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The influence of arginine vasopressin upon the production of adenosine 3',5'-monophosphate by adenyl cyclase from the kidney

SUTHERLAND, RALL AND MENON[1] have reported the preparation of an enzyme system, adenyl cyclase, which catalyzes the conversion of adenosine triphosphate into adenosine 3',5'-monophosphate. Adenyl cyclase as isolated from cardiac muscle and, to a lesser extent, from the liver was found to be influenced by catecholamines in that hormonal stimulation enhanced the production of Ado-3',5'-*P* in the presence of the enzyme system[2]. In view of the studies in this laboratory which have demonstrated that stimulation of dog-kidney particulate preparations with arginine vasopressin (the anti-diuretic hormone of man, the dog, and other species) results in increased production of Ado-3',5'-*P* in these systems[3], we have now investigated the influence of arginine vasopressin upon adenyl cyclase as isolated from the dog kidney.

Dogs were anesthetized with sodium pentobarbital and the kidneys were rapidly excised. Cortical and medullary tissue were separated and then the general procedure, as described by SUTHERLAND, RALL AND MENON, for the isolation of the adenyl cyclase system from cerebral cortex, heart, skeletal muscle, and liver was followed for the obtaining of a soluble preparation in 1.8% Triton (Triton X-100, Rohm and Haas Company, Philadelphia, Pennsylvania, U.S.A.). This preparation, while yet at 3°, was immediately assessed for its ability to respond to arginine vasopressin.

1 ml of the cold adenyl cyclase preparation was added to 1.5 ml of a cold (3°) solution of such composition that the resulting medium contained the following additional components: 1.2 mM ATP, 2 mM $MgSO_4$, 4 mM caffeine, 6 mM NaF, and 24 mM Tris buffer (pH 7.4). Next, 0.25 unit of synthetic arginine vasopressin (prepared in Switzerland by Sandoz, Inc., distributed by the Pharmaceutical Department of Sandoz, Inc., Hanover, New Jersey, U.S.A.) was introduced into those preparations which were to be stimulated. Within 5 sec after stimulation those preparations which were to serve as "zero-time" controls were plunged into a boiling-water bath in which they were allowed to remain for 15 min. At the end of this time these "zero-time" controls were frozen, thawed, centrifuged (15 000 × *g*, 3°, 15 min), and the supernatants were obtained. The remainder of the stimulated preparations and their controls were incubated in a Dubnoff metabolic, shaking incubator for 15 min at 30°. At the end of the incubation period these preparations were plunged into a boiling-water bath and the procedure described for the "zero-time" controls was followed for the obtaining of these supernatants. Aliquots of each of the supernatant solutions were then examined for their content of Ado-3',5'-*P*.

The determination of Ado-3',5'-*P* was conducted using a procedure which depends upon it initiating a sequence of events which results in NADPH being produced in an amount which is directly dependent upon the amount of cyclic nucleotide which was presented to the system[3]. Briefly, Ado-3',5'-*P* enhances the conversion of "inactive" liver α-glucan phosphorylase (EC 2.4.1.1) into "active" liver phosphorylase and the latter in the presence of glycogen then promotes the production of glucose 1-phosphate. The glucose 1-phosphate is enzymically converted into glucose 6-phosphate which in the presence of NADPH is enzymically converted into 6-phosphogluconolactone with the concomitant production of NADPH. This NADPH is determined spectrophotofluorometrically. When supernatants from

stimulated and unstimulated preparations were examined and compared it was found that stimulation of adenyl cyclase with arginine vasopressin increased the production of Ado-3′,5′-*P* in the presence of this enzyme system as isolated from the dog kidney. Data from a typical experiment are presented in Table I.

TABLE I

THE INFLUENCE OF ARGININE VASOPRESSIN UPON THE PRODUCTION OF ADO-3′,5′-*P* IN THE PRESENCE OF ADENYL CYCLASE FROM DOG KIDNEY

200 μl of the 15 000 × *g* supernatant obtained from enzyme preparations (or their controls) or 200 μl of a standard solution of Ado-3′,5′-*P* were added to 0.4 ml of a preparation of the liver phosphorylase system obtained as the 11 000 × *g* supernatant from dog-liver homogenate, according to KALL, SUTHERLAND AND BERTHET[4]. The resulting preparation was made up to 1.1 ml with a solution of composition such that the resulting medium contained the following additional components: 9 mM NaF, 1.8 mM ATP, 6 mM caffeine, 2.3 mM $MgSO_4$, 0.1 M phosphate buffer (pH 7.4), 0.9 mM AMP, 36 mM histidine, 0.9 mg/ml bovine plasma albumin, 1.8% glycogen, and the enzymes glucose-6-phosphate dehydrogenase (EC 1.1.1.49) (45 μg or 3.2 units per ml) and phosphoglucomutase (EC 2.7.5.1) (182 μg or 4 units/ml)*. After incubation, first at 32° for 10 min and then at 37° for 30 min, 1.0 ml of 0.3 M sodium phosphate–0.3 M potassium hydrogen phosphate (3 : 1) was rapidly added to each solution. Solutions were incubated at 63° for 10 min, centrifuged (20 000 × *g*, 3°, 5 min) and 1.5 ml of the resulting supernatant were added to 1.0 ml of alkaline peroxide (6 N NaOH 5.3% H_2O_2). Solutions were then incubated for 10 min at 60°, diluted with distilled water, and examined spectrophotofluorometrically for their content of NADPH according to the alkaline peroxide method of LOWRY, ROBERTS AND KAPPHAHN[5]. The amount of NADPH indicated below is that which resulted in the reaction medium described.

Adenyl cyclase preparation	*Hormonal stimulation*	*Resulting NADPH (μmoles)*
Cortex**	yes	0.155
		0.175
Cortex	no	0.080
		0.080
Cortex, "zero time"	yes	0.090
		0.090
Preparation medium only	yes	0.093
Medulla***	yes	0.129
Medulla	no	0.103
Medulla, "zero time"	yes	0.100
Preparation medium only	yes	0.099
2 mμmoles Ado-3′,5′-*P*§		0.145
4 mμmoles Ado-3′,5′-*P*		0.203

* Glucose-6-phosphate dehydrogenase (5 mg or 350 units/ml) and phosphoglucomutase (10 mg or 220 units/ml) were obtained from C. F. Boehringer and Soehne, Mannheim, Germany, distributed by California Corporation for Biochemical Research, Los Angeles, California, U.S.A.

** A 1-ml aliquot was equivalent to 61.5 mg of tissue.

*** A 1-ml aliquot was equivalent to 77.22 mg of tissue.

§ Ado-3′,5′-*P* was obtained from Swartz BioResearch, Inc., Orangeburg, New York, U.S.A.

Adenyl cyclase from the kidney cortex appeared to be more sensitive to stimulation by arginine vasopressin than that isolated from the medulla. In support of the studies of ORLOFF AND HANDLER[6] in which they found that an anti-diuretic factor and Ado-3′,5-*P* behaved in a manner similar to each other with respect to water movement in the toad bladder, we suggest that anti-diuretic factors, such as arginine vasopressin, influence the adenyl cyclase enzyme system with the result

that the latter becomes more effective in catalyzing the formation of Ado-3',5'-*P*.

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Biotin- and adenosine triphosphate-dependent activation of propionyl apocarboxylase

As reported previously, [2-^{14}C] biotin is oxidized to labeled CO_2 and acetoacetate by cell-free preparations of a soil bacterium grown on biotin as sole carbon source[1,2]. A biotin-activating enzyme obtained from the bacterial extracts appears to catalyze the following reactions, as shown by the ATP-dependent conversion of biotin to biotin hydroxamate, stimulated by CoA, and by a [^{32}P]pyrophosphate–ATP exchange which is inhibited by CoA:

$$\text{Biotin} + \text{ATP} \overset{Mg^{2+}}{\rightleftharpoons} \text{biotinyladenylate} + \text{pyrophosphate} \quad (1)$$

$$\text{Biotinyladenylate} + \text{CoA} \rightleftharpoons \text{biotinyl-CoA} + \text{AMP} \quad (2)$$

The present report is concerned with the possibility that carboxyl activation occurs as a step in the incorporation of biotin into proteins as well as in biotin oxidation. In agreement with the findings of KOSOW AND LANE[3], we have shown that activation of the apoenzyme of propionyl-CoA carboxylase (EC 6.4.1.3) and the binding of [^{14}C]biotin to proteins in cell-free extracts of biotin-deficient liver are dependent upon the presence of ATP (Table I)[4]. The carboxylase activity (as measured by $^{14}CO_2$ fixation) when biotin is omitted is a measure of the residual holocarboxylase present in the biotin-deficient extracts. Since the omission of ATP or glutathione gives a similar value, it may be concluded that the effect of biotin in activating the apocarboxylase is almost entirely ATP-dependent and also requires the presence of glutathione, presumably to stabilize the apoenzyme. The overall apoenzyme-activating reaction, which is stimulated by the presence of Mg^{2+} but not consistently by CoA, may be formulated as follows: