When 5-HT, a substrate for MAO-A alone in rat heart, liver and brain was used, double reciprocal plots of initial velocity against substrate concentration with 0, 5, 15 and 50 μM of mexiletine intersected at the same point on the velocity axis. Dixon analysis confirmed that the inhibition produced was competitive with K_i values of 5.5, 5.0 and 3.6 μM for heart, liver and brain respectively. Mexiletine also competitively inhibited the deamination of tyramine by all three tissues.

When benzylamine was used as substrate, mexiletine competitively inhibited its deamination by the heart but not by the liver, where benzylamine is metabolized by MAO-B alone. The same pattern of inhibition was also seen with β-phenylethylamine. Here, mexiletine competitively inhibited the deamination of β-phenylethylamine by MAO-A in the rat heart, but had no effect on the deamination in either liver or brain where this substrate is metabolized by MAO-B. In all cases, control experiments with d- and l-amphetamine produced closely similar results. Preincubation of the homogenates with mexiletine before the addition of substrate did not increase the magnitude of the resulting inhibition. Preincubation of heart and liver homogenates, before the addition of 5-HT, with mexiletine (10^{-4} M) and pargyline (10^{-5} M) protected the MAO-A in both tissues from irreversible inhibition by over 80%.

Mexiletine appears to be a reversible competitive inhibitor that is selective for MAO-A in rat heart, liver and brain, as defined by its substrate-specificity. In this respect mexiletine closely resembles amphetamine.

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References

BLASCHKO, H., RICHTER, D. & SCHLOSSMAN, H. (1937). The oxidation of adrenaline and other amines. *Biochem. J.*, **31**, 2187–2196.

MANTLE, T.J., TIPTON, K.F. & GARRETT, N.J. (1976). Inhibition of monoamine oxidase by amphetamine and related compounds. *Biochem. Pharmac.*, **25**, 2073–2077.

PUGH, C.E.M. & QUASTEL, J.H. (1937). Oxidation of amines by animal tissues. *Biochem. J.*, **31**, 2306–2321.

SINGH, B.N. & VAUGHAN WILLIAMS, E.M. (1972). Investigations of the mode of action of a new antidysrhythmic drug, Kö 1173. *Br. J. Pharmac.*, **44**, 1–9.

A model to test the relative potencies of phosphodiesterase inhibitors in brain (*in vivo*)

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Phosphodiesterase occurs in high concentrations in brain (Klainer, Chi, Friedberg, Rall & Sutherland, 1962) and provides the major route for the breakdown of cAMP (Butcher & Sutherland, 1962). Adenylate cyclase has been postulated to be a part of the amine receptor (Rodbell, 1971) and in consequence one would expect enhancement of aminergic mechanisms in the CNS by the use of drugs which inhibit phosphodiesterase. Many drugs possess such properties and have been found to be potent *in vitro* inhibitors. The brain, however, presents specific physical barriers to drugs depending on their structure and hence it is important to assess their potency *in vivo*.

To this end groups of 3 male albino Wistar rats were lightly anaesthetized and 20 μl of [^{14}C]-cAMP (specific activity 278 mCi/m mol) injected intraventricularly by the method of Noble, Wurtman & Axelrod (1967) and killed at 0, 1.5, 3, 6, 15, 30, 60 and 120 min after the injection. The brain was removed rapidly and placed in liquid nitrogen. The [^{14}C]-cAMP content was determined by a modification of the combined methods of Schultz & Daly (1973) and Krishna, Weiss & Brodie (1968). A decay curve for the injected [^{14}C]-cAMP was obtained. Animals in groups of 6 were then pretreated for 30 min with 2.5, 5, 10, 20 and 40 mg/kg i.p. of the phosphodiesterase inhibitor, ICI 63197 (Nahorski & Rogers, 1975) and killed in pairs at 8, 12 and 20 min as indicated to be optimum by the decay curve. To compare all these results a variance analysis was used splitting the data in smaller groups when the interaction between the involved factors was significant. To study the potency of the phosphodiesterase inhibitory effect compared with control and ICI 63197 the means were compared using the Student *t* test when the variance analysis was significant ($P < 0.05$). All doses of ICI 63197 gave a significant increase in [^{14}C]-cAMP ($P < 0.01$ between doses—analysis of variance, and $P < 0.05$ at lowest dose compared with control, Student *t* test). Other drugs (diazepam, theophylline, trifluoperazine and desipramine) were compared on a similar schedule with ICI 63197. Both diazepam (2.5, 5 and 10 mg/kg i.p.) and theophylline (5, 10 and 30 mg/kg i.p.) showed evidence of phosphodiesterase inhibitory activity. Diazepam significantly increased the concentration of [^{14}C]-cAMP with increasing dosage ($P < 0.01$ analysis of variance) but was only

significantly different from control at 10 mg/kg ($P < 0.01$ Student t). It was significantly less potent than ICI 63197 ($P < 0.01$ analysis of variance) and a 10 mg/kg dose of diazepam was equipotent with a 5 mg/kg dose of ICI 63197. The theophylline effect also increased with dose ($P < 0.01$ analysis of variance). It was less potent than ICI 63197 ($P < 0.01$ analysis of variance), both 20 mg/kg and 40 mg/kg giving the same difference from control values as 10 mg/kg of ICI 63197.

Desipramine (3.75, 7.5 and 15 mg/kg i.p.) and trifluoperazine (1.25, 2.5 and 5 mg/kg, i.p.) both of which have been shown to have phosphodiesterase inhibiting properties *in vitro* (Janiec, Korczak-Dziuba & Herman, 1974) showed the same potency as the lowest dose used of ICI 63197 but showed no dose response relationship.

The model appears to be a convenient one to evaluate the *in vivo* potency of potential central phosphodiesterase inhibition.

References

BUTCHER, R.W. & SUTHERLAND, E.W. (1962). Adenosine 3',5'-phosphate in biological materials. I. Purification and properties of cyclic 3',5'-nucleotide phosphodiesterase and use of this enzyme to characterize adenosine 3',5'-phosphate in human urine. *J. Biol. Chem.*, **237**, 1244–1250.

JANIEC, W., KORCZAK-DZIUBA, K. & HERMAN, Z.S. (1974). Effect of phenothiazine neuroleptic drugs and tricyclic antidepressants on phosphodiesterase activity in rat cerebral cortex. *Psychopharmacologia (Berl.)*, **37**, 351–358.

KLAINER, L.M., CHI, Y.-M., FREIDBERG, S.L., RALL, T.W. & SUTHERLAND, E.W. (1962). Adenyl cyclase IV. The effects of neurohormones on the formation of adenosine 3',5'-phosphate by preparations from brain and other tissues. *J. Biol. Chem.*, **237**, 1239–1243.

KRISHNA, G., WEISS, B. & BRODIE, B.B. (1968). A simple sensitive method for the assay of adenyl cyclase. *J. Pharmac. exp. Ther.*, **163**, 379–385.

NAHORSKI, S.R. & ROGERS, K.J. (1975). The effect of phosphodiesterase inhibitors on the stimulation and cerebral cyclicAMP formation by biogenic amines *in vitro* and *in vivo*. *Br. J. Pharmac.*, **54**, 272P.

NOBLE, E.P., WURTMAN, R.J. & AXELROD, J. (1967). A simple and rapid method for injecting ^{3}H-norepinephrine into the lateral ventricle of the rat brain. *Life Sci.*, **6**, 281.

RODBELL, M. (1971). Hormones, receptors and adenylcyclase activity in mammalian cells. In: *Colloquium on the role of adenyl cyclase and cyclic 3',5'-AMP in biological systems*, eds. Condliffe, P. & Rodbell, M., pp. 88–95. Fogarty International Center, U.S. government printing office.

SCHULTZ, J. & DALY, J.W. (1973). Cyclic adenosine 3',5'-monophosphate from endogenous adenosine triphosphate and from radioactive adensoine triphosphate formed during a prior incubation with radioactive adenine. *J. Biol. Chem.*, **248**, 843–852.

The effect of chronic administration of D-penicillamine on the rat pancreas

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In response to secretin the exocrine pancreas secretes a watery juice rich in bicarbonate. There is evidence to suggest that this secretion is produced by ductular elements (Bencosme & Lechago, 1971) which comprise less than five per cent of the mass of the gland (Bolender, 1974).

Feeding rats a copper deficient diet containing D-penicillamine (2 g/kg) selectively destroys pancreatic acinar tissue whilst leaving ductular elements functionally intact (Folsch & Creutzfeldt, 1976). After six weeks on this diet weaning rats exhibited a reduced rate of growth and a much reduced pancreas. Light microscopy indicated a non-inflammatory atrophy and fatty infiltration of pancreatic acinar tissue. Ductular elements were unaffected.

Amylase activity was markedly decreased in pancreatic homogenates from penicillamine treated animals whereas the activities of Mg^{2+} and Ca^{2+} stimulated adenosine triphosphatases (ATPases) together with succinic dehydrogenase (SDH) increased (Table 1). The ratio of the mean enzyme activities in penicillamine treated and control animals was about the same for Mg^{2+}-ATPase (2.7), Ca^{2+}-ATPase (3.2) and the mitochondrial marker SDH (2.6), suggesting that the elevated ATPase activities could have resulted from a relative increase in the concentration of mitochondria in the tissue.

The hormone responsiveness of the duct cells was investigated by determining the effects of secretin on cyclic AMP levels *in vitro*. In unstimulated glands the cyclic AMP levels for control and penicillamine treated animals were 4.7 ± 0.6 (24) and 23.8 ± 6.0 (24) μmoles cyclic AMP/kg protein ($P < 0.004$) and in the presence of secretin (0.25 C.U./ml) 10.9 ± 1.2 (24) and 77.8 ± 10.1 (24) μmoles cyclic AMP/kg protein ($P < 0.0005$). The magnitude of this stimulation was 2.3 and 3.3 times in control and penicillamine treated animals respectively, indicating that the sensitivity of the gland to secretin is not decreased by penicillamine treatment.