ADENOSINE 3',5'--CYCLIC MONOPHOSPHATE DEPENDENT PROTEIN KINASE AND PHOSPHOPROTEIN PHOSPHATASE ACTIVITIES IN RAT ISLETS OF LANGERHANS

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SUMMARY: cAMP-dependent protein kinase and phosphoprotein phosphatase activities have been found in isolated beta islets of Langerhans. A natural protein substrate for these enzymes has also been found.

Adenosine 3',5'--cyclic monophosphate (cAMP) has been reported (1,2) to play an important role in insulin secretion. cAMP is known to promote protein phosphorylation through the activation of protein kinases. Rasmussen (3) and Malaisse et al (4) have proposed theories for the mechanism of insulin secretion based on cAMP activated protein kinase phosphorylation of the beta islet microtubular-microfilamentous system.

To our knowledge the activity of protein kinases and their activation by cAMP have not been studied in the islets of Langerhans. The present work describes a study of protein kinase activity, its dependence on cAMP and its substrate specificity in the beta islet cells of the rat pancreas.

METHODS AND MATERIALS:

Islet Isolation and Homogenization

Rat islets of Langerhans were isolated by the centrifugation method of Lacy and Kostianovsky (5). Collagenase from Worthington was used for digestion. The islets were homogenized at 0-4° in 0.05M sodium acetate buffer, pH7.0 employing a glass homogenizer and a Teflon pestle. The homogenate was centrifuged at 100,000 x g for 60 min, the supernatant fluid

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was removed and an aliquot was assayed for protein content by the biuret method (6).

Protein Kinase Assay

Protein kinase activity was measured in a medium containing 10μ moles sodium acetate buffer, pH 6.0, 1 m μmole (γ-^{32}p)ATP, 0.06μ mole ethylene glycol bis (β-aminoethyl ether) N,N'-tetraacetic acid, 2.0 μmoles magnesium acetate, 2.0μ moles sodium fluoride, 0.4μ mole theophylline and 7-90 μg islet protein, with or without 1.0 m μmole cAMP in a total volume of 0.2 ml. In experiments in which an exogenous phosphate acceptor was used 200μ g of calf thymus histone (Sigma) was added to the assay medium. Assays were conducted at 30°. Reactions were terminated by pipetting 25μ l of the reaction mixture onto 2 cm x 2 cm squares of Whatman I chromatography paper. These papers were washed with trichloroacetic acid, ethanol, ether and counted as described by Reimann, et al (7).

(γ-^{32}P)ATP was prepared from 10mCi (^{32}P)-orthophosphate (New England Nuclear) by the method of Glynn and Chappell (8).

RESULTS:

Time Course of Phosphorylation

As demonstrated in Figure 1 islets of Langerhans possess a cAMP-dependent protein kinase(s) which is liberated into the medium upon homogenization. Furthermore a protein fraction is liberated which can serve as a substrate for the protein kinase(s). The addition of histone (200μ g) did not result in an appreciable increase in phosphorylation above levels obtained using the endogenous protein alone. Phosphorylations were linear only within 30-60 sec. Therefore, a 30 sec incubation time was used throughout this study.

Effect of Varying Concentrations of Endogenous Substrate

Figure 2 shows that the rate of phosphorylation remains proportional to the amount of supernatant protein through 90μ g.

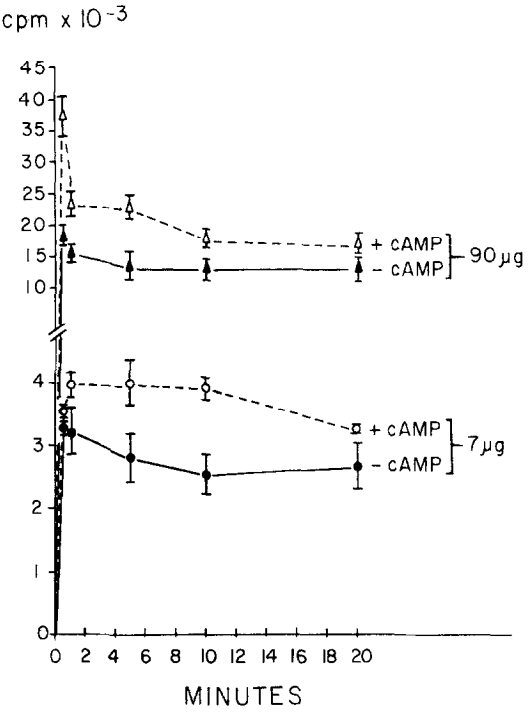

FIGURE 1 Time Course of Phosphorylation of Supernatant Protein from the Homogenization of Beta Islets Langerhans

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Incubations utilizing 7µ g or 90µ g of protein were conducted in the absence (●-7µ g; ▲-90µ g) or presence (○-7 µg; △-90 µg) of cAMP as described under "Methods and Materials". Incubations including 200 µg of histone as an exogenous phosphate acceptor did not differ significantly from the results attained in the absence of histone. Results are from two experiments.

Presence of Phosphoprotein Phosphatase Activity

A slow decline of phosphoprotein radioactivity may be noted in Figure 1. This indicates the presence of a phosphoprotein phosphatase in the supernatant. Table 1 summarizes the phosphoprotein phosphatase activity. As expected a higher supernatant protein content gave a higher percent decline in radioactivity.

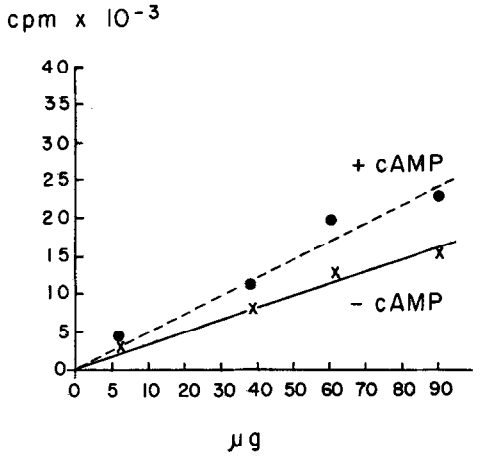

FIGURE 2 Effect of Increasing Concentrations of Supernatant Protein on the ^{32}P Incorporation.

The indicated amounts of supernatant protein from the homogenization of beta islets were incubated with (γ-^{32}P)ATP for 30 sec as described in "Methods and Materials" in the presence (●—●) or absence (X-X) of cAMP.

Table 1

Phosphoprotein Phosphatase Activity

Supernatant protein (μg)	cAMP	Maximum cpm	cpm at 20 min	Per Cent Decreases
7	-	3279	2718	17
	+	3980	3272	18
90	-	18465	13531	27
	+	37854	16590	56

The supernatant protein from the homogenization of beta islets was incubated with (γ ^{32}-P)ATP as described in "Methods and Materials". The maximum amount of phosphorylation (usually 30 sec or 60 sec incubation time) is compared to the cpm at 20 min.

Solubility Characteristics of Phosphorylated Proteins

As can be seen in Table 2 only 20% of the radioactivity was solubilized by heating at 90° for 15 min in 10% trichloroacetic acid, a procedure known to solubilize RNA and DNA. Extraction with chloroform-methanol (2/1) resulted in a decline of about 2% in radioactivity. These results indicate that only

Table 2

Solubility Characteristics of Phosphorylated Components of Beta Islets From Rat Pancreas

Procedure	CPM before treatment	CPM after treatment	Per Cent Change
1. 10% trichloroacetic acid at 90°, 15 min	4977	4003	20
2. Chloroform-methanol (2/1)	4240	4167	2
3. Hydroxylamine	4300	4195	2.5
4. Sodium Hydroxide	4270	460	89

Seven micrograms of supernatant protein from the homogenization of islets of Langerhans was labeled for 30 sec at 30° with (γ^{32}-P)ATP, aliquots were spotted on squares of Whatman 1 paper washed and counted as described in "Methods and Materials". The squares were removed from the scintillation vials, dried and treated in Procedure 1 with 10% trichloroacetic acid for 15 min at 90°. The squares were rinsed briefly in ethanol and ether, dried and counted. In Procedure 2 the squares were washed with chloroform-methanol (2/1); dried and counted. In Procedures 3 and 4 the squares were incubated with 0.8M hydroxylamine in 0.1M sodium acetate buffer (pH5.5) or with 1M NaOH for 10 min at 100° and then dried and counted.

a small portion of incorporation of label occurs in the nucleic acid or phospholipid fractions. Furthermore no label is hydrolyzed in hydroxylamine at pH 5.5 and all radioactivity is converted to (32P) orthophosphate after 10 min at 100° in 1N NaOH, thus indicating that the component phosphorylated is a protein and that the phosphate is covalently bond to the protein in a phosphoester linkage.

DISCUSSION:

The combined activities of a cAMP-dependent protein kinase and a

phosphoprotein phosphatase would provide an enzymatic mechanism for rapid variations in the level of phosphorylation of the microtubular -microfilamentous system of the beta islet. It could also provide through phosphorylation and dephosphorylation of plasma membrane components a sensitive mechanism of control over the passage of substances into and out of the cell.

This study demonstrates the presence of cAMP-dependent protein kinase and phosphoprotein phosphatase acitvities in the beta islet. In addition a protein(s) which serves as a natrual substrate for the protein kinase has also been found. The specificity for this protein(s) by the beta islet protein kinase is indicated by the observation that the addition of histone to the incubation medium did not result in enhancement of phosphorylation. The usual procedures designed to solubilize non-protein substance, primarily nucleic acids and lipoproteins indicate that the predominant species phosphorylated in these experiments is protein. Hydroxylamine treatment did not result in an appreciable reduction in radioactivity, thus indicating that the phosphate is covalently bond to protein in a phosphoester rather than acyl linkage.

Elucidation of the nature of the supernatant protein substrate as well as further characterization of tha beta islet protein kinase(s) and phosphoprotein phosphatase(s) should be of value in understanding the functioning of this very important cell type.

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REFERENCES:

(1) Malaisse, W.J., Malaisse-Lagae, F. and Wright, P.H., Endocrinology 80 (1967) 99.
(2) Sussman, K. E. and Vaughn, G. D., Diabetes 16 (1967) 449.
(3) Rasmussen, H., Science 170 (1970) 404.

(4) Malaisse, W. J., Malaisse-Lagae, F., Walker, M. O. and Lacy, P. E., Diabetes 20 (1971) 257.
(5) Lacy, P. E. and Kostianovsky, M., Diabetes 16 (1967) 35.
(6) Gornall, A. G., Bardawill, C. S. and David, M. M., J. Biol. Chem. 177 (1949) 751.
(7) Reimann, E. M., Walsh, D. A. and Krebs E. G., J. Biol. Chem. 246 (1971) 1986.
(8) Glynn, I. M. and Chappell, J. B., Biochem. J. 90 (1964) 147.