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 ³²P in protein-bound phosphoserine residues.

Most of the ³²P incorporated into microtubular protein, after incubation with [γ -³²P]ATP in the presence or in the absence of added cyclic AMP (final concn. 50 μ 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. Mn²⁺ (final concn. 100 μ 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 [³H]-AMP. Maximum values of 1–4 mmol of bound cyclic [³H]AMP/mol of 'tubulin' monomer (mol.wt.

58000) were obtained. Under similar conditions ³²P 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 μ 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