the degree of α-adrenoceptor blockade, e.g. after phentolamine (0.3 mg/kg), adrenaline (1 μg/kg) produced a mean fall of 20 ± 8 mmHg from a resting level of 67 ± 12 mmHg ($n = 6$): in 13 tests adrenaline never lowered DBP below 24 mmHg. Similar 'adrenaline reversal' could be produced by substituting prazosin (1 mg/kg) for phentolamine. Part of the pressor response to adrenaline is therefore due to activation of prazosin-sensitive α_1-adrenoceptors. The pressor response to noradrenaline is relatively resistant to prazosin (Drew & Whiting, 1979) and may involve post-junctional α_2-adrenoceptors (Docherty, MacDonald & McGrath, 1979). The depressor responses to adrenaline, isoprenaline or salbutamol could be blocked by propranolol but not by practolol; the corresponding chronotropic responses could be blocked by propranolol or practolol. This implicates β_2-adrenoceptors in the depressor response to adrenaline.

Stimulation of the thoracic outflow to the adrenals produced pressor responses which could be reversed to depressor responses after phentolamine, demonstrating 'endogenous adrenaline reversal'.

'Noradrenaline reversal' was not found. Phentolamine could block, but not reverse, the pressor responses to noradrenaline (1 ng/kg–10 μg/kg) or to vasopressor nerve stimulation.

'5-Hydroxytryptamine reversal' could be demonstrated and shown to involve different receptors from adrenaline in both pressor and depressor components. 5-hydroxytryptamine (0.1 mg/kg) produced a biphasic effect on DBP, a dominant but short-lived pressor followed by a depressor component; these were not blocked by prazosin or propranolol, respectively, but the pressor effect could be blocked, selectively, by mianserin (0.01–1 mg/kg) (see Cavero, Lefevre-Borg & Roach, 1980) or lysergic acid diethylamide (1–10 μg/kg).

Nitroprusside (0.01–1 mg/kg) produced a depressor response which reached a similar minimum DBP to that induced by adrenaline and which was resistant to all of the above antagonists.

It is concluded that sufficient vascular tone is present in the pithed rat to demonstrate vasodilation induced by a variety of mechanisms.

References

CAVERO, I., LEFÈVRE-BORG, F. & ROACH, A.G. (1980). Comparison of mianserin with desipramine and maprotiline on blood pressure responses to acetylcholine, histamine and 5-hydroxytryptamine in normotensive rats. *Br. J. Pharmac.*, Proceedings of Br. Pharm. Soc., Dec. 1979.

CLANACHAN, A.S. & MCGRATH, J.C. (1976). Effects of ketamine on the peripheral autonomic nervous system of the rat. *Br. J. Pharmac.*, **58**, 247–252.

DALE, H.H. (1906). On some physiological actions of ergot. *J. Physiol.*, **34**, 163–206.

DOCHERTY, J.R., MACDONALD, A. & MCGRATH, J.C. (1979). Further subclassification of α-adrenoceptors in the cardiovascular system, vas deferens and anococcygeus of the rat. *Br. J. Pharmac.*, **67**, 421–422P.

DREW, G.M. & WHITING, S.B. (1979). Evidence for two distinct types of postsynaptic α-adrenoceptor in vascular smooth muscle *in vivo*. *Br. J. Pharmac.*, **67**, 207–216.

GRUNDY, H.C. & GRUNDY, H.F. (1975). The mechanism of 'adrenaline reversal' in the anaesthetised cat and rabbit. *Br. J. Pharmac.*, **55**, 282–283P.

MCGRATH, J.C. & MACKENZIE, J.E. (1977). The effects of intravenous anaesthetics on the cardiovascular system of the rabbit. *Br. J. Pharmac.*, **61**, 199–212.

NICKERSON, M. (1949). The pharmacology of adrenergic blockade. *Pharmac. Rev.*, **1**, 27–101.

Are the cardiac and smooth muscle activities of papaverine-like compounds due to inhibition of phosphodiesterase?

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It has been claimed that the smooth muscle relaxant activity of papaverine is due to its ability to inhibit phosphodiesterase (Kukovetz & Poch, 1970). In the present investigation, a series of analogues of papaverine were tested for smooth muscle relaxant activity on the guinea-pig lung strip (Drazen & Schneider, 1978), stimulatory activity on the force of contraction of the electrically driven guinea-pig left atrium (Reinhardt, Wagner & Schuemann, 1972) and the effect on cyclic nucleotide content in guinea-pig lung tissue.

It has been shown that papaverine (3×10^{-6}–3×10^{-4} M) causes a dose-dependent increase in the force of contraction of the atria and in the concentration range, 3×10^{-6}–10^{-4} M, relaxes the lung strip. In a concentration of 3×10^{-4} M, this accompanied by a significant elevation of cyclic AMP and cyclic GMP in the lung. However, we have prepared analogues of papaverine which relax the guinea-pig lung strip but do not stimulate the isolated atrium or raise the content of lung nucleotides. In fact, many of these derivatives have an inhibitory effect on the atria. One prime example is 1-(4-chlorophenyl)-6,7-dimethoxy-3,4-dihydroisoquinoline, which produced a 32% reduction in the force of atrial beating in a concentration of 10^{-4} M, in contrast with papaverine which caused an 82% increase in the force of contraction in the same concentration. On the other hand, 6,7-dimethoxy-1-(4-methoxyphenyl)3,4-dihydroisoquinoline had a positive inotropic effect although it was less potent than papaverine (a maximum increase of 38%). Both derivatives were less effective than papaverine in their relaxant action on the lung strip. The results suggest that the cardiac stimulatory effects of papaverine may be due to inhibition of phosphodiesterase but cast doubt on the idea that the smooth muscle relaxant activity derives from the same mechanism. This is in agreement with previous results which showed that the anti-bronchoconstrictor action of papaverine was not related to an effect on the basal levels of cyclic nucleotides in guinea-pig lung (Anderson, Ashfield & Goadby, 1979).

References

ANDERSON, A.A., ASHFIELD, D.J. & GOADBY, P. (1979). Anti-bronchoconstrictor activity of selected phosphodiesterase inhibitors and their effects on lung cyclic nucleotides. Abstracts of the London Meeting of the British Pharmacological Society, 17–19 December, 1979, 43–44.

DRAZEN, J.M. & SCHNEIDER, M.W. (1978). Comparative responses of tracheal spirals and parenchymal strips to histamine and carbachol in vitro. *J. Clin. Invest.*, **61**, 1441–1447.

KUKOVETZ, W.R. & POCH, F. (1970). Inhibition of cyclic -3', 5'-nucleotide-phosphodiesterase as a possible mode of action of papaverine and similarly acting drugs. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **267**, 189–194.

REINHARDT, D., WAGNER, J. & SCHUEMANN, H.J. (1972). Influence of temperature on the sensitivity of the receptors and the contractility of guinea-pig atrium. *Naunyn-Schmiedeberg's Arch. Pharmak.*, **275**, 95–104.

The relationship between the selectivity of agonists for presynaptic α_2-adrenoceptors and their cardiovascular responses in the anaesthetised rat

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Timmermans & Van Zwieten (1977) demonstrated that in pentobarbitone anaesthetised normotensive rats intravenous administration of either clonidine or its congeneric derivatives resulted in similar cardiovascular responses. A decrease in arterial blood pressure was always preceded by an initial pressor response; heart rate was always depressed. The existence of presynaptic α-adrenoceptors (α_2) and their difference from postsynaptic α-adrenoceptors (α_1) is now well established (for review see Starke, 1977). In this study the selectivity of five agonists for α_1 and α_2 adrenoceptors has been related to their cardiovascular profiles.

The selectivity of the α-adrenoceptor agonists for pre- and postsynaptic α-adrenoceptors was assessed as described previously (Doxey, 1979). The cardiovascular responses to the agonists were studied in groups of 4–5 female normotensive rats (>180 g) anaesthetised with pentobarbitone (50–55 mg/kg, i.p.). The trachea was cannulated and arterial blood pressure

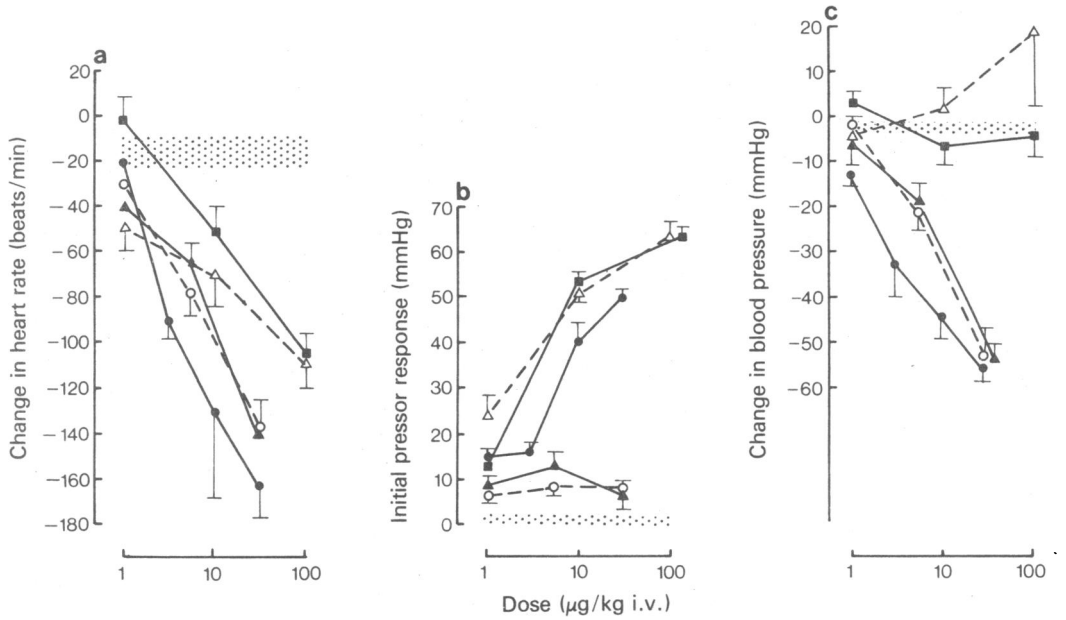

Figure 1 Cardiovascular effects of i.v., clonidine, (1, 3, 10, 30, μg/kg ●), hydroxyguanabenz, (1, 5, 30 μg/kg ○), guanabenz, (1, 5, 30 μg/kg ▲), oxymetazoline, (1, 10, 100 μg/kg △) and St91, (1, 10, 100 μg/kg ■) in pentobarbitone anaesthetised normotensive rats. (1a) heart rate at 15 min; (1b) pressor response at 0.5 min and (1c) secondary hypotensive response at 15 min. Stippled band represents the changes seen in control animals. The results are the mean of a minimum of 4 rats ± s.e. mean.

measured from the carotid artery. Drugs were administered via the cannulated jugular vein.

The respective pre- to postsynaptic ratios for RU 18787 (hydroxyguanabenz), guanabenz, clonidine, oxymetazoline and St91 were 139, 97, 14, 4 and 0.6 in isolated tissue experiments and >288, 222, 34, <1 and <0.1 in pithed rats. All of the compounds studied reduced the heart rate of pentobarbitone anaesthetised rats (Figure 1a). The effects of the compounds on blood pressure were more varied both in terms of the initial pressor response (Figure 1b) and the secondary hypotensive effect (Figure 1c). The difference between the cardiovascular profiles of the compounds studied appeared to be related to their selectivity for pre- and postsynaptic α-adrenoceptors. Compounds which were selective for presynaptic α_2-adrenoceptors (guanabenz and hydroxy-guanabenz) caused minimal initial pressor responses and a prolonged secondary hypotension. In contrast compounds which showed selectivity for postsynaptic α_1-adrenoceptors (oxymetazoline and St91) produced a marked initial pressor response and the secondary reduction in blood pressure was absent. Clonidine occupied an intermediate position.

It has been proposed that the reduction in heart rate produced by clonidine and its analogues, is central in origin and that it is the result of increased vagal activity (Timmermans & Van Zwieten, 1977). If this hypothesis is correct oxymetazoline and St91 reduce heart rate centrally but their intense vasoconstrictor effects mask possible central hypotensive effects. It is not possible however, to exclude a peripheral action on presynaptic α_2-adrenoceptors which results in a reduction in heart rate.

References

DOXEY, J.C. (1979). Pre- and postsynaptic effects of α-agonists in the anococcygeus muscle of the pithed rat. *Eur. J. Pharmac.*, **54**, 185–189.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic systems. *Rev. Physiol. Biochem. Pharmac.*, **77**, 1–124.

TIMMERMANS, P.B.M.W.M. & VAN ZWIETEN, P.A. (1977). Hypotensive and bradycardic activities of clonidine and related imidazolidines, structure activity relationship. *Arch. int. Pharmacodyn.*, **228**, 237–250.

The effect of general anaesthesia on first-pass propranolol uptake in dog lungs *in-vivo*

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We have previously developed a method of estimating first-pass uptake of propranolol in the lungs of intact, conscious dogs. This was done by comparing the ratio of [^{14}C]propranolol to indocyanine green (ICG) injected into the right atrium with the ratio of their concentrations in aortic blood collected over the duration of the first-pass outflow dye curve. In this study, we investigated the effect of general anaesthesia on the process in Labrador dogs weighing between 19.5–31 kg.

The percentage uptake of propranolol at an injected dose of 0.2 mg mixed with 1.875 mg of ICG was measured in three ambulant dogs (Group A—control animals) at least 3 days after insertion of catheters into the right atrium, pulmonary artery and aorta.

Catheters were also inserted and propranolol uptake measured in three dogs under general anaesthesia (Group B). Thiopentone (15 mg/kg i.v.) was used for induction followed by halothane (0.5–1.0%) with nitrous oxide and oxygen. The animals were intubated and ventilated (minute volume 3.5–4.0 l/min.)

A third group of three dogs (Group C) were investigated in the same way. The anaesthetic technique was similar, except that halothane was not used, and the animals received intermittent doses of fentanyl (0.001 mg/kg i.v.) 3–4 times in each experiment as required.

Percentage uptake of propranolol in group A was $52.6 \pm 7.6\%$ (mean $\pm$ s.d., $n = 16$); in group B, $80.5 \pm 3.8\%$ ($n = 13$); and in group C, $63.6 \pm 11.7\%$ ($n = 10$). There were significant differences between the mean values of groups A and B ($P < 0.001$); A and C ($0.01 > P > 0.005$); and B and C ($P < 0.001$).

We conclude that first-pass lung uptake of an intravenously administered bolus of propranolol in conscious dogs is different from that in animals under general anaesthesia. There are also differences in uptake in the two regimes of general anaesthesia investigated. This may contribute to different arterial concentrations of the drug with possible unpredictable pharmacological effects.

A pharmacological comparison of rapid and slow axonal transport in rabbit vagus nerve

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Proteins are transported intra-axonally in a proximo-distal direction in the sensory fibres of rabbit vagus nerve at two distinct rates, viz. 415 mm/day and 24 mm/day (McLean, Frizell & Sjöstrand, 1976). While the properties of rapid axonal transport have been studied in some detail those of slow transport remain largely uncharacterised. The main difference found between the two types is that slow transport differs from fast in its dependence on contact between axon and cell body (McLean *et al.*, 1976).

In this study we have applied three different agents, colchicine, sodium cyanide and tetrodotoxin (TTX) to the vagus nerve *in vivo* in an attempt to distinguish the two types of transport on the grounds of their dependence on microtubules, oxidative phosphorylation and electrical activity, respectively.

Proteins synthesised in the cell bodies of the nodose ganglion of male albino rabbits were radiolabelled by injection first of 1 μCi [^{14}C]-leucine and then, 46 h later, 5 μCi [^{3}H]-leucine into the ganglion with a glass micropipette. As a result, 48 h after the first injection wavefronts of [^{14}C]-labelled slowly-transported proteins and of [^{3}H]-labelled rapidly-transported proteins were present in the cervical vagus nerve about 30 mm from the ganglion. At that time drugs were applied in solution to the nerve trunk in a rayon cuff enclosed in Parafilm®. Any inhibition of axonal transport produced by the drugs colchicine or cyanide led to an accumulation of radiolabelled proteins at the cuff zone over a period of eight hours at which time the animals were killed and the distribution of protein bound radioactivity in the nerve was measured by liquid scintillation counting. Accumulation at the cuff was compared with that produced by a ligature in a separate experiment. Where TTX was used, a stretch of nerve was isolated from electrical activity between the TTX-containing cuff and a distal ligature, and accumulation of proteins was measured at that ligature.

At a concentration of 5 mM, colchicine produced an inhibition of fast and slow transport that was $60.2 \pm 10.2\%$ and $62.2 \pm 10.1\%$ (mean $\pm$ s.e. mean; $n = 6$) of that found at a ligature. At higher and lower

concentrations of colchicine fast and slow transport were equally affected. Cyanide (0.1 M) produced an inhibition of fast and slow transport of 16.0 ± 5.0% and 18.9 ± 4.1% ($n = 6$) of that produced by a ligature. Tetrodotoxin (0.5 mM) was without effect on either slow or fast transport. We have therefore found no significant difference between fast and slow transport in their reaction to the above agents.

This work was supported by the Wellcome Trust.

Reference

McLEAN, W.G., FRIZELL, M. & SJÖSTRAND, J. (1976). Slow axonal transport of labelled proteins in sensory fibres of rabbit vagus nerve. *J. Neurochem.*, **26**, 1213–1216.

Histamine and cardiac function in anaesthetised guinea-pigs

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Administration of histamine or degranulation of mast cells causes stimulation of the guinea-pig working heart *in vitro*. These responses are significantly reduced by treatment with cimetidine (Flynn, Gristwood & Owen, 1979; Gristwood, Owen & Smith, 1980).

In contrast to the data on histamine and cardiac function *in vitro*, relatively little is known about histamine and cardiac function *in vivo*. Guinea-pigs were anaesthetised with sodium pentobarbitone, 90 mg/kg i.p. The trachea was cannulated and the animals maintained by artificial respiration. Airways resistance was determined using a pneumotachograph screen coupled to a differential pressure transducer to monitor tracheal airflow and airflow pressure measured from a side arm to the inflow limb of the system. Blood pressure was measured from one carotid artery, and heart rate derived from the blood pressure pulse triggering an instanteous rate meter. Both femoral veins were cannulated for drug administration and lead II E.C.G. recorded.

Histamine (1.25×10^{-9} to 1×10^{-7} mol/kg) caused dose dependent bronchoconstriction and tachycardia. The bronchoconstriction was inhibited by mepyramine 1×10^{-9} mol kg^{-1} min^{-1}, dose ratio 11.9 (7.9–21.3, 95% confidence limits) and 1×10^{-8} mol kg^{-1} min^{-1}, dose ratio 90.9 (71.4–125.0). Mepyramine had little effect on the tachycardia which was inhibited by cimetidine, 8×10^{-8} mol kg^{-1}, dose ratio 4.6 (3.3–6.5) and 4×10^{-7} mol kg^{-1} min^{-1}, dose ratio 43.4 (30.2–62.3). (Cimetidine experiments were made in animals treated with mepyramine to inhibit the lethal bronchospasm caused by large doses of histamine needed to re-establish the tachycardia in cimetidine-treated animals). Cimetidine did not modify histamine-induced bronchoconstriction.

Intravenous administration of ovalbumin to guinea-pigs sensitised 3 weeks earlier (ovalbumin 100 mg/kg i.p. plus 10 mg/kg s.c.) caused dose-dependent brochoconstriction, tachycardia and unlike histamine alone at the highest challenge doses caused cardiac arrhythmias (heart block, ventricular extrasystoles and occasional ventricular fibrillation). At the lowest challenge dose 100 μg/kg, tachycardia occurred without bronchoconstriction whereas after 500 μg/kg both tachycardia and bronchoconstriction occurred. The tachycardia was abolished by cimetidine (4×10^{-7} mol kg^{-1} min^{-1}) and the bronchonconstriction significantly reduced by mepyramine (2.5×10^{-5} mol mg/kg). Ovalbumin (10 mg/kg) caused almost total cessation of airflow into the lungs which was partially reduced by mepyramine (2.5×10^{-5} mol/kg) and tachycardia, 77 ± 10 bts/min, $n = 6$ which was refractory to either mepyramine (2.5×10^{-5} mol/kg) or cimetidine (up to 2×10^{-6} mol kg^{-1} min^{-1}). Failure of cimetidine to inhibit tachycardia during severe anaphylaxis would suggest involvement of additional mediators. The incidence of heart block was abolished by mepyramine and the incidence and severity of ventricular arrhythmias by cimetidine, 4×10^{-7} mol kg^{-1} min^{-1} (reduced by 66 ± 13%, $n = 6$), and 2×10^{-6} mol kg^{-1} min^{-1} (reduced by 82 ± 15%, $n = 6$).

These studies show that histamine can cause cardiac stimulation *in vivo* by interaction with H_2-receptors. Systemic anaphylaxis caused changes in cardiac function varying from tachycardia at low challenge to arrhythmias after severe challenge. Treatment with cimetidine reduced the cardiac consequences of systemic anaphylaxis.

References

FLYNN, S.B., GRISTWOOD, R.W. & OWEN, D.A.A. (1979). Differentiation of the roles of histamine H_1- and H_2-receptors in the mediation of the effects of histamine in the isolated working heart of the guinea-pig. *Br. J. Pharmac.*, **65**, 127–139.

GRISTWOOD, R.W., OWEN, D.A.A. & SMITH, I.R. Inhibition by cimetidine of cardiac stimulation due to mast cell degranulation (Abstract submitted to this meeting).

Induction of responsiveness to phentolamine by incubation of guinea-pig ileum with clonidine *in vitro*

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Incubation with opiate of guinea-pig isolated ileum (Collier, Cuthbert & Francis, 1980; Hammond, Schneider & Collier, 1976; North & Karras, 1978; Villarreal, Martinez & Castro, 1977) or of cultured neuroblastoma × glioma (NG 108–15) hybrid cells (Sharma, Klee & Nirenberg, 1975, 1977) produces dependence, expressed as increased responsiveness to a specific antagonist or increased activity on withdrawal of opiate. In NG 108-15 cells, induction with a muscarinic or α-adrenoceptor agonist of comparable dependence has also been demonstrated (Nathanson, Klein & Nirenberg, 1978; Sabol & Nirenberg, 1979). Because of these observations and because α-adrenoceptor stimulants, such as clonidine, resemble opiates in acutely inhibiting neurally-evoked contraction of the ileum (Gillan, Kosterlitz, Robson & Waterfield, 1979), we have tested whether incubation with clonidine induces dependence in the isolated ileum and investigated some of the properties of the dependence thus observed.

Incubation and test procedures were as previously described for opiate dependence in the isolated ileum (Collier *et al.*, 1980). Additionally, clonidine hydrochloride and phentolamine hydrochloride were used.

Figure 1 shows that responsiveness to phentolamine is induced by incubation of the isolated ileum with clonidine in the presence of hexamethonium for 24 h at 22°C. The degree of responsiveness was di-

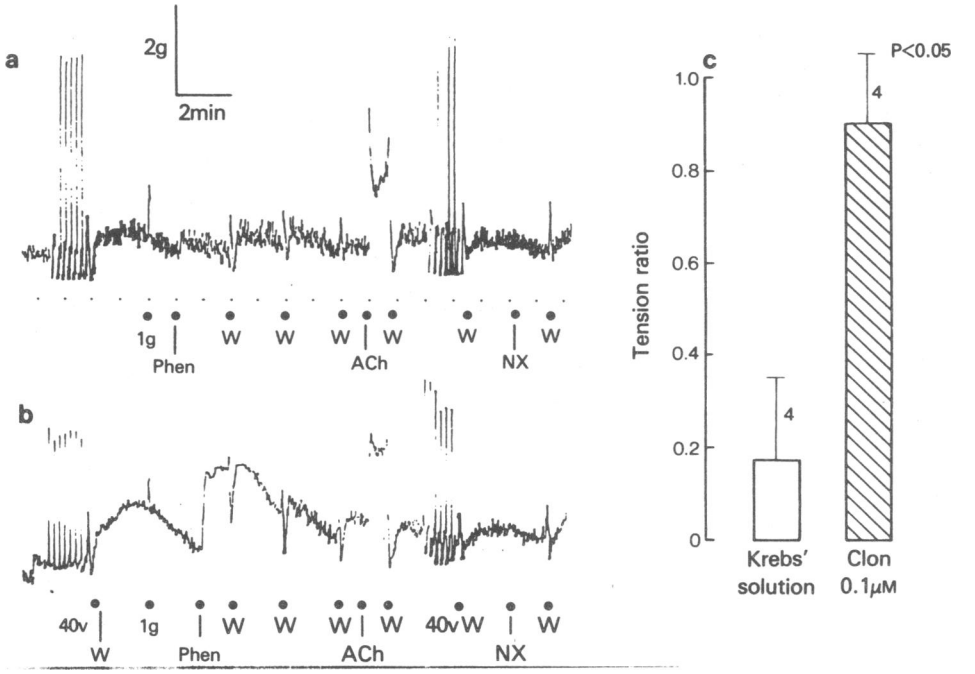

Figure 1 Effect of incubation for 24 h at 22°C in Krebs solution containing hexamethonium (70 μM), either (a) alone, or (b) with clonidine (0.1 μM). After incubation, pairs of segments were set up for test at 37°C in fluid equivalent to that used for incubation. Thirty minutes after equilibration at 37°C, electrical stimulation (40 V) was applied to determine the viability of each tissue, followed by a wash (W) to remove anything released. After a calibration tension (1 g), phentolamine (1 μM, Phen) was added, and washed (W) one minute later. Sensitivity to acetylcholine was then determined by applying (0.01 μM, ACh). Electrical stimulation was again applied and then specificity of the phentolamine response was tested by applying naloxone (30 nM, NX). The histogram (c) shows the effect of clonidine (Clon) incubation on responsiveness to challenge with phentolamine (1 μM). Responsiveness to phentolamine (Tension ratio) is expressed as the ratio of the tension elicited by phentolamine to that elicited by acetylcholine (0.01 μM). Columns indicate mean ± s.e. mean of 4 paired experiments. Significance of difference between treatments was determined using Student's '*t*' test.

rectly related to incubation concentration of clonidine. As with opiate dependence, hyoscine (0.5 μM) blocked the response to challenge with phentolamine. The contracture elicited by phentolamine challenge could be suppressed with clonidine (1.0 μM) or normorphine (1.0 μM). Likewise, in preparations dependent on normorphine, clonidine suppressed withdrawal contracture to naloxone. Addition of phentolamine (1.0 μM) to the incubation fluid inhibited induction of dependence. Naloxone (0.03 μM) did not elicit a contracture from clonidine-dependent preparations.

These observations show that dependence can be induced in neurones of the post-ganglionic myenteric plexus by incubating the isolated ileum with an inhibitory agonist that binds with the α-adrenoceptor receptor. This phenomenon parallels opiate dependence in the ileum, although the receptors are different.

References

COLLIER, H.O.J., CUTHBERT, N.J. & FRANCIS, D.L. (1980). Effects of time and drug concentration on the induction of responsiveness to naloxone in guinea-pig ileum exposed to normorphine *in vitro*. *Br. J. Pharmac.* (in press).

GILLAN, M.G.C., KOSTERLITZ, H.W., ROBSON, L.E. & WATERFIELD, A.A. (1979). The inhibitory effects of presynaptic α-adrenoceptor agonists on contractions of guinea-pig ileum and mouse vas deferens in the morphine-dependent and withdrawn states produced *in vitro*. *Br. J. Pharmac.*, **66**, 601–608.

HAMMOND, M.D., SCHNEIDER, C. & COLLIER, H.O.J. (1976). Induction of opiate tolerance in isolated guinea-pig ileum and its modification by drugs. In *Opiates and Endogenous Opioid Peptides*. ed. Kosterlitz, H.W. pp. 169–176. Amsterdam: Elsevier/North-Holland Biomedical Press.

NATHANSON, N.M., KLEIN, W.L. & NIRENBERG, M. (1978). Regulation of adenylate cyclase activity mediated by muscarinic acetylcholine receptors. *Proc. Natl. Acad. Sci. U.S.A.*, **75**, 1788–1791.

NORTH, R.A. & KARRAS, P.J. (1978). Opiate tolerance and dependence induced *in vitro* in single myenteric neurones. *Nature*, **272**, 73–75.

SABOL, S.L. & NIRENBERG, M. (1979). Regulation of adenylate cyclase of neuroblastoma × glioma hybrid cells by α-adrenergic receptors. *J. Biol. Chem.*, **254**, 1921–1926.

SHARMA, S.K., KLEE, W.A. & NIRENBERG, M. (1975). Dual regulation of adenylate cyclase accounts for narcotic dependence and tolerance. *Proc. Natl. Acad. Sci. USA.*, **72**, 3092–3096.

SHARMA, S.K., KLEE, W.A. & NIRENBERG, M. (1977). Opiate-dependent modulation of adenylate cyclase. *Proc. Natl. Acad. Sci. USA.*, **74**, 3365–3369.

VILLARREAL, J.E., MARTINEZ, J.N. & CASTRO, A. (1977). Validation of a new procedure to study narcotic dependence in the isolated guinea-pig ileum. In *Problems of Drug Dependence, 1977*, pp. 305–314. Washington D.C.: Committee on Problems of Drug Dependence Inc.

The effect of environmental temperature on the thermoregulatory response to peripheral histamine administration in the rat

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The hypothermic effects of systemically administered histamine are well established (Lomax & Green, 1975). Previously it has been suggested that peripheral vasodilation was responsible for this hypothermia. In the rat the tail is a major thermoregulatory organ and it may have been expected that vasodilation of the tail was involved in the histamine induced heat loss. However, it has recently been shown that the tail is not involved in this hypothermia as vasoconstriction probably occurs (Bedford, Bertram & Morris, 1979). In an attempt to try to establish other changes associated to the hypothermia an apparatus was designed in which oxygen consumption and respiratory rate could also be measured. The rat was placed in a restraint box and core and tail temperature measured as previously described (Cox, Kerwin & Lee, 1978) but modified so that air was drawn through the box at 100 ml/min and then through an oxygen analyser (Servomex 0.A.250). The box could be made airtight periodically and the respiratory rate measured with a pressure transducer (Statham P23 Dc) and a Grass polygraph recorder.

The core temperature of adult male Sprague–Dawley rats (250–350 g) to acidified saline, and histamine acid phosphate (B.D.H.), 40 mg/kg i.p. and 80 mg/kg i.p. in rats held at 22°C and placed in restraint boxes at 22°C (Group 1), rats held at 17°C for 18 h and placed in restraint boxes at 17°C (Group II) and rats held at 22°C and placed in restraint boxes at 17°C (Group III) are given in Table 1. (mean ± s.e. mean, $n = 6 - 9$).

The core temperature changes in Group I and II were similar. After histamine there was an initial

Table 1

	Core temperature		
	Group I	*Group II*	*Group III*
Saline treated			
Max Hyperthermia (15 min)	+0.16 ± 0.06	+0.02 ± 0.05	+0.15 ± 0.06
Max Hypothermia (75 min)	−0.11 ± 0.12	−0.28 ± 0.07	−0.15 ± 0.11
Histamine acid phosphate (40 mg/kg)			
Max. Hyperthermia	+0.38 ± 0.07	+0.20 ± 0.06	−0.07 ± 0.09
Max. Hypothermia	−0.56 ± 0.19	−0.41 ± 0.07	−1.02 ± 0.15
Histamine acid phosphate (80 mg/kg)			
Max. Hyperthermia	+0.51 ± 0.13	+0.26 ± 0.10	+0.02 ± 0.09
Max. Hypothermia	−0.48 ± 0.11	−0.84 ± 0.12	−1.03 ± 0.20

hyperthermia followed by hypothermia. However, in Group III the hyperthermia was absent and the hypothermia exaggerated.

The oxygen consumption and respiratory rate was measured for 1 h before and 2 h after treatment in Group II. Saline treated rats showed a 0.2 ± 5.0% increase in cumulative oxygen consumption post injection and an average respiratory decrease of 1.5 ± 5.3%. Histamine acid phosphate treatment at 40 mg/kg produced a 13.4 ± 4.2% cumulative decrease in oxygen consumption post injection with a 9.7 ± 5.3% decrease in average respiratory rate, whilst at 80 mg/kg the cumulative oxygen consumption decrease was 23 ± 5.9% with an average respiratory decrease of 16 ± 3.1%.

These results suggest that the hypothermia may be due to a decreased heat production and that the environmental temperature before and during experimentation can influence the response.

References

BEDFORD, C.D., BERTRAM, J.A. & MORRIS, I.D. (1979). Effect of histamine upon core and tail skin temperature of the conscious restrained rat. *Proc. Brit. Pharmacological Soc.*, 12–14th September 1979. page 62.

COX, B., KERWIN, R. & LEE, T.F. (1978). Dopamine receptors in the central thermoregulatory pathways of the rat. *J. Physiol. Lond.* **282**, 471–483.

LOMAX, P. & GREEN, M.D. (1975). Histamine and Temperature Regulation. *In: Temperature Regulation and Drug Action.* (Eds Lomax, P., Schöbaum, E. & Jacob, J.) Karger, Basel.

M & B 22,948—A novel anti-allergic drug

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M & B 22,948 (2-o-propoxyphenyl-8-azapurine-6-one) a novel anti-allergic drug under development as a prophylactic agent for the treatment of asthma in man. M & B 22,948 has high activity in three experimental models of allergic asthma and has shown promising activity in the clinic. M & B 22,948 and disodium cromoglycate produced complete inhibition of reagin-allergen induced passive cutaneous anaphylaxis in the conscious rat at intravenous doses of 0.05 mg/kg and 2 mg/kg respectively. In the anaesthetised cynomolgus monkey (*Macaca fascicularis*) M & B 22,948, administered by aerosol generated from a 200 mg/ml solution using a Bird MK7 modified respirator, almost completely inhibited the allergic bronchospasm produced after challenge with an aerosol of ascaris antigen. M & B 22,948 was 10–30 times more potent than disodium cromoglycate in inhibiting the antigen-induced release of histamine from passively sensitised human lung *in vitro*. Preliminary results in the clinic indicate that M & B 22,948, 5 mg administered by metered-dose aerosol to asthmatic patients, was effective in preventing allergic bronchospasm induced by an aerosol of *Dermatophagoides sp.*

Protection against aspirin-induced gastric lesions in the rat by the H_2-receptor antagonists ranitidine and cimetidine

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Conflicting evidence has been reported for the ability of cimetidine to reduce the formation of aspirin-induced gastric lesions in rats dosed orally with exogenous acid. Carmichael, Nelson & Russell (1978) concluded that cimetidine was ineffective, whereas Guth, Aures & Paulsen (1979) found cimetidine to be effective in this type of test. In view of this situation it is now appropriate to report the effects of the newer H_2-receptor antagonist ranitidine on aspirin-induced lesions, in the absence or presence of exogenous acid, in the rat.

In control experiments groups of at least 10 female rats (Hooded Lister, 100–120 g: received 300 mg/kg aspirin (suspended in 0.5% w/v carboxymethylcellulose, 5 ml/kg) orally. The rats were sacrificed after 5 h, the stomachs removed, and the petechial lesions that occurred in the glandular region of the gastric mucosa were counted. Other groups of rats received either oral ranitidine (0.3, 1, 3 or 10 mg/kg) or cimetidine (3, 10 or 30 mg/kg) 15 min prior to aspirin administration. Both ranitidine and cimetidine produced a dose-related inhibition of lesion formation, the respective oral ED_{50} values (95% confidence limits) being 0.78 (0.56–1.05) and 3.93 (2.67–5.47) mg/kg, showing ranitidine to be five times more potent than cimetidine (seven times more potent on a molar basis) in this respect.

A second series of experiments was carried out to determine the effect of ranitidine on aspirin-induced lesion formation in the presence of exogenous acid. In these experiments both ranitidine (3, 30 or 300 mg/kg) and aspirin were administered orally in 160 mM HCl, and further doses of 160 mM HCl (5 ml/kg) were given at 1, 2, 3 and 4 h after aspirin. The rats were killed after 5 hours. Under these conditions ranitidine was still effective, but the ED_{50} value of 23.22 (14.07–37.97) mg/kg p.o. was higher than that obtained in the absence of exogenous acid.

In the final series of experiments the effect of parenterally administered ranitidine (0.3 and 3 mg/kg s.c.) on aspirin-induced lesion formation was investigated. The ED_{50} values for ranitidine against aspirin-induced lesion formation both in the absence and presence of exogenous acid were respectively 1.06 (0.68–1.69) and 1.65 (0.82–6.21) mg/kg s.c. These experiments show that the effect of ranitidine on aspirin-induced lesions formed in the presence of exogenous acid can be influenced by its route of administration.

Thus it can be concluded that the H_2-receptor antagonists ranitidine and cimetidine protect against the formation of aspirin-induced lesions. Furthermore, the effectiveness of ranitidine in the presence of exogenous acid shows that this protection is at least in part through an H_2-receptor mediated mechanism other than the inhibition of gastric acid secretion.

References

CARMICHAEL, H.A., NELSON, L.M. & RUSSELL, R.I. (1978). Cimetidine and prostaglandin: Evidence for different modes of action on the rat gastric mucosa. *Gastroenterology*, **74**, 1229–1232.

GUTH, P.H., AURES, D. & PAULSEN, G. (1979). Topical aspirin plus HCl gastric lesions in the rat. Cytoprotective effect of prostaglandin, cimetidine and probanthine. *Gastroenterology*, **76**, 88–93.

Noradrenaline and gastric acid secretion by the rat isolated stomach

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Noradrenaline reduces both gastric mucosal blood flow and acid secretion in the intact animal in a number of species by an action at α-adrenoceptors (Holton, 1973). The reduction in acid output may be secondary to the reduced blood flow or be a direct effect on the mucosal cells which have been reported to receive a sympathetic nerve supply in the rat (Costa & Gabella, 1971). We have investigated the effect of noradrenaline on acid secretion by the rat isolated stomach where complications due to cardiovascular effects are avoided.

The isolated stomach was set up as described previously for guinea-pigs (Holton & Spencer, 1976). Drugs were added to the serosal bathing fluid and

where appropriate, tissues were pre-incubated with antagonists for 1 h prior to addition of noradrenaline. The responses are expressed as the mean secretory ratio (R) with standard error and number of observations. R was calculated as the ratio of stimulated to spontaneous secretion in each stomach.

Noradrenaline stimulated acid secretion over the range 5×10^{-7} M to 2×10^{-5} M. Neither methoxamine or phenylephrine (up to 10^{-4} M) stimulated acid output and the response to noradrenaline (5×10^{-6} M, $R = 1.83 \pm 0.072$, $n = 8$) was not significantly inhibited by phentolamine or thymoxamine (up to 10^{-4} M). Respective values were $R = 1.69 \pm 0.093$, ($n = 8$); $R = 1.79 \pm 0.135$ ($n = 7$). Propranolol (2×10^{-5} M) did cause a significant inhibition of the response ($R = 1.49 \pm 0.086$, $n = 8$; $P < 0.01$).

The noradrenaline response does not appear to involve histaminic receptors or cholinoceptors as it was not inhibited by either metiamide (10^{-4} M, $R = 1.69 \pm 0.103$, $n = 6$) or atropine (10^{-5} M, $R = 1.78 \pm 0.069$, $n = 8$).

Catecholamines increase serum gastrin levels in intact rats by acting on β-adrenoceptors (Hsu & Cooper, 1977) and noradrenaline may act *in vitro* by releasing stored gastrin. The mean maximum value of R obtained with pentagastrin was 1.81 ± 0.061 ($n = 11$) and with noradrenaline $R = 2.03 \pm 0.127$ ($n = 6$); the difference between them not being significant. Straight lines were fitted to the central part of the dose response curves for pentagastrin and noradrenaline giving regression coefficients of 0.529 and 0.700 respectively. Analysis showed these not to be significantly different. Metiamide has been reported to inhibit gastrin stimulated acid secretion in the rat isolated stomach (Bunce, Parsons & Rollings, 1976) whereas we have shown it to be without effect on the noradrenaline response. We tested this apparent distinction by observing the action of cimetidine (1.3×10^{-4} M) on both pentagastrin (2.17×10^{-7} M) and histamine (5.4×10^{-5} M) in the same stomachs. With pentagastrin alone the value of $R = 1.71 \pm 0.082$ ($n = 5$) and with cimetidine $R = 1.68 \pm 0.098$ ($n = 6$). Control histamine $R = 1.58 \pm 0.106$ ($n = 5$), and with cimetidine $R = 1.12 \pm 0.050$ ($n = 6$). Thus, there was a significant inhibition of histamine but not pentagastrin in our preparation.

In conclusion, noradrenaline behaves similarly to isoprenaline (Canfield & Price, 1979) in stimulating acid secretion by an action at β-adrenoceptors in the rat isolated stomach. This does not appear to involve either histamine or cholinergic receptors but we cannot exclude the possibility that it results from the release of stored gastrin.

References

BUNCE, K.T., PARSONS, M.E. & ROLLINGS, N.A. (1976). The effect of metiamide on acid secretion stimulated by gastrin, acetylcholine and dibutryl cyclic adenosine 3', 5' monophosphate in the isolated whole stomach of the rat. *Br. J. Pharmac.*, **58**, 149–156.

CANFIELD, S.P. & PRICE, C.A. (1979). Isoprenaline and gastric acid secretion in the rat. *Proc. Physiol. Society, Birmingham.*

COSTA, M. & GABELLA, G. (1971). Adrenergic innervation of the alimentary canal. *Z. Zellforschung mikrosk Anat.*, **122**, 357–377.

HOLTON, P. (1973). Catecholamines and gastric secretion. In **'Pharmacology of Gastrointestinal Motility and Secretion'** Ed. Holton. Vol 1, Pergamon Press, Oxford.

HOLTON, P. & SPENCER, J. (1976). Acid secretion by guinea-pig isolated stomach. *J. Physiol.* **255**, 465–479.

HSU, W.H. & COOPER, C.W. (1977). Serum gastrin in the rat: cholinerigic and adrenergic effects. *Proc. Soc. Exp. Biol. Med.*, **154**, 401–406.

Modification of radioreceptor assay for 1,25-dihydroxyvitamin D using gut cytosolic receptor from adult male or immature Japanese quail

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Recently a sensitive and highly specific radioreceptor assay for 1,25-dihydroxyvitamin D using cytosolic receptor-chromatin prepared from duodenal mucosae of egglaying Japanese quail, has been reported (Abel de la Cruz *et al.*, 1980). Unlike previous chicken radioreceptor assays for this hormone (Brumbaugh *et al.*, 1974), the quail are not fed with a rachitogenic diet prior to harvesting the mucosae.

We now report that gut mucosae from either adult male ($\simeq$6 months) or immature mixed sex (4 weeks) Japanese quail can also be used as a basis for this assay. Like the egglaying hens, these birds were fed normal Purina game bird Layena and Startena diets prior to killing. Specific ($\simeq 90\%$) and nonspecific binding were determined for the receptors. Receptors from both groups of birds were saturable with 1,25-dihy-

droxyvitamin D_3, and Scatchard plots normally showed linearity with Kd's of approximately 1.4 to 1.8×10^{-9} M. Occasional evidence for a separate population of high affinity receptors was also found. Isotope dilution curves produced useful ranges of 5 to 80 pg of 1,25-dihydroxyvitamin D_3 for receptors from both groups of birds, with 5 pg per tube as the lowest detectable concentration. These data are quite similar to those reported for egglaying hen receptor by Abel de la Cruz, *et al.* (1980). The assay can be used for determination of circulating 1,25-dihydroxyvitamin D in plasmas of several vertebrate species (Pollard *et al.*, 1980).

It is concluded that gut mucosae from Japanese quail in any normal physiological condition may be used as a basis for the 1,25-dihydroxyvitamin D radioreceptor assay.

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References

ABEL DE LA CRUZ, L., SHIEH, J., POLLARD S.K. & KENNY, A.D. *Fed. Proc.*, 1980, in press.

BRUMBAUGH, P.F., HAUSSLER, D.H., BURSAE, K.M. & HAUSSLER, M.R. *Biochemistry*, 1974, **13**, 4091–4097.

POLLARD, S.K., ABEL DE LA CRUZ, L., SHIEH, J. & KENNY, A.D. *Fed. Proc.*, 1980, in press.

Inhibition of phospholipase A activity in the perfused rat kidney by steroid anti-inflammatory drugs

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The precursor fatty acids for prostaglandin biosynthesis arise mainly from phospholipids under the influence of the enzyme phospholipase A (Nijkamp, Flower, Moncada & Vane, 1976). Availability of substrate has been shown to be the rate-limiting step in prostaglandin formation. Studies with isolated perfused guinea-pig lungs have suggested that steroid anti-inflammatory drugs have an inhibitory effect on phospholipase A (Blackwell, Flower, Nijkamp & Vane, 1978). In the present study phospholipase A activity and prostaglandin production were measured in the isolated perfused rat kidney. The effects of the steroid anti-inflammatory drugs dexamethasone, betamethasone and hydrocortisone were determined.

A single rat kidney was isolated perfused as described by Armstrong, Blackwell, Flower, McGiff, Mullane & Vane (1976). Phospholipase A activity was measured using a double-isotope assay. Di[1-^{14}C]-palmitoyl L-α phosphatidyl choline and [9,10^{3}H]-palmitic acid (0.1μCi total) were injected into the renal artery. The perfusate was collected for 5 min. The labelled fatty acids were extracted with 50 ml n-hexane. The solvent was evaporated to dryness and the $^{14}C/^{3}H$ ratio estimated after liquid scintillation counting. Prostaglandin E_2 and $F_{2\alpha}$ formation was measured by cascade superfusion of the rat stomach strip and rat colon (Gilmore, Vane & Wyllie, 1968).

Preliminary experiments demonstrated that all the ^{14}C radioactivity in the perfusate co-chromatographed with [1-^{14}C] palmitic acid on thin-layer chromatography. The perfused rat kidney exhibited a low basal hydrolysis of labelled phosphatide (2–4%) which remained constant from 30 min to 5 h after commencement of perfusion. Infusion of dexamethasone, betamethasone or hydrocortisone produced a time-dependent, concentration-dependent inhibition of phospholipase A activity and prostaglandin production. After 1 h of steroid infusion the ID_{50} values for inhibition of phospholipase A were: dexamethasone (2.5 μg/ml), betamethasone (2.5 μg/ml) and hydrocortisone (23 μg/ml). Progesterone (50 μg/ml) had no effect on phospholipase A. Simultaneous administration of progesterone (1–5 μg/ml) produced a competitive reduction of the inhibitory effects of dexamethasone, betamethasone and hydrocortisone. Phospholipase A activity was increased following 20 s gentle mechanical vibration of the kidney by means of an electrically driven vibrator. This increase in phospholipase A activity resulted in a similar increase in prostaglandin formation. The increased phospholipase A activity and prostaglandin production were inhibited by the anti-inflammatory steroids.

These results indicate that changes in phospholipase A activity result in corresponding changes in prostaglandin production by the isolated perfused rat kidney. The ability of dexamethasone, betamethasone and hydrocortisone to inhibit phospholipase A was similar to their relative anti-inflammatory potencies (Nijkamp, Flower, Moncada & Vane, 1976).

References

ARMSTRONG, J.M., BLACKWELL, G.J., FLOWER, R.J., MCGIFF, J.C., MULLANE, K.M. & VANE, J.R. (1976).

Genetic hypertension in rats is accompanied by a defect in renal prostaglandin catabolism. *Nature*, **260**, 582–586.

BLACKWELL, G.J., FLOWER, R.J., NIJKAMP, F.P. & VANE, J.R. (1978). Phospholipase A_2 activity of Guinea-pig isolated lungs: stimulation and inhibition of anti-inflammatory steroids. *Br. J. Pharmac.*, **62**, 79–82.

GILMORE, N., VANE, J.R. & WYLLIE, J.H. (1968). Prostaglandins released by the spleen *Nature*, **218**, 1135–1138.

NIJKAMP, F.P., FLOWER, R.J., MONCADA, S. & VANE, J.R. (1976). Partial purification of rabbit aorta contracting substance-releasing factor and inhibition of its activity by anti-inflammatory steroids. *Nature*, **263**, 479–482.

Plasma propranolol concentrations in rats with adjuvant induced arthritis

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It has previously been reported that after a single oral dose of propranolol, patients in the active stage of an inflammatory disease with a raised erythrocyte sedimentation rate (ESR) had much higher plasma drug levels than did healthy subjects (Schneider, Bishop & Hawkins, 1979).

In order to investigate this further propranolol was given orally (2 mg) or intravenously (0.25 mg) to two groups of female Wistar rats weighing 200–250 g. In one group, an adjuvant-induced arthritis had been produced by injecting 0.075 ml of a complete Freund's adjuvant into a hind foot-pad (Newbould, 1963). The drug was administered when the inflammation had spread to a second site, and the ESR increased to above 3 mm in the first hour. After oral administration, rats were sacrificed after 20, 30 or 40 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 5 h or 6 h, and following iv dosing after 10, 20, 30 or 40 min, 1 h, 1.5 h, 2 h or 2.5 h. Animals were anaesthetized and bled from the aorta into 3.8% sodium citrate solution. Plasma was separated, and the propranolol estimated fluorimetrically (Shand, Nuckolls & Oates, 1970).

The mean area under the plasma propranolol concentration-time curve (AUC) after oral administration in the arthritic rats was approximately 10 times that in control animals, and after i.v. administration, was approximately doubled.

The apparent volume of distribution calculated from the i.v. data was approximately halved in the arthritic rats and it seems likely that this is due to an increase in the plasma protein binding (Evans, Nies & Shand, 1973).

If the volume of distribution is assumed to be the same after oral as after i.v. dosing, an increase in systemic bioavailability of approximately five fold still remains. Perrier & Gibaldi (1974) have shown that in drugs which are subject to extensive first-pass metabolism, changes in hepatic clearance are reflected quantitatively by changes in the AUC after oral administration. If this is the case, first-pass hepatic clearance in the arthritic rats is only one-fifth as efficient as in the control animals. There is evidence that liver damage occurs in adjuvant arthritis since Whitehouse & Beck (1973) have shown that both microsomal enzymes and glucuronidation are impaired.

The mechanism of these changes is not known, but since Walle, Fagan, Conradi, Walle & Gaffney (1979) have demonstrated that in man, pre-systemic glucuronidation plays an important part in the preliminary metabolism of propranolol, it may be that it is in this system that the malfunction occurs.

References

EVANS, G.H., NIES, A.S. & SHAND, D.G. (1973). The distribution of propranolol. III. Decreased half-life and volume of distribution as a result of plasma binding in man, monkey, dog and rat. *J. Pharmac. Exp. Ther.*, **186**, 114–122.

NEWBOULD, B.B. (1963). Chemotherapy of arthritis induced in rats by mycobacterial adjuvant. *Br. J. Pharmac.*, **21**, 127–136.

PERRIER, D. & GIBALDI, M. (1974). Clearance and biologic half-life as indices of intrinsic hepatic metabolism. *J. Pharmac. exp. Ther.*, **191**, 17–24.

SCHNEIDER, R.E., BISHOP, H. & HAWKINS, C.F. (1979). Plasma propranolol concentration and the erythrocyte sedimentation rate. *Br. J. Clin. Pharmac.*, **8**, 43–47.

SHAND, D.G., NUCKOLLS, E.M. & OATES, J.A. (1970). Plasma propranolol levels in adults with observations in four children. *Clin. Pharmac. Ther.*, **11**, 112–120.

WALLE, T., FAGAN, T.C., CONRADI, E.C., WALLE, U.K. & GAFFNEY, T.E. (1979). Presystemic and systemic glucuronidation of propranolol. *Clin. Pharmac. Ther.*, **26**, 167–172.

WHITEHOUSE, M.W. & BECK, F.J. (1973). Impaired drug metabolism in rats with adjuvant-induced arthritis: a brief review. *Drug. Metab. Dispos.*, **1**, 251–255.

Non-steroid anti-inflammatory drugs which potentiate leukocyte migration in carrageenin-induced inflammation

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Indomethacin has a differential effect on leukocyte migration *in vivo*; low doses significantly enhance migration while higher doses cause a reduction in leukocyte numbers in inflammatory exudates (Eakins, Higgs, Moncada, Mugridge & Vane, 1980). We have now examined a number of non-steroid anti-inflammatory drugs for their effects on leukocyte migration and prostaglandin biosynthesis in carrageenin-induced inflammation.

Inflammatory exudates were collected 24 h after the subcutaneous implantation of carrageenin-impregnated polyester sponges in rats (Higgs, Flower & Vane, 1979). Drugs or vehicle were given orally to groups of five animals, at the time of sponge implantation, plus additional doses at 5–8 h and 21 hours. Total leukocyte numbers in exudates were estimated on a blind basis and prostaglandin concentrations were determined by bio-assay.

All the drugs tested produced a dose-dependent reduction in leukocyte numbers and prostaglandin concentrations in inflammatory exudates (Table 1). With the exception of BW755C, low doses of each drug significantly ($P < 0.05$) increased leukocyte migration by 20–70% of control values. Indomethacin, aspirin and flurbiprofen potentiate migration at doses which reduce prostaglandin generation and this effect may indicate a diversion of arachidonic acid metabolism towards the generation of chemotactic lipoxygenase products. This cannot, however, account for the effects of phenylbutazone or sodium salicylate, which do not significantly reduce prostaglandin production at doses which increase leukocyte numbers. An alternative explanation is that these drugs may potentiate lipoxygenase at some doses (Siegel, McConnel & Cuatrecases, 1979).

BW755C is approximately equi-potent in reducing oedema, prostaglandin generation and leukocyte migration; this may be because BW755C inhibits both lipoxygenase and cyclo-oxygenase (Higgs *et al.*, 1979), in contrast to the other drugs tested which are thought to be selective cyclo-oxygenase inhibitors. All the drugs tested do, however, reduce leukocyte migration at high doses and this may be due to dual cyclo-oxygenase lipoxygenase inhibition.

BW755C was synthesized at the Wellcome Research Laboratories by Dr. F.C. Copp and Dr. C.V. Denyer.

References

EAKINS, K.E., HIGGS, G.A., MONCADA, S., MUGRIDGE, K.G. & VANE, J.R. (1980). The effects of indomethacin and BW755C on leukocyte migration and prostaglandin production in carrageenin-induced inflammation. *Br. J. Pharmac.* In press.

HIGGS, G.A., FLOWER, R.J. & VANE, J.R. (1979). A new approach to anti-inflammatory drugs. *Biochem. Pharmac.*, **28**, 1959–1961.

SIEGEL, M.I., MCCONNEL, R.T. & CUATRECASAS, P. (1979). Aspirin-like drugs interfere with arachidonate metabolism by inhibition of the HPETE peroxidase activity of the lipoxygenase pathway. *Proc. Nat. Acad. Sci.*, **76**, 3774.

Table 1 Effects of non-steroid anti-inflammatory drugs on leukocyte migration and prostaglandin production *in vivo*

Drug	ED_{50} (mg/kg × 3/24 h) Inhibition of prostaglandin synthesis	Inhibition of leukocyte migration	Leukocyte-potentiating doses (mg/kg × 3/24 h)
Indomethacin	0.15	5.70	0.5–1.0
Aspirin	15.0	130.0	5.0–20.0
Sodium Salicylate	47.0	140.0	1.0–5.0
Phenylbutazone	2.8	52.0	0.05
Flurbiprofen	0.032	0.47	0.05
BW755C*	21.0	8.0	—

* 3-amino-1-[m-(trifluoromethyl)-phenyl]-2-pyrazoline.

Polymorphonuclear leucocyte aggregation: a possible bioassay for lipoxygenase products

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Products of the lipoxygenase pathway of arachidonic acid (AA) metabolism have been shown to possess potent chemotactic and chemokinetic properties towards polymorphonuclear leucocytes (PMNs) (Goetzl & Sun, 1979). Other chemotactic agents, such as synthetic and complement derived peptides, have also been shown to cause transient aggregation of PMNs (O'Flaherty & Ward, 1978). AA causes aggregation of PMNs and it has been suggested that this is due to product(s) of the lipoxygenase pathways (Ford-Hutchinson, Bray & Smith, 1979). Our communication at this meeting describes the production of chemokinetic substances, possibly hydroxy fatty acids, from rat PMNs exposed to calcium ionophore A23187 (Bray, Ford-Hutchinson & Smith). Supernatants from these experiments were tested for their ability to cause PMN aggregation.

The aggregation assay was carried out in a Payton aggregometer. The cells were suspended (500 µl aliquots) and stirred (800 rev/min, 37°C) at a concentration of 1×10^7 cells/ml in Eagle's minimum essential medium buffered to pH 7.4 with 30 mM N'-2-hydroxy-ethyl-piperazine-N'-2-ethane sulphonic acid. Rat cells (>85% PMNs) were obtained from peritoneal exudates 24 h after the injection of 12% sodium caseinate and human PMNs were obtained from peripheral blood by dextran sedimentation followed by purification on Ficoll-Hypaque.

Neither PGE_1, PGE_2, PGI_2 nor TXB_2 produced aggregation of rat PMNs at doses up to 1 µg/ml. However, dilution of supernatants (up to 1:1000) from rat or human PMNs exposed to calcium ionophore A23187 (10^{-5} M) for 4 min caused a rapid transient aggregation response, peaking at 45 s, towards rat or human PMNs. The pattern of inhibition of generation of this aggregatory response was very similar to that observed for the production of chemokinetic activity (Bray, Ford-Hutchinson & Smith) with inhibition by nordihydro-guaiaretic acid and 5, 8, 11, 14 eicosatetraynoic acid but only partial or no inhibition with high doses of conventional non-steroidal anti-inflammatory drugs such as indomethacin and aspirin. It is suggested therefore that PMN aggregation is a rapid and sensitive bioassay for products of the lipoxygenase pathways of AA metabolism.

References

BRAY, M.A., FORD-HUTCHINSON, A.W. & SMITH, M.J.H. Generation of chemokinetic activity from rat polymorphonuclear leucocytes treated with calcium ionophore A23187. *Communication this meeting.*

FORD-HUTCHINSON, A.W., BRAY, M.A. & SMITH, M.J.H. (1979) The aggregation of rat neutrophils by arachidonic acid: a possible bioassay for lipoxygenase activity. *J. Pharm. Pharmacol.*, **12**, 868–869.

GOETZL, E.J. & SUN, F.F. (1979) Generation of unique mono-hydroxyeicosatetraenoic acids from arachidonic acid by human neutrophils. *J. Exp. Med.*, **150**, 406–411.

O'FLAHERTY, J.T. & WARD, P.A. (1978). Leucocyte aggregation induced by chemotactic factors. *Inflammation*, **3**, 177–194.

Effects of partially-purified guinea-pig SRS-A on plasma protein extravasation in guinea-pig skin

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Slow reacting substance of anaphylaxis (SRS-A) has relatively few defined pharmacological properties (Brocklehurst, 1979). Although crude SRS-A has been reported to increase cutaneous vascular permeability (Orange, Stechschulte & Austen, 1969) it has been pointed out that this effect may have been influenced by the presence of other materials (Parker, 1979). In view of the availability of both a specific SRS-A antagonist (FPL55712; Augstein, Farmer, Lee, Sheard, & Tattersall, 1973), and more purified preparations of SRS-A (Lee, Fuher, Holroyde, Mann & Bantick, 1979), we have examined the ability of guinea-pig SRS-A to increase vascular permeability (measured as extravasation of intravenously injected [^{125}I]-HSA) in guinea-pig skin.

Partially-purified SRS-A (12–100 units; 1 unit SRS-A equivalent to 5 ng histamine on guinea-pig ileum) caused dose-related increases in plasma protein exudation (IPPE). The potency of SRS-A relative to histamine on IPPE observed in the present study was similar to that reported for contraction of guinea-pig ileum (Sheard, personal communication). The response was not significantly reduced by doses of

mepyramine (20–400 ng) which completely abolished histamine responses of comparable magnitude. Conversely, the effect was inhibited by up to 60–70% by doses of locally injected FPL55712 (1–70 μg) which had no significant effect on histamine responses. FPL55712 in doses > 12 μg/site was itself inflammatory. Additive effects were seen when histamine and SRS-A were injected together whereas SRS-A and PGE2 showed synergistic interaction.

All the above SRS-A responses were measured 1 h after intradermal injection. Studies of the time course of SRS-A action demonstrated an initial peak response 0.5 h after injection which, in certain batches only, was followed by a marked delayed response maximal at 2 hours. Unlike the early peak response, the late component was not potentiated by superinjection of PGE_2. The reasons for batch variation in the delayed response are uncertain at present but may be a consequence of the existence of several slow reacting substances whose relative proportions vary between preparations.

We thank Fisons Ltd., Loughborough, for the purified SRS-A and the gift of FPL55712.

References

AUGSTEIN, J., FARMER, J.B., LEE, T.B., SHEARD, P. & TATTERSALL, M.L. (1973). Selective inhibitor of slow reacting substance of anaphylaxis. *Nature New Biol.*, **245**, 215–217.

BROCKLEHURST, W.E. (1979). Slow-reacting substance of anaphylaxis—a commentary. In '*Prostaglandins and Inflammation*'. Eds. Rainsford, K.D. & Ford-Hutchinson, A.W. Birkhauser Verlag, Basel.

LEE, T.B., FUHER, G., HOLROYDE, M.C., MANN, J. & BANTICK, J.R. (1979). An improved technique for the partial purification of SRS-A. *J. Pharm. Pharmac.*, **31**, 866–867.

ORANGE, R.P., STECHSCHULTE, D.J. & AUSTEN, K.F. (1969). Cellular mechanisms involved in the release of slow reacting substance of anaphylaxis. *Fed. Proc.*, **28**, 1710–1714.

PARKER, C.W. (1979). Prostaglandins and slow-reacting substance. *J. Allergy Clin. Immunol.*, **63**, 1–14.

Inhibition of histamine-induced mouse pinnal oedema by reserpine

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The anti-inflammatory action of calcitonin may be due to a selective inhibition of the effects of histamine but not 5-hydroxytryptamine (5-HT) upon vascular permeability (Strettle, Bates & Buckley, 1980). Reisterer & Jacques (1969) demonstrated that the inhibition of dextran-induced paw oedema by calcitonin was reversed by propranolol; this effect could not readily be explained in terms of an anti-histamine effect of calcitonin. Therefore we have investigated the effects of agents which interfere with adrenergic transmission upon changes in vascular permeability induced by histamine.

The effects of histamine and 5-HT upon vascular permeability were studied by a modification (Strettle *et al.*, 1980) of the method of Church & Miller (1975).

Under light ether anaesthesia, groups of 10 mice of either sex (20–40 gm CFLP strain) were injected via the tail vein with 0.2 ml of a 10 mg/ml solution of pontamine sky blue. Immediately, one pinna was pierced with a 21 gauge hypodermic needle through a drop of histamine or 5-HT. The contralateral pinna was pierced through a drop of the vehicle. After 30 min the mice were killed, the pinnae were removed and the surface area of the blue spot produced by leakage of the dye was measured. The response to the agonist was taken as the response of the test pinna minus the response of the control pinna.

Histamine and 5-HT produced concentration related leakage of dye which could be fully antagonized by their respective antagonists mepyramine (0.025–10 μmol/kg) and methysergide (0.03–3.0 μmol/kg).

Reserpine (8.2 μmol/kg) injected i.p. 4, 8, 16 or 24 h prior to the dye gave 78 ± 8% (mean ± s.e. mean), 87 ± 5%, 96 ± 3% and 96 ± 3% inhibitions of the response to histamine but reserpine did not reduce the response to 5-HT at 24 hours.

The inhibition of the response to histamine did not appear to be related to the fall in body temperature because a maximum inhibition could be observed in normothermic reserpinized animals.

The response to histamine was enhanced (50% and 80%) by propranolol (0.1 and 1.0 μmol/kg i.p. 30 min prior to dye) but inhibited up to a maximum of 65 ± 8% by phentolamine (3.5–35 μmol/kg i.p. 30 min. before the dye).

The response to histamine was inhibited up to a maximum of 63 ± 13% by guanethidine (100

μmol/kg 4 h prior to dye) but 3–4 di-iodotyrosine (4.6 mM in the drinking water for 4 days) and p-chlorophenylalanine (1.56 mmol/kg i.p. daily for 3 days) did not significantly affect the response to histamine.

In conclusion, reserpine inhibited the increase in vascular permeability induced by histamine but not 5-HT. The maximum effect of reserpine could not be mimicked by other drugs which deplete mono amines or block the release of catecholamines.

References

CHURCH, M.K. & MILLER, P. (1975). Simple models of anaphylaxis and of histamine and 5-hydroxytryptamine induced inflammation using the mouse pinna. *Br. J. Pharmac.*, **55**, 315P.

REISTERER, L. & JAQUES, R. (1969). Reduction of increased vascular permeability by calcitonin. *Pharmacology*, **2**, 35–63.

STRETTLE, R.J., BATES, R.F.L. & BUCKLEY, G.A. (1980). Evidence for a direct anti-inflammatory action of calcitonin: inhibition of histamine induced mouse pinnal oedema by porcine calcitonin. *J. Pharm. Pharmac.*, in press.

Inhibition of histamine induced bronchoconstriction by prostaglandin $F_2\alpha$—a sympathetic mechanism

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Sympathetic reflexes, facilitated by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), have been described in various vascular beds in animals (Ducharme, Weeks and Montgomery, 1968; Kadowitz *et al.*, 1973; Brody & Kadowitz, 1974). In addition vagosympathetic bronchodilator reflex has recently been demonstrated in response to intravenous administration of prostaglandin $F_{2\alpha}$ (Clay & Hughes, 1978).

The aim of this present study was to determine whether prostaglandin $F_{2\alpha}$ excited a sympathetic reflex to airway smooth muscle, of sufficient magnitude to inhibit histamine induced bronchoconstriction. Bronchoconstriction was assessed using a sensitive measurement of respiratory resistance. The effects of $PGF_{2\alpha}$ on histamine induced increases in resistance were evaluated before and after guanethidine (4 mg/kg) and propranolol (1 mg/kg).

Male Dunkin Hartley guinea pigs (450–650 gm) were anaesthetized with a combination of diazepam (3 mg/kg i.p.) and fentanyl and fluanisone (1 ml/kg i.m.), paralysed with gallamine (4 mg/kg i.v.) and maintained under artificial respiration after tracheostomy. Respiratory resistance was measured by a forced oscillation technique. An oscillation frequency of 6 Hz was used; resistance was measured during a 10–15 s period of apnoea at end expiration and was commenced 5 s after histamine injection. $PGF_{2\alpha}$ was administered 30 s before histamine.

In 18 tests in 16 animals histamine (1.5 μg) produced a 114% increase in respiratory resistance (131 ± 10 to 280 ± 12 cm H_2O l^{-1} sec). Thirty seconds following the intravenous administration of $PGF_{2\alpha}$ (0.5 μg/kg) the same dose of histamine only produced a 26% increase in resistance (141 ± 8 to 178 ± 10 cm H_2O l^{-1} sec.); this represents a 77% inhibition of histamine induced bronchoconstriction ($P < 0.001$). Control resistance increased non-significantly with the dose of $PGF_{2\alpha}$ used.

In 17 tests in 8 animals pre-treated with i.v. guanethidine (4 mg/kg), $PGF_{2\alpha}$ (0.5 μg/kg) produced a 50% inhibition of histamine induced bronchoconstriction. This was a significant reduction in inhibitory activity compared to untreated animals ($P < 0.05$).

In 8 tests in 4 animals, pre-treated with propranolol (1 mg/kg), $PGF_{2\alpha}$ (0.5 μg/kg) produced a 12% inhibition of histamine induced bronchoconstriction which was not significant.

$PGF_{2\alpha}$ at doses insufficient to change baseline resistance values, inhibits histamine induced bronchoconstriction by a β-sympathetic reaction. The source of this effect appears to be a combination of neuronal and extraneuronal catecholamine release. The latter presumably depends on a direct action of $PGF_{2\alpha}$ on the adrenal medulla.

We conclude that β-sympathetic modulation of airway smooth muscle reactivity is achieved by $PGF_{2\alpha}$. We believe this to be a more important physiological event than direct constricting activity in the guinea-pig.

References

BRODY, M.J. & KADOWITZ, P.J. (1974). Prostaglandins as modulators of the autonomic nervous system. *Fed. Proc.*, **33**, 48–60.

CLAY, T.P. & HUGHES, J.M.B. (1978). Evidence for a vago-sympathetic bronchodilator reflex initiated by prostaglandin $F_{2\alpha}$. *Brit. J. Pharmac.* **(64),** 422–423P.

DUCHARME, D.W., WEEKS, J.R. & MONTGOMERY, R.G. (1968). Studies on the mechanism of the hypertensive effect of prostaglandin $F_{2\alpha}$. *J. Pharmacol. E-P. Ther.*, **160,** 1–10.

KADOWITZ, P.J., GEORGE, W.J., JOINER, P.D. & HYMAN, A.L. (1973). Effect of prostaglandins E_1 and $F_{2\alpha}$ on adrenergic responses in the pulmonary circulation. *Adv. Biosc.*, **9,** 501–506.

Differential modulation of delayed hypersensitivity

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Differential modulation by a range of antirheumatoid and immunosuppressant agents on the induction and elicitation of delayed hypersensitivity responses was investigated in murine models.

Male CD-I mice were used in groups of 8–10 in these experiments. Mice were sensitized to methylated bovine serum albumen (MBSA) by s.c. injection of 1 mg emulsified in 0.2 ml. Freund's complete adjuvant. Fifteen days later mice were challenged in one hind footpad with MBSA (20 μg in 0.05 ml saline). Compounds were administered either at the time of sensitisation (days 0–4) or before challenge (days 12–15). Other mice were sensitised to *B. pertussis* (BP) by s.c. injection of 10^9 killed organisms in 0.2 ml saline and footpad challenge was with 10^8 organisms in 0.05 ml saline twelve days later. Here, compounds were administered only before challenge (days 9–12). After challenge, in both systems, hind paw swelling was measured at 24 and 48 h and the difference between injected and non-injected hind paws was taken as a measure of delayed hypersensitivity. Vehicle and drug treated groups were compared using Student's *t*-test.

The anti-inflammatory agents indomethacin (0.1, 0.5, 2.5 mg/kg/day) and phenylbutazone (1, 5, 25 mg/kg/day) inhibited the response to BP, but only phenylbutazone inhibited the elicitation of the response to MBSA. Indomethacin, but not phenylbutazone, potentiated the induction of the MBSA response.

The response to BP was also inhibited by prednisolone (1, 5, 25 mg/kg/day) and dexamethasone (0.01, 0.1, 1.0 mg/kg/day) but not by hydrocortisone (1, 5, 25 mg/kg/day). The elicitation of the MBSA response was potentiated by hydrocortisone and inhibited by prednisolone and dexamethasone. The steroids did not affect the induction of the MBSA response.

The immunosuppressant agents azathioprine (2, 10, 50 mg/kg/day) and cyclophosphamide monohydrate (1, 5, 25 mg/kg/day) inhibited the response to BP; cyclophosphamide inhibited the induction and elicitation of the MBSA response whilst azathioprine only inhibited its induction.

Of other clinically active agents, chloroquine diphosphate (1, 10, 100 mg/kg/day) potentiated the response to BP but was without effect on either arc of the MBSA response. Sodium aurothiomalate monohydrate (2, 10, 50 mg/kg/day) and D-penicillamine (1, 10, 100 mg/kg/day) inhibited the BP response and the elicitation of MBSA reaction. Levamisole hydrochloride (1, 5, 25 mg/kg/day) inhibited the induction of the MBSA response but was without effect on either reaction involving challenge. All compounds were administered p.o. except sodium aurothiomalate (s.c.).

It was found that the reaction to BP was inhibited by a wider range of agents than that to MBSA, which may reflect the larger proportion of an underlying non-immune inflammatory reaction to BP.