of cyclic AMP in stimulating insulin release may be to promote microtubular function by increasing the phosphorylation of microtubular proteins.

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Adenosine 3':5'-Cyclic Monophosphate and Adenylate Cyclase in Subcellular Fractions of Rat Brain

By P. ISAAC and D. G. GRAHAME-SMITH (*Medical Unit, St. Mary's Hospital Medical School, London W.2, U.K.*)

A cyclic-AMP (adenosine 3':5'-cyclic monophosphate)-binding protein prepared from bovine adrenal cortex has been used to measure cyclic AMP by saturation analysis (Brown *et al.*, 1971).

Rat brains were homogenized and fractionated as described by Gray & Whittaker (1962). Crude mitochondrial fractions from 1g wet wt. of brain contained 3pmol of cyclic AMP, all of which could be hydrolysed by incubation with a preparation of rat brain phosphodiesterase. No cyclic AMP was found in the high-speed supernatant. Subfractionation of crude brain mitochondrial fractions on a discontinuous Ficoll gradient revealed the following distribution of cyclic AMP: myelin fraction, 11.5%; synaptosomal fractions, 77.2%; mitochondrial fraction, 11.3%.

The action of sodium fluoride (10mM), in the presence of theophylline, Mg^{2+} and ATP, to stimulate adenylate cyclase activity was used to study the distribution of this enzyme activity in the same fractions. The following distribution was obtained: myelin fraction, 37.3%; synaptosomal fractions, 49.8%; mitochondrial fraction, 12.9%.

There have been several reports that brain monoamines variably increase the production of cyclic AMP in brain slices. Production of cyclic AMP by crude mitochondrial fractions has therefore been studied in the presence of noradrenaline, dopamine (3,4-dihydroxyphenethylamine), 5-hydroxytryptamine and histamine (10mM). No significant change in the production of cyclic AMP could be demonstrated, although sodium fluoride reliably produced a tenfold increase. Adenosine and acetylcholine were also inactive in crude mitochondrial

fractions. We conclude that these monoamines do not stimulate synaptosomal adenylate cyclase and that their action on brain slices may be either post-synaptic or on non-neuronal tissue. However, one cannot exclude a loss of hormonal response engendered by the tissue preparation.

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The Effect of Putative Transmitters and other Agents on Phosphoprotein Turnover in Respiring Slices of Guinea-Pig Cerebral Cortex

By M. REDDINGTON and R. RODNIGHT [*Department of Biochemistry, Institute of Psychiatry (British Postgraduate Medical Federation, University of London), De Crespigny Park, London SE5 8AF, U.K.*]

The application of electrical pulses to respiring slices of cerebral cortex increases the rate of incorporation of [^{32}P]orthophosphate into protein-bound phosphoserine residues, probably located in a membrane protein (Heald, 1957; Trevor & Rodnight, 1965; Jones & Rodnight, 1971). Electrical stimulation of brain slices under similar conditions also increases the cyclic AMP (adenosine 3':5'-cyclic monophosphate) content of the tissue (Kakiuchi *et al.*, 1969). Since membrane fragments prepared from cortical tissue contain a bound protein kinase, stimulated by cyclic AMP and mediating the phosphorylation of a membrane protein (Weller & Rodnight, 1970, 1971), it appears possible that the increased turnover of protein-bound phosphoserine on stimulation results from an increased content of cyclic AMP in the tissue. If this interpretation is correct other agents, such as biogenic amines and adenine derivatives, which increase the cyclic AMP content of respiring cortical slices (Shimizu *et al.* 1970; Sattin & Rall, 1970), might also be expected to have a similar effect on phosphoprotein turnover.

To investigate this hypothesis slices of guinea-pig cerebral cortex were cut and incubated with [^{32}P]orthophosphate as described by Jones & Rodnight (1971). Incorporation of ^{32}P into protein-bound phosphoserine was taken as the radioactivity (c.p.m.) in the alkali-labile P of the tissue proteins relative to the radioactivity (c.p.m.) in the acid-soluble P of the tissue. Exposure of slices to noradrenaline (0.5mM), histamine (0.1mM) or serotonin (5-hydroxytryptamine) (1 μ M) for 10min increased the rate of incorporation of ^{32}P into protein by 10-15% ($P < 0.005$), but had no significant effect on the relative specific radioactivity of the γ -P of ATP. In a comparable series of experiments electrical stimulation for 10s increased incorporation by 15-20% ($P < 0.0025$)

with a 10% decrease in ATP (γ -P) radioactivity. By contrast adenosine and AMP, added at 0.125mM concentration for 5 min, had no effect on the turnover of protein-bound phosphoserine, despite their more potent effect in increasing the cyclic AMP content of the tissue (Sattin & Rall, 1970). However, rather higher concentrations of adenosine inhibit protein kinase activity in preparations of membrane fragments from brain (Weller & Rodnight, 1971), and it is possible that the compound may exert a similar effect on protein phosphorylation in the intact tissue.

These observations support the hypothesis that some post-synaptic responses to neurotransmitter release may be mediated through cyclic-AMP-dependent protein kinase activity in the receptor cell. Whether these are events occurring at the post-synaptic membrane or intracellularly remains to be investigated.

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Partial Reactions of D-Glucose 6-phosphate-1L-myoInositol 1-Phosphate Cyclase from Rat Testis

By A. RASHEED, D. L. CORINA and J. E. G. BARNETT
(Department of Physiology and Biochemistry, University of Southampton, Southampton SO9 5NH, U.K.)

From a study of hydrogen movements during the cyclization, we have previously proposed (Barnett & Corina, 1968) that the sequence of events in the enzyme reaction is oxidation by NAD^+ of C-5 of D-glucose 6-phosphate to give 5-oxo-D-glucose (D-xylulo-5-hexosulose) 6-phosphate, an aldol condensation between C-6 and C-1 of this molecule to give 1L-myoinosose-2 1-phosphate, and, finally, reduction by the NADH first formed to give 1L-myoinositol 1-phosphate. The NADH and oxidized intermediates were tightly bound to the enzyme and could not be isolated.

We have found that the NAD^+ can be removed from the enzyme by using charcoal, and that on readdition of NAD^+ (K_m 5×10^{-5} M) the enzyme retains 75% of the original activity, but has modified

properties. By using the charcoal-treated enzyme lacking NAD^+ we have been able to incorporate hydrogen from $[^3\text{H}]\text{NADH}$ into 5-oxo-D-glucose 6-phosphate. The label was located in the D-[5- ^3H]-glucose 6-phosphate formed by the methods previously described (Barnett & Corina, 1966).

For kinetic measurements carrier glucose 6-phosphate was added, the reaction mixture was reduced with sodium borohydride, the D-glucitol 6-phosphate was hydrolysed, separated by paper chromatography, benzoylated and purified on t.l.c., and the D-glucitol hexabenzoylate was assayed by its E_{232} value and its radioactivity counted.

The incorporation of radioactivity was linear with time up to 2h, and after 4h about 7% of the label from the NADH was incorporated. The incorporation was abolished by pretreatment with trichloroacetic acid, and 90% inhibited by 0.2mM-2-deoxy-D-glucose 6-phosphate, which has K_i 2×10^{-5} M for the cyclization of glucose 6-phosphate. No label was incorporated if untreated enzyme containing bound NAD^+ was used.

Incubation of the substrate analogue 5-oxo-D-glucitol 6-phosphate (L-sorbose 1-phosphate) with the charcoal-treated enzyme and $[^3\text{H}]\text{NADH}$ gave D-[5- ^3H]-glucitol 6-phosphate.

The enzyme-catalysed reaction of the hypothetical intermediates of the enzyme to give substrate strongly suggests that the mechanism previously proposed for the enzyme is correct.

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Pullulanase Synthesis in *Klebsiella (Aerobacter) aerogenes*

By A. C. R. DEAN, S. C. GRAY, G. C. HOPE and I. M. JOHNSON
(Physical Chemistry Laboratory, University of Oxford, Oxford OX1 3QZ, U.K.)

Bacterial pullulanase, an inducible enzyme, has proved valuable in the analysis of starch-type polysaccharides (Manners, 1966) and has potential industrial uses. Initially reported by Bender & Wallenfels (1961) in a strain of *Klebsiella aerogenes* that they had isolated, pullulanase has also been detected in *K. aerogenes* A.T.C.C. 9621 (N.C.I.B. 8021) grown in appropriate conditions (Brown *et al.*, 1965). We have investigated the precise conditions necessary for its optimum synthesis by *K. aerogenes* N.C.I.B. 418 grown in chemostat continuous culture. In these conditions the enzyme remains entirely cell-bound. When maltose, maltotriose or pullulan was used as sole carbon source in carbon-limited conditions the steady-state values of pullulanase specific