IN VIVO REGULATION OF ADIPOSE TISSUE PROTEIN KINASE BY ADENOSINE 3',5'-MONOPHOSPHATE MEDIATED GLUCAGON STIMULATION

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Received October 22,1976

SUMMARY

In vivo administration of glucagon produced an increase in the dissociation of protein kinase subunits. This dissociation went parallel with elevated adenosine 3',5'-monophosphate concentrations in the rat adipose tissue. These changes were concomitantly accompanied by a decrease in cyclic AMP-binding activity. Protein kinase activity, measured in the presence and absence of the cyclic nucleotide, was reduced at 5 and 15 minutes of glucagon administration and increased at 30 minutes.

Cyclic AMP has been suggested to be the second messenger which mediates the action of a variety of hormones in different animal tissues (1). According to a widely accepted notion (2) cyclic AMP exerts its diverse physiological effects as second hormonal messenger by activating protein kinases, enzymes which catalyze the phosphorylation of proteins and appear to play an important role in cellular regulation (3). The action of cyclic AMP is involved in binding to a regulatory subunit of protein kinase for dissociation of the holoenzyme into a binding subunit and a catalytic subunit (4).

Regulation of protein kinases by hormones has been reported for few tissues. Shen et al.(5) have reported influences of insulin and epinephrine on the activity of glycogen synthetase in muscle. Changes in the activity of cyclic AMP-dependent protein kinase by insulin and epinephrine have been demonstrated in rat diaphragm by Walaas et al.(6,7) and in adipose tissue by Soderling et al. (8). Ovarian protein kinase activity in pubescent rats was markedly enhanced by incubating intact ovaries with luteinizing hormone (9). An important role for protein kinase in ACTH action on the adrenocortical cell using isolated adrenal cells has been demonstrated (10). Beta-adrenergic stimulation with isoproterenol increased cyclic AMP concentrations,

decreased the concentration of unsaturated cyclic AMP receptor sites and increased cyclic AMP-independent protein kinase in uterine preparations from ovariectomized rats (11).

Only a few studies have been reported by in vivo experiments. In this regard results have been obtained with either ACTH or glucagon on protein kinases of adrenal gland (12,13) and hepatic enzyme(14,15) respectively.

It was the aim of the present work to examine the in vivo effect of glucagon on protein kinase and cyclic AMP-binding activities as well as on the levels of cyclic AMP in rat adipose tissue.

MATERIALS AND METHODS

Young male Wistar rats weighing 100-150 g, were fasted overnight before the day of experiment. Rats were sacrificed by neck dislocation. The epididymal fat pads were removed and a 25% homogenate was prepared in 0.15 M KCl (w/v). The homogenate was centrifuged during 30 minutes at 25,000 x g at 4°C and the aqueous portion below the floating fat layer was removed and used as the source of protein kinase.

Protein kinase was assayed essentially as described by Corbin and Krebs (16) using calf thymus histone as protein substrate. The assay mixture, in a final volume of 0.1 ml,contained: 50 mM potassium phosphate buffer pH 6.5, 3 mM magnesium acetate, 200 µg histone, 2 x 10^{-4} M γ-^{32}P-ATP and the enzyme source in varying amounts (10-100 µg protein). Assays were performed at 30°C for 5 min. ATP was the final addition to start the reaction. The reaction was stopped by adding 0.5 ml 10% TCA with 0.1 ml of bovine serum albumin (10 mg/ml). Precipitated proteins were collected by centrifugation and then dissolved in 1 ml 0.1 N NaOH. Proteins were reprecipitated, washed twice with 1 ml 5% TCA, dissolved in 0.1 ml 98% formic acid and counted in 10 ml of Instagel in a liquid scintillation counter. These studies were carried out using adipose tissue rather than isolated adipocytes since most of the protein kinase of adipose tissue has been found to be within the adipocytes (16).

Cyclic AMP binding protein was measured by the method of Anderson et al. (17). Adipose tissue was homogenized with 4 volumes of 50 mM Tris-HCl, pH 7.5, and centrifuged at 25,000 x g for 30 min and supernatants were used for measuring this activity. Assay conditions were maintained essentially as previously

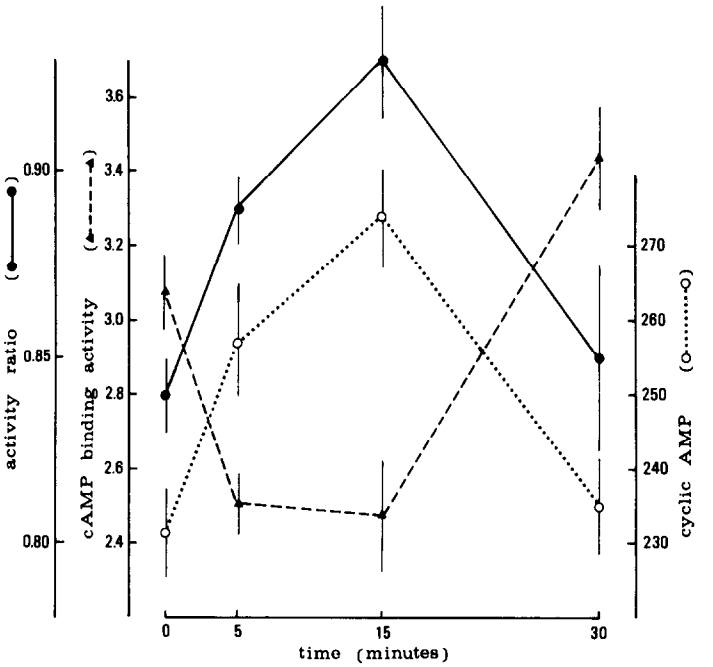

Figure 1. Time course of glucagon effect on cyclic AMP concentration (pmoles/g wet weight of tissue), protein kinase activity ratio and cyclic AMP-binding specific activity (pmoles ^{3}H-cyclic AMP/mg protein). Data are given as mean ± standard error (indicated by vertical bars). n= 10.

described (18). Non specific binding was determined by adding 10 mmol unlabeled cyclic AMP to the assay mixture (19).

Cyclic AMP levels were measured by competitive protein binding assay (20,21) and deproteinization prior to assay was carried out as described by Cooper et al. (22) by TCA method.

Protein concentration was determined by the method of Lowry et al. (23).

Crystalline glucagon was obtained from Ely Lilly; calf thymus histone type II A, cyclic AMP (sodium salt) and other reagents were obtained from Sigma Chemical Co., St Louis, U.S.A., ^{3}H-cyclic AMP, ammonium salt (sp.act. 27.5 Ci/mmol) and γ-^{32}P-ATP, ammonium salt (sp.act. 16.2 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, U. K.

Statistical analysis of the data was done using Student's t test (24). In all experiments, the means ± standard error are given.

Table 1

Effect of glucagon on protein kinase activity

protein kinase activity	time (minutes)			
	0	5	15	30
minus cAMP	448 ± 23	294 ± 24	381 ± 45	683 ± 46
plus cAMP	536 ± 25	331 ± 46	449 ± 43	797 ± 47

Data are given as mean ± standard error; n= 10
Protein kinase values are given as units (18)

RESULTS AND DISCUSSION

Variations in cyclic AMP concentrations, protein kinase and cyclic AMP-binding activities were determined at different time intervals after the subcutaneous administration of glucagon (600 µg/100 g body weight). Results are given in Fig. 1 showing a significant increase in cyclic AMP concentrations which reach a plateau between 5 and 15 minutes and decrease sharply at 30 minutes. This pattern of variation runs parallel with similar increments in the dissociation of protein kinases which was estimated by the protein kinase ratio. The binding of ^{3}H-cyclic AMP which measures in vivo receptor saturation decreases in an inverse manner attaining the lowest level at 5-10 minutes.

We must point out that the position of equilibrium of the equation that explains the molecular mechanism of action of cyclic AMP is regulated among other factors by the concentration of cyclic AMP in tissue. Changes in the levels of endogenous cyclic AMP following hormonal modulation can lead to variations in the "activity ratio" (which has been defined (8) as the ratio of activity in the absence of cyclic AMP to the activity on its presence) and in opposite changes in nonsaturated cyclic AMP binding sites. Our results show that in fact this is the case with rat adipose tissue since the administration of glucagon produces an apparently coordinate increase in cyclic AMP levels and protein kinase dissociation as well as a reduction in cyclic AMP-binding activity.

Protein kinase activity in the absence of the cyclic nucleotide diminished sharply at 5 minutes and highest values

were reached at 30 minutes as it can be seen in Table 1. The protein kinase activity measured under standard assay conditions in the presence of an optimal concentration of cyclic AMP shows a similar pattern of variations. We must point out that the reduction of protein kinase activity may be a rather general event after hormonal stimulation since it has been reported in all tissues examined (1,3,5). The present findings demonstrate that a similar situation takes place in the case of rat adipose tissue.

Acknowledgements

The authors are grateful to Prof. A. M. Municio for helpful suggestion and encouragement of these studies and to Miss M. D. Aragones for her skillful assistance.

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