responses, rapidly for angiotensin and prostaglandin E_2 but slowly for potassium.

Tyrode containing 2,4-dinitrophenol (0.05 mmol) caused little reduction of the phasic responses to angiotensin and prostaglandin E_2 but a progressive reduction of tonic responses to $4.0 \pm 1.9\%$ ($n=6$) and $3.6 \pm 2.5\%$ ($n=6$) respectively after 65 minutes. The corresponding potassium response was reduced to $42.0 \pm 6.7\%$ ($n=6$) ($P<0.01$).

Angiotensin, prostaglandin E_2 and potassium responses were recorded following a 30 min exposure to Tyrode solution containing imidazole (0.05 mol) or theophylline (0.3 mmol) and plotted as a percentage of the maximum response against log concentration. Imidazole caused a non-specific potentiation of all responses. Theophylline caused a reduction of responses to all agonists but the ED_{50} log concentration shift was significantly greater for angiotensin (0.45 ± 0.05, $n=6$) and prostaglandin E_2 (0.64 ± 0.16, $n=6$) than for potassium (0.14 ± 0.03, $n=6$) ($P<0.001$). Isoprenaline (5×10^{-9}–1.6×10^{-7} mol) added 30 s before the agonists caused a dose dependent decrease in the responses, the reduction of the potassium responses was significantly less than those of angiotensin or prostaglandin E_2 ($P<0.001$).

These findings support suggestions that potassium causes depolarization of smooth muscle (Goodman & Weiss, 1971) associated with calcium influx and subsequent intracellular release (Cheng, 1976). Responses to angiotensin and prostaglandin E_2 may consist of two phases, an initial transient associated with depolarization and a sustained tonic response independent of depolarization but dependent upon metabolic energy. This supports other reports of the energy dependence of prostaglandin (Coceani & Wolfe, 1966) and angiotensin (Crocker & Wilson, 1975) responses. This tonic phase of the angiotensin response is more dependent upon extracellular calcium than that of prostaglandin E_2. Increases in cyclic AMP induced by isoprenaline or theophylline had little effect upon potassium responses but markedly reduced angiotensin or prostaglandin E_2 sustained responses which may be related to an effect upon calcium distribution.

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Substrate selective inhibition of monoamine oxidase by mexiletine

B.A. CALLINGHAM

Department of Pharmacology, University of Cambridge, Hills Road, Cambridge, CB2 2QD

Mexiletine (1-methyl-2-(2,6-xylyloxy)-ethylamine hydrochloride, Kö 1173) is an effective antiarrythmic agent in man and other animals, with a local anaesthetic potency comparable with that of lignocaine, to which it shows a structural similarity (Singh & Vaughan Williams, 1972). However, mexiletine is also an α-substituted monoamine, which suggested that it could inhibit monoamine oxidase (MAO), since many compounds with this structure are known to possess this property (Blaschko, Richter & Schlossmann, 1937; Pugh & Quastel, 1937; Mantle, Tipton & Garrett, 1976).

The hearts, livers and brains of male Wistar rats of 300–350 g body weight, were homogenized in 1 mM potassium phosphate buffer, pH 7.4. The homogenates were centrifuged at low speed to remove unbroken cells and nuclei, and the supernatant fractions used for all experiments. MAO activity was assayed radiochemically with [^{3}H]-tyramine, [^{3}H]-5-HT, [^{14}C]-β-phenylethylamine and [^{14}C]-benzylamine as substrates.

The effects of mexiletine were measured *in vitro* by addition to aliquots of the tissue homogenates either 20 min before or at the same time as the addition of substrate. All incubations were carried out in an atmosphere of oxygen at 37°C, and repeated at least 5 times.

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.

Supported by a grant from the British Heart Foundation. My thanks are due to Boehringer Ingelheim, Ltd., for generous supplies of mexiletine.

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

A. CHIU, D. ECCLESTON & T. PALOMO (introduced by C.M. YATES)

MRC Brain Metabolism Unit, Department of Pharmacology, University of Edinburgh

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