The electrolyte composition of the basic Ringer solution was: NaCl, 111 mM; KCl, 3 mM; $CaCl_2$, 1·8 mM; $NaHCO_3$, 2·38 mM. In other experiments chloride was replaced, completely, by various anions to make up sulphate, gluconate and isethionate Ringer solutions. In all Ringer solutions, sucrose was included to bring the measured osmolality to 224 m-osmole/kg H_2O. After an equilibration period, theophylline ($2{\cdot}5 \times 10^{-3}$M) was added to the inner bathing solution.

The stimulatory effect of theophylline on active sodium transport (measured as I_{sc}) across skins mounted in chloride Ringer was similar to that described by others (Baba, Smith & Townshend, 1967; Cuthbert & Painter, 1968a).

However, theophylline also caused clear changes—increased I_{SC} and decreased R—in skins mounted in sulphate, gluconate and isethionate Ringer solutions, i.e., in the absence of chloride. Typical examples are depicted in Fig. 1.

We conclude that the presence of chloride is not necessary to demonstrate the influence of theophylline on active sodium transport; and we consider it likely that the effect of theophylline in chloride-free solution is mediated via accumulation of cyclic 3′, 5′-AMP.

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The mechanism of potentiation of inotropic responses to phenylephrine by theophylline

R. MASSINGHAM† (introduced by P. A. NASMYTH), *Department of Pharmacology, St. Mary's Hospital Medical School, London, W.2*

Phenylephrine (10–200 μg) sometimes produced small inotropic effects in the isolated perfused heart of the frog. Thirty to sixty minutes after the inclusion of theophylline hydrate (5 mM) in the perfusion fluid, all these doses of phenylephrine produced inotropic effects which were bigger than pre-existing ones and which could be blocked by propranolol ($3{\cdot}5 \times 10^{-6}$M). Iminazole (10–50 mM) prevented the inotropic effect of phenylephrine.

It seems unlikely that the potentiation of the inotropic effect of phenylephrine was due to inhibition of uptake, since the uptake site has a low affinity for phenylephrine (Iversen, 1964). Furthermore theophylline potentiated inotropic responses to tyramine (100–400 μg) in parallel with inotropic responses to phenylephrine, whereas cocaine ($2{\cdot}25 \times 10^{-5}$M) in the presence of theophylline potentiated the effects of phenylephrine but inhibited those of tyramine.

Bianchi (1961) has shown that caffeine increases intracellular calcium ions in skeletal muscle, but increasing the concentration of Ca^{2+} in the perfusion fluid from 1·5 mM, by varying amounts, up to 4·5 mM only increased the amplitude of the heart beat. It did not affect the response induced by phenylephrine.

Since the concentrations of theophylline and iminazole used have been shown to inhibit and activate phosphodiesterase respectively (Butcher & Sutherland, 1962), the results described here indicate that the potentiation of the inotropic effects of phenylephrine by theophylline is mediated by its action on phosphodiesterase and indirectly support the hypothesis that adenylcyclase is part of the β-effector system (Robison, Butcher & Sutherland, 1967).

† M.R.C. Scholar.

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A reinvestigation of the substrate specificity of pig kidney diamine oxidase

W. G. BARDSLEY*, C. M. HILL and R. W. LOBLEY (introduced by H. SCHIEDEN), *Department of Physiology, University of Manchester, Manchester* 13, and *Department of Chemistry, University of Salford, Salford*

Pig kidney diamine oxidase (Histaminase, E.C. No. 1.4.3.6) was purified 600-fold and the substrate pattern was reinvestigated using known substrates and several other compounds. All substrates used were purified as hydrochlorides by recrystallization until homogeneous by thin layer chromatography, and pure as judged by melting point. Satisfactory infrared and proton magnetic resonance spectra were recorded for all compounds synthesized. The oxidation of substrates was followed manometrically and the results did not always agree with those previously reported (Blaschko & Chrušciel, 1959; Zeller, Fouts, Carbon, Lazana & Voegtli (1956). The differences are probably attributable to the use of more highly purified materials. Oxidation was expressed as a percentage of the rate for cadaverine as measured concurrently.

Ortho- and para-isomers of bis(aminomethyl) benzene were synthesized. The rate of oxidation of the para-isomer was found to be 50% of that of cadaverine, whereas the ortho- and meta-isomers were not substrates but were good inhibitors of cadaverine oxidation.

Ortho-, meta- and para-isomers of bis(2-aminoethyl) benzene were also synthesized. The ortho- and para-isomers were not oxidized and the meta-isomer was a poor substrate (7%).