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

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SUMMARY

In vivo administration of glucagon caused an increase in the dissociation of protein kinase subunits which was accompanied by elevated adenosine 3',5'-monophosphate concentrations in the rat liver. Concomitantly, there was a decrease in non saturated adenosine 3',5'-monophosphate binding sites. A reduction in protein kinase activity measured in the presence of the cyclic nucleotide was apparent at 5 minutes of glucagon administration while enzyme activity assayed in the absence of adenosine 3',5'-monophosphate was already increased after one minute. Glucose, given through an intragastric tube, caused no changes in the effect of glucagon on hepatic protein kinase.

Although protein kinases have been the object of great interest in recent years, regulation of their activity by hormones has been reported for few tissues (1-5). With the exception of studies in rat adrenal glands (3) all other results have been obtained by in vitro stimulation of incubated organs or tissue.

According to the model postulated by Brostrom et al. (6), the mechanism of cyclic AMP* mediated hormonal activation of protein kinases is expressed by the following equation:

$$\text{cyclic AMP} + \text{RC} \rightleftarrows \text{R. cyclic AMP} + \text{C} \qquad (1)$$

The holoenzyme (RC) is inactive; the nucleotide binds to the regulatory unit (R), thereby releasing the catalytic unit (C) which can then phosphorylate its substrate(s). Since the free catalytic unit does not require the

*Abbreviations: cyclic AMP, adenosine 3',5'-monophosphate; EDTA, ethylenediamine-tetraacetate disodium salt.

presence of cyclic AMP for activity it is usually called "cyclic AMP-independent protein kinase". Two other types of protein kinase activities, "total protein kinase" and "cyclic AMP-dependent protein kinase"*, can be determined when the enzyme is assayed in the presence and in the absence of the cyclic nucleotide.

The position of equilibrium in equation 1 is regulated among other factors by the concentration of cyclic AMP in tissues. Modifications in the levels of endogenous cyclic AMP secondary to hormonal modulation can be expected to result in variations in "activity ratio" of protein kinase** and in opposite changes in nonsaturated cyclic AMP binding sites.

The work presented in this publication demonstrates that such is the case for the rat liver. Subcutaneous injection of glucagon to intact animals causes an apparently coordinated increase in cyclic AMP levels and protein kinase dissociation as well as a reduction in cyclic AMP-binding activity. Evidence is also introduced indicating that the total hepatic protein kinase activity measurable in crude preparations varies with time of treatment and that the administration of glucose does not affect the activation of protein kinase due to glucagon.

MATERIALS AND METHODS

Young male Sprague-Dawley rats, weighing 100-120 g, were maintained on a pelleted protein-free diet for five days and fasted overnight before the day of experiment. At this time the animals weighed 70-90 g. These conditions were selected in order to obtain information that could be compared with previous studies (9). The rats were sacrificed by neck

*"Total protein kinase" is operationally defined in the author's laboratory as the protein kinase activity measured under standard assay conditions in the presence of an optimal concentration of cyclic AMP. Maximal activation in rat liver is usually obtained with 5μM cyclic AMP (7). "Cyclic AMP-dependent protein kinase" is, operationally, the activity obtained by substracting the values determined in the absence of cyclic AMP from the total protein kinase. These definitions are at variance from those used by others (1,3) and are presented here because of the conceptual difference.

**This has been defined as the ratio of activity in the absence of cyclic AMP to the activity in its presence (8).

dislocation; a small fragment of liver was rapidly removed after sacrifice and frozen in liquid nitrogen. The remaining liver was excised immediately, rinsed briefly in cold saline, blotted and weighed. The livers were minced and homogenized in 4 volumes of SPE buffer (0.25 M sucrose in 10 mM phosphate buffer, pH 6.5, and 4 mM EDTA). In experiments to be reported, it has been established that the position of equilibrium of equation 1 does not change in this buffer for periods of over one hour after centrifugation. The homogenates were centrifuged at 26,000xg for 10 minutes at 0° and the supernatants removed. Cyclic AMP-binding and protein kinase activities were assayed by procedures already described (9). Hepatic cyclic AMP levels in the frozen fragments were measured as before (10). Protein concentration was determined by the method of Lowry et al. (11). Glucagon was obtained from Eli Lilly and Co.; calf thymus histone and cyclic AMP were purchased from Sigma Co.; [^{3}H]cyclic AMP and Y-[^{32}P]ATP were from Schwartz-Mann and New England Nuclear, respectively.

RESULTS AND DISCUSSION

Changes in cyclic AMP concentration, protein kinase activities and cyclic AMP receptor saturation were studied simultaneously at various time intervals after the subcutaneous administration of 0.5 mg of glucagon per 100 g of body weight. In some experiments, not all three parameters were examined. Figure 1 shows that, at this dose, there is a rapid increase in cyclic AMP which reaches a plateau between 5 and 10 minutes and decreases slowly to about one-half its maximum value at 30 minutes. This is paralleled by similar increments in the dissociation of protein kinase as estimated by the protein kinase activity ratio. *In vitro* binding of [^{3}H] cyclic AMP, which measures *in vivo* receptor saturation, decreases in an inverse manner attaining the lowest levels at 5-10 minutes. There is an apparent rough correspondence between these three parameters, as would be predicted from equation 1. Furthermore, the temporal changes in cyclic AMP are closely correlated to

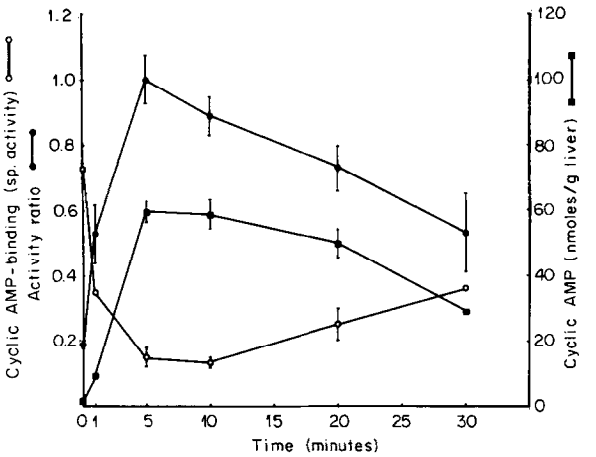

Figure 1: Time course of glucagon effect on cyclic AMP concentration, protein kinase activity ratio and cyclic AMP binding specific activity.

Data are given as mean ± standard error (indicated by vertical bars) of two to five animals per point. The standard error falls within the character for those points where no standard error is indicated. Differences between values at 5 and 10 minutes are not statistically significant.

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those in protein kinase activity ratio and cyclic AMP-binding activity.

Elevated activity ratios are apparently the result of both high protein kinase activity in the absence of cyclic AMP and low total protein kinase present in the enzyme preparations, as demonstrated in table 1. Protein kinase in the absence of the cyclic nucleotide is already increased about 3-fold at one minute after glucagon administration, reaching maximum values at 5 and 10 minutes. The total protein kinase, on the other hand, shows a decrease in specific activity which accounts for approximately 20 to 50% of the corresponding increases in activity ratio shown in figure 1. The statistical significance of changes in total protein kinase activity was examined by a one way analysis of variance (table 2); the value of F was significant at the 0.01 level. Scheffé's method of multiple comparisons (12) was then applied and the average levels at 0, 1 and 30 minutes were found to differ from those at 5, 10 and 20 minutes at the 0.05 level of significance.

Reduction of total protein kinase activity may be a rather general event

TABLE 1

EFFECT OF GLUCAGON ON PROTEIN KINASE SPECIFIC ACTIVITY

Animals were treated as indicated in text. Data are given as mean ± standard error, with number of animals per group indicated in parethesis.

Protein Kinase Assay	Time (minutes)					
	0	1	5	10	20	30
Minus Cyclic AMP	67±8 (4)	220±46 (2)	251±12 (5)	256±17 (5)	175±9 (5)	198±49 (2)
Plus Cyclic AMP	350±37 (4)	413±8 (2)	256±32 (5)	293±17 (5)	241±23 (5)	372±1 (2)

TABLE 2

ONE WAY ANALYSIS OF VARIANCE
(Total protein kinase specific activities)

Source of variation	d.f.	Sum of squares	Mean squares	F
Among groups	5	73353.81	14670.76	4.66**
Within groups	17	53482.80	3146.05	
Total	22	126836.61		

after hormonal stimulation since it has been observed in all tissues examined, (1, 3, 5) with the possible exception of rat fat pads and isolated fat cells (4). The present findings demonstrate that a similar situation obtains in the rat liver, following in vivo glucagon stimulation. This phenomenon has been attributed to translocation of dissociated protein kinase to the microsomal fraction in uteri from castrated rats (5) and to inactivation of released catalytic subunits by the heat stable inhibitor protein present in extracts from rat diaphragm (1).

In complementary experiments, 3 ml of a 70% glucose solution was given by gavage to rats; control animals received the same volume of saline after intubation. Glucagon, 0.5 mg/100 g weight, was injected subcutaneously to both groups at zero time. Determination of protein kinase activity both in the presence and absence of cyclic AMP at 5 and 10 minutes showed the same values for either glucose-fed or control animals, indicating that glucose does not interfere with the cyclic AMP-mediated glucagon activation of the enzyme. These results confirm previous in vitro studies from this laboratory (9) on the role of the cyclic nucleotide during glucose repression of enzyme induction.

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REFERENCES

1. Walaas, O., Walaas, E., and Grønnerød, O. (1973) Eur. J. Biochem. 40, 465-477.
2. Shen, L. C., Villar-Palasi, C., and Larner, J. (1970) Physiol. Chem. Physics, 2,536-544.
3. Ichii, S. (1972) Endocrinol. Japon., 19,229-235.
4. Soderling, T. R., Corbin, J. D., and Park, C. R. (1973) J. Biol. Chem., 248,1822-1829.

5. Korenman, S. G., Bhalla, R. C., Sanborn, B. M., and Stevens, R. H. (1974) Science, 183,430-432.
6. Brostrom, M. A., Reimann, E. M., Walsh, D. A., and Krebs, E. G. (1970) Adv. Enzyme Regul., 8,191-203.
7. Chambaut, A. M., Leray, R., and Hanoune, J. (1971) FEBS Letters, 15,328-334.
8. Corbin, J. D., Soderling, T. R., and Park, C. R. (1973) J. Biol. Chem., 248,1813-1821.
9. Sudilovsky, O. and Pitot, H. C. (1972) Proc. Soc. Exptl. Biol. and Med., 144,113-121.
10. Sudilovsky, O., Pestaña, A., Hinderaker, P. H., and Pitot, H. C. (1971) Science, 174,142-144.
11. Lowry, O. H., Rosenbrough, N. J., Farr, A. L., and Randall, R. (1951) J. Biol. Chem., 193,265-275.
12. Scheffé, H. (1959) The Analysis of Variance, pp. 1-477, John Wiley and Sons, Inc., New York.