The Possible Significance of Adenosine 3':5'-Cyclic Monophosphate-Stimulated Protein Kinase Activity Associated with Purified Microtubular Protein Preparations from Mammalian Brain

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It has been reported that cyclic AMP (adenosine 3':5'-cyclic monophosphate) stimulates the phosphorylation of microtubular protein (i.e. colchicine-binding) purified from soluble extracts of ox brain by a protein phosphokinase closely associated with the purified protein (Goodman *et al.*, 1970). These observations have now been confirmed and extended for microtubular proteins purified from soluble extracts of pig, rabbit and rat brain. Protein kinase activity was usually assayed by an adaptation of the filter-disc method of Tao *et al.* (1970), or by the procedure of Weller & Rodnight (1971) by measuring ^{32}P in protein-bound phosphoserine residues.

Most of the ^{32}P incorporated into microtubular protein, after incubation with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ in the presence or in the absence of added cyclic AMP (final concn. $50\mu\text{M}$), was present in the major band (mol.wt. 58000) seen after electrophoresis of incubated samples in polyacrylamide gels according to the method of Weber & Osborn (1969). However, electrophoresis in tris-buffered gels containing sodium dodecyl sulphate and urea, which results in the separation of two main forms of 'tubulin' monomer (see Bryan & Wilson, 1971; Feit *et al.*, 1971; Olmsted *et al.*, 1971), indicates that the faster component appears to be preferentially labelled.

Colchicine and vincristine, which are known to bind to microtubular proteins, did not show consistent effects, although in general vincristine stimulated kinase activity in the absence of added cyclic AMP. Mn^{2+} (final concn. $100\mu\text{M}$) markedly inhibits microtubular kinase activity, which thus resembles the 'intrinsic' kinase of synaptosome membranes (Weller & Rodnight, 1971), but differs from the soluble histone kinase studied by Miyamoto *et al.* (1969).

Microtubular protein preparations from pig and rabbit brain also bind cyclic AMP, as determined by the method of Tao *et al.* (1970) with cyclic $[\text{H}^3]\text{AMP}$. Maximum values of 1–4 mmol of bound cyclic $[\text{H}^3]\text{AMP}/\text{mol}$ of 'tubulin' monomer (mol.wt.

58000) were obtained. Under similar conditions ^{32}P incorporation into serine residues seldom exceeds 20 mmol/mol of the monomer. The extent to which these relatively low values represent exchange reactions remains to be determined.

Dibutyryl cyclic AMP (6-*N*,2'-*O*-dibutyryl-adenosine 3':5'-cyclic monophosphate) (final concn. $100\mu\text{M}$) markedly stimulates the growth of neurites in chick-embryo sympathetic-ganglia cultures grown in the absence of added nerve growth factor. The growth-promoting effects of both cyclic AMP and nerve growth factor are abolished by low concentrations of drugs known to prevent microtubule assembly (see also Prasad & Hsie, 1971). The possibility that cyclic AMP might stimulate neurite growth through the phosphorylation of free or membrane-bound (see Lagnado *et al.*, 1971) microtubule subunits, and indeed that nerve growth factor may act partly through stimulation of adenylate cyclase activity, will be discussed in the light of experiments still in progress.

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An Effect of Insulin on the Adenosine 3':5'-Cyclic Monophosphate Phosphodiesterase and Guanosine 3':5'-Cyclic Monophosphate Phosphodiesterase Activities in the Perfused Langendorff and Working Hearts of Normal and Diabetic Rats

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It was shown in a previous study (Chain *et al.*, 1969) that, in the perfused working rat heart rendered insulin-deficient by injection of anti-insulin serum or

streptozotocin into the intact animal before excision of the heart, glycogen synthesis from $[U-^{14}C]$ glucose was decreased by over a half whereas glucose oxidation to $^{14}CO_2$ was not affected. The rate of glycogen synthesis could be restored to normality by the addition of insulin (2munits/ml) to the perfusion medium. Insulin has been shown to activate *in vitro* adipose-tissue cyclic AMP (adenosine 3':5'-cyclic monophosphate) phosphodiesterase (EC 3.1.4.-) (Loten & Sneyd, 1970) and purified ox heart cyclic AMP phosphodiesterase (Senft *et al.*, 1968). A direct effect of insulin on cyclic AMP phosphodiesterase or cyclic GMP (guanosine 3':5'-cyclic monophosphate) phosphodiesterase has been postulated but is still controversial (Sutherland & Robinson, 1969; Thompson & Appleman, 1971).

The heart-perfusion method, apparatus and preparation of the streptozotocin-diabetic rats were essentially as described by Chain *et al.* (1969). Cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activities were assayed in 1000g sediment and supernatant by the method described by Butcher & Sutherland (1962), with some modifications; cyclic GMP phosphodiesterase activity was assayed by using cyclic GMP as substrate in place of cyclic AMP. There were consistent and significant decreases (25–35%) in both activities in the streptozotocin-diabetic perfused rat heart. The decreases in these activities were more evident in nuclear and membrane fractions. Insulin (10munits/ml) in the perfusate significantly increased both cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activities in normal diabetic perfused hearts.

The patterns of decrease or increase in phosphodiesterase activity are similar whether we use cyclic AMP or cyclic GMP as substrate. The increase in the phosphodiesterase activities with insulin is more marked in the 1000g-sediment fraction. This is the first report of the stimulation of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activities in an isolated perfused organ by insulin. No stimulation of the phosphodiesterase activities by insulin was observed when the homogenate was treated with insulin (3units/ml) *in vitro*. Senft *et al.* (1968) described an activation of purified ox heart cyclic AMP phosphodiesterase *in vitro* by insulin. The regulation of cyclic AMP phosphodiesterase and cyclic GMP phosphodiesterase activities may control the tissue concentrations of cyclic nucleotides, the latter in turn regulating the rate of glycogen synthesis and degradation as suggested by Senft *et al.* (1968). The increase in cyclic nucleotide phosphodiesterase activities here demonstrated may therefore be partially responsible for the increased glycogen synthesis found in the perfused insulin-treated rat heart (Chain *et al.*, 1969). The magnitude of the increase in glycogen synthesis in normal and diabetic perfused hearts (Chain *et al.*, 1969) suggests, how-

ever, that there might be some other additional factor(s) also contributing to the stimulatory action of insulin on glycogen synthesis in the perfused rat heart.

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Glucose-Mediated Insulin Release from Mouse Islets of Langerhans: Adenylate Cyclase

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Cyclic AMP (adenosine 3':5'-cyclic monophosphate) has been implicated in mediating insulin release from the β -cells of the pancreas. There is virtually no delay between the arrival of glucose at the β -cell and insulin secretion. It is reasonable to assume therefore that the glucose effect may be mediated by direct interaction with the plasma membrane. Insulin release takes place in two distinct phases (Grodsky *et al.*, 1967; Blackard & Nelson, 1971). The first phase is over within 10min, but the second phase is more prolonged. It is the events underlying this first phase that are the subject of the present investigation. The effects of glucose on mouse islet adenylate cyclase were investigated since it may be expected that this enzyme could be one of the primary points of interaction.

Large numbers of islets (5000–18000) were isolated from mouse pancreas by the collagenase technique. After homogenization, the islets were centrifuged and the 1000g pellet was assayed for adenylate cyclase activity. The pH optimum of the enzyme was found to be 7.6. The apparent K_m was approx. $2 \times 10^{-4}M$ for ATP. The effects of various substances and hormones on the initial velocity of the reaction under the same conditions of enzyme and substrate concentration (0.4mM-ATP) were tested. The cyclase was stimulated by the following substances: NaF (2.4 and 8mM), glucagon (0.3, 0.6 and $1.6\mu M$), pancreozymin (0.15, 0.3 and $0.6\mu M$), secretin (0.3 and $0.6\mu M$). The enzyme was inhibited by Ca^{2+}