The cyclic AMP-dependent protein kinase (isoenzymes I and II) was measured using a synthetic peptide analog of the porcine hepatic phosphorylation site sequence (Leu-Arg-Arg-Ala-Ser-Leu-Gly). Epinephrine caused a time- and dose-dependent activation of the hepatic cyclic AMP-dependent protein kinase and inactivation of pyruvate kinase. Maximal activation of the protein kinase occurred within 1 min with an increase in the protein kinase activity ratio from 0.06 ± 0.01 to 0.18 ± 0.02. Both the total cyclic AMP-dependent protein kinase activity and the component stimulated by epinephrine were inhibited more than 90% by the heat-stable inhibitor protein of the protein kinase. The apparent epinephrine-stimulated protein kinase activity ratio was stabilized by the presence of salt in the extraction buffer, suggesting that this hormone preferentially activates the cyclic AMP-dependent protein kinase isozyme II in isolated hepatocytes.

Application of selected α- and β-agonists and antagonists failed to reveal a strong correlation between epinephrine-mediated changes in protein kinase activity ratio, pyruvate kinase activity, and gluconeogenesis from lactate. Activation of the protein kinase by 10 μM epinephrine was partially blocked by the β-antagonist, propranolol (10 μM) and the α-antagonists, phenoxybenzamine (10 μM) and phentolamine (10 μM). The inactivation of pyruvate kinase and stimulation of gluconeogenesis were preferentially blocked by the α-antagonists. Compared with epinephrine, the β-agonist, isoproterenol (0.1 mM), had a weaker effect on the activation of the protein kinase, inhibition of pyruvate kinase activity, and stimulation of gluconeogenesis. Nevertheless, the isoproterenol effects were each specifically blocked by propranolol. The effects produced by the α-agonist, phenylephrine, were similarly affected by α- and β-antagonists as were those of epinephrine; the inactivation of pyruvate kinase and stimulation of gluconeogenesis were largely blocked by phenoxybenzamine. Other α-agonists, oxymetazoline, naphazoline, and tetrahydrozoline, were without effect. Overall, our data suggest that epinephrine-stimulated hepatocyte gluconeogenesis may be mediated by activation of the cyclic AMP-dependent protein kinase.

Early studies by Exton and Park (reviewed in Ref. 1) provided evidence that hepatic gluconeogenesis from pyruvate and lactate was under hormonal control by glucagon and epinephrine. It was proposed that the regulation of gluconeogenesis by these hormones was mediated by cyclic AMP. More recently, Taunt and his co-workers (2, 3) reported that the activities of hepatic fructose-1,6-bisphosphatase, phosphofructokinase, and pyruvate kinase were under hormonal control. These observations were consistent with the idea that the regulation of gluconeogenesis may involve the coordinated phosphorylation (interconversion) of a number of hepatic glycolytic and gluconeogenic enzymes in a manner analogous to the classical mechanism proposed for glycogen metabolism (reviewed in Ref. 4).

The demonstration by Engstrom and his co-workers (5) that hepatic pyruvate kinase was phosphorylated and inactivated by the cyclic AMP-dependent protein kinase in vitro further encouraged the development of the idea that protein phosphorylation was important in the regulation of gluconeogenesis.

The concept that glucagon regulates the interconversion of hepatic pyruvate kinase by a cyclic AMP-dependent protein phosphorylation mechanism is supported by evidence from many studies (6-11). By analogy, it might be expected that control of hepatic pyruvate kinase by epinephrine would be mediated by the β-adrenergic receptor since this receptor is believed to be linked to the adenyl cyclase. However, several studies with adrenergic agonists and antagonists have indicated that the control of gluconeogenesis by epinephrine is mediated by an α-adrenergic mechanism independent of cyclic AMP (12-15). This model implies that phosphorylation of pyruvate kinase is not essential for adrenergic stimulation of gluconeogenesis or alternatively, interconversion of pyruvate kinase may also be under α-adrenergic control by a hitherto unknown mechanism. The goal of this study was to investigate the relationship between the cyclic AMP-dependent protein kinase activity ratio, the activity of pyruvate kinase and the rate of gluconeogenesis with the expectation that this information would contribute to our understanding of the role of pyruvate kinase interconversion in the control of hepatic gluconeogenesis by epinephrine.

MATERIALS AND METHODS

Chemicals—Sodium L-1-[14C]lactate was purchased from American-Searle; [32P]orthophosphate from Australian Atomic Energy Commission; l-epinephrine bitartrate, 1-methyl-3-isobutyl xanthine, dichloroisoproterenol-HCl; and l-isoproterenol d-bitartrate from Sigma; dl-propranolol-HCl from Ayerst Laboratories; phenoxybenzamine-HCl from Smith, Kline and French Laboratories; phentolamine mesylate and naphazoline-HCl from CIBA-GEIGY; butoxamine-HCl from Burroughs Wellcome & Co.; phenylephrine-HCl

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from Winthrop Laboratories; oxymetazoline-HCl from Schering Corp.; and tetrahydrozoline-HCl from Pfizer Laboratories.

**Hepatocyte Incubation and General Analyses**—Isolated liver parenchymal cells were prepared from fed (185 g) rats by a modification of the procedure of Berry and Friend (16) with omission of hyaluronidase and substitution of Krebs-Henseleit saline buffer (pH 7.4) (17). Buffers were equilibrated with 95% O2, 5% CO2 before use. Isolated hepatocytes (50 mg, wet weight/ml) were incubated in glass vials (2 ml) in a total volume of 1.2 ml and shaken in a water bath (37°C) at 100 oscillations/min. Incubations were commenced by the addition of cells to vials containing CaCl2 (1.3 mM) and where appropriate, 50 µM methyl-isobutyl xanthine and antago-

ists. Each vial was assayed with the O2/C02 mixture, stopped, and placed in the water bath and preincubated for 15 min. After the 15-

min preincubation, [3-14C]lactate and, where appropriate, agonists were added. Samples (0.2 ml) were removed after a further 1-min incubation (for cyclic AMP, protein kinase, and pyruvate kinase analyses). Measurements of gluconeogenesis and glucose output were made on aliquots (0.2 ml) of the hepatocyte suspension added to 0.1 ml of 6% (w/w) HClO4. After thorough mixing, the pH was adjusted to pH 7 using 1 M KOH. Denatured protein and potassium perchlorate were removed by centrifugation. Glucose output was assessed by measuring the concentration of glucose in neutralized samples using the glucose oxidase-peroxidase method (18) at the beginning and during the incubation. The basal rate (mean ± SE) of glucose output (µmol glucose/mg wet weight/h) was 4.9 ± 0.7. Epinephrine failed to reduce it, indicating that the background radioactiv-

ity was either neutral or positively charged in the presence of 30% acetic acid. Since the level of background radioactivity was 4.9 ± 0.7 nmol/h/mg, wet weight, determined after 15 min incubation in the presence of 1.73 mM [3-14C]lactate (mean ± SE for 10 experiments). Incubations for cyclic AMP determinations contained unlabeled lactate and cyclic AMP was measured by the protein binding assay of Gilman (20).

Unless otherwise indicated, the data are presented as the mean ± SE of multiple incubations using a single cell preparation.

**Enzyme Assays**—Aliquots of the hepatocyte suspension (0.2 ml) were added to an equal volume of ice cold extraction buffer (containing Triton X-100) and stored on ice in Eppendorf microfuge tubes. The extraction buffer (pH 7.2) contained 50 mM potassium phosphate, 10 mM Na3 EDTA, 2 mM methyl-isobutyl xanthine, 2% Triton X-100, 30 mM 2-

methyl-2-pentenyl thiol, and 20 mM NaF. The purpose of adding the above components to the phosphate buffer was to minimize phosphodies-
terase, phosphoprotein phosphatase, and protease activities in the hepatocyte extracts (21). The final concentration of these components in the hepatocyte extracts was half the above values. Extracts were centrifuged for approximately 1 min in an Eppendorf microfuge to sediment any microsomal material.

**Enzyme Analysis**—Pyruvate kinase activity was measured essentially as described by Felu et al. (7). (Aliquots (usually 25 µl) of the cell extract were assayed at room temperature (22°C) by recording at 340 nm the oxidation of NADH in a Gilford spectrophotometer. The pyruvate kinase assay mixture contained 50 mM glycylglycine buffer at pH 7.4, 0.1 M MgCl2, 10 mM Na2 EDTA, 10 mM NaF, 0.1% Triton X-100, and 2 units of lactate dehydrogenase in a total volume of 2.06 ml. The reaction was started by adding a unlabeled lactate and NADH (0.15 mM).

Cyclic AMP-dependent protein kinase activity was measured using the synthetic peptide substrate corresponding to part of the phospho-
lipid (phosphorylation site sequence) of the porcine hepatic pyruvate kinase (22). Studies on the substrate specificity of the cyclic AMP-dependent protein kinase have shown that because of its higher Kcat, this peptide (Leu-

Arg-Arg-Ala-Ser-Leu-Gly) is a better substrate than the commonly used synthetic peptide, and 10 µl of hepatocyte extract + 5 µM cyclic AMP in a total volume of 100 µl. All components in the hepatocyte extract were therefore present in the protein kinase reaction mixture at a dilution of 10-fold. Incubations were terminated by adding 0.5 ml of 30% acetic acid and the reaction mixtures were transferred quantitatively to anion exchange columns (4 ml, AG 1-8x, acetate, Bio-Rad). The phosphorylated peptide was eluted directly into liquid scintillation vials with 30% acetic acid and counted using Cerenkov radiation (21). Weighing apparatus, apparent efficiency, 50%; Nuclear Chicago Model III) as described previously (24).

The heat-stable inhibitor protein of the cyclic AMP-dependent protein kinase was isolated from bovine heart muscle and purified according to the procedure of Walsh et al. (25).

**RESULTS**

**Measurement of Epinephrine-mediated Activation of Cyclic AMP-dependent Protein Kinase**—In this study, the “activity ratio” (26) of the cyclic AMP-dependent protein kinase has been used to estimate the hormonal activation of the enzyme. The activity ratio is defined as the ratio of protein kinase activity measured in the absence and presence of added cyclic AMP and reflects the extent of dissociation of the enzyme. The procedures for measuring hormone-mediated changes in the protein kinase activity ratio used in this study were based on those of Corbin and his colleagues (21).

Since the peptide substrate of the cyclic AMP-dependent protein kinase has not been used previously in hepatocyte extract studies, it was considered germane to demonstrate that the protein kinase activities measured corresponded to those found by other workers. Fig. 1 shows that the Triton X-

100 hepatocyte extracts contained the two major protein kinase isoenzyme activity peaks when chromatographed on DEAE-cellulose as observed by others using protein sub-

strates (21). Details of the synthesis and characterization of the synthetic peptide substrate together with the results of a study of the protein kinase assay conditions using the peptide as substrate are given in the supplement 2 which contains Figs. 2 and 3. Three protein kinase assays were performed on every extract. One assay contained both synthetic peptide substrate and cyclic AMP; the second peptide, without cyclic AMP; and the third, a control without either peptide or cyclic AMP. In this way, a measure of the endogenous phosphorylation, pep-
tide phosphorylation minus cyclic AMP and the cyclic AMP-

dependent peptide phosphorylation were obtained for each extract. The level of background radioactivity observed in the absence of synthetic peptide was approximately 6% of the total protein kinase activity. The background radioactivity was stable to both alkali and acid treatment (1 M, room temperature 15 h), indicating that it probably did not contain O-phosphoserine or O-phosphothreonine side chain linkages. Fur-

thermore, repeated passage through anion exchange columns failed to reduce it, indicating that the background radioactiv-

ty was either neutral or positively charged in the presence of 30% acetic acid. Since the level of background radioactivity was unaffected by any of the hormone treatments, it was not characterized further.

There was a gradual rise in the protein kinase activity ratio if extracts were stored on ice (Fig. 4, Panel A) and for this reason, enzyme assays were staggered in time so that the total elapsed time after extraction (less than 15 min) was kept approximately constant for all hepatocyte incubations. The epinephrine-mediated increase in the protein kinase activity ratio was observed when aliquots of the cell suspension were added directly to the protein kinase assay tubes containing Triton X-100 (at room temperature). In this way, protein

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1 The abbreviations used are: methylisobutyl xanthine, 1 methyl 3-isobutyl xanthine; EGTA, ethylene glycol bis(β-aminoethyl ether)-N,N'-tetraacetic acid.

2 Some of the data, including Figs. 2 and 3, are presented as a miniprint supplement immediately following this paper. These data are from a study of the assay conditions employed in measuring the cyclic AMP-dependent protein kinase activity in extracts of hepatocytes with the synthetic peptide substrate. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, Md. 20014. Request Document No. 77M-1724, cite author(s) and include a check or money order for $1.00 per set of photocopies.
Control of Protein Kinase and Pyruvate Kinase

Serine protein kinase released from the hepatocytes would immediately be in contact with synthetic peptide substrate and \([\gamma^3P]ATP\). When this procedure was followed, values of 0.09 and 0.17 were obtained for the protein kinase activity ratio in cells treated with saline (0.9% NaCl solution) and 10 μM epinephrine, respectively. These corresponded to values of 0.06 and 0.24 which were obtained using the conventional procedure. It is clear that epinephrine caused a measurable increase in the protein kinase activity ratio even when the protein kinase activity was determined as near as practicable to the time of extraction.

The total protein kinase activity (measured in the presence of saturating cyclic AMP) and the epinephrine-stimulated activity (measured in the absence of added cyclic AMP) were inhibited by approximately 90% in the presence of the specific heat-stable inhibitor (25) of the cyclic AMP-dependent protein kinase (Fig. 4, Panel B). These results indicate that essentially all of the synthetic peptide phosphorylation measured was catalyzed by the cyclic AMP-dependent protein kinase.

**Effect of Extraction Buffer Salt Concentration on Apparent Epinephrine Stimulation of Protein Kinase Activity Ratio—**It has been reported that the two cyclic AMP-dependent protein kinase isozymes differ in their susceptibility to dissociation by salt (21). The salt concentration present in the hepatocyte extracts employed in this study (44 mM K’ and 92 mM Na’) by flame photometry) was slightly less than that used by Cherrington et al. (21) but essentially represented a compromise choice between the differing ionic requirements of the two isozymes for stability in the extraction buffer.

When extracts of hepatocytes were prepared by the procedure of Cherrington et al. (21) using buffer containing 150 mM KCl, the increase in the protein kinase activity ratio over basal caused by epinephrine was highest (Table I). In contrast, when extracts were prepared in buffer containing low levels of salt according to the procedure of Birnbaum and Fain (27), the epinephrine-mediated increase in the protein kinase activity ratio was minimal (Table I). Similar salt-dependent effects on the apparent epinephrine-stimulated protein kinase activity ratio were observed when hepatocyte extracts were prepared by...
pared using buffers containing Triton X-100 (results not shown).

**Epinephrine Time Course and Dose-Response for Activation of Protein Kinase; Inhibition of Pyruvate Kinase and Stimulation of Gluconeogenesis**—Activation of the protein kinase was maximal by 30 s with half-maximal activation occurring at approximately 20 s (Fig. 5). No activation of the protein kinase was observed if epinephrine was added after extracts were prepared. Inactivation of pyruvate kinase was also time-dependent with half-maximal inactivation occurring after 30 s (Fig. 5). The corresponding time courses of gluconeogenesis at different levels of epinephrine are shown in Fig. 6, Panel B.

Increasing concentrations of epinephrine decreased the activity of pyruvate kinase (measured at subsaturating levels of phosphoenolpyruvate) in parallel with the activation of the protein kinase (Fig. 7). The highest level of epinephrine tested (10 μM) was nearly saturating with respect to protein kinase activation and pyruvate kinase inactivation. The dose-response curves suggest that half maximal effects occur in the epinephrine concentration range of 0.1 to 1 μM. The corresponding dose-response curves for gluconeogenesis ([14C]glucose + [14C]glycogen synthesis) and glucose output are shown in Fig. 6 Panel A.

**Effect of a- and β-Antagonists on Action of Epinephrine**

The effects of propranolol and phenoxybenzamine were tested on the epinephrine-mediated changes in the following parameters; cyclic AMP, protein kinase activity ratio, pyruvate kinase activity, glucose output, and gluconeogenesis (Fig. 8). Both 0.1 mM propranolol and 10 μM phenoxybenzamine appeared to block the stimulation of the protein kinase, whereas epinephrine stimulation of gluconeogenesis was more strongly affected by 10 μM phenoxybenzamine. The results of a series of experiments in which these adrenergic antagonists were tested on the action of epinephrine on pyruvate kinase and the protein kinase are summarized in Table II. The extent of inhibition by propranolol (0.1 mM) was variable from one hepatocyte preparation to another and complete inhibition was never observed. The a-adrenergic antagonist phenoxybenzamine (10 μM) inhibited the protein kinase activation by approximately the same extent as 0.1 mM propranolol but was also variable in its effect. However, when both 10 μM phenoxybenzamine and 0.1 mM propranolol were added together, the epinephrine stimulation of the protein kinase was consistently reduced to control levels. Another a-adrenergic antagonist, phentolamine (0.1 mM) also inhibited the activation of the protein kinase. The adrenergic antagonists (α or β) alone at the above concentrations had no significant effect on the basal protein kinase activity (Fig. 8) nor was the total protein kinase activity measured in the presence of cyclic AMP altered by the adrenergic antagonists carried over into the protein kinase assays from the hepatocyte extracts.

In general, when protein kinase activation was strongly inhibited by adrenergic antagonists, the epinephrine-mediated inhibition of pyruvate kinase was relieved. These effects of the α-adrenergic antagonists, phenoxybenzamine, and phen-
Control of Protein Kinase and Pyruvate Kinase

tolamine on the protein kinase activation were dose-dependent (Fig. 9) and correlated with the changes in pyruvate kinase activity. It is evident from the dose-response results with the \( \alpha \)-and \( \beta \)-antagonists that the protein kinase activation and pyruvate kinase inactivation were not affected equally. Propranolol (0.1 mM) and phenoxybenzamine (0.1 mM) both produced approximately the same degree of inhibition of the protein kinase activation and yet phenoxybenzamine had a more dramatic effect on pyruvate kinase.

The \( \beta \)-antagonist dichloroisoproterenol, which was also tested, had no effect on the epinephrine-mediated activation of the protein kinase and inactivation of pyruvate kinase over the range 1 to 10 \( \mu \)M. However, 1 mM dichloroisoproterenol had marked effects reducing the epinephrine-stimulated protein kinase activity ratio from 0.14 \( \pm \) 0.01 to 0.07, compared to the control of 0.05, and reversing the epinephrine-inhibited pyruvate kinase activity from 6.4 \( \pm \) 0.3 units to 12.8 units, compared to the control value 14.3 \( \pm \) 0.3 units. At high concentrations (1 mM), both \( \beta \)-antagonists inhibited basal gluconeogenesis (65 and 45% inhibited by dichloroisoproterenol and propranolol, respectively) possibly as a result of toxic effects on the hepatocytes or because the basal level of cyclic AMP contributes to the control of basal gluconeogenesis under the conditions employed.

The \( \alpha \)-adrenergic antagonist butoxamine (1 \( \mu \)M to 1 mM) (28) appeared to have no significant effect against epinephrine.

**FIG. 8.** Effect of adrenergic antagonists on the epinephrine-mediated changes in cyclic AMP levels, protein kinase activity ratio, pyruvate kinase activity, glucose output, and gluconeogenesis in isolated hepatocytes. The data represent the results of a single liver cell preparation experiment in which four parameters were measured. For cyclic AMP determinations were taken from a second experiment without [U-\( ^{14} \)C]lactate present. Cyclic AMP, protein kinase, and pyruvate kinase were determined 1 min after epinephrine addition; glucose output and gluconeogenesis, 30 and 15 min, respectively. Hepatocytes were incubated with saline (CON), 10 \( \mu \)M epinephrine (EPI), 0.1 mM propranolol (PRO), and 0.1 mM phenoxybenzamine (POB) as shown.

**TABLE II**

<table>
<thead>
<tr>
<th>Additions</th>
<th>EPI</th>
<th>EPI PRO</th>
<th>EPI POB</th>
<th>EPI PRO + POB</th>
<th>EPI PHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein kinase activity ratio</td>
<td>0.06 ( \pm ) 0.01</td>
<td>0.16 ( \pm ) 0.02</td>
<td>0.13 ( \pm ) 0.01</td>
<td>0.12 ( \pm ) 0.02</td>
<td>0.07 ( \pm ) 0.01</td>
</tr>
<tr>
<td>( -cAMP/+cAMP )</td>
<td>(n = 7)</td>
<td>(n = 6)</td>
<td>(n = 5)</td>
<td>(n = 4)</td>
<td>(n = 2)</td>
</tr>
<tr>
<td>Pyruvate kinase activity (units/g, wet wt.)</td>
<td>10.6 ( \pm ) 1.2</td>
<td>4.5 ( \pm ) 0.8</td>
<td>5.1 ( \pm ) 0.7</td>
<td>7.4 ( \pm ) 1.3</td>
<td>9.8 ( \pm ) 0.8</td>
</tr>
</tbody>
</table>

Hepatocytes were incubated with saline as control (CON) or 10 \( \mu \)M epinephrine (EPI), 0.1 mM propranolol (PRO), 10 \( \mu \)M phenoxybenzamine (POB), 0.1 mM phentolamine (PHT). Antagonists were added at the beginning of the incubation; saline or epinephrine were added after 15 min. Samples were removed 1 min after addition of epinephrine or saline for the measurement of protein kinase and pyruvate kinase activities. Values given are means \( \pm \) S.E. for multiple hepatocyte preparations (n) or the average of duplicate preparations. One unit of pyruvate kinase activity equals 1 \( \mu \)mol/min. Activities of each enzyme were determined as described under "Materials and Methods."
increase was blocked by 0.1 mM propranolol but not by 10 μM. The values given represent the combined results for at least four liver cell preparations. Enzyme activities and the gluconeogenic rate have been expressed as a percentage of the control values obtained for each cell preparation and as the fraction of the peak II activity observed. The stimulatory effect of phenoxybenzamine and lack of effect of propranolol completely blocked the epinephrine-mediated changes in the activities of pyruvate kinase or the protein kinase upon addition of high concentrations of salt, possibly resulting from reassociation of the protein kinase II subunits. Since we observed the maximal apparent stimulation of the hepatocyte protein kinase by epinephrine when extracts were prepared with high concentrations of salt, it would appear that this hormone selectively stimulates the protein kinase isozyme II. A correspondingly small response to epinephrine was seen when the extraction buffer contained low concentrations of salt, possibly resulting from reassociation of the protein kinase isozyme II subunits. Earlier, Corbin and his colleagues (21) reported that inclusion of 150 mM KCl in the extraction buffer was disadvantageous in preserving the effect of glucagon on the protein kinase activity ratio in hepatocyte extracts. However, their data suggest that glucagon activates both isozymes since, even in the absence of added KCl in the extraction buffer, the stimulation of the protein kinase activity ratio over basal was half the maximum obtained in the presence of added KCl. We also observed that the concentration of salt in the extraction buffer strongly influenced the basal protein kinase activity ratio as reported previously (21). The apparent selective activation of the cyclic AMP-dependent protein kinase isozyme II by epinephrine in hepatocytes is of particular interest in the light of a recent report by Corbin et al. (29) in which it was proposed that the protein kinase, particularly the Peak II isozyme, is compartmentalized with the membrane adenylate cyclase in rat hepatocytes. There is also the possibility that the hepatocyte population is not homogeneous with respect to its protein kinase isozyme composition.

Since epinephrine activated the cyclic AMP-dependent protein kinase in isolated hepatocytes, we attempted to relate this to other epinephrine-mediated changes within the hepatocytes, including cyclic AMP concentration (predominantly β-receptor mediated (13, 14)), pyruvate kinase inactivation (receptor unknown), and gluconeogenesis (proposed to be predominantly α-receptor-mediated (13–15)). Surprisingly, we observed that the epinephrine-mediated activation of the cyclic AMP-dependent protein kinase was partially blocked by phenoxybenzamine (or phentolamine) as well as propranolol. Moreover, combination of both phenoxybenzamine and propranolol completely blocked the epinephrine-medi-
 stimulated effects on the protein kinase, pyruvate kinase, and gluconeogenesis, suggesting that both α- and β-receptor mechanisms may be involved. The idea that both receptors are involved is consistent with an earlier report in which the stimulation of gluconeogenesis by phenylephrine was found to be enhanced by the presence of a phosphodiesterase inhibitor (21). We observed that the β-agonist, isoproterenol, activated the protein kinase, inhibited pyruvate kinase, and stimulated gluconeogenesis; these effects were fully blocked by propranolol and unaffected by phenoxybenzamine. Phenylephrine was the only α-agonist that had any effect on the protein kinase activity ratio and pyruvate kinase activity; oxymetazoline, naphazoline, and tetrahydrozoline were without effect. Phenylephrine inhibited pyruvate kinase activity and stimulated gluconeogenesis and both of these effects were inhibited by phenoxybenzamine.

The molecular basis of the apparent α-receptor influence on gluconeogenesis is unclear. In the case of the stimulation of hepatic glycogenolysis by phenylephrine, there is strong evidence implicating calcium (30, 31); however, gluconeogenesis is apparently not stimulated by the calcium ionophore nor calcium as does glycogenolysis (21). Although our data establish a correlation between the activation of the protein kinase and inhibition of pyruvate kinase by epinephrine, they do not provide direct evidence that either of these effects are essential for this hormone’s stimulatory effect on gluconeogenesis. Indeed Rognstad and Katz (32) have recently claimed that epinephrine does not inhibit the metabolic flux through pyruvate kinase, implying that control of this enzyme may not be necessary for the stimulation of gluconeogenesis. Nevertheless, at the molecular level, further support for the concept that regulation of hepatic gluconeogenesis involves coordinated phosphorylation of several enzymes is accumulating. Fructose-1,6-bisphosphatase has been reported to be phosphorylated by the cyclic AMP-dependent protein kinase (33). Thus, the proposal that the control of gluconeogenesis in fed rats by epinephrine occurs independent of the cyclic AMP-dependent protein kinase remains to be substantiated.

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REFERENCES


Additional references are found on p. 5154.
Control of Protein Kinase and Pyruvate Kinase

Peptide Synthesis and purification

The synthetic peptide (Leu-Arg-Arg-Ala-Glu-Asp-Glu) was synthesized by the Merrifield solid phase peptide synthesis procedure (11). Peptide synthesis was monitored by reverse phase high-performance liquid chromatography (HPLC), FAB mass spectrometry (MS), and NMR spectroscopy. The purified peptide was stored at -20°C before use.

Protein kinase assay conditions

Fig. 2. Effect of cyclic AMP on protein kinase activity in an extract of hepatocytes incubated with dihydrotestosterone. The protein kinase activity was assayed as described under Methods except that the assay was performed for 5 min at 30°C in the presence of cyclic AMP (3 mM).

Fig. 3. A: Effect of various concentrations of protein kinase inhibitors on the protein kinase activity in an extract of hepatocytes incubated with dihydrotestosterone. The protein kinase activity was assayed as described under Methods except that the assay was performed for 5 min at 30°C in the presence of cyclic AMP (3 mM).

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