Inhibition of Adenosine 3':5'-Cyclic Monophosphate Phosphodiesterase

By J. B. SMITH and D. C. B. MILLS. (*Medical Research Council Thrombosis Research Group, Department of Pharmacology, Royal College of Surgeons of England, Lincoln's Inn Fields, London W.C.2, U.K.*)

We have compared the activities of the following drugs as inhibitors of cyclic AMP (adenosine 3':5'-cyclic monophosphate) phosphodiesterase from three sources: theophylline, caffeine, papaverine, dipyridamole and RA 233 [2,6-bis(diethanolamino)-4-piperidinopyrimido[5,4d]pyrimidine]. The enzyme preparations were ox heart phosphodiesterase [Boehringer Corp. (London) Ltd.], dialysed rabbit brain supernatant (Cheung, 1967) and dialysed human platelet supernatant (Marquis, Vigdahl & Tavormina, 1969). Enzyme activities were measured in 0.10 ml incubation mixtures containing 5 mM-magnesium sulphate, 20 mM-tris-HCl buffer, pH 7.4, and 3 μ M-cyclic [8-³H]AMP (3 Ci/mmol). The reactions were started by the addition of enzyme and stopped after 10 min at 37°C by the addition of 1.0 ml of 0.5 M-HClO₄ containing 1000 c.p.m. of cyclic [8-¹⁴C]AMP as a standard for recoveries. Cyclic [³H]AMP remaining was determined by the method of Krishna, Weiss & Brodie (1968). The enzyme concentration was chosen to give 80–90% conversion in the absence of inhibitors.

Papaverine and dipyridamole were more active than caffeine and theophylline as inhibitors of phosphodiesterase activity in all three preparations, and theophylline was more active than caffeine. RA 233 and papaverine were particularly effective against the human platelet phosphodiesterase.

Prostaglandin E₁ inhibits the aggregation of platelets induced by ADP (Kloeze, 1967) and increases the incorporation of radioactivity into cyclic AMP in human platelets containing labelled nucleotides (Marquis *et al.* 1969; Mills, Smith & Born, 1970). Both of these effects are increased by phosphodiesterase inhibitors, and the activities of the five drugs tested as potentiators of prostaglandin E₁ were closely related to their relative abilities to inhibit the phosphodiesterase from platelets when a concentration of 3 μ M-cyclic AMP was used for the assay.

These results support the theory that inhibition of ADP-induced platelet aggregation by a number of drugs is mediated by their effects on platelet cyclic AMP concentrations.

Cheung, W. V. (1967). *Biochemistry, Easton*, 6, 1079.
Kloeze, J. (1967). In *Prostaglandins*, p. 241. Ed. by Bergström, S. & Samuelsson, S. A. London: Interscience Publishers (Inc.) Ltd.

Krishna, G., Weiss, B. & Brodie, B. B. (1968). *J. Pharmac. exp. Ther.* 163, 379.
Marquis, N. R., Vigdahl, R. L. & Tavormina, P. A. (1969). *Biochem. biophys. Res. Commun.* 36, 965.
Mills, D. C. B., Smith, J. B. & Born, G. V. R. (1970). *Proc. 18th Symp. Blood, Detroit* (in the Press).

Evidence for Oscillatory Properties in the Concentrations of Adenosine 3':5'-Cyclic Monophosphate Produced by Cardiac Sarcoplasmic Reticulum

By I. M. GILLIBRAND. (*Cardiac Department, St Bartholomew's Hospital, London E.C. 1, U.K.*)

It has been postulated (Butcher, Ho, Meng & Sutherland, 1965) that changes in intracellular concentrations of cyclic AMP (adenosine 3':5'-cyclic monophosphate) may indicate a mechanism by which adrenergic hormones may act on the adenylate cyclase system. Measurements of cyclic AMP in whole tissues is difficult, because of the problem of membrane permeability (Krause, Halle, Kallabis & Wollenberger, 1970). Homogenized cardiac sarcoplasmic-reticulum fractions (Hess, Briggs, Shinebourne & Hamer, 1968) were used because of their high adenylate cyclase specific activity.

In the method of Krishna, Weiss & Brodie (1968) with 1 μ Ci of [8-¹⁴C]ATP/determination on duplicate samples, the error of the method was calculated to be approx. $\pm 2\%$. The results indicate that the concentrations of cyclic AMP produced by the membrane system can both increase (adenylate cyclase activity) and decrease (phosphodiesterase activity) to an extent far greater than the calculated percentage error of the method. Duplicate measurements at regular intervals over a period of 20 min showed a steep rise from zero to a maximum value, followed by a minimum value, a second maximum and a second minimum, so that the values for maxima and minima successively tended towards a median value. In accordance with oscillatory properties, the rising gradients were in proportion to the maximum value, as also were the decreasing gradients to the minimum value.

Preliminary results on the effect of 10 μ M-ouabain-2.0 mM-Ca²⁺ showed an oscillation of increased amplitude but similar frequency.

The results agree with the theoretical work of Spangler & Snell (1961), in which it was calculated that, if two enzymes each influence the product of the other, they will produce sustained oscillations, in for example a membrane having non-equilibrium kinetics. In addition, Mueller & Rodin (1968) proposed an allosteric gate pump mechanism for the control of energy levels, in which electrochemical energy in a transmembrane ion gradient, high-

energy electrons of the redox system and high-energy phosphate bonds in ATP are closely coupled. Therefore the specific determination of an intermediary compound between two such enzymes in a structurally organized system may be a direct way of measuring the interactions (Jardetzky, 1966) and the related permeability properties occurring within the membrane itself.

This work was supported by a grant from the British Heart Foundation.

- Butcher, R. W., Ho, R. J., Meng, H. C. & Sutherland, E. W. (1965). *J. biol. Chem.* **240**, 4515.
 Hess, M. L., Briggs, F. N., Shinebourne, E. & Hamer, J. (1968). *Nature, Lond.*, **220**, 79.
 Jardetzky, O. (1966). *Nature, Lond.*, **211**, 969.
 Krause, E.-G., Halle, W., Kallabis, E. & Wollenberger, A. (1970). *J. molec. cell. Cardiol.* **1**, 1.
 Krishna, G., Weiss, B. & Brodie, B. B. (1968). *J. Pharmac. exp. Ther.* **163**, 379.
 Mueller, P. & Rodin, D. O. (1968). *Nature, Lond.*, **217**, 713.
 Spangler, R. A. & Snell, F. M. (1961). *Nature, Lond.*, **191**, 457.

The Effects of Theophylline on Mucosubstance Secretion by Rous Sarcoma Cells

By D. M. COE, J. T. GALLAGHER, J. S. GIBSON and J. C. MARSDEN. (*Department of Biology, University of York, York, U.K.*)

Cell cultures of rat submaxillary gland maintain the secretion of mucosubstances for many months (Gallagher & Marsden, 1969). The rate of secretion is stimulated 28% ($P < 0.02$) by 0.1 mM-adrenaline and 111% ($P < 0.001$) by vasopressin (0.5 i.u./ml.). Similar observations have been made on mucosubstance release by Rous sarcoma cells.

The action of both adrenaline and vasopressin is believed to involve cyclic AMP (adenosine 3':5'-cyclic monophosphate) (Robison, Butcher & Sutherland, 1968).

At 5 mM the dibutyryl derivative of cyclic AMP stimulates the secretion of mucosubstances by Rous sarcoma cells nearly threefold. Derivatives of cyclic AMP have also been found to stimulate secretion in rat parotid gland (Babad, Ben-Zvi, Bdolah & Schramm, 1967) and in rabbit pancreas (Ridderstrap & Bonting, 1969), and in both cases stimulation could also be achieved with theophylline, which, by inhibiting phosphodiesterase, elevates the concentrations of endogenous cyclic AMP (Humes, Rounbehler & Kuehl, 1969). In Rous sarcoma cells, however, 5 mM-theophylline caused a marked inhibition (47%; $P < 0.1$) of mucosubstance release; only at lower concentrations (1.7 mM) was a stimulation (68%; $P < 0.1$) noted. Thus the effect

of theophylline appears to involve rather more than simple inhibition of the breakdown of cyclic AMP.

Theophylline has been found to inhibit protein synthesis in rat adrenal gland (Halkerston, Feinstein & Hechter, 1966), and we have found that puromycin at a concentration of 100 μ g/ml inhibits virtually completely the incorporation of D-[1- ^{14} C]-glucosamine into mucosubstances by cell cultures; a similar effect on the synthesis of exportable protein by cycloheximide has been reported for guinea-pig pancreas (Jamieson & Palade, 1968). These observations suggest that the use of theophylline to mimic the effects of cyclic AMP should be approached with caution where additional protein synthesis may also be involved.

- Babad, H., Ben-Zvi, R., Bdolah, A. & Schramm, M. (1967). *Eur. J. Biochem.* **1**, 96.
 Gallagher, J. T. & Marsden, J. C. (1969). *Biochem. J.* **112**, 32P.
 Halkerston, I. D. K., Feinstein, M. & Hechter, O. (1966). *Proc. Soc. exp. Biol. Med.* **122**, 896.
 Humes, J. L., Rounbehler, M. & Kuehl, F. A. (1969). *Analyt. Biochem.* **32**, 210.
 Jamieson, J. D. & Palade, G. E. (1968). *J. Cell Biol.* **39**, 580.
 Ridderstrap, A. S. & Bonting, S. L. (1969). *Pflügers Arch. ges. Physiol.* **313**, 62.
 Robison, G. A., Butcher, R. W. & Sutherland, E. W. (1968). *A. Rev. Biochem.* **37**, 149.

The Transformation of Lymphocytes by Adenosine 3':5'-Cyclic Monophosphate, and Consequent Changes in Histone Microstructure

By MARGARET E. CROSS and MARGERY G. ORD. (*Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.*)

Cyclic AMP (adenosine 3':5'-cyclic monophosphate) will transform thymocytes (MacManus & Whitfield, 1969) at concentrations in the range 0.01–1.0 μ M. At 0.1–10 μ M no stimulation of human lymphocytes by cyclic AMP was observed (Hirschhorn, Grossman & Weissmann, 1970). In contrast with this it is reported here that cyclic AMP at a concentration of 0.01–0.1 μ M will stimulate cultures of lymphocytes prepared from pig peripheral blood as described by Forsdyke (1968). The timing and extent of the initial polydisperse RNA synthesis (0–1 h), subsequent rRNA synthesis (4–5 h) (Monjardino & MacGillivray, 1968), eventual DNA synthesis (approx. 72 h) and mitosis (approx. 90 h) are identical for cyclic AMP and phytohaemagglutinin. Chlorpromazine at a concentration that is reported to inhibit the adenylate cyclase membrane system (Wolff &