On the basis of these observations, the non-adrenergic inhibitory innervation of the rat stomach is unlikely to be purinergic.

I am grateful for the following gifts of drugs; dipyridamole (Boehringer-Ingelheim Co. Ltd.) hexobendine (Osterreichische Stickstoffwerke A.G.) and 2-2'pyridylisatogen tosylate (Dr D.F. Weetman, School of Pharmacy, Sunderland Polytechnic).

References

BURNSTOCK, G. (1972). Purinergic nerves. *Pharmac. Rev.*, **24**, 510-581.

HEAZELL, M.A. (1974). The innervation and Receptor. Population of the Rat Gastric Fundus. *Ph.D. Thesis. University of London.*

HOOPER, M., SPEDDING, M., SWEETMAN, A.J. & WEETMAN, D.F. (1974). 2-2' pyridylisatogen tosylate: an antagonist of the inhibitory effects of ATP on smooth muscle. *Br. J. Pharmac.*, **50**, 458-459P.

SATCHELL, D.G., LYNCH, ANGELA, BOURKE, P.M. & BURNSTOCK, G. (1972). Potentiation of the effects of exogenously applied ATP and purinergic nerve stimulation on the guinea-pig taenia coli by dipyridamole and hexobendine, *Eur. J. Pharmac.*, **19**, 343-350.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344-349.

Comparison of the effects of selective α- and β-receptor agonists on intracellular cyclic AMP levels and glycogen phosphorylase activity in guinea-pig liver

D. OSBORN
(introduced by D.H. JENKINSON)

University College London, Department of Pharmacology

(−)-Isoprenaline (a selective β-receptor agonist) and (±-amidephrine (a selective α-receptor agonist) have been shown to increase glucose release from guinea-pig liver slices (Haylett & Jenkinson, 1972a,b). The effects of these agents on intracellular cyclic AMP levels and glycogen phosphorylase activity have now been measured under similar experimental conditions. The slices were exposed to the agonists for 2 min, at which time increased glucose release was substantial but not maximal. Cyclic AMP was determined by a protein binding technique (Tovey, Oldham & Whelan, 1974) in samples extracted from slices by TCA precipitation of acid insoluble material, after freezing the tissue in liquid nitrogen. The supernatant was passed through a 3.0 x 0.5 cm column of Dowex 50 (H^+ form) to remove the TCA and substances that might interfere with the cyclic AMP assay. Glycogen phosphorylase activity was measured by the method of Danforth, Helmreich & Cori (1962). Glucose release was measured by the procedure of Park & Johnson (1949).

Doses of agonist which cause near maximal glucose release–isoprenaline 20 nM, amidephrine 20 μM–both produce comparable increases in phosphorylase activity. However while the cyclic AMP level in slices treated with this dose of isoprenaline was significantly greater than the control values ($P < 0.05$), there was little change in the cyclic AMP level in slices treated with amidephrine (Figure 1). Glucose release in the 2 min exposure to amidephrine was 53.8 ± 11.8% ($n = 16$) above control release, as compared with 25.9 ± 8.1% ($n = 15$) after isoprenaline. The increase in phosphorylase activity was shown to be dose-related between 4 and 20 μM amidephrine. The response to 20 μM amidephrine, but not that to 20 nM isoprenaline, was abolished in the presence of 40 μM phentolamine.

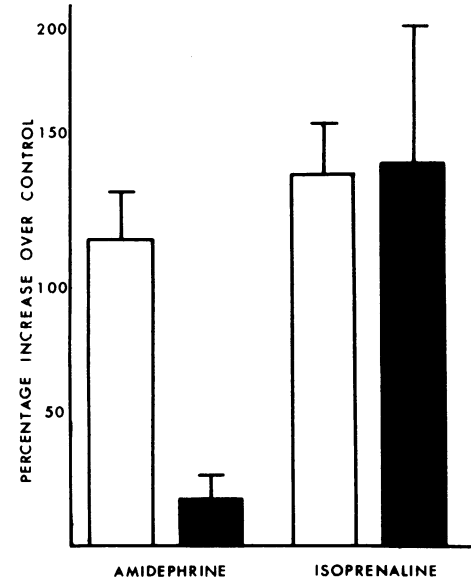

Figure 1 Effect of amidephrine (20 μM) and isoprenaline (20 nM) on glycogen phosphorylase activity (□) and tissue level of cyclic AMP (■). Each histogram represents the mean of at least 7 experiments; the vertical bar represents one s.e. mean.

These results suggest that amidephrine activates glycogen phosphorylase by an α-receptor mediated mechanism not associated with a rise in the intracellular level of cyclic AMP.

I note the receipt of an MRC Training award, and thank Mrs M. Butler for her excellent technical assistance.

References

DANFORTH, W.H., HELMREICH, E. & CORI, C.F. (1962). Effect of contraction and epinephrine on the phosphorylase activity of frog sartorius muscle. *Proc. Nat. Acad. Sci.*, 48, 1191-1199.

HAYLETT, D.G. & JENKINSON, D.H. (1972a). Effects of noradrenaline on potassium efflux, membrane potential, and electrolyte levels, in tissue slices prepared from guinea-pig liver. *J. Physiol.*, 225, 721-750.

HAYLETT, D.G. & JENKINSON, D.H. (1972b). The receptors concerned in the action of catacholamines on glucose release, membrane potential, and ion movements in guinea-pig liver. *J. Physiol.*, 225, 751-772.

PARK, J.T. & JOHNSON, M.J. (1949). A submicro determination of glucose. *J. biol. Chem.*, 181, 149-151.

TOVEY, K.C., OLDHAM, K.G. & WHELAN, J.A.M. (1974). A simple direct assay for cyclic AMP in plasma and other biological samples using a protein binding technique. *Clin. Chim. Acta*, 56, 221-234.

Cyclic AMP in HeLa cells stimulated with cholera enterotoxin and methylxanthines

J.J. KING & D.V. MAUDSLEY*

Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts, U.S.A.

There are two general approaches being used to elucidate the regulatory role of cyclic AMP in cell growth. One is to add cyclic AMP or its analogues to the system under study and observe its effects upon various parameters of cell activity. The other approach is to alter the intracellular levels of cyclic AMP by modulation of adenyl cyclase or phosphodiesterase activity. We report here our attempts to manipulate endogenous cyclic AMP in cells in tissue culture.

HeLa cells were grown in suspension culture in Jokliks medium containing 7% calf serum to a density of 8×10^5 cells/ml. Cyclic AMP levels were measured as described previously (Albano, Barnes, Maudsley, Brown & Ekins, 1974). In cells incubated in the presence of 8 mM theophylline cyclic AMP levels were increased by cholera enterotoxin (1 μg per 10^6 cells) but not by prostaglandin E_1 (10 μg) or adrenaline (10 μg). In the absence of theophylline, toxin produced little or no increase in cyclic AMP. A characteristic of toxin stimulation of cyclic AMP is the existence of a lag period between the addition of the toxin and the increase in cyclic AMP (Pierce, Greenough & Carpenter, 1971). In HeLa cells the lag period is of 5-10 min duration which is much shorter than it is for several other systems such as adrenal cells (Haksar, Maudsley & Péron, 1975).

Theophylline alone in concentrations up to 32 mM or 3-isobutyl-1-methylxanthine in concentrations up to 2 mM did not increase the basal levels of cyclic AMP. These concentrations of theophylline and 3-isobutyl-1-methylxanthine, however, did reduce the uptake of [^{3}H]-leucine. Caffeine had similar effects and of the agents used 3-isobutyl-1-methylxanthine was the most potent and caffeine the least active. Thus, while the relative potencies of the methylxanthines approximate their known ability to inhibit cyclic nucleotide phosphodiesterases the effects upon transport may not be mediated by cyclic AMP. In this system, therefore, methylxanthines do not appear to be useful for the manipulation of basal cyclic AMP levels. Furthermore, these results also indicate that in HeLa cells cyclic AMP produced in response to toxin is turned over more rapidly than cyclic AMP in the resting cell.

References

ALBANO, J.D.M., BARNES, G.D., MAUDSLEY, D.V., BROWN, B.L. & EKINS, R.P. (1974). Factors affecting the saturation assay of cyclic AMP in biological systems. *Anal. Biochem.*, 60, 130-141.

HAKSAR, A., MAUDSLEY, D.V. & PERON, F.G. (1975). Stimulation of cyclic adenosine 3'5'-monophosphate and corticosterone formation in isolated rat adrenal cells by cholera enterotoxin. Comparison with the effects of ACTH. *Biochim. Biophys. Acta*, 381, 308-323.

PIERCE, N.F., GREENOUGH, W.B. & CARPENTER, C.C. (1971). Vibrio cholerae enterotoxin and its mode of action. *Bacteriol. Rev.*, 35, 1-13.