Glucocorticoids and Transaminase Activity

VI. COMPARISON OF THE ADAPTIVE INCREASES OF ALANINE- AND TYROSINE-α-KETOGLUTARATE TRANSAMINASES*

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The activity of alanine-α-ketoglutarate transaminase (l-alanine:2-oxoglutarate aminotransferase, EC 2.6.1.2) is increased markedly in the livers of diabetic or fasted rats (1). The induction of this enzyme also occurs when either intact or adrenalectomized rats are fed diets high in protein or given corticosteroid injections (2-4). The adaptive response of this enzyme in glucocorticoid-sensitive tissues, such as thymus (5) and Walker carcinoma 256 (5), is of particular interest since most studies of adaptive enzymes have been restricted to liver. Lin and Knox (6) and Sereni, Kenney, and Kretchmer (7) have shown that tyrosine-α-ketoglutarate transaminase (l-tyrosine:2-oxoglutarate aminotransferase, EC 2.6.1.5) is increased several fold in the livers of rats treated by injection with cortisol. Before undertaking studies directed toward an understanding of the mechanism of enzyme induction and the physiological significance of the altered activity of these enzymes, further information on the comparative responses of alanine and tyrosine transaminases seemed desirable. The present report describes a comparative study of these two transaminases with special reference to (a) the time course of the response after the injection of cortisol, (b) induction by substrates, (c) effects of adrenalectomy on the endogenous and cortisol-induced levels, (d) effects of fasting, high protein diets, and alloxaan diabetes, and (e) their response in cortisol-sensitive tissues. Differences in the specificity and nature of induction of these two adaptive enzymes were noted. A preliminary report of certain aspects of this work has appeared (8).

EXPERIMENTAL PROCEDURE

Animals—Male rats weighing from 75 to 125 g, obtained from the Holtzman Rat Company, Madison, Wisconsin, were used in all experiments. Bilateral adrenalectomy was performed by the technique described by Grollman (9), and the animals were maintained by replacing drinking water with a solution of 1% sodium chloride. Walker carcinoma 256 was obtained from Dr. K. Sugiuara, Sloan-Kettering Institute, New York. Tumors which were 12 to 14 days old were cut under aseptic conditions into solid pieces about 1 mm, and these were transplanted by trocar into the right flank of each rat.

Diet and Compounds—Rockland rat diet was fed ad libitum to the animals. In the studies on the relationship of dietary pro-
toil to tyrosine transaminase activity, variations in the protein content were made at the expense of the sucrose in the purified diet (1) and by lowering the fat content at the highest level of protein.

*p-Hydroxyphenylpyruvic acid was purchased from K and K Laboratories, Inc. Injectable commercial preparations of hydrocortisone acetate (cortisol) and deoxycorticosterone acetate were used. l-Tyrosine was prepared for injection by suspension in 1% carboxymethyl cellulose.

ASSAY PROCEDURES—Procedures for preparation of tissue homogenates, assay of alanine transaminase, and protein were the same as described previously (1). Tryptophan pyrrole activity was determined by the method described by Knox (10). The colorimetric procedure for tyrosine transaminase described by Canellakis and Cohen (11) was modified in the following manner. Buffer-substrate reagent sufficient for 36 samples, prepared before use from refrigerated individual stock solutions, contained 12 ml of 0.1 M α-ketoglutaric acid (adjusted to pH 7.4), 10 ml of 0.2 M sodium phosphate buffer, 4.0 ml of 0.5 M diethyldithiocarbamate (7), and 50 ml of 0.25 M sucrose. To 1.9 ml of the buffer-substrate reagent in a 30-ml beaker was added 0.3 ml of tissue homogenate (1:10 dilution for normal rat liver, 1:20 dilution for livers of cortisol- or tyrosine-treated animals). If larger volumes of the tissue homogenate were used, such as for thymus or tumor, the volume of the sucrose solution in the reagent mixture was correspondingly reduced. Individual blanks were prepared for each sample by adding 0.3 ml of 100% trichloroacetic acid (w/v) solution to the buffer-substrate reagent plus tissue homogenate before incubation. The standard was prepared by adding 150 μg (0.3 ml) of a freshly prepared aqueous solution of p-hydroxyphenylpyruvic acid to 1.9 ml of the buffer-substrate solution. All samples, standards, and blanks, in duplicate, were first incubated while being shaken for 3 minutes at 38° in a Dubnoff incubator. After this period in the incubator, 0.6 ml of 0.01 M l-tyrosine was added to each beaker and incubation was continued with constant agitation for 10 minutes. The reaction was stopped by the addition of 0.3 ml of 10% trichloroacetic acid to the beakers containing the samples and standard. The solutions were filtered through Whatman No. 1 paper. To 1 ml of the clear filtrate, 2 ml of color reagent were added. The color reagent was prepared immediately before use and contained 0.6 ml of 3% ammonium molybdate in 5 N HCl, 1.0 ml of 1% KH₂PO₄, and 1.6 ml of water. After development for 1 hour at room temperature, the color was read in a Beckman model DU spec-
intraperitoneal injection of cortisol; after 12 hours, the activity after color development. Tissues were kept on ice and assayed trophotometer at 850 mμ. This procedure avoided turbidity after color development. Tissues were kept on ice and assayed within 1 hour after they were obtained, or they were frozen immediately in a solution of acetone and Dry-Ice and kept on Dry-Ice until ready for assay.

RESULTS

Time Course of Response—Several days are required to induce a maximal increase in alanine transaminase by treatment with cortisol (Fig. 1). Maximal activity of this enzyme was reached within 48 hours and persisted for at least 5 days after a single subcutaneous dose of 100 mg of cortisol per kg of body weight. Lin and Knox (12) previously reported that maximal response of tyrosine transaminase was obtained within 5 hours after a single intraperitoneal injection of cortisol; after 12 hours, the activity had essentially returned to normal. This pattern of response is similar to that observed previously with tryptophan pyrrolosase (13).

The gradual increase in activity in alanine transaminase over a period of 2 days compared with the rapid response of tyrosine transaminase may reflect the relatively slow turnover of this enzyme. Based on the degradation of tyrosine transaminase after the induction, the apparent half-life of this enzyme has been estimated to be less than 3 hours (12). Since it is possible that small amounts of cortisol are retained at the site of subcutaneous injection, determination of the half-life of alanine transaminase after such treatment is unsatisfactory. However, the rate of loss of alanine transaminase activity after adrenalectomy (14) or after elevation of enzyme activity by feeding high protein diets indicates that the half-life of this transaminase is about 40 hours. Recently, Segal and Hopper (15) estimated that this enzyme has a half-life of 2 days, based on the increase in activity in rat liver after cortisol treatment.

Response to Cortisol in Adrenalectomized Rats—When intact and adrenalectomized rats were treated with the same amounts of cortisol, the tyrosine transaminase response in the livers of the adrenalectomized animals was significantly greater than that noted in the intact rats (Fig. 2). A single injection of 1.25 mg of cortisol per kg produced less than a 50% rise in tyrosine trans-

![Fig. 1. Rate of increase in hepatic alanine transaminase activity following a single injection of cortisol. A single dose of 100 mg per kg of cortisol was administered subcutaneously and the rats were killed on the subsequent days indicated below. Each point is the average of individual assays on four animals per group. The vertical lines through each point represent the standard deviation.](http://www.jbc.org/)

![Fig. 2. Comparison of the response of tyrosine- and alanine-α-ketoglutarate transaminase and tryptophan pyrrolosase to cortisol in intact and adrenalectomized rats. Enzyme activity is expressed as millimoles of product formed per g of protein per hour. Each point represents the average of individual assays on four animals per group.](http://www.jbc.org/)

<table>
<thead>
<tr>
<th>Corticosteroid treatment*</th>
<th>Tyrosine transaminase activity†</th>
<th>Alanyl transaminase activity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.37 ± 0.07</td>
<td>0.30 ± 0.07</td>
</tr>
<tr>
<td>Cortisol, 5 mg per kg, 1 day</td>
<td>1.7 ± 0.54</td>
<td>0.41 ± 0.71</td>
</tr>
<tr>
<td>Cortisol, 5 mg per kg, 1 day, + deoxycorticosterone, 1 mg per kg, 4 days</td>
<td>1.2 ± 0.59</td>
<td>3.7 ± 0.91</td>
</tr>
<tr>
<td>Deoxycorticosterone, 1 mg per kg, 4 days</td>
<td>0.48 ± 0.06</td>
<td>0.48 ± 0.2</td>
</tr>
<tr>
<td>Cortisol, 5 mg per kg, 1 day</td>
<td>1.6 ± 0.22</td>
<td>4.5 ± 0.78</td>
</tr>
<tr>
<td>Cortisol, 5 mg per kg, 4 days</td>
<td>1.6 ± 0.27</td>
<td>2.9 ± 0.39</td>
</tr>
</tbody>
</table>

* Cortisol was injected intraperitoneally and deoxycorticosterone subcutaneously; the animals were killed 5 to 6 hours after the final injection.
† Micromoles of product formed per mg of protein per hour; average values ± standard deviation.
‡ Treatment was started on the fourth day following adrenalectomy.

Impaired tyrosine transaminase response in adrenalectomized rats treated with multiple doses of corticosteroids.

The data shown in Table I suggest that the loss of the mineralocorticoid following ablation of the adrenals may be responsible for the increased sensitivity of tyrosine transaminase to cortisol. When deoxycorticosterone acetate was administered to rats without adrenals, the response of tyrosine transaminase to small doses of cortisol was about the same as that obtained in the intact rats. Furthermore, the finding that daily administration of cortisol for 4 days is not as effective as the single dose of the steroid in rats without adrenals is puzzling. Previously it was reported that in intact rats treated with deoxycorticosterone acetate,
hepatic alanine transaminase levels were lowered to 30% of the normal value (1). However, administration of deoxycorticos-
terone acetate did not depress the endogenous activity of either
tyrosine transaminase (Table I) or tryptophan pyrroline in rat
liver (16).

Substrate Induction—Both tyrosine transaminase (6) and tryp-
tophan pyrroline (16, 17) can be increased in activity by treat-
ment with their substrates, tyrosine or tryptophan, and with
other nonspecific amino acids as well as with cortisol. The find-
ing that the hepatic activity of alanine transaminase in both in-
tact and adrenalectomized rats was related to the protein content
of the diet (1) suggested that the substrates of this enzyme might
also be effective as inducing agents. The data obtained when
the substrates of alanine transaminase were injected or fed in the
diet are shown in Table II. Some toxicity occurred in animals
treated by injection with 5 or 10 mmoles of alanine per kg daily
for 7 days as indicated by a loss in body weight. The 50% rise
in the activity of alanine transaminase cannot be related specif-
cally to the injection of alanine, since a loss of body weight is a
sufficient stress to cause such a slight increase in the activity of
this enzyme. The combination of alanine and glutamic acid,
when given intraperitoneally for 1 week, did not induce appreci-
able changes in the activity of this transaminase.

Effects of Conditions That Enhance Rate of Gluconeogenesis—
In previous studies it was found that in addition to the marked
rise in hepatic activity of alanine transaminase noted after cor-
tisol treatment, 5- to 7-fold increases in the activity of this en-
zeyme occurred in fasted and diabetic animals and in rats fed high
protein diets (1).

The data presented in Fig. 3 show the effects of each of these
conditions on the levels of tyrosine transaminase in rat liver.
The values after 24 hours of fasting were markedly increased
(2.5-fold) over the control level. When food was withheld for a
period of 4 days, a 7-fold increase in activity was observed. The
effects of depriving of food on the activity of tyrosine trans-
aminase occurred earlier and were of greater magnitude than
those observed for alanine transaminase in a similar experiment
(1). In relation to the group of animals fed a diet lacking pro-
tein, the ratios of the values when animals were fed diets contain-

TABLE II
Effect of substrate administration on hepatic alanine
transaminase activity

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Dose</th>
<th>Route</th>
<th>Alanine transaminase activity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>5 mmoles/kg</td>
<td>Intraperitoneal</td>
<td>4.4 (3)</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>10 mmoles/kg</td>
<td>Intraperitoneal</td>
<td>6.8 (4)</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>10 mmoles/kg</td>
<td>Intraperitoneal</td>
<td>6.3 (4)</td>
</tr>
<tr>
<td>None</td>
<td>2.5 mmoles/</td>
<td>Intraperitoneal</td>
<td>5.0 (4)</td>
</tr>
<tr>
<td>L-Alanine</td>
<td>5% In diet</td>
<td>In diet</td>
<td>6.7 (3)</td>
</tr>
<tr>
<td>L-Glutamic acid</td>
<td>5% In diet</td>
<td>In diet</td>
<td>7.8 (5)</td>
</tr>
</tbody>
</table>

* Micromoles of product formed per mg of protein per hour.
The values given are averages for the numbers of animals shown
in parentheses.

For tyrosine transaminase, thymus tissue and Walker carei-
noma 256 were diluted 1:3, and 1.0 ml of these tissue homogenates
was added to the buffer-substrate reagent. Otherwise the enzyme
assay was similar to that for liver, except that the incubation
period was 60 minutes. The assay for alanine transaminase was
 carried on l:10 homogenates of thymus gland and Walker
tumor. Average values for five animals in each group are given.

TABLE III
Effect of cortisol and tyrosine on alanine and tyrosine transaminase
activity in corticosteroid-responsive tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Alanine transaminase*</th>
<th>Tyrosine transaminase*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cortisol/ control</td>
<td>Tyrosine transaminase/ control</td>
</tr>
<tr>
<td>Liver</td>
<td>11</td>
<td>3.8</td>
</tr>
<tr>
<td>Thymus</td>
<td>20</td>
<td>0.35</td>
</tr>
<tr>
<td>Walker carcinoma 256</td>
<td>2.8</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>1.0 mg per kg per day</td>
<td>0.63</td>
</tr>
</tbody>
</table>

* Ratio of treated to control transaminase activity (micromoles
of product formed per mg of protein per hour).
† Cortisol, 30 mg per kg per day, was injected subcutaneously
for 14 days.
‡ L-Tyrosine, 600 mg per kg per day, was injected intraperi-
toneally for 14 days.

Response in Target Tissues—The response of alanine and tyro-
sine transaminase to treatment with cortisol or tyrosine has been
compared in liver, thymus gland, and Walker carcinoma 256. Both enzymes were measured in the same tissues from tumor-
bearing animals. Daily subcutaneous injections of cortisol for 14
days caused a 95% reduction in the size of the thymus gland, and
tumor growth was markedly impaired. The prolonged adminis-
tration of cortisol resulted in significant increases in the activity
of alanine transaminase in each of these tissues (Table III). Also,
tyrosine transaminase activity was increased 2- to 3-fold in the
livers of rats treated with cortisol or tyrosine for 14 days.
However, in tumor and thymus, the level of tyrosine transamin-
ase appeared to be somewhat lowered by administration of tyrosine while remaining unaltered in tumor tissue from the steroid-treated rats. The amount of thymus tissue available after cortisol was administered for 2 weeks was not sufficient to carry out an accurate determination of tyrosine transaminase activity. Although liver tyrosine transaminase is known to respond maximally to a single intraperitoneal injection of the inducing agent, this mode of treatment with cortisol or tyrosine also was without effect on the level of this transaminase in thymus tissue or the Walker tumor when measured after 5 hours.

**DISCUSSION**

Several differences between the adaptive enzymes, alanine- and tyrosine-α-ketoglutarate transaminase, are apparent. In contrast to the induction of tyrosine transaminase within a few hours, at least 2 days are required after treatment with cortisol before the maximal response of alanine transaminase is obtained. The route of administration of cortisol, as well as the duration of treatment (5 days), was previously shown to be of importance in obtaining maximal increases in activity of alanine transaminase in liver and tumor. Although the subcutaneous route has been found to be the most effective for inducing a response of alanine transaminase, maximal stimulation of tyrosine transaminase is obtained when tyrosine or cortisol is given intraperitoneally. In an experiment in which doses of 3 mmoles of L-tyrosine per kg of body weight or 30 mg of cortisol per kg were given subcutaneously, the increase in tyrosine transaminase activity after 5 hours was about half of that seen after the intraperitoneal injection of the same amounts of these agents.

The route of administration and duration of treatment with the inducing agent should be considered before it is concluded that an enzyme is noninducible. The urea cycle enzymes (18) and threonine dehydrase (19) do not respond within 5 hours to a single intraperitoneal dose of cortisol but are significantly increased in activity in the livers of rats treated with this steroid for several days (19, 20). Similarly, other enzymes previously shown to be unresponsive to a single dose of cortisol (12) may respond after several days of treatment by the subcutaneous route.

In rats 6 weeks of age or older, adrenalectomy results in as much as a 70% fall in hepatic alanine transaminase activity (12) within 48 hours. Another inducible enzyme, tryptophan pyrrolase, also has been reported (17) to decrease (40%) in activity in liver after removal of the adrenals. In confirmation of the findings of Lin and Knox (6), tyrosine transaminase activity in the livers of immature rats was not reduced after adrenalectomy (Table I); in mature rats, the level of this enzyme also was unaffected by adrenalectomy.

The failure of tyrosine transaminase in liver to show a significant drop in activity after adrenalectomy is in contrast to the substantial decrease in activity of alanine transaminase and tryptophan pyrrolase in such animals. The lack of effect of adrenalectomy on hepatic tyrosine transaminase activity is not consistent with the greater response of this transaminase to small doses of cortisol in adrenalectomized rats. The several factors which may be balanced to maintain normal levels of this enzyme in the liver of adrenalectomized rats are not known. Since tyrosine does not induce a response of tyrosine transaminase in adrenalectomized animals (6), factors other than substrate levels must be considered.

The unique responsiveness of hepatic tyrosine transaminase to small doses of cortisol in the adrenalectomized rat indicates some function of the adrenals in regulating the activity of this enzyme. It is notable that in the adrenalectomized animal, the daily administration of deoxycorticosterone reduces the sensitivity of the response of this enzyme to cortisol. These data and the similar effects obtained with repeated doses of cortisol suggest some common action of these corticosteroids which could involve the transport of ions or amino acids in liver. These findings reveal some of the complex relationships between corticosteroids which affect the induction of enzymes. The increased responsiveness of tyrosine transaminase to cortisol in adrenalectomized rats could be associated with impaired capacity to maintain the level of a repressor.

Previously, we reported on the marked response of alanine transaminase, but not aspartic-α-ketoglutarate transaminase, in liver, thymus tissue, and Walker carcinosarcoma 256 in animals treated daily with cortisol for 1 week (5). Since both of these enzymes are present in the cell sap, it was concluded that the specific rise in alanine transaminase is not due to concentration of this enzyme in residual thymus tissue or Walker tumor. Since tyrosine transaminase in thymus and Walker tumor did not respond after subcutaneous or intraperitoneal injections of tyrosine, further consideration should be given to the possibility that the low activity is attributable to an artifact. The activity of tyrosine transaminase in both thymus and Walker tumor is unusually low and consequently difficult to measure with accuracy.

Three conditions associated with an enhanced rate of gluconeogenesis, namely, a high protein intake, fasting, and diabetes, stimulated tyrosine transaminase in liver to an extent comparable to that resulting from cortisol or tyrosine treatment. Similar changes in alanine transaminase in liver were noted in rats subjected to the same conditions. Schimke recently reported that feeding a high protein diet (18) and fasting (21) resulted in significant increases in the activity of the urea cycle enzymes in liver. These observations and our data are interpreted as indicating that the increase in enzyme activity follows some common effect produced by these treatments. Catabolism of labile protein stores accompanies each of these conditions, which are associated with stress. The metabolic pools of amino acids in liver would be correspondingly enlarged, thus favoring the synthesis of the adaptive enzymes. That the pool size of amino acids in liver may be related to alanine transaminase activity is suggested by the several fold increases in activity of this enzyme noted after cortisol administration (3), feeding a high protein diet (1), fasting (1), alloxan diabetes (1), hypophysectomy (4), and aging (14, 22) and its depression in activity by as much as 50% in pregnant (23), partially hepatectomized, or tumor-bearing animals (24, 25).

It is well established that tyrosine transaminase can be induced by tyrosine in the livers of intact, but not adrenalectomized, rats (6), whereas tryptophan pyrrolase can be induced in either intact or adrenalectomized animals (14). The experiments in which substrates of alanine transaminase were tested as inducing agents of this enzyme, at near toxic doses and by various routes of administration, were uniformly negative. The relatively slow turnover rate of alanine transaminase or the requirement for adequate levels of precursor amino acids during the long period required for induction of this enzyme may be partly responsible for the lack of substrate induction.

1 H. R. Harding, F. Rosen, and C. A. Nichol, to be published.
Among the enzymes that are inducible, differences in the rate of response, effect of adrenalectomy, half-life, or inducibility by substrates can be recognized. These differences require consideration in further studies directed toward some understanding of the regulation of enzyme synthesis as well as the physiological significance of such adaptive responses.

Studies by Feigelson and Greengard (26) have demonstrated that new enzyme synthesis and activation by the hematin cofactor contribute to the increased activity of tryptophan pyrroloase in response to tryptophan. Recently, we have obtained evidence that the increase in tryptophan pyrroloase in the livers of adrenalectomized or hypophysectomized rats treated by injection with tryptophan represents mainly activation of this enzyme rather than its synthesis (27). This observation is considered in detail in the accompanying paper (28) which also deals with the unusual specificity shown by tryptophan and other indoles as inducers of tyrosine transaminase.

SUMMARY

1. A period of about 48 hours is required to attain maximal activity of alanine-α-ketoglutarate transaminase in liver after subcutaneous treatment of rats with a single injection of cortisol. In contrast, tyrosine-α-ketoglutarate transaminase reaches a maximal value in liver between 4 and 5 hours after an intraperitoneal dose of cortisol.

2. The increases in hepatic tyrosine-α-ketoglutarate transaminase levels observed in adrenalectomized rats are significantly greater than those obtained for intact animals treated with the same dose of cortisol. Adrenalectomy does not alter the sensitivity of the alanine-α-ketoglutarate transaminase or tryptophan pyrroloase response to cortisol treatment.

3. Increases in hepatic alanine-α-ketoglutarate transaminase could not be demonstrated after treatment with the substrates of this enzyme by two routes of administration. This transaminase differs, therefore, from tyrosine-α-ketoglutarate transaminase and tryptophan pyrroloase, which can be induced in intact rats treated with their amino acid substrates.

4. Both alanine- and tyrosine-α-ketoglutarate transaminases are 3 to 7 times higher than normal in the liver of rats made diabetic by alloxan, deprived of food, or fed high protein diets. Each of these conditions enhances the rate of gluconeogenesis.

5. Although appreciable increases (3- to 20-fold per mg of protein) in alanine transaminase activity can be induced in cortisol-responsive tissues, such as thymus gland and Walker carcinoma 256, the low level of tyrosine-α-ketoglutarate transaminase in these tissues was not significantly altered after cortisol or tyrosine administration.

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REFERENCES

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