Immunological Studies of Serine Dehydratase and Ornithine Aminotransferase Regulation in Rat Liver in Vivo*

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Previous studies of serine dehydratase (EC 4.2.1.13) and ornithine aminotransferase (EC 2.6.1.13) adaptation in rat liver showed that in rats on a high protein diet, glucocorticoid administration increased serine dehydratase activity while simultaneously reducing the activity of ornithine aminotransferase. The present study examines the role of enzyme synthesis in the expression of these and other dissimilar adaptive characteristics of the two enzymes.

Both enzymes were purified to crystallinity and used to prepare specific antibodies. Changes in the rate of synthesis of each enzyme during adaptation were then measured immunochemically.

In rats fed ad libitum, the synthetic rates for both enzymes exhibited circadian rhythm, although enzyme levels remained relatively constant. The circadian cycle for ornithine aminotransferase synthesis was in phase with the cycles for body weight and relative liver weight (maxima at 9 a.m., minima at 9 p.m.) but was approximately 12 hours out of phase with the cycle for serine dehydratase synthesis. 9α-Fluoro-11β,17β-dihydroxy-16α,17α-isopropylidenedioxyprogna-1,4-diene-3,20-dione (triamcinolone), injected at 9 a.m., increased serine dehydratase synthesis and simultaneously decreased the synthesis of ornithine aminotransferase. When triamcinolone was injected at 9 p.m., however, serine dehydratase synthesis was not stimulated, although the reduction of ornithine aminotransferase synthesis was still produced.

These results suggest that: (a) circadian cycling of synthesis may be a general phenomenon in enzyme regulation even though for enzymes with relatively long half-lives, such cycling may not be reflected as fluctuations in enzyme levels; (b) such circadian rhythmicity may also involve cyclic changes in the responsiveness of the enzyme-forming system to regulatory stimuli; (c) whereas the adaptive behavior of serine dehydratase typifies that of amino acid-catabolizing enzymes in general, the responses of ornithine aminotransferase denote a functional association of this enzyme with anabolic processes. On this basis, the possibility that ornithine aminotransferase plays a pivotal role in the regulation of urea cycle activity and nitrogen balance is discussed.

EXPERIMENTAL PROCEDURE

Materials—Male Sprague-Dawley rats, age 21 days, were purchased from Charles River Laboratories, Wilmington, Mass., and were housed five per cage under conditions of constant temperature (23°) and
regulated lighting (light, 7 a.m. to 7 p.m.; dark, 7 p.m. to 7 a.m.). A standard laboratory animal diet (Wayne Lab Blox, Allied Mills, Chicago, Ill.) or the appropriate test diet was available with water ad libitum.

Pelleted diets containing 90% or 85% casein were purchased from Teklad Mills, Madison, Wis. All diets were isocaloric; the carbohydrate content (cornstarch) was adjusted to compensate for the changes in fat content. The diets were otherwise nutritionally adequate. The following substances were obtained from the designated sources: triamcinolone acetate (25 mg/ml), Lederle Laboratories, Pearl River, N.Y.; t-[4,5-3H]leucine, 45 Ci/mmol, Schwarz/Mann, Orangeburg, N.Y.; Scintisol-Complete, Isolab Inc., Akron, Ohio. All other reagents were of the highest purity obtainable commercially, and all reagent solutions were prepared with glass-distilled water.

Enzyme Assays—Ornithine aminotransferase activity was assayed by measuring the A$_{412}$ of the adduct formed between the reaction product, $\Delta^4$-pyrroline-5-carboxylate, and o-aminobenzoic acid as previously described (4). The millimolar extinction coefficient was 2.71. One A$_{412}$ unit corresponds to that amount of enzyme causing an increase in A$_{412}$ of 1.00/min under the conditions of the assay. In the regulation studies, this assay was performed on sonified 40% liver homogenates prepared as described under "Preparation of Extracts," and the A$_{412}$ values were converted to micromoles of product formed.

Serine dehydratase activity was assayed by coupling the reaction to lactate dehydrogenase and following the decrease in NADH at 340 nm. The assay was similar to that previously described (1), except that phosphate buffer was used instead of Tris, and dithiothreitol and EDTA were added to the reaction mixture to final concentrations of 10$^{-4}$ M and 10$^{-5}$ M, respectively. The A$_{340}$ activity unit corresponds to that amount of enzyme causing a decreased A$_{340}$ of 1.00/min under the conditions of the assay. In the regulation studies, this assay was performed on the supernatants from sonicated 40% homogenates that had been centrifuged at 200,000 × g for 30 min, and the A$_{340}$ values were converted to umoles of product formed, using the molar extinction coefficient for NADH.

Enzyme Purification—Approximately 100 rats were fed an 85% casein diet for 5 days after which they were killed by cervical dislocation. The livers were removed, chilled in ice-cold buffer (0.1 M Tris/0.15 M KCl, 10$^{-4}$ M dithiothreitol, and 10$^{-5}$ M pyridoxal-5'-phosphate, pH 7.4, at room temperature), was frozen at -180°C and subsequently used to prepare crude preparations described above. The supernatant was discarded, the pellet was dissolved in 1 ml of Buffer B, and the solution was clarified by centrifugation at 200,000 × g for 30 min at 4°C. The supernatant was adjusted to 20% saturation with solid (NH$_4$)$_2$SO$_4$ (pH 7.0), and the precipitate was removed by centrifugation at 4°C, and saturated (NH$_4$)$_2$SO$_4$ (pH 7.0) was added dropwise, with swirling, until a faint opalescence appeared. The preparation was then placed in a covered ice bath as before until crystallization was complete. This cycle was repeated a third time; the final pellet was dissolved in 2 ml of Buffer B.

Electrophoresis of thrice crystallized preparations of serine dehydratase and ornithine aminotransferase in polyacrylamide gels gave single bands (Fig. 1). The more diffuse band exhibited by ornithine dehydratase crystallization. The rate of crystallization was increased by stirring the preparation periodically with a thin glass rod. Crystallization was usually complete in 4 days.

FIG. 1. Polyacrylamide gel electrophoresis of thrice crystallized ornithine aminotransferase (left) and serine dehydratase (right). The origins of the gels coincide exactly with the bottoms of the tube caps.
collected. The fractions containing antibody activity, detected by Ouchterlony double diffusion analysis, were pooled and treated with saturated ammonium sulfate as described above. The precipitated antibody was resuspended in a minimal volume of the column buffer and dialyzed against this buffer. The resulting preparations were stored in 1-ml aliquots at −20°C.

Each antibody preparation was standardized against the appropriate pure enzyme as follows. Different amounts of enzyme were incubated with a constant amount of antibody in a constant volume, and the amount of enzyme (catalytic activity) that remained free in the supernatant after centrifuging down the antibody-antigen complex was determined. The largest amount of added enzyme that was fully precipitated under these conditions marked the equivalence point of the antibody (expressed as Amax or Amin enzyme activity units/unit volume).

Treatments for Regulation Studies—At 37 days of age, rats were randomized by weight (range, 130 to 180 g), average, 150 to 160 g) and on the following day were changed from Lab Blox to the 60% casein diet. Subsequent treatments, specific to each experiment, are described in the figure and table legends. Triamcinolone (5 mg) was given intramuscularly in a single injection (0.2 ml). Forty minutes prior to sacrifice, each rat was injected intraperitoneally with 100 μCi of [3H]leucine in 0.2 ml. Rats were killed by cervical dislocation; livers were rapidly removed, chilled in ice cold buffer (the same used in the preparation of liver extracts, see below), weighed, and frozen. The frozen livers were homogenized in 1.5 volume of buffer (0.1 M Tris/10 mM dithiothreitol/10 mM EDTA/100 mM pyridoxal-5-phosphate, pH 7.4), using a Potter-Elvehjem homogenizer. Each homogenate was sonified in an ice bath for a total of 4 min using a Branson sonifier delivering 120 watts at the probe tip. The temperature of the homogenate was not allowed to exceed 2°C during the sonication. Each homogenate was then centrifuged at 200,000 × g for 45 min at 4°C. This procedure fully released ornithine aminotransferase from the mitochondria. Unless otherwise specified, the samples were kept in an ice bath during subsequent operations.

Preparation of Liver Extracts—Each liver was thawed and homogenized in 1.5 volume of buffer (0.1 M Tris/10 mM dithiothreitol/10 mM EDTA/100 mM pyridoxal-5-phosphate, pH 7.4) using a Potter-Elvehjem homogenizer. Each homogenate was sonified in an ice bath for a total of 4 min using a Branson sonifier delivering 120 watts at the probe tip. The temperature of the homogenate was not allowed to exceed 2°C during the sonication. Each homogenate was then centrifuged at 200,000 × g for 45 min at 4°C. This procedure fully released ornithine aminotransferase from the mitochondria. Unless otherwise specified, the samples were kept in an ice bath during subsequent operations.

Immunochromatographic Procedures—Each liver extract, prepared as described above, was assayed for ornithine aminotransferase and serine dehydratase, and was then heated to 55°C, maintained at that temperature for 1 min, and cooled rapidly to 0°C in an ice water bath. The resultant suspensions were centrifuged at 200,000 × g for 30 min at 4°C, the supernatants were again assayed for enzyme activity, and were then used for immunological analysis.

The heating procedure, which was necessary to prevent nonspecific precipitation of radioactive protein, had no effect on ornithine aminotransferase activity, but reduced serine dehydratase activity by 40 to 50%. When the liver extracts before and after heating were reacted against constant amounts of antibody for each enzyme, the antibody equivalence points for both enzymes were found to be unchanged. Thus, the loss in serine dehydratase activity on heating was accompanied by an equivalent loss of enzyme protein, and the remaining enzyme was unchanged in its antigenic properties. It was, therefore, possible to correct for this heat-induced loss of enzyme in the subsequent immunochromatographic estimation of serine dehydratase synthesis.

The immunoprecipitation reaction mixtures contained 200 Amax units of ornithine aminotransferase or 120 Amax units of serine dehydratase, a 75% excess of specific antibody (in 0.2 ml), and buffer (the same as that used to prepare the liver extracts) in a final volume of 4.0 ml. The standard amount of enzyme activity in each reaction mixture was provided either solely by the appropriate volume of liver extract or, when necessary, by a combination of the maximum permissible volume of liver extract (up to 5.7 ml) and purified carrier enzyme. The antibody-antigen mixtures were incubated for 10 to 18 hours in an ice bath, and the resulting precipitates were collected by centrifuging for 30 min at 1500 × g. Assays of the supernatants from these incubations for enzyme activity indicated that less than 1% of either enzyme remained in the supernatant. The precipitates were washed four times with 2.0-ml aliquots of 0.9% NaCl, dissolved in Scintisol, transferred quantitatively to scintillation vials, and counted. Control samples containing liver extract, buffer, and serum from nonimmunized goats were treated as described above. The counts observed in these controls did not differ from background radiation.

Antibody specificity was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10) of radioactive immunoprecipitates prepared as described above. The results of the electrophoretic analysis (Fig. 2) show that each antibody precipitated a single radioactive protein that corresponded in mobility to that of the appropriate antigen.

The incorporation of [3H]leucine into total liver protein was determined by a modification of the technique of Mans and Novelli (12). Aliquots of the sonicated homogenate were dried on 9-cm Whatman No. 1 filter circles which had been folded to fit securely in the grooves of a Coplin jar. The papers were soaked for 10 min each in three changes of 10% trichloroacetic acid followed by two changes of 95% ethanol. The papers were dried under a heat lamp and combusted in a Packard model 306 sample oxidizer.

Measurement of radioactivity was done in a Beckman LS-200B liquid scintillation counter. Counting efficiency was determined by adding a known quantity of [3H]leucine to the scintillation vials containing the combusted protein samples, and to Scintisol solutions of the immunoprecipitates. Radioactivity, corrected for background, was converted to dpm/g of liver, for each of the antibody-precipitated enzymes and for the total liver protein; the data were expressed finally as the ratio of the radioactivity in each enzyme to that in the total liver protein. (The radioactivity incorporated into total liver protein was approximately 1.5 × 107 dpm/g of liver on the average.) Changes in this ratio in response to dietary and hormonal stimuli were considered to represent changes in the rate of synthesis of the specific enzyme relative to changes in the rate of total protein synthesis. This method of expression (13) corrects for treatment-related changes in the size of the free leucine pool and for variations in the uptake of labeled leucine among different animals. In the present experiments, the latter consideration proved to be of greater importance, since we did not observe a significant systematic change in total protein labeling with any of the treatments.

All values are expressed as means and standard errors. The numbers of animals per data point are shown in parentheses in the figures. The vertical bars at all data points indicate standard errors.

RESULTS

In rats that had been fed a 60% casein diet for 5 days (Fig. 3, solid lines), distinct circadian rhythms were observed in the patterns of serine dehydratase and ornithine aminotransferase synthesis measured over the following 72-hour interval, but the cycles for the two enzymes were not in phase. Thus, serine dehydratase synthesis (Fig. 3A) was at a maximum near the beginning of the dark phase, reached a minimum 2 to 3 hours before the beginning of the light phase, and began to increase again after 2 to 3 more hours. Ornithine aminotransferase synthesis (Fig. 3C) was at a maximum near the beginning of the light phase, reached a minimum at the time the lights went out, and began to increase again after approximately 6 hours of darkness. The amplitude of the oscillation for ornithine aminotransferase synthesis decreased progressively during the observation interval, whereas the cycle for serine dehydratase synthesis did not. The catalytic activities of the two enzymes did not show cyclic behavior in these rats (Fig. 3, B and D).

When rats that had been on the 60% casein diet for 5 days were given a single triamcinolone injection at 9 a.m. (zero time), the rate of serine dehydratase synthesis increased substantially and the circadian rhythm seen in the uninjected rats was retained (Fig. 3A, dashed lines). The maximum rate of synthesis measured over the following 72-hour interval, but the cycles for the two enzymes were not in phase. Thus, serine dehydratase synthesis (Fig. 3A) was at a maximum near the beginning of the dark phase, reached a minimum 2 to 3 hours before the beginning of the light phase, and began to increase again after 2 to 3 more hours. Ornithine aminotransferase synthesis (Fig. 3C) was at a maximum near the beginning of the light phase, reached a minimum at the time the lights went out, and began to increase again after approximately 6 hours of darkness. The amplitude of the oscillation for ornithine aminotransferase synthesis decreased progressively during the observation interval, whereas the cycle for serine dehydratase synthesis did not. The catalytic activities of the two enzymes did not show cyclic behavior in these rats (Fig. 3, B and D).

In contrast to the positive response of serine dehydratase,
ornithine aminotransferase synthesis and activity decreased after triamcinolone administration (Fig. 3, C and D). Because the triamcinolone was given during the descending phase of the ornithine aminotransferase circadian cycle, the repression of ornithine aminotransferase synthesis was most clearly manifested as a blockage of the increase in synthesis during subsequent cycles, although a more rapid decline during the first cycle is also evident (Fig. 3C). The reduction of synthesis was accompanied by a decrease in ornithine aminotransferase activity, but this decrease did not begin until 18 hours had elapsed following the triamcinolone injection (Fig. 3D). The average rate of ornithine aminotransferase synthesis in the triamcinolone-injected rats was about half that in the uninjected rats despite the delayed decrease in activity, the average activity in injected rats was also about half that in uninjected rats.

Table I, in conjunction with Fig. 3, shows the effects of changing the time of triamcinolone administration from 9 a.m. to 9 p.m. on the responses of both enzymes over the subsequent 12 hours. When triamcinolone was given during the phase of increasing serine dehydratase synthesis (9 a.m.), further stimulation of serine dehydratase synthesis occurred (Fig. 3A). However, if this treatment was given at 9 p.m., during the phase of declining serine dehydratase synthesis, this decline was not affected (Table I). The repressive effect of triamcinolone given at 9 a.m. on ornithine aminotransferase synthesis was only marginally apparent over the following 12 hours because the synthetic rate was also declining in the untreated rats during this period (Fig. 3C). Administration of triamcinolone at 9 p.m., during the phase of minimum ornithine aminotransferase synthesis (see Fig. 3C), produced a further decline in synthesis over the next 12 hours (Table I), in contrast to the marked increase in synthetic rate that occurred in the untreated rats during this interval (Fig. 3C and Table I).

With the use of standardized antibody preparations, immunoenzymochemical titrations of both enzymes were conducted on liver extracts from rats given the dietary and hormonal treatments described above. None of the treatments altered the antibody equivalence point for either enzyme from that of the purified enzyme standard, indicating that the observed changes in enzyme activity resulted from changes in the amount of enzyme protein rather than from changes in catalytic efficiency. This agrees with earlier immunofluorescence studies showing elevated ornithine aminotransferase levels in the livers of rats on a 60% casein diet (14). Correspondence between catalytic activity and enzyme amount has also been obtained in other studies of enzyme adaptation (11, 15-19).

Fig. 4 shows that for rats on the 60% casein diet without the triamcinolone injection, the ratios of liver weight to body weight and the body weights also fluctuated according to a circadian cycle; the decreases occurred during the light phase and the increases occurred during darkness. The rats gained weight during the experiment but there was no upward trend in the liver to body weight ratio. The greatest food consumption in these rats occurred at the beginning of the dark phase and preceded the circadian increase in liver to body weight ratio and body weight (Fig. 4).

The administration of triamcinolone to the rats on the 60% casein diet was followed by a loss of body weight, and an increase in the liver to body weight ratio; these changes were
FIG. 3. Synthesis rates and catalytic activities of serine dehydratase and ornithine aminotransferase measured over a 72-hour period in rats prefed a 60% casein diet for 5 days (solid lines), and in rats given this dietary treatment plus a single injection of triamcinolone at 0 hours (9 a.m.) (dashed lines). The rats were maintained on the 60% casein diet throughout the experiment. The left ordinate refers to Panels A and C, whereas the right ordinate refers to Panels B and D. The horizontal bar indicates period of light (open rectangles) and darkness (solid rectangles).

TABLE I

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Serine dehydratase</th>
<th>Ornithine aminotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Synthesis</td>
<td>Activity</td>
</tr>
<tr>
<td>Control, killed 9 p.m.</td>
<td>5.60 ± 0.40</td>
<td>139 ± 11</td>
</tr>
<tr>
<td>Control, killed 9 a.m.</td>
<td>2.23 ± 0.33</td>
<td>162 ± 11</td>
</tr>
<tr>
<td>Triamcinolone 9 p.m., killed 9 a.m.</td>
<td>2.91 ± 0.25</td>
<td>283 ± 15</td>
</tr>
</tbody>
</table>

Comparison of the data in Figs. 3 and 4 indicates that: (a) in rats gaining weight on a 60% casein diet, the circadian cycles for liver to body weight ratio and for body weight were approximately in phase with the cycle for ornithine aminotransferase synthesis, and approximately 12 hours out of phase with the cycle for serine dehydratase synthesis; and (b) the administration of triamcinolone dampened the circadian cycles for liver to body weight ratio, and body weight, but the cycle for serine dehydratase synthesis was retained.

DISCUSSION

The circadian changes in the synthetic rates of serine dehydratase and ornithine aminotransferase (Fig. 3, A and C) were not clearly manifested as circadian changes in enzyme...
levels (Fig. 3, R and D) because of the relatively long half-lives of these enzymes (approximately 20 hours for serine dehydratase, Ref. 8, and 1.9 days for ornithine aminotransferase, Ref. 20). The levels of amino acid-catabolizing enzymes with short half-lives such as tyrosine aminotransferase (1.5 hour) and tryptophan oxygenase (2 to 3 hours) (13), do, however, show pronounced circadian fluctuations (21, 22), which are presumably reflections of similar oscillations in their synthetic rates. It appears, therefore, that circadian cycling may be a ubiquitous component of gene expression whether or not this cycling is expressed as fluctuations in the concentrations of the final gene product. The retention of the circadian cycle for serine dehydratase synthesis in rats receiving a large dose of triamcinolone suggests that cyclic changes in serum glucocorticoid levels (23-25) are not responsible for the oscillations of enzyme synthesis. This suggestion is in agreement with evidence showing that the circadian fluctuations in tyrosine aminotransferase levels are not glucocorticoid dependent (21, 22, 26).

The change in inducibility of serine dehydratase by triamcinolone injected at 9 a.m. or 9 p.m. is positively correlated with the circadian change in the synthetic rate of the enzyme in uninjected rats (Fig. 3 and Table I). Studies of tyrosine aminotransferase have also revealed circadian changes in the responsiveness of this enzyme to glucocorticoids (27, 28) and theophylline (29). These observations suggest that the circadian cycle for enzyme synthesis may involve: (a) cyclic changes in the enzyme-forming system itself that render it periodically unable to synthesize enzyme endogenously or respond to an inducer; or (b) changes in the concentrations of other agents such as glucagon, insulin, or cyclic AMP, that may also be involved in the regulation of these enzymes (27, 30, 31-33). It is not yet possible to distinguish unequivocally
between these two possibilities, but the observation that the circadian cycling of tyrosine aminotransferase levels and inducibility persists in cultured liver cells (28) does suggest that such cycling is generated intracellularly rather than at the level of the whole organism.

The circadian cycle for ornithine aminotransferase synthesis is not in phase with the cycles for serine dehydratase (Fig. 3), tyrosine aminotransferase, and tryptophan oxygenase (21, 22) but does coincide with the cycles for liver to weight ratio and body weight (Fig. 4) and for liver DNA synthesis (34). The stimulation of ornithine aminotransferase synthesis appears, therefore, to be temporally associated with the acceleration of anabolic processes, whereas the reverse is the case for serine dehydratase.

The linking of ornithine aminotransferase and serine dehydratase to anabolic and catabolic processes, respectively, is also suggested by the manner in which the responses of these enzymes to a pharmacological dosage of glucocorticoid correlate with other metabolic consequences of such treatment. Thus, the overriding effect of glucocorticoid administration is the shift to negative nitrogen balance (35), accompanied by a rapid onset of hepatomegaly and loss of body weight, and a subsequent reduction of food intake (Fig. 4). Negative molecular effects in the liver include: a reduction of DNA synthesis (35); a reduction in the levels of 3-P-glycerate dehydrogenase (36); a reduction in the synthesis of phosphoenolpyruvate carboxykinase (30); and a reduction in the synthesis of ornithine aminotransferase (Fig. 3 and Table 1). On the other hand, the synthesis of serine dehydratase (Fig. 3), tyrosine aminotransferase, and tryptophan oxygenase (13) is increased under these conditions.

In considering the sequence of events involved in the transition from positive to negative nitrogen balance after triamcinolone administration, it is evident that the changes in the synthesis of serine dehydratase and ornithine aminotransferase (Fig. 3 and Table 1) precede the reduction in body weight and food intake (Fig. 4). This suggests that the changes in these enzymes are part of the processes by which negative nitrogen balance is produced.

The adaptive behavior of ornithine aminotransferase under these conditions suggests a possible role for this enzyme as a participant in the control of urea cycle activity. Since the equilibrium of the reaction catalyzed by ornithine aminotransferase greatly favors ornithine catabolism (4, 5), the action of this enzyme would tend to deplete the ornithine pool, thereby limiting urea cycle activity and favoring nitrogen retention. Conversely, the increased nitrogen excretion accompanying the onset of negative nitrogen balance would require the elevation of urea cycle activity, which would be favored by the increase in ornithine availability resulting from ornithine aminotransferase suppression.

This scheme is compatible with the observed reciprocal relationship between the responses of ornithine aminotransferase and serine dehydratase to glucocorticoid administration, but it does not account for the fact that both enzymes increase in rats changed from a low to a high protein diet (Table 1) and forces the animals into negative nitrogen balance (Fig. 4) even though the dietary protein provides countervailing stimuli to both responses. This suggests that either: (a) glucocorticoid and adaptive stimuli from the diet are in competition for common regulatory sites, where they exert opposing effects, and glucocorticoid is more efficient in its interaction with these sites; or (b) the two types of stimuli act at different regulatory sites, but the site affected by glucocorticoid plays a more central role in regulation, and the consequences of its activation supersede those produced by diet-regulator interactions.

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REFERENCES

Immuochemical studies of serine dehydratase and ornithine aminotransferase regulation in rat liver in vivo.
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