Interactions of Diet and Cortisone in the Regulation of Adaptive Enzymes in Rat Liver*

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SUMMARY

The responses of the adaptive enzymes, serine dehydrase (L-serine hydro-lyase (deaminating), EC 4.2.1.13), ornithine transaminase (L-ornithine:2-oxoacid aminotransferase, EC 2.6.1.13), glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate:NADP oxidoreductase, EC 1.1.1.49), and glucokinase (ATP:o-glucose 6-phosphotransferase, EC 2.7.1.2), in rat liver were studied in relation to changes in the level of dietary protein and treatments with cortisone. The administration of cortisone markedly altered the responses of the enzymes to the diet, and the effects of the hormone depended both on the route of its administration (intraperitoneal or intramuscular injection) and on the dietary regimen of the rats. The response of each enzyme to the regulatory stimuli (diet and cortisone) showed elements of both uniqueness and similarity with respect to the responses of the other three enzymes to these stimuli. The results indicate that the regulatory system for each enzyme responds to a specific set of effectors (e.g. amino acids or carbohydrate or both, of dietary or endogenous origin) and that cortisone alters the capacity of the regulatory systems to interact with the appropriate effectors.

Earlier studies showed that two enzymes of amino acid catabolism, serine dehydrase and ornithine transaminase, are induced in rats forcibly fed amino acids (1, 2) and that this induction is inhibited when the rats are simultaneously fed carbohydrate (3, 4). Evidence, recently obtained, indicates that these effects involve true changes in the rate of synthesis of enzyme protein (5).

Although the presence of glucocorticoids is not required for the induction of these enzymes (1), cortisone treatment permits the dietary induction of these enzymes in the presence of a normally inhibitory amount of dietary carbohydrate (6). Cortisone alone does not cause their induction, however, under these conditions. These findings indicate that cortisone exerts a regulatory influence on the responses of these enzymes but is not a primary initiatory stimulus.

The present investigation explores further the inter-relationships of diet and cortisone in controlling adaptive enzymes in rat liver under ad libitum feeding conditions. The results obtained indicate that the participation of cortisone in hepatic enzyme regulation involves the interaction of cortisone with the regulatory system for each enzyme, which results in a modification in the response of that system to the other regulators.

EXPERIMENTAL PROCEDURE

Materials

Male Sprague-Dawley rats were obtained at an age of 5 weeks (body weight, 100 to 150 g) from the animal facilities of the Biology Division at Argonne, and were housed under conditions of constant temperature (25°C) and regulated lighting (12 hours of light and 12 hours of dark). They had free access to food and water. Adrenalectomies were performed on some of these rats, when they were 5 weeks old, at the Hormone Assay Laboratories, Inc., Chicago. They were given both food and 1% NaCl solution ad libitum thereafter, and were placed on experiment on the 7th day after adrenalectomy.

Pelleted diets, containing 0.70 or 0.60% protein, were obtained from General Biochemicals; they contained nutritionally adequate amounts of fat, carbohydrate, vitamins, and minerals. The carbohydrate (corn starch) content was adjusted to compensate for the changes in protein (casein) content. A standard laboratory animal diet, containing 24% protein (Wayne Lab Blox, Allied Mills, Inc., Chicago, Illinois), was also given in some cases.

Cortisone acetate (0.9% NaCl suspension) was obtained from Merck. The reagents for the enzyme assays were of the highest purity obtainable from Calbiochem and Sigma, and all reagent solutions were made with glass-distilled water.

Treatments

All injections, diet changes, and dissections were performed between 8 a.m. and 11 a.m. All diet changes were made without intervening periods of fasting. All rats were fed Wayne Lab Blox prior to Day zero. Cortisone was given intramuscularly into the hind leg or intraperitoneally.
**FIG. 1.** Serine dehydrase (A) and ornithine transaminase (B) in adrenalectomized rats given a 60% protein diet and intact rats given cortisone acetate or fed a diet containing different levels of protein. •—•, adrenalectomized rats were given a 60% protein diet and 1% NaCl solution *ad libitum* throughout the experiment; X—X, rats were fed Wayne Lab Blox *ad libitum* throughout the experiment; O—O, rats were fed a protein-free diet *ad libitum* beginning at zero time and continuing throughout the experiment.

### Table: Preparation of Samples

<table>
<thead>
<tr>
<th>Diet</th>
<th>Serine dehydrase</th>
<th>Ornithine transaminase</th>
</tr>
</thead>
<tbody>
<tr>
<td>60% protein, adrenalectomized</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>24% protein (Lab Blox)</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>24% protein (Lab Blox) + cortisone, intramuscular</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>0% protein</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

### Enzyme Assays

**Serine Dehydrase** Freshly thawed Fraction S3 was diluted 10- or 20-fold (depending on the activity of the sample) with ice-cold buffer (pH 8.4) which was 0.1 M in Tris, 0.15 M in KCl, and 10^{-4} M in pyridoxal phosphate. An aliquot (0.05 ml) of diluted Fraction S3 was then immediately added to 0.95 ml of assay medium in a 1-ml cuvette with a 1-cm light path. (The cuvette, its contents, and the cuvette holder were warmed previously to 37°.) Immediately after addition of the sample, the cuvette contents were mixed with a “plumper” (Calbiochem). The change in absorbance at 340 nm was determined in a Gilford model 2000 multiple sample absorbance recorder equipped with a water-jacketed cuvette chamber set at 37°. The maximum linear slope was used to calculate enzyme activity.

The final concentrations of reagents in the assay mixture were Tris, 0.10 M; KCl, 0.15 M; L-serine, 0.20 M; pyridoxal phosphate, 0.4 mm; NADH, 0.57 mm; and lactic dehydrogenase (rabbit muscle), 1.0 μg per ml of reaction mixture. The total volume per assay was 1.0 ml, and the pH was 8.4. This procedure is a modification of that described by Pitot and Pries (7).

**Ornithine Transaminase**—The final concentrations of com-
Fig. 2. Effects of intramuscular (I.M.) or intraperitoneal (I.P.) injections of cortisone on the response of serine dehydrase (A) and ornithine transaminase (B) to dietary protein changes. •—•, a diet containing 60% of protein was fed ad libitum beginning at zero time; •—•, single daily injections of cortisone acetate were given intramuscularly (5 mg per injection) for the remainder of the experiment to rats receiving the 60% protein diet; ▲—▲, same regimen as described above, except that cortisone was given by intraperitoneal injection; ○—○, a protein-free diet was fed ad libitum beginning at zero time; •—•, a protein-free diet was fed ad libitum beginning at zero time. Beginning on the 3rd day after zero time, single daily intramuscular injections of cortisone were given (5 mg per injection) for the remainder of the experiment; ○—○, same regimen as described above, except that cortisone was given by intraperitoneal injection.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Serine dehydrase</th>
<th>Ornithine transaminase</th>
</tr>
</thead>
<tbody>
<tr>
<td>60% protein</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>60% protein + cortisone, intraperitoneal</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>60% protein + cortisone, intramuscular</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>0% protein</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>0% protein, 3 days: 0% protein + cortisone, intraperitoneal, 11 days</td>
<td>10</td>
<td>7</td>
</tr>
</tbody>
</table>
Fig. 3. Effects of preliminary treatment with high protein diet on the response of serine dehydrase (A) and ornithine transaminase (B) to cortisone. ●—●, as in Fig. 1; ○—○, as above for 9 days. Beginning on Day 9, intramuscular injections of cortisone were given as described previously and were continued to the end of the experiment.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Serine dehydrase</th>
<th>Ornithine transaminase</th>
</tr>
</thead>
<tbody>
<tr>
<td>60% protein, 9 days; 60% protein + cortisone, intramuscular, 5 days</td>
<td>15</td>
<td>7</td>
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Error Calculations

Each point on the curves is an average of the values from five rats. The average standard error (expressed as a percentage) for each curve is given in the heading for each figure. These values were obtained by calculating the standard error of the mean for each experimental point, dividing the standard error by the mean to obtain the percentage error at each point (these percentages did not differ from each other by more than 10%), totaling these percentages for each curve, and dividing by the number of experimental points on the curve.

RESULTS

When intact rats receiving a 24% protein diet (Lab Blox) were given cortisone by intramuscular injection, serine dehydrase increased progressively during the experiment but the level of ornithine transaminase remained unchanged (Fig. 1). Both enzymes increased markedly, however, when adrenalectomized rats were changed from the 24% protein diet to the 60% protein diet (Fig. 1). The magnitude of this response in adrenalectomized rats was similar to that in intact rats on the same diet regimen (see Fig. 2, below). The response of serine dehydrase in adrenalectomized rats receiving the high protein diet was much greater than it was in intact rats receiving both the 24% protein diet and cortisone treatment.

Fig. 2 shows the effects of changes in dietary protein level combined with the administration of cortisone by different routes (intramuscular or intraperitoneal) on the responses of these enzymes in intact rats. When the rats were changed from a 24% protein diet to a 60% protein diet at zero time a large increase in both enzymes resulted, as was described previously (11-15). The increase, with 60% protein, in serine dehydrase was enhanced by the intramuscular injection of cortisone, but the response of ornithine transaminase to the 60% protein diet was completely blocked by intramuscular cortisone. As in Fig. 1, however, cortisone treatment did not decrease ornithine transaminase below its pretreatment level, although this was not the lowest level to which the enzyme could be reduced. Both enzymes were reduced below their pretreatment levels when the rats were changed to a protein-free diet (Figs. 1 and 2).

When rats previously fed a protein-free diet for 3 days were given cortisone intramuscularly, serine dehydrase increased gradually up to Day 13 (Fig. 2). This response was small when compared to the responses to dietary protein or to the combined administration of protein and cortisone. The pronounced effects of cortisone after intramuscular injection were not obtained when the same dose of cortisone was given by intraperitoneal injection (Fig. 2). Ornithine transaminase showed variable in-
Enzyme Regulation in Rat Liver

The effect of cortisone on ornithine transaminase shown in this study suggests that this hormone blocks the interaction of the inductive stimulus (amino acids) with the regulatory system for this enzyme. The level of ornithine transaminase in rats fed the 24% protein diet (Fig. 1 and zero time in Fig. 2) reflects an apparent basal rate of production for this enzyme, the regulation of which is unaffected by cortisone. This basal level of production must be mediated by amino acids, since removal of dietary protein causes the enzyme to decrease to negligible levels. Thus, ornithine transaminase may exist in three distinct physiological states: (a) a depleted state, in which the enzyme has virtually disappeared owing to the lack of amino acid stimuli; (b) a noninduced state, in which the enzyme is present in substantial amounts owing to the presence of an adequate but not super-

The results of this study (Figs. 1 to 4) and of studies reported earlier (1-6) suggest that amino acids are primary effectors in the induction of serine dehydrase and ornithine transaminase. Cortisone, on the other hand, is not involved in the primary induction phenomenon, but instead appears to alter the capacity of the regulatory systems for these enzymes to respond to amino acids. Thus, cortisone is not necessary for the induction of serine dehydrase (Fig. 1) and is not able to induce or sustain this enzyme without a source of amino acids (Figs. 2 and 4). Cortisone and amino acids appear to act synergistically to enhance the induction of serine dehydrase, as shown in Fig. 2, where the combined treatment with both cortisone and the high protein diet produced a far greater increase in serine dehydrase than did treatment with cortisone or high protein alone (Figs. 1 and 2).

The level of glucokinase was not changed when rats were changed from a 24% protein diet to a 60% or a 0% protein diet, but in response to intramuscular injections of cortisone the enzyme increased, whether the rats were on the 60% or the 0% protein diet. Glucokinase also increased temporarily when cortisone was given intraperitoneally to rats on the 60% protein diet, but no increase followed intraperitoneal cortisone treatment when the rats were fed the 0% protein diet.

DISCUSSION

The inhibitory effects of intraperitoneal cortisone treatment during the first one-half of the experiment, but these effects were not sustained (Fig. 2).

The increase in serine dehydrase resulting from feeding the high protein diet reached a plateau after the 2nd day, and the enzyme remained at a relatively constant level for the rest of the experiment (Fig. 2). When rats on this diet were given intramuscular injections of cortisone, beginning on Day 9, a second pronounced increase in serine dehydrase followed (Fig. 3). This treatment had the opposite effect on ornithine transaminase, however, and caused that enzyme to decrease progressively after Day 9. By Days 13 and 14 (Fig. 3), the level of ornithine transaminase had decreased to that maintained by feeding the 24% protein diet (Fig. 1); this was also the level at which cortisone held the enzyme in the presence of the high protein diet (Fig. 2). The decrease in ornithine transaminase produced by cortisone treatment after the enzyme had been previously raised to high levels (Fig. 3) is in sharp contrast to the results shown in Figs. 1 and 2, in which cortisone treatment did not result in a decrease in the noninduced enzyme.

When rats receiving both the high protein diet and cortisone (intramuscular or intraperitoneal) were shifted to the protein-free diet on Day 3, serine dehydrase decreased rapidly in the rats given intraperitoneal cortisone, but in those receiving intramuscular cortisone this decline was generally slower (Fig. 4). Intramuscular cortisone treatment could not sustain the enzyme at high levels in the absence of dietary protein, however, and by Day 14 the enzyme had decreased to a point which corresponded to the maximum level produced by intramuscular cortisone treatment in rats on a protein-free diet (Day 13, Fig. 2). This indicates that the latter value was probably the highest attainable by the administration of cortisone to rats on a protein-free diet.

Fig. 5 shows the effects of cortisone treatment and dietary protein changes on the levels of glucose 6-phosphate dehydrogenase and glucokinase. Glucose 6-phosphate dehydrogenase increased slightly when the rats were shifted from the 24% protein diet to the 60% protein diet, but this treatment did not change the level of glucokinase. The intramuscular administration of cortisone to rats receiving the 60% protein diet caused glucose 6-phosphate dehydrogenase to rise substantially above the level in the 60% protein group by the end of the experiment. No increase followed, however, when cortisone was given to rats on the protein-free diet. The intraperitoneal injection of cortisone did not alter the response of this enzyme from that in the rats receiving the 60% protein diet alone.

The level of glucokinase was not changed when rats were changed from a 24% protein diet to a 60% or a 0% protein diet, but in response to intramuscular injections of cortisone the enzyme increased, whether the rats were on the 60% or the 0% protein diet. Glucokinase also increased temporarily when cortisone was given intraperitoneally to rats on the 60% protein diet, but no increase followed intraperitoneal cortisone treatment when the rats were fed the 0% protein diet.
The inset in Fig. 5B (glucokinase) is a graph of the data for 60% protein + cortisone, intramuscular, and 60% protein + cortisone, intraperitoneal, taken from the main figure and equalized with respect to the time administration of cortisone was begun.

The observation that glucose 6-phosphate dehydrogenase increased when the level of dietary protein was raised and only responded to cortisone in the presence of amino acids suggests that amino acids are as important in the regulation of this enzyme as in the regulation of serine dehydrase and ornithine transaminase. This agrees with other evidence that dietary proteins are important in the regulation of glucose 6-phosphate dehydrogenase (17–21). Recent studies have shown, however, that this enzyme is not induced in fasted rats refed a carbohydrate-free diet containing adequate protein (22). Thus, it appears that the regulatory system for glucose 6-phosphate dehydrogenase functions by interacting with both amino acids and carbohydrate, and becomes unresponsive when either is absent. Several studies have examined the possible involvement of hormones in the regulation of glucose 6-phosphate dehydrogenase (17–21). They indicate that this enzyme is controlled by com-
complex dietary-hormonal interactions. Fig. 5 indicates that an interaction of this nature occurred in the present study, producing subtle, but definite, effects on the responsiveness of this enzyme. The expression of the interaction among amino acids, carbohydrate, and cortisone apparently changed as their relative concentrations in the liver changed during the experiment.

With glucokinase, the diet-hormone interactions were equally complex. Thus, intramuscular cortisone exerted a positive effect on glucokinase, which was not markedly influenced by the protein content of the diet. These results agree with an earlier report of the stimulation of glucokinase by glucocorticoid treatment (27) where it was also found that glucokinase was induced by glucose in adrenalectomized rats and that administration of hydrocortisone to rats on a carbohydrate-free diet only slightly stimulated the enzyme. These results (27) are analogous to those obtained in Figs. 1 to 4 (involving the interaction of amino acids and cortisone in the regulation of serine dehydrase). They indicate that glucose is the primary inducer of glucokinase and that cortisone enhances the response of this enzyme to glucose. In this connection, a conclusion of the earlier study (27) was that "...hydrocortisone seemed to facilitate the inductive effect of exogenous glucose..." with respect to the response of glucokinase. Although carbohydrate appears to be of paramount importance in relation to the primary stimulation of this enzyme, the effects of intraperitoneal cortisone, shown in Fig. 5, indicate the possible participation of amino acids (of either exogenous or endogenous origin) in the regulation of glucokinase by glucocorticoids.

Table I is a brief summary of the regulatory effects of amino acids, carbohydrate, and cortisone on the four adaptive enzymes studied in this report. When the regulatory responses of these enzymes are compared, a continuum of changes in regulatory properties emerges which progresses from (a) a primary dependence on amino acids and a positive response to cortisone (serine dehydrase) through (b) a primary dependence on amino acids and a negative response to cortisone (ornithine transaminase) and (c) a dependence on the simultaneous presence of amino acids, carbohydrate, and cortisone (glucose 6-phosphate dehydrogenase) to (d) a primary dependence on carbohydrate and a positive response to cortisone (glucokinase). Cortisone itself is not a primary effector in any of the cases studied, but instead exerts its effects through interaction with other regulators.

At present, the primary locus of metabolic regulation, at least in the case of microorganisms, is thought to be associated with the process of transcription (28). The complex, multifaceted nature of regulation in rat liver, as disclosed by the results of the present investigation, however, supports the suggestion (29, 30) that alternative or additional regulatory sites exist in mammalian systems, especially with reference to the actions of glucocorticoids.

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REFERENCES
Interactions of Diet and Cortisone in the Regulation of Adaptive Enzymes in Rat Liver
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