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COMMUNICATIONS

Receptor occupation and *in vivo* pharmacological effects in rats

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The degree of receptor occupation by a drug can be assessed by *ex vivo* receptor binding (Leysen & Laduron, 1978) after measurement of pharmacological activity in the same animal.

Rats were systemically treated with the unlabelled drug, at time of peak effect the pharmacological activity was measured and immediately thereafter the animals were sacrificed and membrane preparations incubated with a labelled ligand, followed by a filtration procedure (Leysen, Gommeren & Laduron, 1978). The reduction of specific labelling of membranes from treated versus non-treated rats, is a measure of the degree of receptor occupation and can be compared directly with the intensity of the *in vivo* observed effect. Long-acting compounds such as the narcotic lofentanil (Leysen & Laduron, 1978), the neuroleptic spiperone (Leysen, Gommeren & Laduron, 1978) and the dopamine agonist bromocryptine are suitable for such investigations. Lofentanil (0.63 μg/kg i.v.) induced surgical analgesia in rats (tail-withdrawal reaction--TWR), Niemegeers, Schellekens, Van Bever & Janssen (1976), but no significant occupation of opiate receptors labelled with [^{3}H]-fentanyl (Leysen & Laduron, 1978) in brain areas and spinal cord was observed. Lofentanil (1.25 μg/kg) produced a significant occupation of 30% of the CNS opiate receptors, and maximal occupation was reached at 10 μg/kg, i.e. 20 times the lowest ED_{50} in the TWR and 15 times the ED_{50} inducing muscular rigidity.

With spiperone the degree of dopamine receptor occupation in the striatum measured with [^{3}H]-spiperone binding (Leysen, Gommeren & Laduron, 1978) corresponded to the inhibition of spontaneous locomotion measured in the Doppler motility test (Niemegeers & Janssen, 1978) from 20% at 10 μg/kg to 85% at 160 μg/kg. However, four times lower spiperone doses antagonized apomorphine induced stereotypy in rats.

Thus, it seems that occupation of a minor part of the entire receptor population may be responsible for pronounced pharmacological effects *in vivo*. This has been further investigated for the dopaminergic system using bromocryptine followed by *ex vivo* or *in vivo* [^{3}H]-spiperone binding (Laduron & Leysen, 1977). Such studies may help to clarify the apparent discrepancy between the observation of pronounced *in vivo* supersensitivity and rather minor modifications in receptor density after long-term neuroleptic treatment of animals.

References

LADURON, P. & LEYSEN, J. (1977). Specific *in vivo* binding of neuroleptic drugs in rat brain. *Biochem. Pharmacol.*, **26**, 1003–1007.

LEYSEN, J.E. & LADURON, P.M. (1978). Receptor binding properties *in vitro* and *in vivo* of some long-acting opiates. *Arch. Int. Pharmacodyn.*, **232**, 243–246.

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. Pharmacol.*, **27**, 307–316.

NIEMEGEERS, C.J.E., SCHELLEKENS, K.H.L., VAN BEVER, W.F.M. & JANSSEN, P.A.J. (1976). Sufentanil, a very potent and extremely safe intravenous morphine-like compound in mice, rats and dogs. *Arzneim. Forsch.*, **26**, 1551–1816.

NIEMEGEERS, C.J.E. & JANSSEN, P.A.J. (1978). *In vivo* interaction of haloperidol and apomorphiine. *Arch. Int. Pharmacodyn.*, **236**, 43–50.

Assessment of neurotransmitter uptake *in vivo*

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Studies of the action of antidepressant agents at the molecular level frequently involve measurement of radiolabelled neurotransmitter uptake into nerve terminals. These experiments are often performed *in vitro* and in general the quantitative correlation of the results so obtained with the therapeutic effectiveness of the agents examined is poor (Coppen, Rama Rao, Swade & Wood, 1979). In this type of study two important transport processes are circumvented: absorption from the gastrointestinal tract and transport across the blood-brain barrier.

Attempts have been made to resolve this by measuring uptake *ex vivo* ie using established *in vitro* methodology on material prepared from drug-tested animals. Using Wy 25093, a selective 5-HT uptake inhibitor (Dickison, Diggory, Wood & Wyllie, 1980), it has become apparent that the results obtained using this technique may be influenced by the re-distribution of the drug during the isolation procedure and may not necessarily reflect events prior to death. We describe here a method for measuring the rate of [^{3}H]-5-HT uptake *in vivo*.

Rats were anaesthetized with pentobarbitone (50 mg/kg i.p.) immediately prior to use. [^{3}H]-5-HT (1 μCi) was administered into the lateral ventricle via a stereotaxically implanted cannula. Rats were decapitated at various intervals after injection, subcellular fractions isolated by the method of Gray & Whittaker (1962) and radioactivity counted and corrected for quenching.

During the first hour after administration there was a progressive increase in the level of radioactivity in the crude mitochondrial fraction (P_2). This was largely due to a progressive increase in uptake into the synaptosomal fraction (P_2B). The radioactivity incorporated in the synaptosomal fraction was retained by a 0.45 μm filter and was osmotically sensitive. These features are indicative of an association with nerve terminals.

Pretreatment of the animals with imipramine (100 mg/kg p.o.), 1 h prior to administration of the radiolabel resulted in a reduction of the total radioactivity in the brain (Table 1). This was probably due to an inhibition of amine uptake into nerve terminals because the degree of reduction achieved was related to the proportion of nerve terminals per fraction

Table 1 The effects of imipramine on the accumulation of [^{3}H]-5-HT into subfractions of rat brain

Treatment	*Uptake: d/min per fraction*** *Homogenate*	*Mitochondria* (P_2)	*Synaptosomes* (P_2B)
Saline control	115,000 ± 5000	51,000 ± 3000	45,000 ± 1500
Imipramine (100 mg/kg p.o.)	86,000 ± 3000†	23,000 ± 2000††	11,000 ± 1000††
Imipramine (10^{-5} M) *in vitro**	119,000 ± 6000	49,000 ± 2500	43,500 ± 2000
Nerve terminals as % of fraction	15%	40%	85–95%

† $P < 0.01$. †† $P < 0.001$.

* Imipramine was added to the homogenate otherwise the animals were identical to the control group.

** Uptake is expressed per 10 mg cortical protein.

(synaptosomal fraction > mitochondrial fraction > homogenate).

No significant change in radioactivity per fraction was observed following the addition of imipramine to the incubation medium *in vitro*. It is unlikely therefore that the effects of imipramine could be accounted for by a redistribution of the drug during the homogenization procedure.

References

COPPEN, A., RAMA RAO, V.A., SWADE, C. & WOOD, K. (1979). Inhibition of 5-hydroxytryptamine reuptake by amitriptyline and zimelidine and its relationship to their therapeutic action. *Psychopharmacology*, **63**, 125–129.

DICKISON, S.E., DIGGORY, G.L., WOOD, M.D. & WYLLIE, M.G. (1980). The effects of a selective uptake blocker on 5-HT turnover in the CNS. *Br. J. Pharmac.*, proceedings in press.

GRAY, E.G. & WHITTAKER, V.P. (1962). The isolation of nerve endings from brain: an electron microscopic study of cell fragments derived by homogenization and centrifugation. *J. Anat. Lond.*, **96**, 79–87.

Detection of electrical stimulation in the medial forebrain bundle at the level of the lateral hypothalamus: effects of haloperidol and cocaine on the intensity gradient

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The present study examined the effects of haloperidol and cocaine on the detection of electrical stimulation of the medial forebrain bundle, and on the intensity gradient.

Rats were implanted with monopolar electrodes in the medial forebrain bundle at the level of the lateral hypothalamus (MFB–LH: coordinates: AP + 4.6, V − 3.4, L 1.6) (Pellegrino & Cushman, 1967), and were trained to discriminate electrical stimulation in a three-operant procedure which combines significant features of drug discrimination procedures (Colpaert, Niemegeers & Janssen: Shannon & Holtzman, 1976). The electrical stimulation consisted of a 5.0 s train of pairs of negative-going rectangular 0.2 msec pulses. The P_1–P_2 interval was 1.5 msec; one pair of pulses occurred every 50 msec, thus yielding a frequency of 40 pulses per second. Constant current intensity was 200 μA in all animals.

Tests for generalization of lower intensities were carried out at 60 to 140 μA following treatment (*t*—30 min: subcutaneous: 1 ml/100 g body weight) with either saline, 0.02 mg/kg haloperidol, or 10 mg/kg cocaine.

The intensity gradient obtained after saline treatment could be fitted by a linear function in a log-probit plot. Following cocaine, response control by the electrical stimulation deteriorated in that there was a decrease in the detection of the training stimulation: this decrease occurred in the absence of a proportional increase of inappropriate responding under the no-stimulation condition. The cocaine treatment also acted to steepen the intensity gradient and to decrease apparent sensitivity. Haloperidol produced no significant effect on either the detectability of the training stimulation or the intensity gradient.

The cocaine data observed here suggest that increasing catecholamine availability by inhibiting reuptake or promoting release may interfere with the detection of electrical stimulation in the MFB–LH. The detectability of this stimulation may thus depend upon the activation of catecholamine neurones.

References

COLPAERT, F.C., NIEMEGEERS, C.J.E. & JANSSEN, P.A.J. (1976). On the ability of narcotic antagonists to produce the narcotic cue. *J. Pharmacol. Exp. Ther.*, **197**, 180–187.

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

SHANNON, H.E. & HOLTZMAN, S.G. (1976). Evaluation of the discriminative effects of morphine in the rat. *J. Pharmacol. Exp. Ther.*, **198**, 54–65.

The effects of chronic nicotine administration and withdrawal on 5-hydroxytryptamine synthesis and uptake by rat brain synaptosomes

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Previous studies have shown that chronic nicotine administration and its withdrawal causes a reduction in hippocampal 5-hydroxytryptamine (5-HT) levels in rats (Benwell & Balfour 1979). The present investigation was designed to investigate the biochemical basis of this effect.

Male Sprague–Dawley rats (Charles River), housed in groups of three, were treated subcutaneously with daily injections of nicotine (0.4 mg/kg) or saline for 39 days (Benwell & Balfour 1979). On day 40, half the nicotine-treated rats received nicotine while the remainder were withdrawn and received saline in place of nicotine. The controls received saline as usual. The rats were killed by cervical dislocation 30 min after their last injection and the brains were rapidly removed and dissected according to the procedure of Glowinski & Iversen (1966) into the hypothalamus, hippocampal formation and the remainder which was pooled as residual brain. Homogenates containing synaptosomes were prepared from these brain regions and were used to study the effects of chronic nicotine-treatment on synaptosomal processes involved in 5-HT formation and recapture.

5-HT formation from exogenous L-tryptophan was reduced when hippocampal synaptosomes, prepared from nicotine-treated and withdrawn rats, were incubated in media containing 10 μM tryptophan (Table 1), although the change was only statistically significant for the nicotine-treated group. These treatments also reduced tryptophan uptake by hippocampal synaptosomes (Table 1) suggesting that this, rather than reduced tryptophan hydroxylase activity, was the reason for the reduced 5-HT formation. No changes in tryptophan uptake or metabolism were observed in either of the other brain regions studied. Also the treatments had no effect on the 5-HT reuptake process in any of the regions. The results suggest therefore that chronic nicotine treatment specifically lowers hippocampal 5-HT levels by reducing tryptophan uptake and thus 5-HT formation in hippocampal 5-HT nerve-endings.

Table 1 The effects of chronic nicotine administration and its withdrawal on L-tryptophan uptake and metabolism by hippocampal synaptosomes

	Tryptophan uptake (*p moles/mg protein/min*)	*5-HT formation*
Control	146 ± 11 (8)	0.477 ± 0.03 (8)
Nicotine-treated	101 ± 7** (7)	0.336 ± 0.02** (6)
Nicotine-withdrawn	103 ± 10** (8)	0.419 ± 0.05 (8)

Tryptophan uptake was measured using a slight modification of the method of Karobath (1972); 5-HT formation by the method of Ichiyama, Nakamura, Nishizuka and Hayaishi (1970). The results are the means ± s.e. means of the numbers of observations in parenthesis.

** Significantly different from control (Student's *t*-test) $P < 0.01$.

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References

BENWELL, M.E.M. & BALFOUR, D.J.K. (1979). Effects of nicotine administration and its withdrawal on plasma corticosterone and brain 5-hydroxyindoles. *Psychopharmacology*, **63**, 7–11.

GLOWINSKI, J. & IVERSEN, L.L. (1966). Regional studies of catecholamines in rat brain—1. *J. Neurochem.*, **13**, 655–669.

KAROBATH, M. (1972). Serotonin synthesis with rat brain synaptosomes. *Biochem. Pharmacol.*, **21**, 1253–1263.

ICHIYAMA, A., NAKAMURA, S., NISHIZUKA, Y. & HAYAISHI, O. (1970). Enzymic studies on the biosynthesis of serotonin in mammalian brain. *J. biol. Chem.*, **245**, 1699–1709.

Inhibitory 5-Hydroxytryptamine receptors located on dopamine nerve terminals in the rat striatum

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5-Hydroxytryptamine (5-HT) may exert an inhibitory effect on central dopamine (DA) neurones, since striatal DA turnover increases after dorsal raphé nucleus lesions (Herve, Simon, Blanc, Lisoprawski, Le Moal, Glowinski & Tassin, 1979).

We have investigated the possibility that this inhibition is due, at least in part, to a modulatory effect of 5-HT on the amount of DA released from the dopaminergic nerve terminals in the striatum. Slices of rat striatum (0.25 × 0.25 mm) were loaded with [^{3}H]-DA and superfused with Krebs solution at 37°C. Two 4 min pulses of potassium (25 mM) were given 32 (S_1) and 60 (S_2) min after the start of superfusion. Drugs were added immediately after S_1. The ratio (S_2/S_1) was calculated for drug treated slices and expressed as a percentage of the control ratio.

Compounds were also tested for their ability to inhibit the uptake of [^{3}H]-DA into nerve terminals (Blackburn, Foster, Greenwood & Howe, 1978) since this could cause apparent changes in stimulated DA release.

The 5-HT agonists (except 5-methyl and N-methyl tryptamine) produced significant (Mann-Whitney U test), dose-related decreases in potassium-evoked tritium release and pIC_{25} values are shown in Table 1. The agonists had little effect on DA uptake (Table 1). Two 5-HT antagonists, cinanserin and methergoline, enhanced evoked tritium release at concentrations above the pIC_{50} for inhibition of DA uptake. However concentrations below their pIC_{50}'s (10^{-6} M and 10^{-7} M respectively) antagonised the inhibitory effect of 10^{-6} M 5-methoxytryptamine without themselves significantly modifying evoked tritium release. Tetrodotoxin (10^{-7} M) did not antagonise 5-methoxytryptamine (10^{-6} M) indicating that interneurones are unlikely to be involved in its inhibitory effect. Thus, these results suggest that there are inhibitory 5-HT receptors on dopaminergic nerve terminals in the striatum which may modulate DA release *in vivo*.

References

BLACKBURN, T.P., FOSTER, G.A., GREENWOOD, D.T. & HOWE, R. (1978). Effects of viloxazine, its optical enantiomers and its major metabolites on biogenic uptake mechanisms *in vitro* and *in vivo*. *Eur. J. Pharmacol.*, **52**, 367–374.

HERVE, D., SIMON, H., BLANC., G., LISOPRAWSKI, A., LE MOAL, M., GLOWINSKI, J. & TASSIN, J.P. (1979). Increased utilisation of dopamine in the nucleus accumbens but not in the cerebral cortex after dorsal raphe lesion in the rat. *Neurosci. Letts.*, **15**, 127–133.

Table 1 Effect of 5-HT agonists and antagonists on [^{3}H]-DA release from rat striatal slices and inhibition of [^{3}H]-DA uptake into striatal synaptosomes

Compound	*Inhibition of [^{3}H]-DA release (Mean pIC_{25} ± s.e. mean)*	*Inhibition of [^{3}H]-DA uptake (pIC_{50})*
5-Hydroxytryptamine	6.1 ± 0.2	<5.0
5-Methoxytryptamine	6.5 ± 0.4	<5.0
5-Methoxy N-N-dimethyltryptamine	6.5 ± 0.4	Not active
5-Methyltryptamine	*	<5.0
N-Methyltryptamine	*	<5.0
Cinanserin	—	5.4
Methergoline	—	6.4

* Compounds caused an enhancement of K^+-evoked tritium release which was not directly proportional to the concentration.
—No inhibition at any dose tested.

Involvement of the periaqueductal grey in dopamine-mediated circling behaviour

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Outflow pathways from zona reticulata of substantia nigra mediate circling behaviour to dopamine agonists in rats with a previous unilateral 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway. The nigro-tectal and nigro-thalamic pathways are not necessary for circling although unilateral lesions of the ventromedial nucleus of thalamus exert a modulatory action (Reavill, Leigh, Jenner & Marsden, 1979; Jenner, Leigh, Marsden & Reavill, 1979). The zona reticulata of substantia nigra also projects to areas of midbrain reticular formation (Hopkins & Niessen, 1976). We report a histological examination of connections between the substantia nigra and various nuclei of the brainstem reticular formation, and the effect of lesioning the latter on 6-OHDA-induced circling behaviour.

Horseradish peroxidase (HRP; Sigma type VI; 0.05–0.10 μl 30% solution in 0.01 M phosphate buffer pH 7.4) was injected into nucleus reticularis gigantocellularis (NG: A −3.8; L 0.8; V −7.7) or into nucleus tegmenti pedunculopontinus (NP; A −0.8; L 1.8; V −2.8) according to Pellegrino & Cushman (1971). Anaesthetized rats were perfused 24–48 h later with 25 ml 0.09% saline, 500 ml modified Karnovsky fixative and 500 ml 0.1 M phosphate buffer pH 7.4 containing 5% sucrose. Frozen brain sections were taken into saturated sodium sulphate and stained according to Hardy & Heimer (1971).

HRP injection into NP labelled cells in a number of areas including the medial, caudal and rostral aspects of the substantia nigra zona reticulata. HRP injection into NG labelled cells in the midbrain, in the lateral aspect of the periaqueductal grey (PAG), in the region of NP and in the deep grey strata of the superior colliculus.

Unilateral 6-OHDA hydrobromide (8 μg/3 μl 0.9% saline plus 2 μg ascorbic acid) lesions of the left lateral hypothalamus (A 4.6; L 1.9; V −3.0; de Groot, 1959) caused contraversive circling to apomorphine hydrochloride (0.5 mg/kg s.c.) and ipsiversive circling to (+)-amphetamine sulphate (3 mg/kg i.p.).

Subsequent electrolytic lesioning (4 mA, 8 s) of the left NG had no effect on apomorphine induced turning (before NG lesion 14.3 ± 1.0 turns/min, following NG lesion 17.8 ± 3.0 turns/min; $P > 0.05$) or on amphetamine-induced circling (before NG lesion 7.4 ± 0.5 turns/min, following NG lesion 8.6 ± 0.8 turns/min; $P > 0.05$). Kainic acid (0.5 μg/0.5 μl 0.9% saline) induced lesions of the left NP also did not alter apomorphine-induced turning (before NP lesion 18.8 ± 2.1 turns/min, following NP lesion 15.8 ± 2.1 turns/min; $P > 0.05$) or amphetamine-induced circling (before NP lesion 8.5 ± 1.0 turns/min, following NP lesion 8.8 ± 1.4 turns/min; $P > 0.05$). However, electrolytic lesions of the left PAG (A 0.6, L 1.0, V −1.4; Pellegrino & Cushman, 1971) markedly reduced, and in some animals abolished, apomorphine circling (before PAG lesion means were 15.0 ± 1.0 turns/min, after PAG lesion means were 7.7 ± 2.2 turns/min; $P < 0.01$). Amphetamine-induced circling was not altered (before PAG lesion 11.0 ± 1.1 turns/min, following PAG lesion 16.3 ± 2.6 turns/min; $P > 0.05$). The reduction of apomorphine-induced circling was due to a reduction in postural asymmetry with no alteration in locomotor hyperactivity.

We suggest that cell bodies or fibres of passage in the lateral periaqueductal grey are important in mediating the postural component of circling behaviour caused by dopamine agonists in animals with prior 6-OHDA lesions.

References

DE GROOT, J. (1959). The rat forebrain in stereotaxic coordinates. *Vehr. K. Ned. Akad. Wet.*, **52**, 11–40.

HARDY, H. & HEIMER, L. (1977). A safer and more sensitive substitute for diamino-benzidine in the light microscopic demonstration of retrograde and anterograde axonal transport of HRP. *Neurosci. Lett.*, **5**, 235–240.

HOPKINS, D.A. & NIESSEN, L.W. (1976). Substantia nigra projections to the reticular formation, superior colliculus and central grey in the rat, cat and monkey. *Neurosci. Lett.*, **2**, 253–259.

JENNER, P., LEIGH, N., MARSDEN, C.D. & REAVILL, C. (1979). Dopamine mediated circling behaviour is modulated by lesions of the ventromedial nucleus of the thalamus. *Br. J. Pharmacol.*, **67**, 432P.

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

REAVILL, C., LEIGH, N., JENNER, P. & MARSDEN, C.D. (1979). Dopamine-mediated circling behaviour does not involve the nigro-tectal pathway. *Exp. Brain Res.*, **37**, 309–316.

Behavioural and biochemical studies following injection of capsaicin into the substantia nigra of the rat

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Capsaicin (8-methyl-N-vanillyl-6-nonenamide) has been used as a tool to deplete substance P from spinal neurones following either peripheral (Jessell, Iversen & Cuello, 1978) or intrathecal administration (Yaksh, Farb, Leeman & Jessell, 1979). However, other work would predict that 5-hydroxytryptamine (5-HT) as well as substance P mechanisms might be involved in the pharmacological activity of capsaicin (see Virus & Gebhart, 1979, for review). To date focal injections of capsaicin have not been used to deplete substance P supraspinally, and we have investigated biochemical and behavioural actions of injecting this agent into the substantia nigra of the rat.

Capsaicin was dissolved in dimethylsulphoxide (DMSO) and stereotaxically infused at a dose of 30 μg in 1 μl into the substantia nigra reticulata of female Porton rats (coordinates A 1.8, L ±2.5, V −2.8 mm; De Groot, 1959). Sham-operated animals received bilateral injections of DMSO or saline (1 μl). Other animals underwent surgery for the implantation of guide cannulae (outer diameter 0.65 mm) bilaterally 2 mm above the substantia nigra.

Locomotor activity was assessed in activity cages in DMSO and capsaicin-injected animals on days 1, 3 and 6 after injection. Capsaicin injected rats showed an increased spontaneous locomotor activity ($P < 0.01$) on each of these times when compared to DMSO injected animals. (DMSO animals behaved indistinguishably from those rats injected with saline only). Administration of (+)-amphetamine sulphate (1.5 mg/kg, i.p.) enhanced the locomotor activity of DMSO-injected animals significantly more than capsaicin-treated animals on day 3 after injection ($P < 0.01$). Injection of capsaicin (1–30 μg) bilaterally into the substantia nigra of conscious rats through guide cannulae did not produce any immediate behavioural response.

We had utilised capsaicin initially in an attempt to selectively deplete substance P containing neurones within the substantia nigra reticulata. However, biochemically the levels of nigral substance P remained unchanged after local injection of capsaicin into this area. Substance P levels in DMSO treated animals were 25.2 ± 2.9 ng/mg (n = 6–8) protein compared to 26.5 ± 1.8 ng/mg protein in animals 3 days after injection with capsaicin.

In contrast concentrations of both 5-HT and its metabolite 5-hydroxyindole-acetic acid were decreased in the substantia nigra on days 1 and 3 after capsaicin injection (to 60–77% of control DMSO values, $P < 0.01$). 5-HT levels also remained lower on day 6 ($P < 0.05$). Concentrations of homovanillic acid were significantly elevated in the striatum and nucleus accumbens on day 1 following capsaicin injection into the substantia nigra ($P < 0.05$).

Injection of capsaicin into the substantia nigra of rats induces increased spontaneous motor activity which lasts for 6 days. Biochemically the effect is associated with decreased nigral 5-HT function and enhanced metabolism of dopamine in the nigrostriatal and mesolimbic pathways. The pharmacological actions of capsaicin appear complex, and does not appear as a suitable agent to deplete substance P supraspinally following focal injection.

D.D. is a student of the Parkinson's Disease Society.

References

DE GROOT, J. (1959). The rat forebrain in stereotaxic coordinates. *Verh. K. Ned. akad. Wet.*, **52**, 11–39.

JESSELL, T.M., IVERSEN, L.L. & CUELLO, A.C. (1978). Capsaicin-induced depletion of substance P from primary sensory neurones. *Brain Res.*, **139**, 354–358.

VIRUS, R.M. & GEBHART, G.F. (1979). Pharmacological actions of capsaicin: apparent involvement of substance P and serotonin. *Life Sci.*, **25**, 1273–1284.

YAKSH, T.L., FARB, D.H., LEEMAN, S.E. & JESSELL, T.M. (1979). Intrathecal capsaicin depletes substance P in the rat spinal cord and produces prolonged thermal analgesia. *Science*, **206**, 481–483.

A method for screening the effects of drugs on manual co-ordination in the marmoset

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Various methods have been reported which attempt to quantitate hand and finger movements in primate species. Such studies have mainly involved the effects of neurological modifications and of low levels of radiation on manipulation in the rhesus monkey (Beck & Chambers, 1970, Davis, McDowell, Deter & Steele, 1956, Leary & Ruch, 1955). Ettlinger & Kalsbeck (1962) used retrieval of sugar lumps from a moving belt to quantitate visual reaching in rhesus monkeys. This method has been developed to quantitate the effects of drugs on manual co-ordination in the marmoset, a primate species gaining popularity in toxicological and pharmacological studies.

Marmosets (Hapale jacchus, 300–400 g, mixed sexes) were trained to retrieve small cubes of apple from a moving belt which was positioned in front of their home cages. Three types of response per trial could be categorised as follows; successful retrievals, which resulted in the animal's consumption of the reward, unsuccessful attempts when the animal reached for but failed to retrieve the reward and no attempts when the subject did not reach out to retrieve the reward. Subjects were tested daily and when steady performance levels had been attained, after 4–6 weeks training, drugs were administered intramuscularly in an injection volume of 0.5 ml/kg. Two injections were given per week; if drug was administered on Tuesday, the control vehicle was given on Thursday and vice versa. Scores in each response category on drug days were compared to scores obtained during the rest of the week, by means of a Chi Squared test.

Diazepam and chlorpromazine, with lowest effective doses of 0.1 mg/kg and 0.2 mg/kg respectively, disrupted performance baselines in a dose related manner, when tested 30 min post administration. Decrements in human performance involving manual co-ordination have been shown with doses of diazepam similar to those used in the present study (Borland & Nicholson, 1974).

The test has also been used to determine the time activity profile of atropine. The lowest effective dose of atropine, 0.05 mg/kg, caused a decrement in performance up to 60 min post injection but not subsequently.

This test provides a method for screening, in a primate species, drugs which disrupt motor co-ordination where time activity profiles can be obtained quickly after a 4–6 week training period.

References

BECK, C.H. & CHAMBERS, W.W. (1970). Speed, accuracy and strength of forelimb movement after unilateral pyramidotomy in rhesus monkeys. *J. Comp. Physiol. Psychol. Monograph.*, **70**, 1–22.

BORLAND, R.G. & NICHOLSON, A.N. (1974). Immediate effects on human performance of a 1,5-benzodiazepine (clobazam) compared with the 1,4-benzodiazepines, chlordiazepoxide hydrochloride and diazepam. *Br. J. clin. Pharmac.*, **2**, 215–221.

DAVIS, R.T., MCDOWELL, A.A., DETER, C.W. & STEELE, J.P. (1956). Performance of rhesus monkeys on selected laboratory tasks presented before and after a large single dose of whole body X radiation. *J. Comp. Physiol. Psychol.*, **49**, 20–26.

ETTLINGER, G. & KALSBECK, J.E. (1962). Changes in tactile discrimination and in visual reaching after successive and simultaneous bilateral posterior parietal ablations in the monkey. *J. Neurol. Neurosurg. Psychiat.*, **25**, 256–268.

LEARY, R.W. & RUCH, T.C. (1955). Activity, manipulation drive and strength in monkeys subjected to low level irradiation. *J. Comp. Physiol. Psychol.*, **48**, 336–342.

Demonstration of withdrawal hyperexcitability following administration of benzodiazepines to rats and mice

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Recent debate on the liability of benzodiazepines to induce physical dependence has prompted us to examine some of these drugs in our halothane-induced sleeping time model. We have previously demonstrated that when hypnotics such as barbiturates or meprobamate are withdrawn following administration of very large doses acutely (Turnbull & Watkins, 1976a) or small doses chronically (Turnbull & Watkins, 1976b) a CNS hyperexcitability is produced and this can be quantitated by measuring the decreased sensitivity of animals to halothane. This decrease was often followed by a rebound increase in sensitivity to halothane before return to normal. In the present experiments the excitability of the CNS was monitored following acute administration of very large doses of nitrazepam and diazepam to rats and during and after withdrawal of chronic administration of low doses of diazepam to mice.

In the acute experiments, female Wistar rats weighing approximately 150 g were injected i.p. with nitrazepam (total dose of 105 mg/kg or 225 mg/kg over a 10 h period) or diazepam (105 mg/kg/10 h) in 1% Tween 80. Halothane sleeping time, measured according to the method of Turnbull & Watkins (1976a) was determined at 4 h intervals beginning 6 h after the last injection and the results expressed as % ± s.e. mean of control. A significant decrease in sensitivity to the anaesthetic was found 18 h after the last injection of diazepam (sleeping time 77 ± 7 of control) and the higher dose of nitrazepam (sleeping time 76 ± 5 of control) followed in both cases by a 'rebound' increase in sensitivity to halothane at 36 h with nitrazepam (129 ± 9 of control) and 28 h with diazepam (130 ± 8 of control). Sensitivity to halothane had returned to normal by the third day after drug withdrawal.

In the chronic experiment, female CBA mice (20 g) were dosed orally with 1 mg/kg twice daily for 28 consecutive days with diazepam in 0.5% Tween 80. Control mice received vehicle only. At regular intervals throughout the study, sub-groups were removed for determination of their sensitivity to halothane (sleeping time following 5 min exposure to 2L/min of 3% halothane in O_2) and to the convulsant 3-mercaptopropionic acid (3-MPA) (EC_{50} with 95% confidence limits for tonic extensor seizures). This testing was performed 6 h after the first daily dose of diazepam. After day six, animals became completely tolerant to the anticonvulsant and halothane-potentiating effects of diazepam. 24 h after drug withdrawal on day 28, a hyperexcitability of the CNS became apparent with a significant reduction in 3-MPA convulsant threshold (Control 33.9 (30.9–36.1); Treated 26.0 (24.1–27.9) mg/kg i.p.) and halothane sleeping time (Control 84 ± 6, Treated 38 ± 8 (mean time in s ± s.e. mean)).

We have thus demonstrated that acute administration of high doses or chronic administration of low doses of benzodiazepines leads to a withdrawal hyperexcitability of the CNS as indicated by the change in sensitivity to halothane.

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References

TURNBULL, M.J. & WATKINS, J.W. (1976a). Determination of halothane-induced sleeping time in the rat: effect of prior administration of centrally active drugs. *Br. J. Pharmac.*, **58**, 27–35.

TURNBULL, M.J. & WATKINS, J.W. (1976b). Change in sensitivity to halothane in the rat following administration of sodium barbitone in the drinking water. *Br. J. Pharmac.*, **58**, 285–286P.

Dopaminergic supersensitivity and cyclic GMP in rat striatum

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Cyclic GMP is thought by some workers to be involved in membrane depolarisation and the subsequent release of neurotransmitter (Ferendelli, Rubin & Kinscherf, 1976). Since supersensitivity may be a modification of neuronal excitability with changed permeability to Ca^{2+}, the present experiments examine the relationship between dopaminergic supersensitivity, induced either by a unilateral lesion of the nigrostriatal pathway (Ungerstedt, 1971) or by prolonged blockade of dopamine receptors with haloperi-

dol, and the *in vitro* accumulation of cyclic GMP in response to dopamine, depolarisation and Ca^{2+}. Two months after the lesion or 48 hours after the last dose of haloperidol, 350 μM, neostriatal slices from these animals were preincubated (45 min at 37°C) in Krebs solution (pH 7.4) and then incubated (10 min) in Krebs solution containing one of the test substances (Kruger, Forn, Walters & Roth, 1976; and Ferendelli, Rubin & Kinscherf, 1976). Cyclic GMP was subsequently assayed by radioimmunoassay (Amersham).

The results (Table 1) indicate that in the striata from both lesioned and haloperidol treated rats cyclic GMP concentration was higher than in control striata. Incubation in the presence of dopamine (50 μM) causes a fall on the lesioned side and in the haloperidol treated rats while greater dopamine concentration is needed to produce a significant change in normal striata. Amphetamine causes a fall in cyclic GMP in control slices and on the intact side of the lesioned animals. However, the response to ionic alterations in the medium differ between both supersensitivity models: the responses either to K^+ or to Ca^{2+} are greater on the lesioned side than either the intact side or the control, whereas no increase is demonstrated on the slices from haloperidol treated animals.

These results suggest that the biochemical consequences of dopaminergic supersensitivity involve changes in cGMP concentration and its response to both dopamine and to ions. Similarities and differences are demonstrated between the two models of supersensitivity.

References

FERENDELLI, J.A., RUBIN, E.H. & KINSCHERF, D.A. (1976). Influence of divalent cations on regulation of cyclic GMP and cyclic AMP levels in brain tissue. *J. Neurochem.*, **26**, 74.

KRUGER, B.K., FORN, J., WALTERS, J.R., ROTH R.H. & GREENGARD, P. (1976). Stimulation by dopamine of adenosine 3'5' monophosphate formation in rat caudate nucleus: effect of lesions on the nigro-neostriatal pathway. *Mol. Pharmacol.*, **12**, 639.

UNGERSTEDT, U. (1971). Stereotaxic mapping of the monoamine pathways in the rat brain. *Acta. Physiol. Scand.*, **82**, (suppl. 367) 1.

Table 1 The influence of drugs and ions on cyclic GMP concentrations (pmol/mg protein) mean (s.d.) (n) in tissue slices from DA-supersensitive striata.

Treatment	*Control*	*Intact side*	*Lesion side*	*Chronic haloperidol*
Krebs only	0.79 (0.12) (10)	1.67 (0.52) (7)***	1.44 (0.60) (7)**	0.98 (0.16) (10)*
Dopamine (50 μM)	0.66 (0.14) (6)	1.63 (0.13) (3)	1.23 (0.07) (3)§§	0.44 (0.18) (7)‡‡
Dopamine (100 μM)	0.45 (0.16) (3)‡			
Amphetamine (1 μM)	0.51 (0.11) (4)‡	0.98 (0.19) (3)§	1.52 (0.65) (3)	
Ca^{2+} (3.5 mM)	1.53 (0.44) (5)‡‡	1.38 (0.58) (4)	2.44 (1.32) (4)††	1.12 (0.42) (4)
K† (80 mM)	0.66 (0.07) (6)	1.42 (0.63) (4)	1.84 (0.56) (4)§†	0.51 (0.23) (3)

*, **, *** $P < 0.01$, $P < 0.005$ and $P < 0.001$ respectively compared with control (student *t*-test).
‡, ‡‡ $PP < 0.005$ and $P < 0.001$ respectively compared with the basal level of the same group (student *t*-test).
§, §§ $P < 0.05$ and $P < 0.01$ respectively compared with the basal level of the same tissue (paired *t*-test).
†, †† $P < 0.02$ and $P < 0.01$ respectively greater response (−basal) compared with response on intact side (paired *t*-test).

Presynaptic dopamine like inhibitory receptors on the noradrenergic nerve endings of the rabbit hypothalamus

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Dopamine and dopamine agonists like apomorphine decrease the stimulation-evoked release of noradrenaline from peripheral noradrenergic nerve endings, through activation of specific presynaptic dopamine receptors which are different from the presynaptic α-adrenoceptors (Enero & Langer, 1975; Dubocovich & Langer, 1980). Yet in the central nervous system, dopamine does not inhibit the stimulation-evoked release of noradrenaline (Taube, Starke & Borowski, 1977; Mulder, Wemer & de Langen, 1979). The present experiments were designed to investigate whether the stimulation-evoked release of noradrenaline from the rabbit hypothalamus was affected by potent dopamine agonists such as apomorphine and pergolide (Fuller, Clemens, Kornfeld, Snoddy, Smalstig & Bach, 1979).

Slices of rabbit hypothalamus (0.4 mm thickness) were labelled with [^{3}H]-(±) noradrenaline and superfused with Krebs' solution. Two periods (S_1, S_2) of electrical stimulation (3 or 5 Hz, 2 ms, 26mA, 2 min) were applied with an interval of 40 min and drugs were added 40 min before S_1 or 20 min before S_2.

Both pergolide (0.03 μM) and apomorphine (1 μM) when added before S_2 significantly decreased the release of [^{3}H]-noradrenaline elicited by electrical stimulation at 5 Hz ($S_2/S_1 = 0.60 \pm 0.02$ $n = 6$ $P < 0.05$; $S_2/S_1 = 0.67 \pm 0.03$ $n = 3$ $P < 0.05$, respectively, when compared with the corresponding control at 5 Hz: $S_2/S_1 = 1.02 \pm 0.09$ $n = 6$). This effect was completely antagonized by S-sulpiride (1 μM). This inhibitory effect of the dopamine receptor agonists apomorphine and pergolide remained unaffected when the presynaptic alpha adrenoceptors were blocked by yohimbine 0.1 μM.

In the rabbit hypothalamus, superfused with cocaine (10 μM) exposure to adrenaline (0.01 μM) reduced the stimulation-evoked release ($S_2/S_1 = 0.61 \pm 0.07$, $n = 6$, $P < 0.05$ when compared with the controls at 3 Hz in the presence of cocaine $S_2/S_1 = 1.11 \pm 0.07$ $n = 11$). The α-adrenoceptor antagonist yohimbine (0.1 μM) shifted to the right the concentration-effect curve for the inhibitory effect of adrenaline on noradrenergic neurotransmission, while exposure to S-sulpiride (1 μM) failed to antagonize this inhibitory effect of adrenaline.

Dopamine when added before S_2 failed to modify the stimulation evoked-release of [^{3}H]-noradrenaline at 3 Hz in the presence of cocaine 10 μM ($S_2/S_1 = 1.02 \pm 0.10$ $n = 4$; 0.98 ± 0.09 $n = 4$ and 1.01 ± 0.09 $n = 4$ for 0.01, 0.1 and 1 μM dopamine respectively). These results confirm similar observations in other tissues of the central nervous system. (Taube *et al.*, 1977; Mulder *et al.*, 1979).

The absolute value of the fractional release of [^{3}H]-noradrenaline in S_1 elicited by electrical stimulation at 5 Hz in the presence of cocaine was: $S_1 = 1.68 \pm 0.55 \times 10^{-2}$ ($n = 3$) and the ratio S_2/S_1: 0.97 ± 0.09 ($n = 3$). In the presence of this neuronal uptake inhibitor both pergolide (0.03 μM) and apomorphine (1 μM) failed to reduce the stimulation-evoked release of [^{3}H]-noradrenaline ($S_2/S_1 = 0.98 \pm 0.02$ ($n = 4$) for pergolide and $S_2/S_1 = 0.97 \pm 0.02$ $n = 4$ for apomorphine). These results support the view that there is an interaction between inhibition of neuronal uptake by cocaine and presynaptic inhibitory dopamine-like receptors. Therefore, it appears that the presynaptic inhibitory dopamine-like receptors on the noradrenergic nerve endings of the central nervous system can be only demonstrated under conditions in which neuronal uptake of noradrenaline is not inhibited by drugs.

The present findings support the view that presynaptic inhibitory dopamine-like receptors different from the presynaptic alpha-adrenoceptors, are present on the noradrenergic nerve endings of the rabbit hypothalamus. Since dopamine itself does not seem to inhibit noradrenergic neurotransmission it is unlikely that these presynaptic inhibitory dopamine-like receptors play a physiological role in noradrenergic neurotransmission. Yet, these receptors might be of pharmacological importance since they can be acted upon by dopamine agonists like apomorphine and pergolide to reduce central noradrenergic neurotransmission.

References

DUBOCOVICH, M.L. & LANGER, S.Z. (1980). Dopamine and alpha-adrenoceptor agonists inhibit neurotransmission in the cat spleen through different presynaptic receptors. *J. Pharmacol. Exp. Ther.*, **212**, 144–152.

ENERO, M.A. & LANGER, S.Z. (1975). Inhibition by dopamine of [^{3}H]-noradrenaline release elicited by nerve stimulation in the isolated cat's nictitating membrane. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **289**, 179–203.

FULLER, R.W., CLEMENS, J.A., KORNFELD, E.C., SNODDY, H.D., SMALSTIG, E.G. & BACH, N.J. (1979). Effects of (8β)-8 ((Methylthio)methyl)-6-propylergoline on dopaminergic function and brain dopamine turnover in rats. *Life Sciences*, **24**, 375–382.

MULDER, A.H., WEMER, J. & DE LANGEN, D.J. (1979). Presynaptic receptor-mediated inhibition of noradrenaline release from brain slices and synaptosomes by noradrenaline and adrenaline. In Presynaptic receptors. Eds.: Langer, S.Z., Starke, K. & Dubocovich, M.L., 219–224. Oxford: Pergamon Press.

TAUBE, M.D., STARKE, K. & BOROWSKI, E. (1977). Presynaptic receptor systems on the noradrenergic neurones of rat brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **299**, 123–141.

Effect of various adrenoceptor agonists and antagonists on the spontaneous firing rate of rat locus coeruleus cells

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Electrophysiological studies have shown that the activity of the noradrenergic cells of the locus coeruleus (L.C.) is decreased by intravenous perfusion of the antidepressant desipramine (DMI) in male Wistar rats anaesthetized with chloral hydrate (Figure 1a) (Scuvée-Moreau & Dresse 1979). Two different mechanisms could explain this inhibition: (1) a presynaptic mechanism involving an increased stimulation of the inhibitory α_2 receptors on L.C. perikaryon due to the inhibition by DMI of the uptake of noradrenaline liberated by axonal collaterals, (2) a postsynaptic mechanism involving a uni- or multineuronal feed-

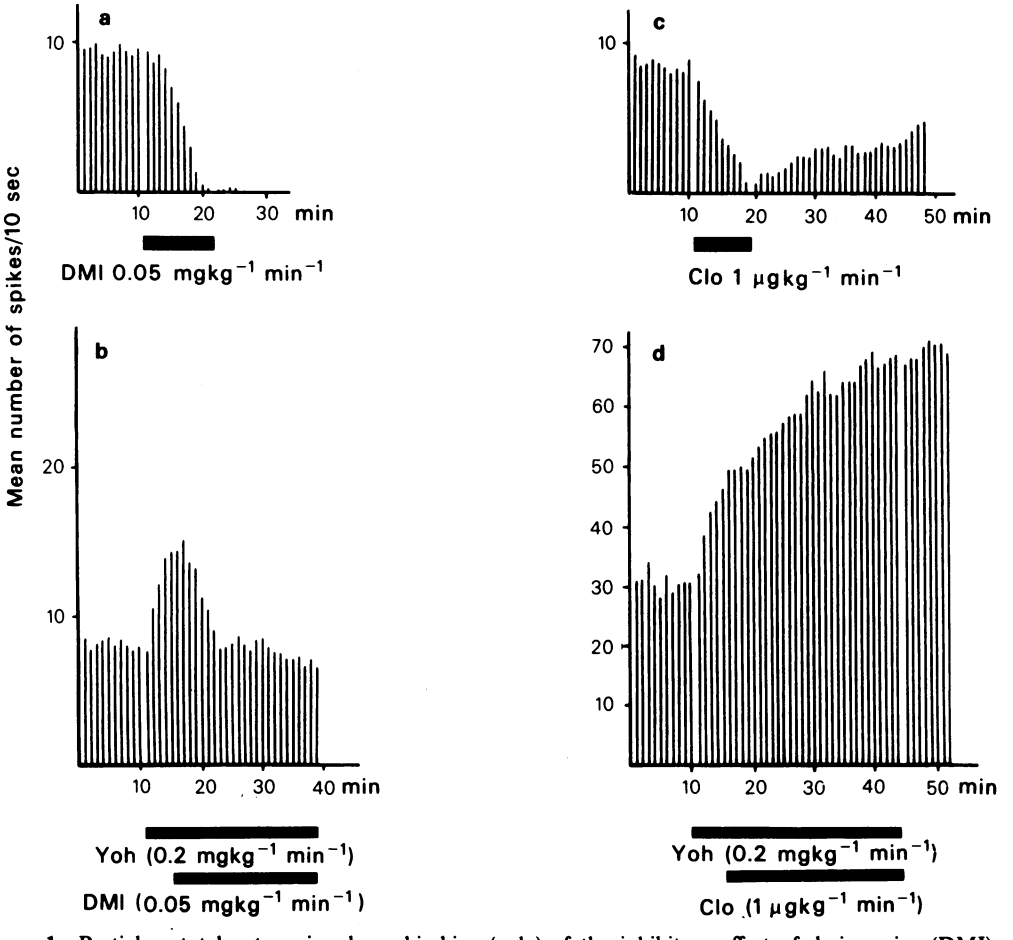

Figure 1 Partial or total antagonism by yohimbine (yoh.) of the inhibitory effect of desipramine (DMI) and clonidine (clo) respectively on the firing rate of locus coeruleus neurones. Concentrations refer to the bases.

back loop due to increased stimulation of α_1 or β receptors.

In order to test these two possibilities, selective agonists and antagonists of the pre and postsynaptic adrenoceptors were perfused alone or in combination with DMI. Six rats at least were used in each experiment. The selective α_2 presynaptic antagonist yohimbine increased the firing rate of L.C. cells and completely antagonized the action of the selective α_2 agonist clonidine (Figure 1 c–d). Perfusion of yohimbine initiated 4 min before and maintained during a DMI perfusion partially antagonized the inhibitory effect of this drug (Figure 1 b). These observations indicate that presynaptic α_2 receptors play a role in the regulation of the spontaneous activity of L.C. neurones and are implicated in the effect of DMI.

Prazosin (0.2 mg kg^{-1} min^{-1}), an α_1 postsynaptic antagonist also increased the spontaneous activity of the L.C. cells and partially antagonized the effect of DMI without interfering with the action of clonidine. This confirms the postsynaptic site of action of prazosin and indicates that α_1 receptors may also be involved in the regulation of the L.C. cells and in the effect of DMI.

Finally, a perfusion of (±) propranolol (0.2 mg kg^{-1} min^{-1}), a β antagonist, also increased the activity of L.C. cells only in 3 out of 6 rats and produced a slight reduction of the effect of DMI.

In conclusion, both pre and postsynaptic mechanisms seem to play a role in the inhibitory effect of acutely perfused DMI on L.C. neurones.

Reference

SCUVÉE-MOREAU, J. & DRESSE, A. (1979). Effect of various antidepressant drugs on the spontaneous firing rate of locus coeruleus and dorsal raphe neurons of the rat. *Europ. J. Pharmacol.*, **57**, 219–225.

Amphetamine inhibits the electrical stimulation-evoked release of [^{3}H]-dopamine from the rabbit caudate nucleus

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Amphetamine releases catecholamines from the central nervous system and inhibits the neuronal uptake of noradrenaline (NA) and dopamine (DA). The present investigation was aimed at studying the effects of amphetamine on the electrical stimulation-evoked release of [^{3}H]-DA in slices of rabbit caudate nucleus that were prelabelled with 3H-DA as described by Starke, Reimann, Zumstein & Hertting (1978). Two periods (S_1, S_2) of electrical stimulation (3 Hz, 2 ms during 2 min with a current of 24 mA) were applied with an interval of 44 min and drugs were added either 20 min before S_1 or S_2.

The percentage of total tissue radioactivity released by electrical stimulation during S_1 was 3.88 ± 0.20% (n = 12) and for S_2: 3.58 ± 0.20% (n = 12). The ratio S_2/S_1 was 0.93 ± 0.03 (n = 12). Amphetamine (0.1 and 1 μM) inhibited the release of [^{3}H]-DA evoked by electrical stimulation giving ratios S_2/S_1 of 0.55 ± 0.05 (n = 19, $P < 0.001$) and 0.43 ± 0.04 (n = 7, $P < 0.001$) respectively. Studies of [^{3}H]-DA uptake in this preparation revealed that at 0.1 μM, amphetamine was not inhibiting the uptake and retention of [^{3}H]-DA.

In slices of rat cortex, amphetamine enhances the release of [^{3}H]-NA evoked by electrical stimulation (Pelayo, Dubocovich & Langer, 1980), similarly in slices of rabbit hypothalamus, exposure to amphetamine (0.1 μM) enhanced the release of [^{3}H]-NA evoked by electrical stimulation. The ratio S_2/S_1 for controls was 0.99 ± 0.05 (n = 6) and for amphetamine (0.1 μM) it was increased to S_2/S_1 = 1.42 ± 0.13 (n = 6, $P < 0.02$).

In the presence of three different concentrations of haloperidol (0.001 μM, 0.01 μM or 0.1 μM), amphetamine (0.1 μM) still inhibited significantly the stimulation-evoked release of [^{3}H]-DA. The corresponding ratios S_2/S_1 in the presence of amphetamine were: 0.58 ± 0.08 (n = 4); 0.53 ± 0.08 (n = 8), and 0.53 ± 0.03 (n = 12).

The inhibition by amphetamine (0.1 μM) of the [^{3}H]-DA release elicited by electrical stimulation was prevented by nomifensine (10 μM) when amphetamine was added before S_2 (S_2/S_1 = 0.74 ± 0.08, n = 9), compared with an S_2/S_1 of 0.85 ± 0.04 (n = 9) in the corresponding controls with nomifensine.

Amfonelic acid (AFA) is a central nervous system stimulant which like amphetamine releases DA. Exposure to AFA (0.1 μM) decreased the evoked release of [^{3}H]-DA elicited by electrical stimulation (S_2/S_1 = 0.65 ± 0.08, n = 6, $P < 0.005$) while at 1 μM the ratio S_2/S_1 was 0.52 ± 0.01, n = 6, $P < 0.001$). Exposure to tyramine 0.05 μM also inhibited the release of [^{3}H]-DA elicited by electrical stimulation (S_2/S_1 = 0.69 ± 0.04, n = 3, $P < 0.005$).

We conclude that amphetamine at a concentration which does not inhibit neuronal uptake of DA and

does not yet enhance the spontaneous outflow of radioactivity acts on DA neurones to inhibit the release evoked by electrical stimulation. On the other hand, amphetamine acts on NA neurones to enhance the release elicited by electrical stimulation. The inhibitory effect of amphetamine on the DA-neurone is not mediated through presynaptic inhibitory DA receptors since these effects were unaffected by haloperidol. The antagonism by nomifensine of these effects of amphetamine coupled with the results of AFA and tyramine suggest that amphetamine may inhibit the stimulation-evoked release of [^{3}H]-DA by an intracellular mechanism that could be shared by AFA and tyramine.

References

PELAYO, F., DUBOCOVICH, M.L. & LANGER, S.Z. (1980). Inhibition of neuronal uptake reduces the presynaptic effects of clonidine but not of α-methylnoradrenaline on the stimulation evoked release of ^{3}H-noradrenaline from rat occipital cortex slices. *Eur. J. Pharmacol.*, in press.

STARKE, R., REIMANN, W., ZUMSTEIN, A. & HERTTING G. (1978). Effect of dopamine receptor agonists and antagonists on release of dopamine in the rabbit caudate nucleus *in vitro*. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **305**, 27–36.

Changes in cGMP in amygdaloid kindling in rats

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Changes in cyclic GMP have been reported in both focal (Raabe *et al.* 1978) and generalized (Ferrendelli, 1976) experimentally induced epilepsy. This study examined cyclic GMP levels in the amygdalae of rats following kindling (Goddard *et al.* 1969, Racine 1972). Stainless-steel bipolar electrodes were chronically implanted into the left amygdala and a daily kindling stimulus (1 s duration: frequency 60 Hz: current 100–400 μa) was applied (Farjo & Blackwood, 1978). After 7–18 stimulations (mean 13.7) generalized convulsions were triggered in six rats. Six control rats were chronically implanted with electrodes but received no stimulus. Three months after completion

Table 1 Cyclic GMP concentration in amygdala (pmol cyclic GMP mg^{-1} protein)

		Experiment	*Basal*	*K*$^+$	*Ca*$^{2+}$	*Dopamine*	*Haloperidol*
Control	*Left*	I	0.77	1.44	1.71	2.14	1.66
		II	1.01	1.77	2.22	3.83	2.39
		III	1.29	1.80	1.72	2.56	2.89
	Right	I	1.33	2.48	1.52	2.26	2.37
		II	1.20	1.28	1.62	1.66	2.04
		III	0.78	1.42	1.02	3.06	1.37
Kindled	*Left*	I	1.03	1.22	2.26	1.93	0.79
		II	1.70	1.66	1.30	1.22	2.28
		III	1.43	1.83	1.16	1.32	1.67
	Right	I	1.28	1.63	2.63	2.71	2.02
		II	3.11	2.52	1.98	2.87	2.95
		III	2.13	1.79	1.93	3.40	3.13

Basal Level: In both left and right amygdala cGMP was higher in kindled than in control animals (Wilcoxon rank sum test $P < 0.05$).
Response to Treatments: Analysis of variance demonstrated a significant increase of cGMP over basal levels in the K^+Ca^{2+} dopamine and haloperidol incubations in the control group ($P < 0.01$) the four treatments caused no significant change in cGMP levels in the kindled group.

of kindling all animals were killed, the right and left amygdala dissected and tissue slices prepared using a McIlwain chopper. Tissue from two animals was pooled and pre-incubated at 37°C for 45 min at pH 7.4 in Krebs buffer (120 mM-NaCl, 4.7 mM-KCl, 25 mM-$NaHCO_3$, 1.2 mM-KH_2PO_4, 1.0 mM-$CaCl_2$, 2.3 mM-$MgSO_4$, 10 mM-glucose). The slices from each tissue sample were then divided into five separate flasks for incubation in either Krebs buffer or buffer containing one of the following modifications: 1. high K^+ (119.7 mM) 2. high Ca^{2+} (5 mM) 3. dopamine 10^{-4} M 4. haloperidol 10^{-5} M. The reaction was stopped after 10 min by the addition of 1 ml ethanol. The suspension was evaporated to dryness and after homogenization in tris-EDTA buffer the cyclic GMP content was measured by radioimmunoassay (Radiochemical Centre, Amersham). The results in Table 1 show higher basal levels of cyclic GMP in the right and left amygdalae from kindled rats compared to sham-operated controls. The incubation of slices in media containing high concentrations of K^+ or Ca^{2+}, or dopamine or haloperidol all of which led to a rise in cyclic GMP over basal levels in control tissue did not cause a response in slices from kindled animals.

These results suggest that lasting changes in cyclic GMP are part of the biochemical response to kindling. Similar findings have been obtained in several brain areas from golden hamsters with hereditary audiogenic epilepsy (T. Palomo, Unpublished).

References

FARJO, I.B. & BLACKWOOD, D.H.R. (1978). Reduction in tyrosine hydroxylase activity in the rat amygdala induced by kindling stimulation. *Brain Res.*, **153**, 423–426.

FERRENDELLI, J.A. (1976). Role of cyclic GMP in the function of the central nervous system. In: Cyclic nucleotides in disease. B. Weiss (Ed) Univ. Press, Baltimore p. 377–390.

GODDARD, G.V., MCINTYRE, D.C. & LEECH, C.K. (1969). A permanent change in brain function resulting from daily electrical stimulation. *Exp. Neurol.*, **15**, 295–330.

RAABE, W., NICOL, S., GUMNIT, R.J. & GOLDBERG, N.D. (1978). Focal penicillin epilepsy increases cyclic GMP in cerebral cortex. *Brain Res.*, **144**, 185–188.

RACINE, R.J. (1972). Modification of seizure activity by electrical stimulation, II motor seizures. *Electroenceph. Clin. Neurophysiol.*, **32**, 281–294.

Effect of anti-grand mal drugs on kindled epilepsy in the rat

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Conflicting reports exist concerning the effectiveness of the commonly used anticonvulsant drugs, phenytoin and carbamazepine, against kindled amygdaloid epilepsy. While some workers have found these drugs to be ineffective against kindled epilepsy in the rat (Racine, Livingstone & Joaquin, 1975; Woda, 1977), others have reported that both possess anticonvulsant properties in this model (Ashton & Wauquier, 1979; Babington & Wedeking, 1973), and that both exert anticonvulsant and antiepileptic effects in kindled seizures in the cat and baboon (Wada, Osawa, Sato, Wake, Corcoran & Troupin, 1976), once adequate plasma levels of each are attained. The aim of the present study was to investigate the anti-epileptic and anticonvulsant effects of phenytoin and carbamazepine, and sodium phenobarbitone, a drug also used in grand mal epilepsy, on kindled amygdaloid epilepsy in the rat. Since differences in drug bioavailability may partly account for the disparate results with phenytoin and carbamazepine (Masuda, Utsui, Shiraishi, Karasawa, Yoshide & Shimuya, 1979), these drugs were administered both i.p. and p.o.

Bipolar electrodes were implanted chronically into the basolateral amygdala of male Sprague–Dawley rats (280–300 g): each animal received daily stimulation for 2 s (biphasic DC pulses; 100 μA; 1 ms; 62.5 Hz—standard stimulation) until reproducible generalized clonic convulsions were induced. Amygdaloid EEG and the incidences of the various convulsive components were recorded. Control convulsions and after-discharges (ADs) were triggered on two successive days; on day three, dose-groups of 8–10 rats received one of the following in the dose range indicated: sodium phenobarbitone (12.5 to 50 mg/kg p.o.); carbamazepine (6.25 to 50 mg/kg i.p., or 12.5 to 200 mg/kg p.o.): sodium phenytoin (12.5 to 100 mg/kg i.p., or 25 to 200 mg/kg p.o.) or drug vehicle (2% Tween/H_2O; 2.5 ml/kg). Standard stimulation was applied 1–4 h later depending on the drug, and 24 h later.

Phenobarbitone produced a clear dose-related decrease in AD duration (ED_{50} = 17 mg/kg at 1 h) which was well correlated with decreased incidences of forelimb clonus, rearing and full convulsions. Marked hypotonia and sedation were apparent at the highest dose.

Carbamazepine, dosed p.o., did not significantly affect AD duration but it did block full convulsions (ED_{50} = 50 mg/kg at 2 h): this was only a weak

effect since the other convulsive components were not decreased. However, carbamazepine, dosed i.p., was much more effective: it caused a dose-related reduction in AD duration (ED_{50} = 12 mg/kg at 1 h) which correlated well with decreased incidences of all the seizure components.

Oral doses of phenytoin were ineffective against either AD activity or the various convulsive symptoms. This contrasts with i.p. administration which resulted in a dose-related blockade of full convulsions (ED_{50} = 50 mg/kg at 1 h), but which did not significantly influence the other convulsive components. The effect on AD duration was not dose-related: there was a significant reduction (40%; $P < 0.05$) at only one dose (50 mg/kg), the other doses being completely ineffective. Marked neurotoxicity became evident at 50 mg/kg i.p.

These findings demonstrate that both phenobarbitone and carbamazepine exert quite potent antiepileptic effects on kindled focal epilepsy which correlate well with their observed anticonvulsant effects. In contrast, phenytoin has only a weak anticonvulsant effect which is not related to a decrease in AD duration. The comparative lack of effect of phenytoin and carbamazepine when dosed p.o. indicates that these drugs are poorly absorbed by this route in the rat.

References

ASHTON, D. & WAUQUIER, A. (1979). Behavioural analysis of the effects of 15 anticonvulsants in the amygdaloid kindled rat. *Psychopharmacology*, **65**, 7–13.

BABINGTON, R.G. & WEDEKING, P.W. (1973). The pharmacology of seizures induced by sensitisation with low intensity brain stimulation. *Pharmacology Biochemistry and Behaviour*, **1**, 461–467.

MASUDA, Y., UTSUI, Y., SHIRAISHI, Y., KARASAWA, T., YOSHIDE, K. & SHIMIZU, M. (1979). Relationships between plasma concentrations of diphenylhydantoin, phenobarbital, carbamazepine and 3-sulphomomethyl-1,2-benzoisoxazole (AD-810), a new anticonvulsant agent and their anticonvulsant or neurotoxic effects in experimental animals. *Epilepsia*, **20**, 623–633.

RACINE, R., LIVINGSTON, K. & JOAQUIN, A. (1975). Effects of procaine hydrochloride, diazepam and diphenylhydantoin on seizure development in cortical and subcortical structures in rats. *Electroenceph. clin. Neurophysiol.*, **38**, 355–365.

WADA, J.A. (1977). Pharmacological prophylaxis in the kindling model of epilepsy. *Arch. Neurol.*, **34**, 389–395.

WADA, J.A., OSAWA, T., SATO, M., WAKE, A., CORCORAN, M.E. & TROUPIN, A.S. (1976). Acute anticonvulsant effects of diphenylhydantoin, phenobarbital and carbamazepine: a combined electroclinical and serum level study in amygdaloid kindled cats and baboons. *Epilepsia*, **17**, 77–88.

The high pressure neurological syndrome: do anti-convulsants prevent it?

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The high pressure neurological syndrome (HPNS) is one of the major limitations in deep sea diving. Divers experience tremors, E.E.G. changes, somnolence and motor incoordination above 30 atmospheres (atm), animals exposed to higher pressures subsequently have both tonic and clonic convulsions (Brauer *et al.*, 1979). Some anaesthetics can ameliorate this syndrome (Green, Halsey & Wardley-Smith, 1977).

We have studied some anti-convulsant, sedative and anti-Parkinsonian drugs to test the hypothesis that some of these compounds would also ameliorate aspects of HPNS. We were interested in including drugs used in the treatment of other neurological disorders.

Male T-O mice (25–30 g) were injected i.p. with the drugs in either acute or chronic regimens. Groups of 8 mice in rotateable cages were placed in a 100 atm pressure chamber. Their rectal temperatures were maintained at 37.0 ± 0.5°C and the P_{O_2} at 0.6 atm. Righting reflex, intention tremor (i.e. tremor during forced movement) and resting continuous tremor were assessed as the pressure increased (compression rate 1.5 atm/min). The control onset pressure for these tremor endpoints, which have their counterparts in the human syndrome, were found to be reproducible (±3 atm).

Details of the drugs, dosages and results are in Table 1. Only primidone, clonazepam and diazepam were noticeably effective. These anti-convulsants have an 'anaesthetic' effect at higher dosages. None of the completely non-anaesthetic drugs such as phenytoin had any beneficial effect on HPNS. We have also carried out preliminary experiments with naloxone, which can prevent drug-related convulsions in mice (Blum *et al*, 1977). However, we have so far failed to demonstrate any protective effects of the antagonist on pressure induced tremors.

These data do not eliminate the possibility that some anti-convulsants may be partially effective in ameliorating HPNS. However, the lack of dramatic effect relative to anaesthetics is evidence against the hypothesis of a direct link between HPNS and other

Table 1 Effect of different drugs on the Intention tremor onset pressure and resting tremor onset pressure in mice

Drug	*Dose (mg/kg)*	*Treatment duration (days)*	*Intention Tremor (atm ± 1 s.e. mean)*	*Resting Tremor (atm ± 1 s.e. mean)*
Controls	(0.9% saline)	1–7	40.6 ± 3.1	70.1 ± 2.5
Phenytoin	25	1–7	75.3 ± 4.8	75.3 ± 4.8
Primidone	500	1	71.6 ± 5.5	97.3 ± 2.7
Ethosuximide	250	1	39.3 ± 3.3	53.3 ± 2.0
Carbamazepine	100	1	17.0 ± 2.0	34.0 ± 3.9
Phenobarbitone	50–75	1	40.5 ± 5.1	67.5 ± 11.6
Clonazepam	0.8–1.6	1	57.3 ± 11.9	95.9 ± 2.4
	1.6	1–7	69.3 ± 8.1	> 100
Diazepam	8.0	1	60.7 ± 0.5	> 100
	8.0	1–7	58.6 ± 4.7	> 100
Phencyclidine	2.5	1	50.5 ± 6.3	71.4 ± 2.0
L-Dopa	320	1–7	45.6 ± 0.6	80.4 ± 5.6

neurological syndromes which also lead to convulsions and motor incoordination in man.

References

BLUM, K., FUTTERMAN, S., WALLACE, J.E. & SCHWERTNER, H.A. (1977). Naloxone-induced inhibition of ethanol dependence in mice. *Nature*, **265**, 49–51.

BRAUER, R.W., MANSFIELD, W.M., BEAVER, R.W. & GILLEN, H.W. (1979). Stages in the development of the HPNS in the mouse. *J. Appl. Physiol. Respir. Envir. Exercise Physiol.*, **46(4)**, 756–765.

GREEN, C.J., HALSEY, M.J. & WARDLEY-SMITH, BRIDGET. (1977). Possible protection against some of the physiological effects of high pressure. *J. Physiol.*, **267**, 46–47P.

Cortical involvement in forepaw myoclonus induced by intrastriatal administration of picrotoxin to rats

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Focal injection of the GABA antagonist picrotoxin into the caudate nucleus of rats causes contralateral forelimb myoclonus (Tarsy, Pycock, Meldrum & Marsden, 1978). The development of myoclonus required intrastriatal picrotoxin administration coupled with concurrent damage to the overlying sensorimotor cortex. Since this mechanism of production of picrotoxin-induced myoclonus was unusual we have repeated some of the original experiments in conjunction with studies utilising [^{14}C]-deoxyglucose (DOG) to visualise focal changes in cerebral glucose utilisation (CGU) so as to clarify the role of striatum in the genesis of myoclonus.

Female Wistar rats (150 ± 10 g) were implanted with bilateral guide cannulae mounted either vertically over the anterior striatum (A +8.5, L ±2.5; Konig & Klippel, 1963) or pointing towards the centre of striatum at an angle of 45° (A +3.7; L ±2.5). Some animals with angled cannulae also had a cannula mounted over, or just touching the sensorimotor cortex (A +8.5, L ±2.5).

Focal unilateral injection of picrotoxin (1 μg in 1 μl 0.9% saline) into sensorimotor cortex (V +3.5) caused contralateral forepaw myoclonus in six of seven rats examined. Angled unilateral injection of picrotoxin (1 μg in 1 μl 0.9% saline) into striatum (V, 0), without sensorimotor cortex damage, produced myoclonus in only two of thirteen animals examined. However, nine of eleven rats receiving angled injection of picrotoxin but with damage to the sensorimotor motor cortex caused by a vertically placed cannula developed myoclonus. The latency to onset of myoclonus in rats receiving cortical administration of picrotoxin was

shorter (mean 3.8 ± 0.8 min) than in those receiving angled injections with concurrent cortical damage (mean 18.6 ± 2.4 min) ($P < 0.001$). Similarly, duration of myoclonus was longer in those receiving cortical injections (mean 69.5 ± 9.3 min) than in those receiving angled injections and with cortical damage (mean 35.7 ± 5.5 min) ($P < 0.01$).

Following the development of sustained contralateral forelimb myoclonus due either to unilateral intrastriatal or intracortical injection of picrotoxin (1 μg) some animals received [^{14}C]-deoxyglucose (25 μCi in 1 ml 0.9% saline i.v.) 30 min prior to death. Autoradiographic examination of brain sections following striatal injection of picrotoxin showed focal increases in CGU in the ipsilateral cortex, external segment of globus pallidus and thalamus but no distinctive change in the striatum. Cortical picrotoxin injection produced a similar pattern. However, there was some increase in CGU in the ipsilateral striatum in these animals.

The data suggest that focal myoclonus can be induced by manipulation of cortical GABA function alone and that the striatum does not play a key role in the initiation of the myoclonus, thus confirming the recent findings of Robin, Palfreyman, Zraika & Schechter (1980). It is probable that diffusion of picrotoxin from the striatal site of injection to sensorimotor cortex is responsible for myoclonus in this model.

References

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

ROBIN, M.M., PALFREYMAN, M.G., ZRAIKA, M.M. & SCHECHTER, P.J. (1980) An analysis of the cortical and striatal involvement in dyskinesia induced in rats by intracerebral injection of GABA-transaminase inhibitors and picrotoxin. *Eur. J. Pharmac.*, **62**, 319–327.

TARSY, D., PYCOCK, C.J., MELDRUM, B.S. & MARSDEN, C.D. (1978). Focal contralateral myoclonus produced by inhibition of GABA action in the caudate nucleus of rats. *Brain*, **101**, 143–162.

Differences between central and peripheral actions of substance P and substance P-(1-9)-nonapeptide

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Substance P is an undecapeptide which is most heavily concentrated in the substantia nigra of mammalian brain, iontophoretic application of substance P in the substantia nigra excites neurones (Davies & Dray, 1976). Consistent with this excitatory role, substance P causes an increase in locomotor activity when injected bilaterally into the ventral tegmentum of chronically cannulated rats (Kelley, Stinus & Iversen, 1979). In contrast to the information available from classical bioassay systems, there is little structure-activity data on the effects of substance P and analogues on central neurones. We have therefore studied the central effects of substance P and some fragments iontophoretically-applied to neurones in the rat substantia nigra and compared them with the effects on the guinea-pig ileum. We also evaluated the activity of most of these compounds in stimulating locomotor activity after injection into the ventral tegmentum. The experimental procedure for both sets of experiments has been previously described (Crossman, Walker, Woodruff, 1974; Kelley, Stinus & Iversen, 1979).

Iontophoretic application of substance P and substance P-(1-9)-nonapeptide (60–100 nA) produced a dose-dependent excitation of slow onset and long duration on 35% (35/101) and 27% (23/86) of neurones respectively. The remaining neurones were unaffected. Substance P-(1-2)-dipeptide, (4-9)-hexapeptide and (5-9)-pentapeptide had no effect on between 18 and 31 neurones. Bilateral injection of substance P or the (1-9)-nonapeptide (10 μg/side) produced a significant stimulation of locomotor activity for 15 minutes. The other fragments were devoid of activity at similar doses.

On the guinea-pig isolated ileum preparation, rat salivation and blood pressure models, substance P produced contractions, salivation and hypotension respectively at low doses. Substance P-(1-9)-nonapeptide was devoid of agonist activity on the ileum at ten thousand times the effective substance P dose and had no activity *in vivo* at one thousand times the effective substance P dose.

Our results are consistent with a functional role for substance P in the substantia nigra and ventral tegmentum but raise the possibility that these receptors may be different from those present in the periphery.

References

CROSSMAN, A.R., WALKER, R.J. & WOODRUFF, G.N. (1974). Problems associated with iontophoretic studies in the caudate nucleus and substantia nigra. *Neuropharmac.*, **13**, 547–552.

DAVIES, J. & DRAY, A. (1976). Substance P in the substantia nigra. *Brain Res.*, **107**, 623–627.

KELLEY, A.E., IVERSEN, S.D. & STINUS, L. (1979). Behavioural activation induced in the rat by substance P infusion into ventral tegmental area: implication of dopaminergic A10 neurones. *Neuroscience Letters*, **11**, 335–339.

N^{α}-Methylhistamine was not detected in brain by a sensitive, specific radioenzymatic assay

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Histamine is found in various mammalian tissues including brain, where it occurs at a concentration of approx. 50 ng/g and is believed to play a neurotransmitter role (Schwartz, 1977). We wished to investigate the possible presence of the sidechain N-methylated derivative of histamine, N^{π}-methylhistamine (nomenclature of Black & Ganellin, 1974), as a 'trace amine' in brain. By modification of the radioenzymatic assay for histamine (in which histamine is converted enzymatically into N^{τ}-[^{3}H]methylhistamine, catalysed by histamine N-methyltransferase (HMT) with S-adenosyl-[methyl-^{3}H]methionine as methyl group donor), a radioenzymatic assay for this substance has been developed. Thus N^{π}-methylhistamine, which is also a substrate for HMT (Barth, Crombach, Schunack & Lorenz, 1978), is converted enzymatically into N^{τ}-[^{3}H]methyl-N^{π}-methylhistamine. A t.l.c. procedure for separation of N^{τ}, N^{π}-dimethylhistamine and N^{τ}-methylhistamine is included in the work-up since any biological sample assayed for N^{π}-methylhistamine may also contain histamine.

Brain homogenates (5 vols. in 0.1 M phosphate buffer, pH 7.8) were divided into two portions, one of which was 'spiked' with N^{π}-methylhistamine (final concentration usually 10 ng/ml). Duplicate aliquots (up to 200 µl) of each portion were incubated with HMT and S-adenosyl-[methyl-^{3}H]methionine (500 mCi/mmole, 5 nmoles) in phosphate buffer as described by Beaven and Horakova (1978) for histamine assay. The reaction was stopped by addition of 0.6 M perchloric acid (200 µl) containing 1 mg/ml each of N^{τ}-methylhistamine and N^{τ},N^{π}-dimethylhistamine. After chloroform extraction from an alkaline aqueous phase, the products were back-extracted into 0.1 M hydrochloric acid (2 × 2 ml). The freeze-dried acid extract was re-dissolved in water (400 µl) and an aliquot (330 µl) applied as a 15 cm band on a silica gel t.l.c. plate (Merck, 0.25 mm, 20 × 20 cm) which was developed in ethanol/water/.880 ammonia (50:50:10 by volume). The plate was divided into quarter-inch bands which were counted for tritium as previously described (Knight & Smith, 1978). Radioactivity which chromatographed with the same R_F as N^{τ},N^{π}-dimethylhistamine (R_F approx. 0.27) and N^{τ}-methylhistamine (R_F approx. 0.42), both run as standards on each plate, was attributed to the tritiated products of enzymatic methylation of N^{π}-methylhistamine and histamine respectively.

However, in such experiments, endogenous N^{π}-methylhistamine was not found in rat, guinea-pig or mouse whole brain. In a typical experiment (guinea-pig brain), the spiked portion of the homogenate gave approx. 5000 d/min tritium as a peak over several t.l.c. bands with R_F identical to N^{τ},N^{π}-dimethylhistamine (thus accounting for approximately 30% of the original 'spike'), while the unspiked portion gave only 'blank' levels, approx. 500 d/min, at the same R_F. As little as 2 ng N^{π}-methylhistamine/ml homogenate, i.e. 10 ng/g brain, could have been detected.

The assay as described is sensitive and specific; the specificity derives from the substrate specificity of HMT and the t.l.c. purification procedure of the product mixture. Pre-extraction of N^{π}-methylhistamine before assay should enhance the sensitivity.

We thank Mr. W. Tertiuk for synthesis of N^{τ},N^{π}-dimethylhistamine.

References

BARTH, H., CROMBACH, M., SCHUNACK, W. & LORENZ, W. (1978). Gastric histamine methyltransferase has a less high acceptor substrate specificity. *Fed. Proc.*, **37**, 392.

BEAVEN, M.A. & HORAKOVA, Z. (1978). In *Handbook of Experimental Pharmacology*, vol **XVIII/2** Ed. M. Rocha e Silva; Springer Verlag, Berlin, pp. 151–173.

BLACK, J.W. & GANELLIN, C.R. (1974). Naming of substituted histamines. *Experientia*, **30**, 111–113.

KNIGHT, R.I. & SMITH, I.R. (1978). Separation and quantification of metabolites of radiolabelled histamine in brain. *Br. J. Pharmac.*, **64**, 460P.

SCHWARTZ, J.-C. (1977). Histaminergic mechanisms in brain. *Ann. Rev. Pharmac. Toxicol.*, **17**, 325–339.

Effects of lithium and calcium on manganese activated pyruvate kinase

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The molecular mode of action of lithium is unknown. Pyruvate kinase is one of a number of magnesium dependent enzymes which have been shown to be inhibited by lithium (Birch, 1978). The enzyme has an absolute requirement for divalent cations and also for potassium. When magnesium is the activator, as it is presumed to be *in vivo*, lithium inhibits competitively with respect to ADP and non-competitively with respect to magnesium, phosphoenolpyruvate and potassium, both in enzymes derived from muscle (O'Brien, Allin, Birch & Hullin, 1977) and from rat brain (Birch, Hullin, Kajda & O'Brien, 1979). Calcium also inhibits the magnesium enzyme, being competitive with respect to magnesium and ADP but non-competitive with respect to phosphoenolpyruvate and potassium.

Mildvan and his colleagues have demonstrated in a comprehensive series of studies (see Mildvan, 1979) that there are two independent divalent cations sites in the enzyme-nucleotide complex, one forming a metal-nucleotide complex and the other at the attachment site of this complex to the enzyme itself. Lithium complexes with adenosine nucleotides though less strongly than magnesium (Birch & Goulding, 1975).

Pyruvate kinase is activated less by manganese than by magnesium and in addition we have shown that under otherwise identical conditions (Kajda, Birch, O'Brien & Hullin, 1979) lithium fails to inhibit the manganese activated enzyme. In contrast calcium inhibition is still present and this is competitive with respect to manganese.

It would thus appear that lithium and calcium compete differently at the divalent metal sites. Lack of inhibition by lithium of the manganese activated enzyme suggests that manganese is less readily displaced by lithium from one of the sites than is magnesium. Since we know that Li-ADP complex is weaker than Mg-ADP complex we propose that lithium competes for magnesium at the other, enzyme bound, site but is unable to compete for manganese at that site and this accounts for its lack of inhibition when the latter metal is present. Calcium appears to compete at both sites with both magnesium and manganese. This is compatible with non-competitive inhibition by lithium with respect to magnesium, lithium's competition with respect to ADP (presumably Mg-ADP) in the magnesium activated enzyme and to the competitive nature of calcium inhibition with regard to magnesium, manganese and ADP.

Though this proposed mechanism is directly relevant only to pyruvate kinase, the possibility of similar competition at sites on other magnesium dependent enzymes which have been shown to be inhibited by lithium (Birch, 1978) leads to the possibility of a molecular explanation of at least a part of the pharmacological action of the ion in the treatment of recurrent affective disorders.

References

BIRCH, N.J. (1978). Lithium in Medicine in *New Trends in Bioinorganic Chemistry*, ed. Williams, R.J.P., da Silva, J.J.R.F., pp. 389–435, London, Academic Press.

BIRCH, N.J. & GOULDING, I. (1975). Lithium—nucleotide interactions investigated by gel filtration. *Analyt. Biochem.*, **66**, 293–297.

BIRCH, N.J., HULLIN, R.P., KAJDA, P.K., O'BRIEN, M.J. (1979). Effects of lithium on purified rat-brain pyruvate kinase. *Brit. J. Pharmacol.*, **64**, 70P–71P.

KAJDA, P.K., BIRCH, N.J., O'BRIEN, M.J. & HULLIN, R.P. (1979). Rat-brain pyruvate kinase: purification and effects of lithium. *J. Inorgan. Biochem.*, **11**, 361–366.

MILDVAN, A.S. (1979). The role of metals in enzyme catalysed substitutions at each of the phosphorus atoms of ATP. In *Advances in Enzymology and Related Areas of Molecular Biology*. ed. Meister, A. pp. 103–126, New York, Wiley–Interscience.

O'BRIEN, M.J., ALLIN, C.N., BIRCH, N.J. & HULLIN, R.P. (1977). Inhibition of rabbit muscle pyruvate kinase by lithium. *Brit. J. Pharmacol.*, **61**, 121P–122P.

'Dident' carbon fibre microelectrodes for electrophysiological and electrochemical studies

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Multibarrel glass microelectrodes with a single carbon fibre (7–8 μm diameter) incorporated into one barrel are suitable for low-noise recording of unit activity in the CNS, combined with iontophoretic ejection of drugs (Armstrong-James & Millar, 1979; Armstrong-James, Fox & Millar, 1980). Recent work (Armstrong-James, Kruk & Millar, submitted) has shown that these microelectrodes can be used not only to eject catecholamines (noradrenaline or dopamine) iontophoretically but also to electrochemically measure the concentration of catecholamine produced at the tip of the microelectrode. The assay is based on the polarographic technique (Adams, 1969; McCreary, Dreilling & Adams, 1974) of electrically oxidising material on the carbon fibre tip (the 'working' electrode) and measuring the resultant current increment. The voltage at the tip of the microelectrode system is 'voltage clamped' to a particular waveform pattern by a reference electrode/auxiliary electrode system. The reference electrode senses the voltage at the microelectrode tip, the auxiliary electrode supplies current to ensure that this follows the desired time course (a fast triangular wave).

In early experiments, the reference electrode was one of the glass barrels of the multibarrel microelectrode, filled with either 2 M KCl or 2 M NaCl. However, the resultant reference electrode had a high impedance, and thus a high noise level. This limited the sensitivity of the overall system for *in vitro* assay of catecholamines to about 10^{-6} M.

We have now produced three or four-barrel microelectrodes with two barrels containing carbon fibres. Thus one carbon fibre can be used as the working electrode, and the other used as the reference electrode. The resultant drop in noise caused by the low impedance of the carbon fibre reference electrode has meant that we have consistently been able to detect dopamine or noradrenaline *in vitro* at concentrations of $\sim 5 \times 10^{-8}$ M in 0.9% w/v saline solution. Because of their characteristic two-pronged appearance under the microscope we have christened these duplex microelectrodes 'didents'.

References

ADAMS, R.N. (1969). *J. Pharm. Sci.*, **58**, 1171–1181.

ARMSTRONG-JAMES, M. & MILLAR, J. (1979). *J. Neuroscience Methods* **1**, 278–287.

ARMSTRONG-JAMES, M., FOX, K. & MILLAR, J. (1980). *J. Neuroscience Methods* (in press).

MCCREERY, R.L., DREILLING, R. & ADAMS, R.N. (1974). *Brain Research* **73**, 23.

Effects of dopamine receptor agonists in the guinea-pig renal vasculature and their antagonism by sulpiride

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The existence of dopamine receptors mediating vasodilatation in the dog kidney is well documented (Goldberg, Volkman & Kohli, 1978). In the present study we have investigated the actions of some dopamine receptor agonists and antagonists in the guinea-pig renal vasculature.

Male or female guinea-pigs (350–450 g) were anaesthetized with urethane (1.2 g/kg). The hydrogen washout technique (Aukland, 1964) was used to measure highly localized inner cortical renal blood flow and drugs were administered into the renal artery, either as injections in 0.1 ml/kg vehicle or as continuous infusions. Drugs were dissolved in either 0.9% NaCl or, in the case of sulpiride, in 0.9% NaCl containing a trace of NaH_2PO_4.

Renal vascular resistance (RVR) was calculated from simultaneous measurement of renal blood flow (RBF) and blood pressure changes. Changes were only attributed to a direct renal vascular action of drugs if the changes were in an opposite direction or proportionally greater than those changes expected during normal autoregulation.

The injection of dopamine (0.5–1600 nmol/kg) into the renal artery of prazosin-pretreated (260 nmol/kg) guinea-pigs resulted in a dose-related increase in RBF and decrease in RVR. The maximum response to dopamine was a 30–40% increase in RBF. The re-

sponse to dopamine was not affected by infusions of vehicle or of (±)-propranolol (1.2 μmol kg^{-1} h^{-1}). This same dose of propranolol produced a complete inhibition of the increased RBF responses to equiactive doses of (±)-isoprenaline. The dopamine receptor agonists (±)-ADTN (Woodruff, 1978) and SKF 38393 (Pendleton, Samler, Kaiser & Ridley, 1978) produced a similar, propranolol-resistant increase in RBF and decrease in RVR. ADTN was approximately equipotent with dopamine and produced the same maximum response. SKF 38393 had a threshold dose approximately 10 times lower than that of dopamine but became approximately equipotent with the latter at higher doses.

The increase in RBF produced by dopamine was unaffected by vehicle infusions but was 70% inhibited by the dopamine receptor antagonist, (±)-sulpiride (8.5 μmol kg^{-1} h^{-1}). S-(−)-sulpiride was between 6 and 10 times more potent than R-(+)-sulpiride in its dopamine-blocking actions in the guinea-pig kidney. The responses to ADTN and SKF 38393 were similarly antagonised by (±)-sulpiride (8.5 μmol kg^{-1} h^{-1}) but the antagonist had no effect on responses to isoprenaline.

The *in vivo* guinea-pig kidney appears to be a useful preparation on which to evaluate the activity of dopamine receptor agonists and antagonists.

References

AUKLAND, K. (1964). Measurement of local blood flow with H_2 gas. *Circ. Res.*, **14**, 164–187.

GOLDBERG, L.I., VOLKMAN, P.H. & KOHLI, J.D. (1978). A comparison of the vascular dopamine receptor with other dopamine receptors. *Ann. Rev. Pharmacol. Toxicol.*, **18**, 51–78.

PENDLETON, R.G., SAMLER, L., KAISER, C. & RIDLEY, P.T. (1978). Studies on renal DA receptors with a new agonist. *Eur. J. Pharmacol.*, **51**, 19–28.

WOODRUFF, G.N. (1978). Biochemical and Pharmacological studies on dopamine receptors. In *Dopamine* ed. Roberts, P.J., Woodruff, G.N. & Iversen, L.L. pp. 89–119. New York, Raven Press.

Effects of centrally-administered 6-hydroxydopamine on hypertension during muscle ischaemia in the unanaesthetized rat

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Muscle ischaemia causes an elevation of blood pressure and heart rate in man (Alam & Smirk, 1938) and in the rat (Redfern, Little & Stoner, 1980). Concomitant with this in the rat is an activation of central noradrenergic neurones (Stoner & Hunt, 1976). The possible involvement of these neurones in the cardiovascular changes was investigated using 6-hydroxydopamine (6-OHDA).

6-OHDA (250 μg in 20 μl) was given intracerebroventricularly (i.c.v.) to male Wistar rats (220–290 g). Control rats received 20 μl of the ascorbate vehicle, i.c.v.; a third group were given the vehicle centrally and 6-OHDA (250 μg) intravenously. Six to thirteen days later a tail artery and vein were cannulated; electrocardiogram (e.c.g.) electrodes and a colon thermocouple were positioned during ether anaesthesia. Baroreflex sensitivity was assessed 1 h after recovery by intravenous infusion of phenylephrine (3–50 μg kg^{-1} min^{-1}) for about 7 min, and the regression line of heart period (HP) against mean arterial pressure (MAP) was constructed. Rubber tourniquets were then applied to the thighs under ether anaesthesia (5 min) to produce bilateral hind-limb ischaemia (Stoner, 1961). Phenylephrine was re-infused 0.5 h and 1 h after tourniquet application.

6-OHDA (i.c.v.) did not affect the normal MAP or baroreceptor-heart rate reflex but significantly reduced the pressor response to muscle ischaemia (Fig. 1). The resetting of the reflex to a higher heart

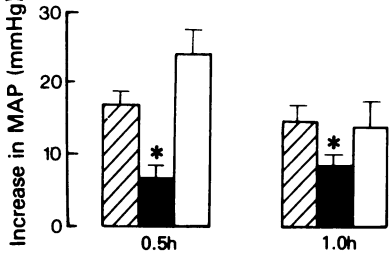

Figure 1 Changes in mean arterial pressure (MAP) at 0.5 h and 1.0 h after beginning of hind-limb ischaemia in unanaesthetized rats. Open columns: ascorbate vehicle i.c.v., $n = 9$; closed columns: 6-OHDA 250 μg i.c.v., $n = 9$; hatched columns: 6-OHDA 250 μg i.v., $n = 6$. The bars represent standard error of the mean. An asterisk denotes significantly different ($P < 0.05$) from the vehicle control (Mann-Whitney U test, two tailed).

rate, and the reduction in reflex gain during muscle ischaemia were unaffected by i.c.v. 6-OHDA. The effect of 6-OHDA was not due to leakage into the general circulation, since the same dose given intravenously was without effect. 6-OHDA given by either route did not alter the sensitivity to phenylephrine.

Our data suggest that central catecholaminergic neurones are involved in the hypertension but not in the changes in the baroreceptor-heart rate reflex during muscle ischaemia in the rat. These neurones have been implicated in other forms of experimental hypertension (Finch, Haeusler & Thoenen, 1972; Doba & Reis, 1974).

W.S.R. is an M.R.C. scholar.

References

ALAM, M. & SMIRK, F.H. (1938). Observations in man upon a pulse accelerating reflex from the voluntary muscles of the legs. *J. Physiol.*, **92**, 167–177.

DOBA, N. & REIS, D.J. (1974). Role of central and peripheral adrenergic mechanisms in neurogenic hypertension produced by brainstem lesions in rat. *Circulation Res.*, **34**, 293–301.

FINCH, L., HAEUSLER, G. & THOENEN, H. (1972). Failure to induce experimental hypertension in rats after intraventricular injection of 6-hydroxydopamine. *Br. J. Pharmac.*, **44**, 356–357P.

REDFERN, W.S., LITTLE, R.A. & STONER, H.B. (1980). Resetting of the baroreceptor heart rate reflex in the unanaesthetized rat during and following a period of hind-limb ischaemia. *XXVIII Int. Congr. Physiol. Sciences: Satt. Symp.: "Homeostasis in Injury and Shock", Proceedings.*

STONER, H.B. (1961). Critical analysis of traumatic shock models. *Fed. Proc.*, **20**, 38–48.

STONER, H.B. & HUNT, A. (1976). The effect of trauma on the activity of central noradrenergic neurones. *Brain Res.*, **97**, 1–15.

Spironolactone and the effect of propranolol in DOCA/salt treated rats

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The adrenal gland has now been implicated in the failure of experimental models to show an adequate antihypertensive response to β-adrenoceptor antagonists. Nijkamp, Van Den Bosch & De Jong (1979) have shown that adrenal cortical hormones have an opposing effect to propranolol in spontaneously hypertensive rats (SHR) whereas Buckingham & Hamilton (1979) reported a masking effect of medullary catecholamines on the antihypertensive effect of several β-adrenoceptor antagonists again in SHR.

We have examined the effect of propranolol, and the mineralocorticoid antagonist, spironolactone, on hypertension in rats implanted subcutaneously with deoxycorticosterone acetate (25 mg) and given 1% saline to drink (DOCA/salt rats).

Propranolol, 20 mg/kg s.c. tds in DOCA/salt male and female Wistar rats caused an inhibition of the development of hypertension which was greatest in females and accompanied by a reduction in fluid intake which was also observed in normotensive animals.

When the dose of propranolol was reduced to 15 mg/kg bd or the drug given in diet at 125 mg kg^{-1} day^{-1} for 10–14 days no hypotensive effect and no reduction in fluid intake were observed. Subsequent administration of spironolactone, 1 mg/kg p.o. caused a hypotensive response. The administration of Triamterine (2–8 mg kg^{-1} day^{-1} p.o.) or chlorothiazide (10–40 mg kg^{-1} day^{-1} p.o.) to DOCA/salt rats receiving propranolol 125 mg kg^{-1} day^{-1} in diet had no effect showing that the hypotensive response to spironolactone was not related to a diuretic or potassium sparing effect.

It has been suggested by Nijkamp *et al.* (1979) that the action of propranolol in SHR is opposed by adrenal corticosteroids which potentiate noradrenaline at the adrenergic nerve terminal. It is possible that spironolactone could directly antagonise such an action but, in view of the variety of steroidal agents which block uptake, this seems unlikely. Any direct effect on steroid metabolism would be complicated by the presence of doca but the possible effect of spironolactone on drug bioavailability and the association of the antihypertensive effect with reduced drinking merit further consideration.

References

BUCKINGHAM, R.E. & HAMILTON, T.C. (1979). Comparison of the anti-hypertensive response to β-adrenoceptor blocking drugs in intact and adrenal-demedullated spontaneously hypertensive rats. *Br. J. Pharmac.*, **68**, 667–676.

NIJKAMP, F.P., VAN DEN BOSCH, R. & DE JONG, W. (1979). An opposing role for the adrenals in the hypotensive effects of propranolol in the spontaneously hypertensive rat. *Eur. J. Pharmac.*, **56**, 187–195.

Removal of endothelium and arterial reactivity to acetylcholine and adenine nucleotides

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It has been suggested that intimal structures play a role in the responsiveness of the blood vessel wall to both vasoconstrictor and vasodilator substances (Pascual & Bevan 1980; Furchgott & Zawadski 1980). The present experiments were performed to investigate the effect of removal of the endothelium upon the reactivity of canine isolated femoral arteries. Control arterial rings and rings from which the endothelium had been removed mechanically were mounted for isometric tension recording in individual organ chambers filled with gassed (95% O_2–5% CO_2) Krebs-Ringer bicarbonate solution at 37°C.

The preparations from which the endothelium had been removed contracted in response to exogenous noradrenaline, nerve stimulation and depolarizing solution with a comparable sensitivity, but with 20% lower maximal amplitude than control rings.

Control rings, previously made to contract with noradrenaline (10^{-7} M) responded to the addition of acetylcholine (10^{-9} to 10^{-6} M), ATP (10^{-7} to 10^{-4} M), ADP (10^{-7} to 10^{-4} M), AMP (3×10^{-5} to 10^{-3} M) or adenosine (3×10^{-5} to 10^{-3} M) with dose-dependent relaxation. Preparations from which the endothelium had been removed failed to relax in response to acetylcholine, ATP, and ADP while the inhibitory responses to AMP and adenosine were comparable to those seen in control arteries.

These experiments confirm that the presence of the endothelium is necessary to obtain relaxation with acetylcholine in arterial tissue (Furchgott & Zawadski 1980); this appears to be also the case for ATP and ADP but not for AMP and adenosine. This in conjunction with the difference in potency between the adenine nucleotides suggests that, as in venous smooth muscle (De Mey *et al.* 1979), ATP and adenosine affect arterial smooth muscle by different mechanisms.

References

DE MEY J., BURNSTOCK G. & VANHOUTTE P.M. (1979). Modulation of the evoked release of noradrenaline in canine saphenous vein via presynaptic receptors for adenosine but not ATP. *Eur. J. Pharmacol.*, **55**, 401–405.

FURCHGOTT R. F. & ZAWADSKI J. (1980). Acetylcholine relaxes arterial smooth muscle by releasing a relaxing substance from endothelial cells. *Fed. Proc.*, **39**, 581.

PASCUAL R. & BEVAN J.A. (1980). Evidence that changes in vascular tone may be initiated from the intima of the rabbit aorta. *In* "Vascular Neuroeffector Mechanisms", ed. by J.A. Bevan *et al.*, Raven Press, New York, pp. 300–304.

Subcellular localization of two forms of amine oxidase in rat aorta

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Rat arteries have been reported to contain two main forms of amine oxidase (Coquil *et al.*, 1973). One form, resembling type A monoamine oxidase, oxidizes tyramine, 5-hydroxytryptamine and noradrenaline, and is highly sensitive to clorgyline and pargyline, but resistant towards carbonyl reagents such as semicarbazide. The other form ozidizes tyramine, but not 5-hydroxytryptamine or noradrenaline, and is inhibited by carbonyl reagents, but not by clorgyline or pargyline. Whereas in several tissues monoamine oxidase has been shown to reside in the outer mitochondrial envelope, the subcellular location of the semicarbazide-sensitive amine oxidase has not been established.

In rat aorta, some 60% of the tyramine oxidase activity was inhibited by semicarbazide (10^{-4} M). The residual activity was little affected by 10^{-3} M semicarbazide, but was almost completely abolished by tranylcypromine (10^{-5} M), a monoamine oxidase inhibitor used as an antidepressant. After fractionation of aorta homogenate by differential centrifugation, the amine oxidase measured with 5-hydroxytryptamine, or with tyramine in the presence of semicarbazide (10^{-4} M), was recovered for the major part (60–65%) in the mitochondrial fraction, with the bulk of cytochrome *c* oxidase. When the mitochondrial fraction was sub-fractionated by density equilibration in

sucrose gradient, the distribution of the semicarbazide-resistant amine oxidase coincided with that of the mitochondrial marker.

About the same percentage (30%) of the semicarbazide-sensitive amine oxidase was recovered in the mitochondrial and microsomal fractions. This repartition was paralleled by that of two putative plasmalemmal constituents, 5'-nucleotidase and Mg^{2+}-ATPase (oligomycin-insensitive). After density equilibration of mitochondrial or microsomal fractions, the semicarbazide-sensitive amine oxidase was again distributed like 5'-nucleotidase and Mg^{2+}-ATPase. Most characteristically, these three enzymes were markedly shifted towards higher densities after digitonin treatment of microsomal fractions, whereas other enzymes, e.g. NADH cytochrome *c* reductase (presumably associated with endoplasmic reticulum elements), were only slightly affected. The marked digitonin shift is a property of plasmalemmal elements from liver (Amar-Costesec *et al.*, 1974) and other tissues. We conclude that the semicarbazide-sensitive amine oxidase of rat aorta is located in the plasma membrane.

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References

AMAR-COSTESEC, A., WIBO, M., THINÈS-SEMPOUX, D., BEAUFAY, H. & BERTHET, J. (1974). Analytical study of microsomes and isolated subcellular membranes from rat liver. IV. Biochemical, physical and morphological modifications of microsomal components induced by digitonin, EDTA, and pyrophosphate. *J. Cell. Biol.*, **62**, 717–745.

COQUIL, J.F., GORIDIS, C., MACK, G. & NEFF, N.H. (1973). Monoamine oxidase in rat arteries: evidence for different forms and selective localization. *Br. J. Pharmac.*, **48**, 590–599.

The release of vasodilators by renin may contribute to the anti-hypertensive activity of captopril

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There is increasing evidence that captopril may have an anti-hypertensive activity which is not wholly dependent on a reduction in circulating angiotensin II. We propose that when angiotensin converting enzyme (ACE) is blocked, renin also induces the release of vasodilator mediators which may participate in the hypotensive action of ACE inhibitors.

Renal venous or carotid arterial blood was drawn at 10 ml/min from chloralose-anaesthetized beagles and used to superfuse strips of bovine coronary artery (BCA), cat terminal ileum (CTI) and a segment of rat colon (RC) according to the blood bathed bioassay technique of Vane (1964). Purified hog renin (1.0–5.0 mg; Pentex) incubated with blood for 1 min passing through a 'delay' coil, or administered intravenously (i.v.) or into the renal artery (i.r.a.) released an angiotensin-like substance, as shown by contraction of the rat colon. This contraction was blocked by the angiotensin antagonist (Sar^1-Ile^8)-angiotensin II (25–50 ng/ml) infused into the blood superfusing the tissues. Concomitant to this release was an increase in blood pressure of 12–31 mm Hg. These effects were generally maintained for 20–50 min, and abolished by the ACE inhibitor captopril (2 mg/kg i.v.).

After captopril, injections of renin, i.v. or i.r.a., produced a fall in blood pressure of 20–40 mm Hg which was maintained for periods up to 1 hour. There was an associated release of a bradykinin-like substance as indicated by contractions of the CTI. Production of this bradykinin-like substance by infusing renin into the delay coil was inhibited by aprotinin (1000–1500 KIU/ml) which also abolished the hypotension usually observed when the blood incubated with renin was returned to the dog.

Renin i.r.a. released both kinin-like material (contractions of CTI) and a prostacyclin-like substance into renal venous blood, as evidenced by relaxations of the BCA. Indomethacin, (5 mg/kg, i.v.) abolished the appearance of the prostacyclin-like substance but not the kinin-like material and reduced (2 experiments) or abolished (1 experiment) the hypotensive effect of renin.

The renin was tested for contamination with kallikrein by incubation with plasma kininogen prepared according to the method of Fasciolo, Espada & Carretero (1963), and the kinins produced were assayed on the CTI. No kallikrein-like activity was observed in this preparation of renin.

The release from blood of a kinin-like substance by renin was originally shown by Ng (1969). We have extended this observation to show that the kinin

formed in the circulation may in turn release a prostacyclin-like substance, and that the hypotensive action of these vasodilators is unmasked by ACE inhibitors. This action of renin, other than that of cleaving angiotensin I from its substrate, may contribute to the antihypertensive effects of captopril, for treatment with captopril is associated with elevations in plasma renin (Gavras, Brunner, Turini, Kershaw, Tifft, Cuttelod, Gavras, Vukovich & McKinstry, 1978).

References

FASCIOLO, J.C., ESPADA, J. & CARRETERO, O.A. (1963). The estimation of bradykininogen content of plasma. *Acta. Physiol. Lat. Am.*, **13**, 215–220.

GAVRAS, H., BRUNNER, H.R., TURINI, G.A., KERSHAW, G.R., TIFFT, C.P., CUTTELOD, S., GAVRAS, I., VUKOVICH, R.A. & MCKINSTRY, D.N. (1978). Antihypertensive effect of the oral angiotensin converting-enzyme inhibitor SQ14,225 in man. *New Eng. J. Med.*, **298**, 991–995.

NG, K.K.F. (1969). Generation of kinin by renin. In: *Prostaglandins, Peptides & Amines*, ed. Mantegazzi, P. & Horton, E.W., pp. 9–13, London: Academic Press.

VANE, J.R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmac. Chemother.*, **23**, 360–373.

What pharmacological properties are necessary for the prevention of early post-infarction ventricular dysrhythmias?

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In a previous communication to this Society (Kane, McDonald & Parratt, 1979) we described a method for the production of experimental dysrhythmias in anaesthetized rats by coronary artery ligation, and demonstrated the effectiveness in this situation of compounds possessing class 1 antidysrhythmic activity (e.g. Lignocaine, ORG6001) and of propranolol. In the present study we have examined whether antidysrhythmic drugs possessing different mechanisms of action (i.e. belonging to classes 3 and 4 in the Vaughan Williams (1974) classification) are similarly effective against these early, life-threatening post-infarction dysrhythmias. The drugs chosen were the calcium antagonists verapamil and nifedipine (class 4 compounds) and melperone, a butyrophenone neuroleptic with antidysrhythmic activity in other experi-

Table 1 Incidence of ventricular fibrillation in all animals (%V.F.) and percentage mortality (%M), together with the total number of ventricular extrasystoles (V.E.) and the duration of ventricular tachycardia (V.T.) in animals surviving 30 min of coronary artery ligation. The percentage incidence of these dysrhythmias is shown in parentheses

	mg/kg	n	*%V.F.*	*%M.*	*V.E.*	*V.T.(s)*
Control		41	54	14	1165 ± 135 (100)	68.1 ± 10.7 (100)
Quinidine	10	8	**0	0	*514 ± 210 (100)	24.5 ± 18.5 **(75)
Melperone	10	8	*12	25	*348 ± 149 (100)	5.0 ***(17)
Sulphinpyrazone	50	8	87	38	1370 ± 235 (100)	82.3 ± 23.5 (100)
Verapamil	0.1	10	60	**50	881 ± 320 (100)	44.5 ± 22.3 (100)
Nifedipine vehicle[a]	1 ml/kg	13	31	31	711 ± 200 (100)	48.8 ± 14.8 (89)
Nifedipine	0.01	10	10	20	301 ± 69 (100)	*11.7 ± 4.0 (100)
Nifedipine	0.05	11	36	18	494 ± 239 (100)	35.4 ± 16.7 (78)

Values are expressed as mean ± s.e. mean for *n* animals. Significance of difference from appropriate control, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. (a) 15% EtOH: 15% PEG 300: 70% H_2O.

mental models which prolongs the cardiac muscle action potential (Arlock, Gullberg & Olsson, 1978), i.e. class 3 activity. Sulphinpyrazone, which has been shown to reduce the incidence of sudden cardiac death following reinfarction (Anturane Reinfarction Trial, 1978) was also examined since it is possible that this may result from an intrinsic antidysrhythmic effect of the compound. All drugs were administered intravenously before coronary artery ligation.

The results are shown in Table 1, where they are compared with those obtained using quinidine, a class 1 compound. Quinidine and melperone were the most effective of these compounds in reducing the severity of the dysrhythmias. The lower dose of nifedipine significantly reduced the duration of ventricular tachycardia. Verapamil had no significant antidysrhythmic activity, and increased the early post-ligation mortality. Sulphinpyrazone had no effect on the dysrhythmias and did not reduce mortality.

These results support our earlier findings that compounds with class 1 activity are very effective in this model. Compounds with class 2 or 3 activity may also be effective. Whether additional protection can be obtained in this situation by combination of drugs with different mechanisms of action warrants further investigation.

References

ANTURANE REINFARCTION TRIAL RESEARCH GROUP (1978). Sulphinpyrazone in the prevention of cardiac death after myocardial infarction. The Anturane Reinfarction Trial. *New Eng. J. Med.*, **298**, 289–295.

ARLOCK, P., GULLBERG, B. & OLSSON S-O.R. (1978). Cardiac electrophysiology of four neuroleptics: melperone, haloperidol, thioridazine and chlorpromazine. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **304**, 27–36.

KANE, K.A., MCDONALD, F.M. & PARRATT, J.R. (1979). Coronary artery ligation in anaesthetised rats as a model for the assessment of antidysrhythmic activity. *Br. J. Pharmac.*, **66**, 436P.

VAUGHAN WILLIAMS, E.M. (1974). Electrophysiological basis for a rational approach to antidysrhythmic drug therapy. *Adv. Drug Res.*, **9**, 69–101.

Interaction of potassium with the two ouabain specific binding sites in guinea-pig heart microsomes

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In guinea-pig atria, in sheep Purkinje fibres and in human heart slices, low doses of ouabain stimulate the Na–K pump, whereas high doses inhibit (Godfraind & Ghysel-Burton, 1979). These two actions are related to the binding of the glycoside to specific high (S) and low (I) affinity sites identified on guinea-pig atria and on a microsomal fraction prepared from guinea-pig and human heart (Godfraind *et al.*, 1980). Since the previous observations in guinea-pig atria showed that KCl increased the maximum binding capacity of the S sites whereas it reduced the affinity of both S and I sites for ouabain (Ghysel-Burton & Godfraind, 1979), we have examined the action of KCl on the binding of ouabain to guinea-pig heart microsomes prepared as previously reported. The enzyme preparations (0.05 unit/ml) were incubated at 37°C in a medium containing 100 mM NaCl, 3 mM $MgCl_2$, 3 mM ATP, 0.1 mM $NaVO_3$, 1 mM EGTA, 20 mM Tris maleate (pH 7.4) various concentrations of [^{3}H]-ouabain (0.19–19 Ci/mmole) and of KCl. [^{3}H]-ouabain specific binding was estimated as described elsewhere (Godfraind *et al.*, 1980). Scatchard plots of [^{3}H]-ouabain binding at equilibrium were linear in the absence of KCl and upward-concave in the presence of KCl, the maximum binding capacity was about 65 pmole ouabain per mg protein whether KCl was present or not.

Dissociation kinetics of [^{3}H]-ouabain were studied after allowing equilibrium binding; their graphical analysis showed a fast and a slow process dependent on KCl concentration. These experiments allowed an estimate to be made of the amount of ouabain bound respectively to the fast (I) and the slow (S) dissociating sites at various concentrations of ouabain in the absence and in the presence of KCl (3 mM). Parameters of binding were calculated from Lineweaver–Burk plots of [^{3}H]-ouabain bound as a function of ouabain concentration. In the absence of KCl, the maximum binding capacity of S sites was equal to 0.4 pmol per mg protein and K_D (apparent dissociation constant) to 4 nM, K_D of I sites being equal to 140 nM. In the presence of KCl (3 mM), the maximum binding

capacity of S sites rose to 1.3 pmol per mg protein and K_D to 130 nM for S sites and to 900 nM for I sites.

The present experiments show that the sensitivities to KCl of the two classes of ouabain specific binding sites are similar in microsomes and in whole tissues.

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References

GHYSEL-BURTON, J. & GODFRAIND, T. (1979). Stimulation and inhibition of the sodium pump by cardioactive steroids in relation to their binding sites and their inotropic effect in isolated guinea-pig atria. *Brit. J. Pharmacol.*, **66**, 175–184.

GODFRAIND, T. & GHYSEL-BURTON, J. (1979). The cardioactive properties of SC4453, a digoxin analogue with a C17β-pyridazine ring. *Eur.. J. Pharmacol.*, **60**, 337–344.

GODFRAIND, T., DE POVER, A. & DI-NUZUZI TONA LUTETE. (1980). Identification with potassium and vanadate of two classes of specific ouabain sites in a $(Na^+ + K^+)$-ATPase preparation from the guinea-pig heart. *Biochem. Pharmacol.*, **28**, 1195–1199.

Interactions of angiotensin and sympathetic reflexes during lower body negative pressure (LBNP) in the cat

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Inhibitors of the renin angiotensin system lower blood pressure and affect cardiovascular reflexes (Clough, Conway, Hatton & Scott, 1979: Gavras, Brunner, Turini, Kershaw, Tifft, Cattelod, Gavras, Vukovich & McKinstry, 1978). The sympathetically-mediated vasoconstrictor reflex response to LBNP is also grossly impaired when these inhibitors are used (Adigun, Clough, Conway & Hatton 1980). The present studies examine the nature of this subtle interaction between angiotensin and the sympathetic nervous system.

Cats were anaesthetized with α-chloralose (80 mg/kg i.p. and respired artificially. The animals were exposed to LBNP at 50 mmHg for 10–15 min and again 30 min later when a converting enzyme inhibitor (CE I SQ 20881) or Saralasin ([Sar^1 ala^8] angiotensin II) was administered during suction. LBNP itself caused transient reductions in central venous pressure (CVP) of 2.2 ± 0.1 cm H_2O ($P < 0.001$) and systemic arterial pressure (BP) 16 ± 0.9 mmHg ($P < 0.001$). While suction was maintained only partial recovery in CVP occurred while BP was restored with 30 seconds. There was a tachycardia of 20 ± 1.2 beats/min ($P < 0.001$) Resting levels of plasma renin activity (ng angiotensin I h^{-1} ml^{-1}) ranged 2.9 ± 0.3 to 3.2 ± 0.4 and rose only slightly after 75 s of suction but significantly after 5 min (to 5.8 ± 0.7 $P < 0.001$) and 10 min (to 6.6 ± 0.8 $P < 0.001$) of suction.

CE1 (1.0 mg/kg i.v.) alone caused only a transient reduction in BP of 15 ± 4.7 mmHg which had recovered within 2 to 5 minutes. The pressor response to AI (200 ng/kg, i.v.) was reduced by 85% 15 min after the injection. CEI administered during suction at 75 s when BP had been restored to control levels caused a fall in pressure of 28 ± 3.2 mmHg to which there was no recovery over the next 10 minutes. CVP was unchanged but heart rate showed a small increase.

In a separate experiment, renal sympathetic efferent nerve activity was recorded before and during suction. LBNP caused a rapid increase in efferent nerve activity within 5 s of approximately 100% which was sustained. CEI given after 6 min of suction reduced BP by 37 ± 5.3 mmHg and sympathetic activity was increased slightly as pressure fell.

Saralasin (8–10 mg/kg/min) alone caused an initial pressor response followed by a depressor effect (−15 ± 4.7 mmHg) which did not show complete recovery at 10 min (5–7 mmHg). When administered during suction at 5 min, Saralasin caused a fall in BP of 37 ± 2.6 mmHg after a short-lived pressor response. Both inhibitors therefore produced a fall in BP during suction which was greater and longer lasting than that observed before suction.

These findings suggest that angiotensin at a level which does not exert a direct vasoconstrictor action, interacts with the sympathetic nervous system to maintain blood pressure when homeostatic reflexes are activated. In the case of CEI interference with these reflexes, involving a peripheral action of angiotensin and the resulting vasodilatation of neurogenic origin, may contribute to its hypotensive effect.

References

ADIGUN, S.A., CLOUGH, D.P., CONWAY, J. & HATTON, R. (1980). The effects of antagonists of the renin angiotensin system on cardiovascular response to lower body

subatmospheric pressure in the anaesthetised cat. *Clin. Sci.*, (In press).

CLOUGH, D.P., CONWAY, J., HATTON, R. & SCOTT, K.L. (1979). The effect of angiotensin converting enzyme inhibitor on baroreceptor reflexes in conscious dogs during sodium depletion. *J. Physiol.*, **295**, 75–76P.

GAVRAS, H., BRUNNER, H., TURINI, G., KERSHAW, G., TIFFT, C., CATTELOD, S., GAVRAS, I., VUKOVICH, R. & MCKINSTRY, D. (1978). Antihypertensive effect of oral angiotensin converting enzyme inhibitor SQ 14225 in man. *N. Eng. J. Med.*, **298**, 991–995.

Attenuation of the rabbit hypothalamo-cardioinhibitory response by α-adrenoceptor blocking drugs

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Electrical stimulation of the rabbit hypothalamus, 1–2 mm from the midline and a similar distance above the lateral mammillary nuclei, evokes strong cardiovascular responses (Evans, 1976). Some resemble those from the cat hypothalamus (Abrahams, Hilton & Zbrożyna, 1960) but in the rabbit, the heart rate (HR) falls. Strong stimulation (250–500 μA, 1 ms, 60 Hz) raises arterial blood pressure (BP) with falls in HR up to 80% due to facilitation of normal baroreflex cardioinhibition (Evans, 1978).

Adult NZW rabbits (2.5–3.5 kg) were anaesthetized with ethyl carbamate (1–1.2 g/kg, i.v.) plus α-chloralose (60–72 mg/kg, i.v.). Stimulation electrodes were placed stereotaxically 1.5 mm lateral and 4 mm caudal to bregma and the depth adjusted for maximum bradycardia during cathodal 250 μA stimulation for 10 seconds.

Administration of phenoxybenzamine, phentolamine or yohimbine (0.01–5 mg/kg, i.v.) resulted in dose-related falls in BP and attentuation of bradycardia (Table 1). Phenoxybenzamine attenuated bradycardia heavily at doses that reduced BP by 20% or less (0.01–0.1 mg/kg). Yohimbine had a weaker action, attenuating bradycardia significantly with negligible effect on BP between 0.1–1 mg/kg. At 3–5 mg/kg it reduced BP by about 15% and attenuated bradycardia heavily. With phentolamine, BP and bradycardia were reduced in similar proportions after doses between 0.01–1 mg/kg.

Attenuation of bradycardia by phenoxybenzamine and yohimbine was not due to less baroreflex cardioinhibition, secondary to low BP. Experiments, in which BP was reduced by haemorrhage, showed that bradycardia was only attenuated 21% after a 20% fall in BP.

None of these drugs attenuated the direct baroreflex cardioinhibition.

The evidence suggests that the facilitatory pathway from the rabbit hypothalamus to baroreflex cardio-

Table 1 Resting mean arterial blood pressure and stimulus-evoked bradycardia, as percentage of control values, after i.v. α-adrenoceptor blocking drugs

Drug		*Cumulated dose (mg/kg)* 0.01	0.1	1	3	5
Phenoxybenzamine	BP	93 ± 3	81 ± 4	60 ± 5	54 ± 1	50 ± 2
	ΔHR	73 ± 8	54 ± 11	21 ± 6	19 ± 3	8 ± 11
Phentolamine	BP	95 ± 1	84 ± 2	63 ± 6	64 ± 3	
	ΔHR	96 ± 3	85 ± 6	57 ± 9	32 ± 8	
Yohimbine	BP		102 ± 3	94 ± 1	84 ± 4	85 ± 4
	ΔHR		95 ± 2	62 ± 6	40 ± 5	29 ± 5

Values are means ± s.e. mean, from 3–7 animals, calculated from the average of 3 replications at each dose level in every animal. Bradycardia (ΔHR) is resting HR minus minimum HR during stimulation, expressed as a percentage of the mean pre-drug response. The attenuation of ΔHR by the drugs is highly significant ($P < 0.01$) in all cases except for Phentolamine 0.01 mg/kg.

inhibitory 'centres' may contain an α-adrenergic synapse.

References

ABRAHAMS, V.C., HILTON, S.M. & ZBROŻYNA, A. (1960). Active muscle vasodilatation produced by stimulation of the brain stem: its significance in the defence reaction. *J. Physiol. (Lond.)*, **154**, 491–513.

EVANS, M.H. (1976). Stimulation of the rabbit hypothalamus: caudal projections to respiratory and cardiovascular centres. *J. Physiol. (Lond.)*, **260**, 205–222.

EVANS, M.H. (1978). Potentiation of a cardioinhibitory reflex by hypothalamic stimulation in the rabbit. *Brain Res.*, **154**, 331–343.

Mechanism of vasodilatation due to adenosine triphosphate and its metabolites in humans

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The vasodilator activity of adenosine triphosphate (ATP) on human skin blood vessels has been known for some time (Duff, Patterson & Shepherd, 1954) although whether its action is direct, acting on vascular smooth muscle or indirect via the release of another vasodilator substance is unclear.

In this study, indirect methods were used to assess the mechanism by which ATP causes cutaneous inflammation in humans. Five subjects received intradermal injections of a range of different doses (0.03–2.56 μmoles) ATP and its breakdown products. The following parameters were assessed: area of erythema at 30 s and 5 min as measured by planimetry, weal (measured by the mean of two perpendicular diameters), duration of erythema and subjective sensations. Histamine (1.63 nmoles) and phosphate-buffered isotonic saline were used as controls. Sigmoid dose-response curves were obtained for ATP. The curves for ADP and AMP were flatter for both areas of erythema and weals. The responses to adenosine, adenine and inosine were not different from those to saline control.

Tachyphylaxis experiments were performed by standard methods. ATP pretreatment induced tachyphylaxis to subsequent injection with ATP or histamine; also histamine induced tachyphylaxis to ATP. Saline control did not induce tachyphylaxis to ATP.

Erythema following intradermal injection of ATP was not blocked by a constricting band. This response is similar to histamine, but different from bradykinin indicating involvement of axon reflex flare. Inhibitor studies were performed following oral pretreatment with diphenhydramine (H_1 antagonist; 200 mg), cimetidine (H_2 antagonist; 800 mg), indomethacin (200 mg) in divided doses over 12 h and doxantrazole (mast cell stabilising agent; 2400 mg) over 48 h prior to the test injections. The ATP responses were significantly inhibited by diphenhydramine ($P < 0.05$) though not to the same degree as histamine ($P < 0.001$). Doxantrazole inhibited the responses to Compound 48/80, which liberates histamine from mast cells, and to the lowest dose of ATP only, but not histamine. Cimetidine or indomethacin pretreatment had no significant effect.

Experimental evidence, therefore, supports a possible role for ATP, from either a neuronal or non-neuronal source, as a mediator of cutaneous inflammation. ATP may work partially by inducing histamine release from mast cells, but also through either a direct effect on cutaneous blood vessels or through the release of other mediators not detected in these studies.

In *in vitro* and animal studies, the mediator responsible for the axon reflex flare elicited by antidromic activity in sensory nerves is unlikely to be any of the classical transmitters such as acetylcholine or catecholamines though ATP and/or substance P may be implicated (Burnstock, 1977). Direct studies assessing ATP in axon reflex flare are needed as are studies on the role of substance P as a possible alternative or co-mediator.

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References

BURNSTOCK, G. (1977). Autonomic Neuroeffector Junctions—Reflex vasodilatation of the skin. *J. Invest. Dermatol.*, **69**, 47–57.

DUFF, F., PATTERSON, G.C. & SHEPHERD, J.T. (1954). A quantitative study of the response to ATP of the blood vessels of the human hand and forearm. *J. Physiol., Lond.*, **125**, 581–589.

Identification of the specific binding of flunarizine to rat aorta

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Flunarizine, the bisfluoroderivative of cinnarizine, inhibits the contraction induced by calcium in depolarized smooth muscle (Van Nueten *et al.*, 1978; Godfraind, 1979). It also reduces the noradrenaline evoked contraction and Ca influx in rat aorta (unpublished observations). The purpose of this work was to analyse the uptake of [^{3}H]-flunarizine by whole aorta and by subcellular fractions of this tissue in order to characterize specific binding sites.

Rat isolated aorta was incubated at 37°C in physiological solution (mM: 112 NaCl, 5 KCl, 25 $NaHCO_3$, 11.5 glucose 1.2 $MgSO_4$, 1 KH_2PO_4, 1.25 $CaCl_2$) gassed with 95% O_2 + 5% CO_2 in the absence or the presence of [^{3}H]-flunarizine at concentrations varying from 10^{-8} M to 5×10^{-7} M.

The uptake of [^{3}H]-flunarizine by the aorta consisted of two components. A nonsaturable uptake had a clearance of 150 l/kg wet wt. A saturable uptake was characterized by a maximum binding capacity of 16 μmole flunarizine/kg wet wt; the concentration for half saturation was equal to 130 nM and the Hill coefficient of the binding was higher than one. This saturable uptake was reversible.

A microsomal fraction was prepared by differential centrifugation of rat aorta homogenates and was incubated at 37°C in a 20 mM Tris-maleate buffer (pH 7.4) with or without [^{3}H]-flunarizine. The reaction was stopped by filtration on glass fibre filters (0.45 μm pore diameter) and the radioactivity retained by the filters was counted by liquid scintillation. The maximum flunarizine uptake was achieved after 4 min incubation and was partly reversible. The binding capacity of the saturable process was equal to about 400 pmole/mg protein and the concentration of flunarizine required for 50% saturation was equal to 70 nM; the Hill coefficient of the binding was also higher than one.

These results indicate that the saturable binding identified in the whole tissue does probably occur on the plasma membrane in which are located the Ca channels that are blocked by flunarizine.

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References

GODFRAIND, T. (1979). Alternative mechanisms for the potentiation of the relaxation evoked by isoprenaline in aortae from young and aged rats. *Eur. J. Pharmacol.*, **53**, 273–279.

VAN NUETEN, J.M., VAN BEEK, J. & JANSSEN, P.A.J. (1978). The vascular effects of flunarizine as compared with those of other clinically and vasoactive substances. *Arzneim. Forsch.*, **28**, 2082–2087.

Comparison of various tryptamine analogues on the human basilar artery and rat aorta *in vitro*

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Recent evidence suggests that the 5-HT induced contraction of certain vascular beds is different from that usually mediated via the classical smooth muscle 'D'-receptor. Some of the vascular tissues in question are the dog carotid (Saxena, 1974), cat pial vessels (Hardebo, Edvinsson, Owman & Svengaard, 1978) and dog saphenous vein (Apperley, Feniuk, Humphrey & Levy, 1980). We have demonstrated that the human basilar arterial strip is much more sensitive to 5-HT than is the rat aortic strip (Forster, Whalley, Mohan & Dutton, 1980) and that antagonists of 5-HT such as methysergide, cyproheptadine and methergoline do not antagonise 5-HT on the human basilar artery *in vitro* in a competitive fashion (Forster & Whalley, 1980).

This study further evaluates 5-HT receptor mechanisms on vascular tissues by comparing the effect of a range of tryptamine analogues on rat aortic and human basilar arterial strips *in vitro*.

Rat aortic and human basilar arterial strips were prepared as described previously (Forster *et al.*, 1980). Dose-response curves were produced to the following compounds: 5-hydroxytryptamine, 5-methoxytryptamine, 5-methyltryptamine, N-methyltryptamine and tryptamine. Experiments were performed in the absence of a monoamine oxidase inhibitor. The results are shown in Table 1.

All the analogues used were full agonists on both types of tissues except 5-methyoxytryptamine which produced a maximum response 60% of the 5-hydroxy-

Table 1 Relative agonist potencies of various tryptamine analogues on the *in vitro* human basilar artery and rat aorta

	Human Basilar Artery		*Rat Aorta*		$\frac{EC_{50}\ Rat\ Aorta}{EC_{50}\ Human\ Basilar\ Artery}$
	EC_{50}[1]	*Potency*[2] *Ratio*	EC_{50}[1]	*Potency*[2] *Ratio*	
5-Hydroxytryptamine	0.015 (0.004–0.053)	1	3.43 (2.49–4.73)	1	229
5-Methoxytryptamine	0.79 (0.56–1.24)	0.019	234 (105.5–521)	0.014	296
N-Methyltryptamine	11.5 (5.95–22.4)	0.0013	20.9 (17.04–25.7)	0.16	1.8
Tryptamine	21.8 (16.9–28.1)	0.0007	23.9 (17.6–32.3)	0.14	1.1
5-Methyltryptamine	11.5 (7.91–16.9)	0.0013	15.6 (12.02–20.2)	0.22	1.4

[1] Results expressed as geometric mean in μM (95% Confidence Limits).
[2] 5-Hydroxytryptamine assigned a potency = 1.
n = 4–8.

tryptamine maximum on the rat aorta. 5-hydroxytryptamine was the most potent compound on both tissues. Tryptamine, 5-methyltryptamine and N-methyltryptamine were essentially equipotent on the rat aorta and human basilar artery. 5-Methoxytryptamine was found to be much more potent on the human basilar artery than the rat aorta. These results provide further evidence to suggest that the receptor sites for 5-HT on the human basilar artery and rat aorta are different and it is interesting to note that Fozard & Mobarok Ali (1978) have previously demonstrated that 5-methoxytryptamine is a potent stimulant of the ileum but is essentially devoid of activity on the rabbit heart.

References

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.*, **68**, 215–224.

FORSTER, C. & WHALLEY, E.T. (1980). Comparison of the 5-hydroxytryptamine-induced contraction of human basilar arterial and rat aortic strips *in vitro*. *Br. J. Pharmac.*, In Press.

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.

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.

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.

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.

An analysis of α_1- and α_2-adrenoceptor mediated pressor effects of adrenaline

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The effects of synthetic agonists have suggested that two populations of α-adrenoceptors on vascular smooth muscle can mediate pressor responses in the pithed rat. One type has a profile of agonist/antagonist 'specificities' similar to smooth muscle 'α_1-adrenoceptors', the other that of neuronal 'α_2-adrenoceptors' (Docherty, MacDonald & McGrath, 1979; Docherty & McGrath, 1980). These receptors may play a physiological role since prazosin-resistant pressor effects of adrenaline are susceptible to yohimbine. This suggests that adrenaline acts on two distinct populations of α-adrenoceptors (Flavahan & McGrath, 1980).

This interpretation of adrenaline's effects relied, however, on the selectivity for α_1-adrenoceptors of prazosin and for α_2-adrenoceptors of yohimbine, which can also antagonise α_1-adrenoceptors (Starke, Borowski & Endo, 1975).

We can now corroborate and extend this hypothesis by substituting corynanthine for prazosin and rauwolscine for yohimbine. Corynanthine and rauwolscine are stereoisomers of yohimbine which are more selective than yohimbine as antagonists at α_1- and α_2-adrenoceptors, respectively (Weitzell, Tanaka & Starke, 1979; Timmermans, Kwa & van Zwieten, 1979; McGrath, 1980).

Male Wistar rats were pithed and were respired with O_2. Drugs were injected via a jugular vein. Successive injections of adrenaline (1 μg/kg) were given to produce reproducible peak arterial diastolic pressor responses, against which antagonists were tested. Successive injections of various antagonists were then given in two groups of experiments, in one of which propranolol (1 mg/kg) was given to eliminate the β_2-adrenoceptor mediated vasodilator effect of adrenaline.

In the absence of propranolol

(a) Corynanthine (1 mg/kg) changed adrenaline's response to a depressor response, i.e. adrenaline reversal.

(b) Rauwolscine (0.2 mg/kg) greatly reduced the pressor response to adrenaline, allowing partial reversal; subsequent prazosin (0.1 mg/kg) completed reversal.

In the presence of propranolol

(a) Corynanthine (1 or 3 mg/kg) produced a similar effect to prazosin (1 mg/kg), reducing adrenaline's pressor response by approximately 40%. The remaining response was little affected by prazosin (1 mg/kg) but was virtually abolished by yohimbine (1 mg/kg). Following prazosin (0.1 mg/kg), corynanthine (10 mg/kg) had little effect.

(b) Rauwolscine (0.2 mg/kg) produced a small reduction (5–20%) of the response to adrenaline, which was less than that produced by yohimbine (1 mg/kg) (50–60%). The remaining response was virtually abolished by prazosin (0.1 mg/kg). Similarly, rauwolscine given after prazosin (0.1 mg/kg) virtually abolished the response. This suggests that the difference between rauwolscine and yohimbine is that yohimbine has some α_1-antagonism.

Thus, for the separation of adrenaline's dual α-adrenoceptor mediated effects corynanthine could substitute for prazosin and rauwolscine was an improvement on yohimbine. The α_1 pressor response to adrenaline (1 μg/kg) could almost equal $\alpha_1 + \alpha_2$ but α_2, alone, produced only half the total. This may simply reflect the non-linear nature of the response but may also reflect the anatomical location of the different receptors (Docherty & McGrath, 1980). Similarly, adrenaline's β-effect could mask the α_2-effect totally and the α_1-effect only slightly less. Caution is indicated in analysing the effects of antagonists with effects at more than one adrenoceptor.

References

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**, 421–422.

DOCHERTY, J.R. & MCGRATH, J.C. (1980). A comparison of pre- and post-junctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus of the rat: evidence for 2 types of post-junctional alpha-adrenoceptor. *Naunyn-Schmiedebergs Arch. Pharmac.* (in press).

FLAVAHAN, N.A. & MCGRATH, J.C. (1980). Blockade by yohimbine of prazosin resistance pressor effects of adrenaline in the pithed rat. *Br. J. Pharmac.*, **69**, 355–359.

MCGRATH, J.C. (1980). Effects of yohimbine analogues on neuronal and smooth muscle α-adrenoceptors in rat vas deferens. (This meeting).

STARKE, K., BOROWSKI, E. & ENDO., T. (1975). Preferential blockade of presynaptic α-adrenoceptors by yohimbine. *Eur. J. Pharmac.*, **34**, 385–388.

TIMMERMANS, P.B.M.W.M., KWA, H.Y. & VAN ZWIETEN, P.A. (1979). Possible subdivision of postsynaptic α-adrenoceptors mediating pressor responses in the pithed rat. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **310**, 189–193.

WEITZELL, R., TANAKA, T. & STARKE, K. (1979). Pre- and postsynaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **308**, 127–136.

Adrenaline-induced cardiovascular depressor effects in conscious dogs

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In anaesthetized rats, central administrations of adrenaline have been shown to induce biphasic blood pressure responses (Ozawa & Uematsu, 1975) in which hypotension follows an initial pressor effect. However, the initial rise in blood pressure has been attributed to a direct action of 'leaked' adrenaline on the peripheral circulation (Struyker Boudier & Bekers, 1975; Borkowski & Finch, 1978) and it would appear that hypotension and bradycardia are the primary effects of centrally administered adrenaline.

McCubbin, Kaneko & Page (1960) considered that central α-adrenoceptor stimulation resulted in hypotension and bradycardia in anaesthetized dogs, while a central β-adrenoceptor involvement with adrenaline-induced cardio-depression has been implicated in conscious rats (Borkowski & Finch, 1977). An attempt has therefore been made to characterize the cardiovascular activity of intracerebroventricular (icv) injections of adrenaline in conscious dogs.

Normotensive male beagle dogs (12.0–18.0 kg) were prepared with an indwelling modified Collison cannula to enable direct injections into the left lateral cerebral ventricle. Catheters were implanted into the left common carotid artery, to facilitate blood pressure recording, and the left jugular vein, to facilitate intravenous injections.

In the conscious dogs, adrenaline (1–40 μg/kg icv), in 50 μl sterile Tyrode solution, induced dose-related reductions in blood pressure and heart rate with no evidence of an initial pressor effect. Central pretreatments with phentolamine (100 μg/kg icv) and yohimbine (100 μg/kg icv) for 30 min did not significantly affect the hypotension or bradycardia induced by the subsequent administration of adrenaline (10 μg/kg icv), suggesting that central α-adrenoceptors are not involved in mediating adrenaline-induced cardiodepression in this species. An involvement of central β-adrenoceptors in these adrenaline induced cardiovascular depressor effects was implicated however, since pretreatments with propranolol (100 μg/kg icv), atenolol (100 μg/kg icv) and metoprolol (50–200 μg/kg icv) reduced the hypotension and abolished the bradycardia induced by adrenaline (10 μg/kg icv).

A central α-adrenoceptor involvement with the hypotension and bradycardia induced by adrenaline (10 μg/kg icv) cannot be ruled out altogether since pretreatment with piperoxan (50–200 μg/kg icv) antagonized these cardiopressor effects in a dose-dependent manner. However, the blockade of adrenaline-induced responses, demonstrated by piperoxan in this study, when other α-adrenoceptor antagonists were ineffective, may be explained if piperoxan is indeed an 'adrenaline-receptor' antagonist as suggested by Hokfelt, Fuxe, Goldstein & Johansson (1974).

Further evidence that adrenaline may be acting on central 'adrenaline-receptors', in eliciting its cardiovascular depressor effects, may be drawn from the inability of either the specific α-adrenoceptor agonist, phenylephrine (1–10 μg/kg icv), or the β-adrenoceptor agonist, isoprenaline (1–10 μg/kg icv) to mimic both adrenaline-induced hypotension and bradycardia.

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References

BORKOWSKI, K.R. & FINCH, L. (1977). Cardiovascular responses to centrally administered adrenaline in spontaneous hypertensive rats. *Br. J. Pharmac.*, **61**, 130P.

BORKOWSKI, K.R. & FINCH, L. (1978). Cardiovascular responses to intraventricular adrenaline in spontaneous hypertensive rats. *Eur. J. Pharmac.*, **47**, 281–290.

HOKFELT, T., FUXE, K., GOLDSTEIN, M. & JOHANSSON, O. (1974). Immunohistochemical evidence of the existence of adrenaline neurons in the rat brain. *Brain Research*, **66**, 235–251.

MCCUBBIN, J.W., KANEKO, Y. & PAGE, I.H. (1960). Ability of serotonin and norepinephrine to mimic the central effects of reserpine on vasomotor activity. *Circulation Research*, **8**, 849–858.

OZAWA, H. & UEMATSU, T. (1975). Centrally mediated cardiovascular responses to intracisternal injections of sympathomimetic amines in anaesthetised rats. *Jap. J. Pharmac.*, **26**, 45–56.

STRUYKER BOUDIER, H.A. & BEKERS, A.D. (1975). Adrenaline-induced cardiovascular changes after intrahypothalamic administration to rats. *Eur. J. Pharmac.*, **31**, 153–155.