The Role of the Adrenals in the Regulation of Phosphoenolpyruvate Carboxykinase of Rat Adipose Tissue*

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SUMMARY

Fasting, as well as adrenalectomy, induced an increased rate of glyceride-glycerol synthesis from pyruvate in rat adipose tissue. When both treatments were combined, the elevation in the rate of glycerogenesis was more than additive. The activity of cytosol pyruvate carboxylase rose in tissues of adrenalectomized rats but not in those of fasted rats. Phosphoenolpyruvate carboxykinase activity, on the other hand, was elevated by both treatments, and reached maximum values in adrenalectomized, fasted rats. The results suggest that the increase in glycerogenesis in fasted, adrenalectomized rats was due to increases in the activities of both pyruvate carboxylase and phosphoenolpyruvate carboxykinase.

The effects of fasting and of adrenalectomy on phosphoenolpyruvate carboxykinase were both counteracted by corticosteroids and by actinomycin D. Corticosteroids were more effective than actinomycin, causing a faster decline and depressing enzyme activity to lower levels. This effect of corticosteroids on phosphoenolpyruvate carboxykinase was prevented by simultaneous administration of actinomycin.

Liver phosphoenolpyruvate carboxykinase was not elevated by adrenalectomy, but liver from the fasted, adrenalectomized animals had a higher activity of this enzyme than did liver from the fasted rats. Contrary to the activity of the adipose tissue enzyme, the liver enzyme activity increased after short time intervals (2½ hours) following corticosteroid treatment.

Glucose is generally considered to be the main source of carbon for the formation of glyceride-glycerol in adipose tissue (1, 2). However, adipose tissue also has the capacity of converting pyruvate to glyceride-glycerol (3–6) via a pathway similar to the initial reactions of gluconeogenesis that occur in liver and kidney. Furthermore, the activity of this pathway as measured by isotope incorporation studies is increased substantially during fasting (4, 5), as is also the activity of phosphoenolpyruvate carboxykinase (EC 4.1.1.32) (4, 6). The activity of this enzyme in rat adipose tissue was maximal after 24 hours of fasting, and fell when fasting was continued after this time (6). It is possible that this subsequent drop in activity is caused by an increased secretion of adrenocortical hormones since Foster, Ray, and Lardy (7) have shown that the fasting response of the liver phosphoenolpyruvate carboxykinase was prevented by high levels of hydrocortisone.

Phosphorylase (EC 6.4.1.1) is also considered to play an obligatory role in gluconeogenesis and glycerogenesis with pyruvate as substrate (8–11). As distinct from phosphoenolpyruvate carboxykinase, the activity of phosphorylase carboxylase in vivo is not generally altered by dietary manipulations (6, 12), and it has been suggested that its activity may be controlled by the concentration of acetyl coenzyme A, an essential cofactor (13, 14). Thus, the rate of gluconeogenesis in kidney slices was enhanced by acetacetate (15), and, similarly, glycerogenesis from pyruvate in adipose tissue was stimulated by the addition of short chain fatty acids (5). Both treatments would be expected to cause the generation of additional acetyl-CoA.

In the present paper the role of the adrenals in the regulation of phosphoenolpyruvate carboxykinase has been studied, together with measurements of pyruvate carboxylase and glycerogenesis from pyruvate in the presence or absence of short chain fatty acids.

EXPERIMENTAL PROCEDURE

Animals—Male albino rats from the Jerusalem breeding center (Wistar origin), 40 to 50 days old, were used throughout the experiments. The animals were fed a high carbohydrate diet ad libitum (Nutritional Biochemical) unless otherwise indicated in the text. Adrenalectomized animals were given 1.0% NaCl in the drinking water and were used for the experiments between the 3rd and 5th postoperative days. Triamcinolone was administered subcutaneously. Actinomycin D and cycloheximide were given intraperitoneally as solutions in 0.15 M NaCl.

Materials—Radioactive compounds were purchased from the Radiochemical Centre, Amersham, England. Cycloheximide...
and hydrocortisone phosphate were from Sigma. Actinomycin D (Lyovac Cosmegen) was from Merck. P-enolpyruvate, IDP, malate dehydrogenase, and NADH were purchased from Sigma.

**Metabolic Study**—Experimental procedure for the metabolic study was the same as previously described (16). Pieces from the distal part of the epididymal fat pad weighing about 100 mg were incubated with shaking in 2 ml of Krebs-Ringer phosphate buffer, pH 7.4, containing 0.5 pmoles of 2-14C-pyruvate. Butyrate (0.5 pmoles) was added where indicated. After a 2-hour incubation at 37°, the reaction was stopped by the addition of 0.3 ml of 1.5 N H2SO4. The tissues were removed and rinsed several times with 0.9% NaCl before extraction with the acidic isopropanol-heptane extraction mixture as described by Dole (17). The heptane phase was washed three times with isopropyl alcohol-0.07 N H2SO4 (4:3), and evaporated to dryness. The residue was hydrolyzed with 0.5 N alcoholic KOH at 70° for 30 min. The long chain fatty acid fraction was extracted with acetic heptane, and the radioactivity in a sample from the residual water phase containing glyceride-glycerol was determined in a liquid scintillation spectrometer (Packard Tri-Carb) with Diotol (18) as the scintillation fluid.

**Enzyme Assays**—Adipose tissue was homogenized in 3 volumes of buffered sucrose (19), and liver tissue in 9 volumes of the same solution. Supernatants prepared by centrifugation at 105,000 x g for 30 min were used for the enzyme assays.

Pyruvate carboxylase (EC 6.4.1.1) was assayed as described by Keech and Utter (20). The method measures the fixation of 14C-labeled NaHCO3 in the presence of ATP, MgCl2, pyruvate, and acetyl-CoA. The exact composition of the reaction mixture and the procedure of the assay have been described previously (11).

P-enolpyruvate carboxykinase was measured by a method similar to that used by Chang and Lano (21), which involves the fixation of 14C-labeled NaHCO3 in the presence of P-enolpyruvate, IDP, MgCl2, GSH, NADH, and malate dehydrogenase.

The complete assay system has been described previously (4). The concentrations of substrates and reactants are optimal; the incorporation rates are linear with time to 30 min, and with enzyme to 0.003 unit of activity. A unit is the amount of enzyme that catalyzes the formation of 1 mumole of product per min at 30°.

Blood glucose was determined enzymatically by the glucose oxidase method (22), and protein was measured on the 105,000 x g (30 min) fraction of adipose tissue by the method of Lowry et al. (23).
Activity of P-enolpyruvate carboxykinase in adipose tissue and liver

P-enolpyruvate carboxykinase was assayed in the supernatant centrifuged at 105,000 \( \times \) g, as described under "Experimental Procedure." Triamcinolone, 5 mg/100 g, body weight, was given subcutaneously; actinomycin D, 50 \( \mu \)g/100 g, and cycloheximide, 0.1 mg/100 g, injected 24 hours before the animals were killed. Values are the means \( \pm \) the standard error of the mean for the number of observations indicated in parentheses.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Adipose tissue</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>27 ( \pm ) 2.3</td>
<td>150 ( \pm ) 25.4</td>
</tr>
<tr>
<td>Fed (8)</td>
<td>160 ( \pm ) 15.8</td>
<td>792 ( \pm ) 39.5</td>
</tr>
<tr>
<td>Fasted 24 hrs (10)</td>
<td>101 ( \pm ) 8.1</td>
<td>448 ( \pm ) 42.1</td>
</tr>
<tr>
<td>Adrenalectomized</td>
<td>220 ( \pm ) 10.3</td>
<td>890 ( \pm ) 97.2</td>
</tr>
<tr>
<td>Fed (23)</td>
<td>20 ( \pm ) 4.7</td>
<td>130 ( \pm ) 28.7</td>
</tr>
<tr>
<td>Fasted 24 hrs + triamcinolone (10)</td>
<td>37 ( \pm ) 6.1</td>
<td>124 ( \pm ) 15.1</td>
</tr>
<tr>
<td>Fed + triamcinolone (8)</td>
<td>51 ( \pm ) 12.1</td>
<td>3.20 ( \pm ) 0.296</td>
</tr>
<tr>
<td>Fasted 24 hrs + actinomycin D (4)</td>
<td>80 ( \pm ) 3.3</td>
<td>1.31 ( \pm ) 0.031</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of triamcinolone with respect to the dose. Adrenalectomized rats were fasted for 24 hours and then given various doses of triamcinolone (in the range of 0.025 mg/100 g to 5 mg/100 g). The animals were killed 24 hours later, and the activity of P-enolpyruvate carboxykinase assayed in adipose tissue (a) and in liver (c). Each point is the mean obtained from six to eight animals given the dose indicated with the standard error represented by the bars.

We have reported (6) that the activity of adipose tissue P-enolpyruvate carboxykinase increase rapidly with fasting up to 24 hours and then declines slowly as the period of fasting is extended to 48 hours. The results of the present study indicate that the adrenals are somehow involved in this decrease, since in adrenalectomized rats, the activity of this enzyme in both adipose tissue and liver increases after 48 hours of fasting (Fig. 2). Since triamcinolone injected at a low dose causes a sharp drop in the activity of P-enolpyruvate carboxykinase in adipose tissue of adrenalectomized rats fasted for 48 hours (Fig. 1), it is probable that in intact animals the physiological output of adrenocortical hormones, which occurs during fasting, may be sufficient to depress the activity of P-enolpyruvate carboxykinase during prolonged fasting periods.

Whether triamcinolone acts directly on adipose tissue P-enolpyruvate carboxykinase or whether it exerts its effect indirectly cannot yet be ascertained. During fasting, a negative correlation was found between the level of the enzyme and the concentration of blood glucose (Fig. 2). A similar correlation has been found by Young et al. (24) for the liver enzyme.

When triamcinolone was administered to adrenalectomized rats that had previously been fasted for 24 hours, the fall in P-enolpyruvate carboxykinase activity in adipose tissue preceded that in liver and preceded any elevation of blood glucose (Table III). Therefore, the effect of triamcinolone on adipose P-enolpyruvate carboxykinase is probably not mediated by its effect on blood glucose.

The difference in response to corticosteroids of liver and adipose tissue was further demonstrated by following the time course of
Adipose Tissue P-enolpyruvate Carboxykinase

Effect of hydrocortisone phosphate on activity of P-enolpyruvate carboxykinase in adipose tissue and liver of adrenalectomized, fed rats

Adrenalectomized, fed rats were given 2.5 mg of hydrocortisone phosphate per 100 g, body weight, intraperitoneally. Animals were killed 24 or 5 hours later. Values are the means ± standard error of the mean for the number of observations shown in parentheses.

<table>
<thead>
<tr>
<th>Time after hydrocortisone phosphate (hrs)</th>
<th>P-enolpyruvate carboxykinase (millimols/g tissue)</th>
<th>Liver (units/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (7)</td>
<td>102 ± 10.2</td>
<td>1.0 ± 0.054</td>
</tr>
<tr>
<td>24 (6)</td>
<td>91 ± 14.3</td>
<td>1.88 ± 0.153</td>
</tr>
<tr>
<td>5 (6)</td>
<td>73 ± 17.9</td>
<td>1.40 ± 0.064</td>
</tr>
</tbody>
</table>

Effect of triamcinolone and actinomycin D on activity of P-enolpyruvate carboxykinase in adipose tissue from adrenalectomized, fed rats

Triamcinolone, 5 mg/100 g, was given subcutaneously and actinomycin D, 50 μg/100 g, was given intraperitoneally to adrenalectomized, fed rats 24 hours before killing the animals. Values are the means ± standard error of the mean for the number of observations indicated in parentheses.

<table>
<thead>
<tr>
<th>Treatment of animals</th>
<th>P-enolpyruvate carboxykinase (millimols/g tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>105 ± 16.7 (4)</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>14 ± 3.26 (5)</td>
</tr>
<tr>
<td>Actinomycin D</td>
<td>34 ± 7.10 (4)*</td>
</tr>
<tr>
<td>Triamcinolone + actinomycin D</td>
<td>35 ± 9.30 (5)*</td>
</tr>
</tbody>
</table>

* Significantly different at the 5% probability level from triamcinolone treated or control animals.

Effect of Actinomycin D and Cycloheximide on P-enolpyruvate Carboxykinase Activity—Actinomycin D causes an effect similar to that of triamcinolone in reducing P-enolpyruvate carboxykinase activity in adipose tissue of adrenalectomized, fed rats (Table V). However, following actinomycin D administration, the activity of the enzyme did not fall to the low level obtained with triamcinolone. Furthermore, actinomycin prevented the decay of enzyme activity by triamcinolone beyond the level obtained with actinomycin D itself (Table V).

In order to compare the rates of enzyme decay brought about by triamcinolone and actinomycin D on both hepatic and adipose tissue P-enolpyruvate carboxykinase, it was necessary to establish that an optimal dose of actinomycin D was used. As described in Fig. 3, a nearly optimal effect was obtained with 10 μg/100 g, both when measured at a short (12 hours) and a long (24 hours) interval of time. Even with excessive amounts of actinomycin D (50 μg/100 g) given to fasted, adrenalectomized rats, the rate of enzyme decay (Table VI) was considerably slower (t1 = 16 hours) than that obtained with triamcinolone (t1 = 9½ hours) (Table III). Actinomycin D treatment, which was adequate to elicit a block of the response of the adipose tissue P-enolpyruvate carboxykinase to fasting, was not effective for the liver enzyme. It was, however, possible to show that repeated injections of actinomycin did prevent the rise in the liver enzyme by fasting.

Cycloheximide, like actinomycin D, prevented the fasting-induced increase in P-enolpyruvate carboxykinase activity in liver and adipose tissue of adrenalectomized animals (Table II). The lower effectiveness of this drug in adipose tissue cannot yet be evaluated since, because of its toxicity, it was not possible to reach an optimal dosage.

DISCUSSION

The results of this study clearly indicate that the adrenals depress P-enolpyruvate carboxykinase activity in adipose tissue.
The removal of the adrenals resulted in an elevation in the activity of this enzyme, while the subsequent administration of corticosteroids abolished this effect (Table II). Also, triamcinolone prevented the induction of the enzyme by fasting and brought about a decline of its level if given during fasting (Tables 11 and III).

The mechanism underlying the effect of triamcinolone on P-enolpyruvate carboxykinase is still debatable. It may be based on an enhanced rate of enzyme destruction, on the prevention of enzyme synthesis, or both. The first possibility is favored by the findings that actinomycin D will also cause a decline in the level of the adipose tissue enzyme from adrenalectomized, fed or adrenalectomized, fasted rats. Actinomycin D was, however, less effective than triamcinolone. The half-life of the enzyme following actinomycin D treatment was 16 hours as compared with 9½ hours with triamcinolone (Tables III and VI). In addition, the final activity of adipose tissue P-enolpyruvate carboxykinase was lower when triamcinolone, rather than actinomycin D, was injected (Table V).

It is generally accepted that actinomycin D blocks RNA-dependent protein synthesis, whereas the decline in the activity of an enzyme subsequent to its rise because of hormone induction is not affected by actinomycin D (25). Therefore, the rate of decay of enzyme activity following actinomycin D treatment may be taken as the rate of degradation in the absence of synthesis. In the present study, on the other hand, triamcinolone treatment brings about a faster rate of enzyme decay than actinomycin; therefore, triamcinolone must accelerate enzyme destruction by inducing a rise in the activity of the degradative system. This induction is sensitive to actinomycin D, because when triamcinolone was given together with actinomycin D, only the actinomycin D effect was evident.

Hormone-mediated depression of enzyme activity was reported by Kenney (26, 27) for the effect of growth hormone on liver tyrosine amino transferase. On the basis of an immunochemical study, Kenney (28) has suggested that growth hormone was preventing enzyme synthesis rather than stimulating its degradation. This conclusion was based on the finding that growth hormone did not affect the rate of enzyme decay; neither did it prevent enzyme induction by other hormones. Only at basal levels, at which the enzyme activity is not susceptible to actinomycin D depression (25, 29), was growth hormone effective (27). However, the effect of triamcinolone on adipose tissue P-enolpyruvate carboxykinase occurs in a system which is sensitive to actinomycin D. It thus seems likely that in adipose tissue a hormone-mediated destruction of P-enolpyruvate carboxykinase is responsible for the effect. The rise in adipose tissue P-enolpyruvate carboxykinase activity caused by adrenalectomy and by fasting, and the additive rise of enzyme activity when adrenalectomy and fasting are combined, may be explained by the removal of the enzyme-degrading system by adrenalectomy and induction of enzyme synthesis by fasting. In spite of the fact that the postulated mechanism of induced enzyme destruction by triamcinolone may explain its depressive effect in both cases (30), in the absence of an immunochemical study, one cannot conclusively distinguish between these two modes of action of the hormone.

REFERENCES
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