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

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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.

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

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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-