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Regulation of Glycogenolysis in Isolated Rat Hepatocytes by the Specific Activation of Type I Cyclic AMP-Dependent Protein Kinase

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SUMMARY

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Incubation of isolated hepatocytes with concentrations of dibutyryl cyclic AMP from 0.1 μM to 10 μM led to a dose-dependent increase in the cyclic AMP-dependent protein kinase activity ratio. A concentration of 10 μM dibutyryl cyclic AMP increased the activity ratio from 0.24 to 0.85. Each concentration led to an optimal protein kinase activation within 5 min of incubation. Incubation of hepatocytes with 5 μM dibutyryl cyclic AMP resulted in a maximal production of glucose compared to control cultures. A concentration of 1 μM dibutyryl cyclic AMP resulted in a near maximal stimulation of glycogenolysis for the initial 60-min incubation period. Concentrations of dibutyryl cyclic AMP from 0.1 μM to 5 μM increased the protein kinase activity ratio from 0.24 to 0.59 and stimulated glycogenolysis in a dose-dependent manner as determined 20 min after the addition of dibutyryl cyclic AMP. Increasing the dibutyryl cyclic AMP to 10 μM increased the activity ratio from 0.59 to 0.85 but did not result in any further increase in glucose production. Concentrations of dibutyryl cyclic AMP from 0.1 μM to 5 μM resulted in the specific activation of type I protein kinase. As the concentration of dibutyryl cyclic AMP in the hepatocytes incubation was further increased to 10 μM, the type II holoenzyme was activated. A similar sequential activation of type I and type II protein kinase occurred with liver cells incubated with increasing concentrations of glucagon. Therefore, maximal stimulation of glycogenolysis was coupled with the selective activation of type I cyclic AMP-dependent protein kinase.

INTRODUCTION

The series of reactions involved in glycogenolysis can be rapidly stimulated in liver by either epinephrine or glucagon. The sequence of reactions begins with, in the case of the liver, α-adrenergic receptor-linked, and in the heart, mixed or β-adrenergic receptor-linked production of cAMP[4] and the subsequent activation of cyclic AMP-dependent protein kinase which catalyzes the adenosine triphosphate-dependent transformation of phosphorylase kinase to its activated form (1-

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[4] The abbreviations used are: cAMP, cyclic AMP; DBcAMP, dibutyryl cAMP.

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4). Phosphorylase kinase catalyzes the phosphorylation of phosphorylase *b* (inactive) to phosphorylase *a* (active) which catalyzes the phosphorylytic cleavage of glucose-1-phosphate from glycogen (1). Glycogen synthase, the enzyme involved in the synthesis of glycogen, is inactivated following phosphorylation by cAMP-dependent protein kinase (5–7). Therefore, an increased concentration of cAMP causes the simultaneous inactivation of glycogen synthase and activation of glycogen phosphorylase.

Cyclic AMP-dependent protein kinase (ATP-protein phosphotransferase E.C. 2.7.1.37) has been characterized as a soluble enzyme composed of two regulatory and two catalytic subunits (8). The inactive holoenzyme dissociates in the presence of cAMP into the active catalytic subunit and a regulatory subunit-cAMP complex: $R_2C_2 + 2cAMP \rightarrow 2(R\text{-}cAMP) + 2C$. There are two major isozyme forms of the holoenzyme referred to as type I and type II. The two isozymes are remarkably similar in different mammalian tissues (9). However, the holoenzymes themselves are quite dissimilar. Isozyme I elutes from DEAE-cellulose at less than 0.1 M NACl while isozyme II elutes at greater than 0.1 M NaCl (10). The type I kinases are more easily dissociated into free regulatory and catalytic subunits by incubation with histone or salt than are the type II kinases, and the separated regulatory and catalytic subunits of isozyme II reassociate more rapidly than the subunits of type I isozyme (10). Also, the type II kinase is capable of autophosphorylation, a process which enhances the net dissociation of the enzyme subunits (10, 11). The differences in the holoenzymes appear to reside in the regulatory subunits. To date, no differences between the catalytic subunits of the two isozymes have been noted (12). The catalytic subunits demonstrate identical specificities and relative rates of phosphorylation with a variety of protein substrates. In fact, catalytic subunits from the multiple protein kinases of rabbit skeletal muscle and rat liver phosphorylate the same serine and threonine residues in histone, protamine, phosphorylase kinase and glycogen synthetase (13).

In view of the apparent lack of substrate specificity and the variable distribution of the isozymes in a variety of tissues, it was of interest to determine if preferential activation of one form of the enzyme could be demonstrated in a tissue containing both forms of the protein kinase. There have been recent reports indicating that the two forms of protein kinase may be selectively compartmentalized (14–17). It is possible that cellular localization of isozymes could provide for either more specific regulation of the enzyme by cAMP or substrate specificity since the catalytic subunit might be generated near its endogenous substrate(s).

We have previously reported that activation of approximately one-half of the total soluble cAMP-dependent protein kinase present in isolated rat hepatocytes by a given concentration of glucagon was sufficient to achieve a maximal rate of glycogenolysis (18). Further activation of protein kinase, measured in supernatant preparations, by increased concentrations of glucagon did not lead to any further production of glucose in these cells. We postulated that since the liver has approximately equal amounts of the type I and type II isozymes, only one form of the enzyme may be involved in the intracellular breakdown of glycogen.

In the studies reported here, we were able to monitor the sequential activation of type I and type II cAMP-dependent protein kinases in isolated liver cells incubated with increased concentrations of glucagon and DBcAMP. The data in this communication indicate that the type I isozyme is predominantly activated with lower doses of either glucagon or DBcAMP. This enzyme activation correlates temporally and quantitatively with the rate of glycogenolysis and suggests that the type I cAMP-dependent protein kinase is the isozyme responsible for regulating glycogenolysis in liver cells.

METHODS

Primary culture in hepatocytes. Hepatocytes were prepared from male Sprague-Dawley rats (200–250 g) fed *ad libitum* a standard Purina rat chow diet and maintained on a 12-hr light-dark cycle at 22°. The livers were perfused with collagenase

(CLS II, 132 U/mg, Worthington Biochemical Corp., Freehold, NJ) via the portal vein and parenchymal cells isolated as previously described (18). The incubation buffer consisted of 3.25% dextran, 123 mM NaCl, 20 mM HEPES (Sigma Chemical Co., St. Louis, MO). 5 mM KCl, 2 mM $MgSO_4$, 2.5 mM K_2HPO_4, 0.154 nM penicillin, 0.8 mM $CaCl_2$, 0.2% bovine serum albumin, and 1 ml antibiotic-antimycotic (100×, Grand Island Biological Co., Grand Island, NY) per 100 ml medium. The incubation buffer was adjusted to a final pH of 7.4 and filtered under sterile conditions through a 0.45 μ Millipore filter.

Each incubation reaction contained 5 ml of cell suspension. The cells were pipetted into 25-ml Erlenmeyer flasks, the bottoms of which has been heated and pushed inward to form a conically raised center which kept the cells from settling. Cell suspensions contained 45–60 mg wet weight cells/ml or 4.4–5.9 × 10^6 cells/ml. Incubations were carried out at 30° and the reaction flasks were swirled in a gyrating incubator at 80 rpm.

Glucose was determined by centrifuging an aliquot of cells and directly assaying the supernatant in a Beckman Glucose Analyzer. Samples for glucose and protein kinase determinations were taken from the same suspension of cells.

Cyclic AMP-dependent protein kinase assay. The conditions for tissue preparation and assay of cAMP-dependent protein kinase were optimized to best preserve the hormonal effects on the enzymes. The procedure was similar to that reported by Cherrington *et al.* (19) and Byus *et al.* (18, 20). A 250-μl aliquot of cells (approximately 15 mg wet weight cells) was centrifuged (40 × *g* for 30 sec at 23°) and the supernatant removed. The pellet was rapidly sonicated in 250 μl of 0.01 M phosphate, pH 6.8, 0.5 mM 3-isobutyl-1-methylxanthine, 20 mM NaF, and 100 mM KCl. The homogenate was centrifuged for 2 min at 10,000 × *g*. The supernatant solution was removed and used as the source of enzyme. The protein kinase activity ratio was determined as previously described (18, 21) by measuring the protein kinase activity in the presence and absence of saturating concentrations of exogenous cAMP. The assay mixture contained 100 μg of mixed calf thymus histone, 10 mM phosphate buffer, pH 6.8, 25 mM $Mg(Ac)_2$, 20 mM NaF, 0.5–1.0 μCi [γ-^{32}P]-ATP (6 mCi/mmole, New England Nuclear, Boston, MA) plus sufficient unlabeled ATP for a concentration of 0.10 mM and 0.025 ml of a 1:1 dilution of the original enzyme extract. Protein kinase activity is expressed as the ratio of ^{32}P incorporated into histones in the absence and presence of 5 μM cAMP.

Under the above conditions, the basal and elevated activity ratios were stable for 60 min in the presence or absence of 5 μM cAMP; the reaction was linear with respect to time and protein concentration and 85–90% of the total protein kinase activity was found in the supernatant fraction.

C_6-Amino alkyl agarose chromatography. C_6-Amino alkyl agarose chromatography relies primarily upon a hydrophobic interaction between the C_6-alkyl chain bound to the resin and hydrophobic regions on proteins to achieve separation (22). This form of column chromatography was originally adapted for use in purifying the protein kinases and their subunits because the catalytic and regulatory subunits do not reassociate if chromatographed rapidly on the C_6-agarose (23, 24). Significant reassociation does occur, however, with DEAE-cellulose.

The C_6-agarose was synthesized from CNBr-activated agarose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) and 1,6-diaminohexane (Aldrich Chemical Co., Milwaukee, WI). Each batch of resin was capable of binding different amounts of liver cell supernatant protein and required 30–75 mM NaCl to elute the free catalytic subunit from the column. For this reason, each new batch of resin was calibrated to determine the amount of supernatant protein bound/ml resin and the initial NaCl concentration needed to elute free catalytic subunit without any detectable reassociation after application of beef heart type II cyclic AMP-dependent protein kinase (21) totally activated by cyclic AMP. In order to prevent reassociation of the catalytic and regulatory subunits, the column had to be equilibrated with buffer at sufficient salt concentrations

to rapidly elute the free catalytic subunit in the flow-through fractions. Type I holoenzyme (R_IC) and type II holoenzyme ($R_{II}C$) were purified 20-fold from rat and beef hearts, respectively, in order to determine their relative abilities to bind to the C_6-agarose. Type I eluted at 120 mM NaCl and type II at 250 mM NaCl. Total activation with cyclic AMP (50 pmole in 250 μl at 30° for 2 min) prior to C_6-agarose chromatography resulted in all of the type I eluting as catalytic subunit with no reassociation. Similar results were obtained with the purified type II enzyme. This procedure was previously worked out for specific activation studies of mitogen-stimulated lymphocytes (see Fig. 1 of reference 20). The percent of the total pool of type I and type II cyclic AMP-dependent protein kinase present as catalytic subunit after C_6-agarose chromatography was the same as the percent of activity detectable in the absence of exogenous cyclic AMP in the standard cyclic AMP-dependent protein kinase assay (18, 20). These data suggested that both assays preserved the physiological activation state of both kinases.

Columns were prepared in disposable plastic 2-ml syringes containing 1.5 ml of resin. The columns were equilibrated with 10 mM $NaPO_4$, pH 6.8, 1 mM EDTA, 5 mM β-mercaptoethanol, and 30–75 mM NaCl (depending upon the particular batch of resin). Two-hundred and fifty microliters of liver cells (15 mg wet weight) were centrifuged gently to remove the culture media. The cells were then sonicated at 4° in 500 μl of the column equilibration buffer and centrifuged for 30 sec in a Beckman Microfuge. Four hundred microliters of supernatant was applied to the column which was rapidly washed with 5–10 ml of the above buffer. (Any free catalytic subunit present in the liver cell supernatant eluted in these flow-through fractions.) A 20 ml linear gradient consisting of 30–75 mM NaCl and 250 mM NaCl was then begun. An aliquot (50 μl) of each fraction was assayed for protein kinase activity as described above in the presence of 5 μM cAMP.

RESULTS

Activation of soluble cAMP-dependent protein kinase by DBcAMP. The effects of concentrations of DBcAMP from 0.01 μM to 10 μM on the activation of protein kinase in liver cells is shown in Fig. 1. Following incubation of the cells for 5 min with DBcAMP, the protein kinase activity ratio increased in a dose-dependent manner. A concentration of 10 μM DBcAMP increased the activity ratio from 0.24 to 0.85. The effect of each concentration of DBcAMP on protein kinase activation was maximal within 5 min of incubation. The protein kinase activity ratio determined in hepatocytes incubated with 10 μM DBcAMP decreased from 0.85 to 0.65 between 5 and 55 min after addition of the cyclic nucleotide. Hepatocytes incubated with 5 and 1.0 μM DBcAMP showed a similar decrease in activity ratio with continued time in culture.

Stimulation of glycogenolysis and activation of protein kinase by DBcAMP. The effects of increasing concentrations of DBcAMP on the production of glucose in liver cells is shown in Fig. 2. Incubation of the hepatocytes with 5 or 10 μM DBcAMP caused the maximal production of glucose compared to control cultures at all times

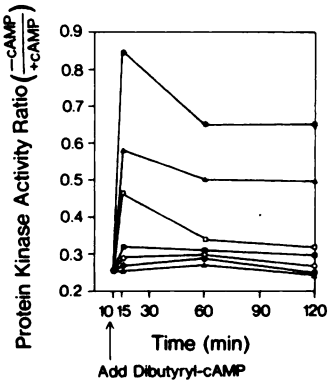

FIG. 1. *The effect of DBcAMP on cAMP-dependent protein kinase activation in isolated liver cells*

Cyclic AMP-dependent protein kinase activation was measured in control cultures (△) and in cultures following the addition of 0.01 μM (●), 0.10 μM (○), 0.5 μM (■), 1.0 μM (□), 5 μM (▲), and 10 μM (★) DBcAMP as described in METHODS. Incubations were carried out at 30° with liver cell concentrations of 50 mg/ml wet weight. The points are the average of duplicate samples not differing by more than ±5%. This experiment was repeated three times and the data shown are from a representative experiment.

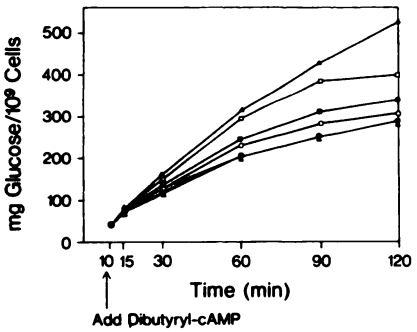

FIG. 2. *The effect of DBcAMP on glycogenolysis in isolated liver cells*

The rates of glycogenolysis were measured in control cultures (△) and in cultures following the addition of 0.01 μM (●), 0.10 μM (○), 0.5 μM (■), 1.0 μM (□), and 5 μM or 10 μM (▲) DBcAMP as described in METHODS. The amount of glucose produced in cultures incubated with 5 μM or 10 μM DBcAMP were identical at all the times indicated and is shown as a single line for both concentrations. The data represented in Figs. 1 and 2 were taken from the same experiment.

studied following the addition of the cyclic nucleotide. A concentration of 1.0 μM DBcAMP caused a near-maximal stimulation of glycogenolysis for the initial 60-min incubation period. The rate of glucose production decreased between 60 and 120 min compared to the stimulation caused by 5 and 10 μM DBcAMP. Lower concentrations of DBcAMP (0.5 and 0.1 μM) also increased the rate of glycogenolysis throughout the 120-min incubation period.

As the concentration of DBcAMP was increased in the incubation media from 0.01 to 5.0 μM, both the change in protein kinase activity ratio from 0.27 to 0.59 (Fig. 1) and the stimulation of glycogenolysis (Fig. 2) increased in a dose-dependent manner when determined 20 min after the addition of DBcAMP. However, when the protein kinase activity ratio was increased from 0.59 to 0.85 following an increase in the concentration of DBcAMP from 5 to 10 μM (Fig. 1), no additional increase in glucose production was observed (Fig. 2). We have reported previously that a similar relationship exists between the degree of protein kinase activation and elevated rates of glycogenolysis following incubation of liver cells with varying concentrations of glucagon (18).

Specific activation of protein kinase isozymes by DBcAMP and glucagon. The activation of type I and type II protein kinase isozymes following incubation of hepatocytes with increasing doses of DBcAMP is illustrated in Fig. 3. C_6-amino alkyl agarose chromatography of supernatants from control (unstimulated) liver cells showed that protein kinase activity was divided between free catalytic subunit and type I and type II holoenzymes in a ratio of approximately 1:1:2 (Fig. 3). As the concentration of DBcAMP was increased to 0.1 and 5 μM, type I protein kinase became activated. This can be seen as a decrease in kinase activity eluting with type I holoenzyme and an increase in free catalytic subunit which appears in the flow-through fractions. No change in the kinase activity eluting as type II holoenzyme occurred. As the concentration of DBcAMP in the hepatocyte incubation was further increased to 10 μM, the type II holoenzyme was activated causing additional protein kinase activity to appear as free catalytic subunit (Fig. 3). A similar sequential activation of type I and type II protein kinase occurred with liver cells incubated with increasing concentrations of glucagon (Fig. 4).

Relationship of type I kinase activation to the stimulation of glycogenolysis. The specific activation of type I and II protein kinase by increasing concentrations of DBcAMP and glucagon in relation to the ability of these agents to stimulate glycogenolysis is shown in Table 1. Incubation of hepatocytes with 0.1 μM DBcAMP for 5 min leads to the activation of 18% of type I kinase and 17% of maximal glucose production within 60 min (Table 1). A concentration of DBcAMP of 5.0 μM activates 85% of type I and results in a maximal stimulation of glycogenolysis. Neither 0.1 nor 5 μM DBcAMP results in any significant activation of type II kinase. A further increase in DBcAMP concentration to 10 μM leads to the activation of 95% of type II kinase without any further stimulation of glycogenolysis (Table 1).

In a similar manner, liver cells incubated with 0.01 μM glucagon were producing glucose at 85% of their maximal rate with 81% of type I kinase activated and only 4% ac-

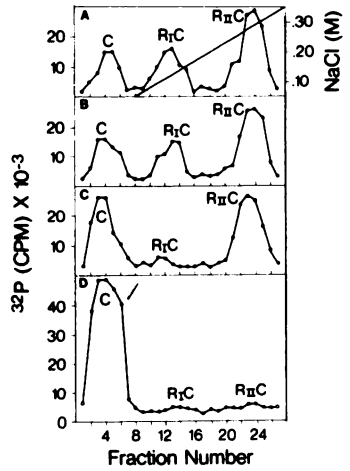

FIG. 3. *Sequential activation of type I and type II cAMP-dependent protein kinase by DBcAMP in isolated liver cells*

Protein kinase activation was measured in control cultures (A) and following the addition of 0.10 μM (B), 5.0 μM (C), and 10.0 μM (D) DBcAMP. Cells were incubated with DBcAMP for 5 min at 30°. One milliliter (50 mg/ml) of cells was removed and spun at 10 × *g* for 1 min. The packed cells were then sonicated for 5 sec in 0.5 ml of 15 mM sodium-potassium phosphate, pH 6.8, 4 mM β-mercaptoethanol, 5 mM $F1^-$, 1.0 mM EDTA, 0.5 mM 3-isobutyl-1-methylxanthine and 50 mM NaCl and centrifuged at 10,000 × *g* for 30 sec. The supernatant (400 μl) was applied to an 0.8 ml C_8-diaminoagarose column equilibrated in the same buffer. The column was washed for 8 fractions (0.50 ml/fraction) with equilibration buffer followed by a linear gradient from 0.05 to 0.35 M NaCl.

Prior experiments performed for each new batch of resin with purified catalytic subunit (C), type I holoenzyme (R_IC), and type II holoenzyme ($R_{II}C$) determined the initial salt concentration to elute the free catalytic subunit in the flow-through fraction and the gradient conditions to separate type I from type II. Fifty microliters of each fraction was assayed for protein kinase activity in the presence of 5 μM cAMP with mixed calf thymus histone as the substrate. The amount of ^{32}P incorporated into histone from [γ-^{32}P]-ATP is shown. The flow-through activity was not stimulated by cAMP (i.e., free catalytic subunit), whereas type I and type II were stimulated 5- to 8-fold by exogenous cAMP. Recovery of activity from each column was greater than 85%.

tivation of type II kinase. As the dose of glucagon was increased to 1 mM, type I and type II kinases were activated 90% and 85%, respectively, resulting in a maximal stimulation of glycogenolysis.

The data suggest that type I kinase is

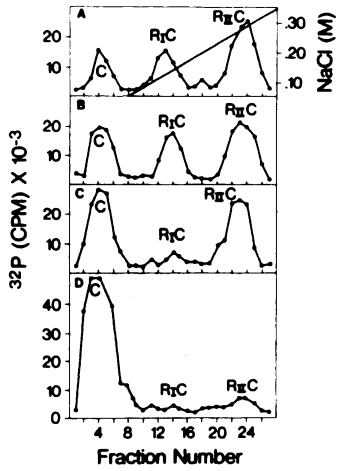

FIG. 4. *Sequential activation of type I and type II cAMP-dependent protein kinase by glucagon in isolated liver cells*

Protein kinase activation was measured in control cultures (A), and following the addition of 0.001 μM (B), 0.01 μM (C), 1.0 μM (D) glucagon. Cells were incubated with glucagon for 5 min at 30°. Column chromatography was performed as described in the legend to Fig. 3 and in METHODS.

preferentially activated as the concentration of either DBcAMP or glucagon is increased, and total activation of this isozyme leads to a maximal increase in the rate of glycogenolysis. Concomitant activation of type II by higher concentrations of DBcAMP or glucagon does not lead to any further increase in the rate of glucose production.

DISCUSSION

We have studied the relationship between the activation of the two isozymes of protein kinase, the activity ratios of the soluble kinases, and rates of glycogenolysis in isolated rat hepatocytes. The data suggest that as the intracellular level of cAMP increases in primary cultured hepatocytes in response to the action of glucagon on adenylate cyclase or through incubation of the cells with a cyclic nucleotide analogue (DBcAMP), specific activation of type I cAMP-dependent protein kinase isozyme occurs. Higher concentrations of glucagon and DBcAMP cause the activation of both kinase isozymes (Figs. 3 and 4). There have been several reports which indicate that, depending upon the assay conditions, type

TABLE 1

Type I and type II cyclic AMP-dependent protein kinase activation and stimulation of glycogenolysis

The percent of the specific protein kinase isozyme activated relative to control values was determined from the C_8-agarose profiles shown in Figures 3 and 4 for each concentration of DBcAMP and glucagon. The percent of glucose production for each level of DBcAMP and glucagon was calculated relative to the maximal amount which the cells could produce when incubated with high concentrations of DBcAMP (10.0 μM) and glucagon (1.0 μM).

Additions	Activity ratios	(% of control kinase isozyme activated) Type I	Type II	(% of maximal glucose production) Glycogenolysis
(μM)				
0.10 DBcAMP	0.27	18	10	17
5.0 DBcAMP	0.59	85	5	100
10 DBcAMP	0.85	90	95	100
0.001 glucagon	0.25	2	2	7
0.01 glucagon	0.54	81	4	86
1.0 glucagon	0.75	90	85	100

I protein kinase has a greater affinity for cAMP than the type II kinase (25). However, the degree of dissociation of both type I and type II holoenzymes by cAMP *in vitro* appears to be dependent upon the MgATP concentration. At a concentration of ATP greater than 1.0 μM *in vitro*, both type I and type II kinase dissociate at the same concentration of cAMP (25). Since the ATP level in normal liver has been reported to be 1.0 mM, the regulatory subunits of type I and type II would be expected to have similar affinities for cAMP and both holoenzymes would dissociate concurrently as the concentration of the cyclic nucleotide increased.

The fact that type I kinase from isolated hepatocytes is activated prior to type II (Figs. 3 and 4) suggests that cAMP is preferentially accumulated in certain areas of the cell in proximity with the type I holoenzyme. Alternatively, type II kinase may have a decreased affinity for cAMP in the cell compared to its actions *in vitro* due to an association with specific organelles (ribosomes, endoplasmic reticulum, membranes) or because of the extent of phosphorylation of the R_{II} regulatory subunit. Rangel-Aldao and Rosen (11) have shown that the phosphorylation of the regulatory subunit from type II kinase (type I kinase cannot be modified in this manner) facilitates the net dissociation of the enzyme by cAMP. While type II kinase is believed to be present in the cell as the phosphoenzyme (11), no studies of this nature have been reported. In primary cultured hepatocytes, type II holoenzyme may exist principally as the dephosphoenzyme imparting a selective advantage for the activation of type I kinase at lower concentrations of cAMP.

The catalytic subunits from both type I and II isozymes are capable of phosphorylating phosphorylase kinase and glycogen synthase, thereby stimulating glycogenolysis while inhibiting glycogenesis. However, activation of type II kinase by the higher doses of DBcAMP and glucagon resulted in only a small additional increase in the rate of glucose formation even though there was a large increase in the amount of free catalytic subunit (Table 1) present in the cytoplasm. It is possible that dissociation of type I protein kinase releases sufficient catalytic subunit to activate all of the phosphorylase kinase present in the cell or that the type I enzyme is in close proximity to the enzymes regulating glycogenolysis.

Recently, Corbin and Keely (17) have compared the activation-deactivation characteristics of protein kinase from rat heart (predominantly type I) and guinea pig heart (predominantly type II) following perfusion with epinephrine and 3-isobutyl-1-methylxanthine. They found that the protein kinase activity ratios of guinea pig heart were less affected by equivalent elevations in cAMP than was the rat heart enzyme. The rat heart enzyme was elevated from a control activity ratio of 0.15 to 0.55 with epinephrine while the guinea pig heart enzyme was activated from a control value of 0.2 to 0.4 suggesting that at similar intracellular levels of cAMP, the type I kinase is preferentially activated.

Schwoch and Hilz (26) have observed differential activation of type I and type II cAMP-dependent protein kinase in the liver of glucagon-treated rats. Type I kinase was found to be activated at lower intracel-

lular concentrations of cAMP. The significance of the distribution of cAMP-dependent protein kinase isozymes in a wide variety of eukaryotic tissues is not known. Type I and type II do, however, have different rates of synthesis and turnover which appear to be related to specific cellular growth conditions which include: progression through the cell cycle (27), cardiac hypertrophy (28), embryonic development (29), and following DBcAMP addition to several cell lines (30). Continued investigation of the specific activation patterns of type I and type II isozymes in relation to their ability to regulate glycogenolysis, lipolysis, gluconeogenesis, and the induction of particular enzymes may help define the unique roles of these isozymes in mediating the actions of cAMP.

Therefore, data presented in this paper along with other studies of differential activation of type I and type II cyclic AMP-dependent protein kinase are consistent with but do not rigorously document the unique relationship between glycogenolysis and type I protein kinase.

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