Studies on the Induction and Repression of Enzymes in Rat Liver

III. INDUCTION OF ORNITHINE &-TRANSAMINASE AND THREONINE DEHYDRASE BY ORAL INTUBATION OF FREE AMINO ACIDS*

CARL PERAINO, ROBERT L. BLAKE,† AND HENRY C. PITOT

From the McArdle Laboratory for Cancer Research, Medical School, University of Wisconsin, Madison 6, Wisconsin

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It is well known that the feeding ad libitum of high protein diets to rats over a period varying from several days to several weeks produces an increase in the activities of enzymes involved in amino acid catabolism (3-8). Studies in our laboratory have shown that at least two amino acid-catabolizing enzymes, threonine dehydrase and ornithine transaminase, can be elevated to high levels within a few hours in the livers of protein-depleted rats fed casein hydrolysate by stomach tube (9-11). In the long term adaptation studies cited above, as well as in our rapid induction experiments, relatively nonspecific dietary inducing agents were used, and no information was obtained on the possible existence of specific dietary requirements for the induction of individual amino acid catabolizing enzymes. To date, tryptophan pyrrolase is the only mammalian enzyme that has received thorough study with respect to its induction requirements (12-17). Other very limited studies on threonine dehydrase induction have yielded contradictory results (18, 19).

The present investigation was designed to determine whether or not a degree of specificity exists in the dietary amino acid requirements for the induction of threonine dehydrase and ornithine transaminase. In these studies the rapid induction of threonine dehydrase and ornithine transaminase was measured as a function of the qualitative and quantitative composition of a mixture of free amino acids administered to rats by stomach tube.

MATERIALS AND METHODS

Male albino rats weighing 130 to 150 g (Holtzman) were fed ad libitum a commercially prepared protein-free diet (General Biochemicals) for 5 days. On the 6th day the protein-depleted rats were given 1 g of protein-free diet by stomach tube at 6:00 a.m. after which they were denied further access to food for 12 hours. (In one experiment (see Table II) a group of protein-depleted rats did not receive the 1-g feeding of protein-free diet, and was deprived of food for 12 hours.) At the end of this period the administration of the amino acid mixtures by stomach tube was begun. In studies involving changes in the qualitative composition of the diet, the quantities of all mixtures fed were such that the intake of each amino acid present in the various mixtures was constant from group to group. All experimental diets were administered in 4 ml of water per rat per feeding. The rats were fed at 0, 6, and 12 hours; the time of day during which the diets were fed was constant from one experiment to the next, and each amino acid mixture was adjusted to pH 7.4 with sodium hydroxide prior to feeding. All of the amino acids used in these experiments were in the L form and were products of General Biochemicals. At intervals during the experimental period (zero time to 18 hours) rats were killed by cervical dislocation, and their livers were assayed for ornithine transaminase and threonine dehydrase by procedures previously described (9, 20, 21).

RESULTS

Effects of Variations in Quantitative Composition of Dietary Amino Acid Mixture—Fig. 1 shows the activities of ornithine transaminase and threonine dehydrase at the end of an 18-hour period during which the four diets described in Table I were fed every 6 hours at various dose levels. With 0.06 g, the induction of ornithine transaminase by any of the diets was negligible when compared with a base level of 20 ± 3 units obtained by depriving the animals of food during the 18 hours. Threonine dehydrase also showed little over-all response at this dose, although the equal weight mixture produced a slight increase (base-line level of threonine dehydrase is 500 ± 50 units). When the dose was increased to 0.1 g, a small but noticeable increase in ornithine transaminase as well as threonine dehydrase occurred in rats receiving the equimolar mixture. The other diets produced no apparent changes in the level of either enzyme. At 0.2 g, the equimolar mix produced a 2-fold increase in ornithine transaminase, while the other mixtures had no significant effect. Threonine dehydrase was also increased by the equimolar mix as well as by the equal weight mix, but not by the rat requirement or liver protein mixtures. At 0.4 g, each of the mixtures produced an elevation of ornithine transaminase activity, with the equimolar mixture still acting as the most effective inducer. This dose also produced an increase in threonine dehydrase in the case
Effects of variations in the quantitative composition of the amino acid mixture and the amount fed on the induction of ornithine transaminase and threonine dehydrase. The composition of each amino acid mixture is given in Table I. Each rat received the amount of mixture designated in Fig. 1 at 0, 6, and 12 hours, and the rats were killed at 18 hours. 

Each value is the average ± the standard error of the mean for five rats. One unit of ornithine transaminase produces 1 pmole of pyrroline carboxylate per g of tissue per hour. One unit of threonine dehydrase produces 1 pmole of α-ketobutyrate per g of tissue per hour.

Table I

<table>
<thead>
<tr>
<th>Amino acid composition of experimental diets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixture</td>
</tr>
<tr>
<td>Rat requirementa</td>
</tr>
<tr>
<td>µmoles/g mixture</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
<tr>
<td>Tryptophan</td>
</tr>
</tbody>
</table>

Fig. 1. Effects of variations in the quantitative composition of the amino acid mixture and the amount fed on the induction of ornithine transaminase and threonine dehydrase. The composition of each amino acid mixture is given in Table I. Each rat received the amount of mixture designated in Fig. 1 at 0, 6, and 12 hours, and the rats were killed at 18 hours. 

The quantitative and qualitative composition of this mixture is based on the daily nutritional requirement of the rat for these amino acids (22).

Consists of equimolar proportions of each of the amino acids nutritionally essential for the rat.

Consists of equal weight proportions of each of the essential amino acids.

Contains the amino acids essential for the rat in proportions based on the concentrations of these amino acids in mammalian liver (23).

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Contains the amino acids essential for the rat in proportions based on the concentrations of these amino acids in mammalian liver (23).

The liver protein mixture did not produce any threonine dehydrase increase. The pattern of induction of ornithine transaminase with 0.6 g was similar to that observed with 0.4 g, except that all values were further elevated by the higher dose. In contrast, threonine dehydrase was further induced only by the equal weight mixture at the 0.6-g level, with 0.6 g of the other amino mixtures producing only as much induction as had the 0.4-g dose. It is interesting that threonine dehydrase was not significantly induced by the liver protein mixture at any of the levels tested.

These results indicate that both ornithine transaminase and threonine dehydrase can be rapidly induced by feeding a mixture of essential amino acids, and that the quantitative composition of the mixture has a pronounced effect on the degree of enzyme induction. It is also apparent that the level at which the mixtures are fed strongly influences their relative inducing ability. The two enzymes also differ somewhat from each other in their responses to alterations in the quantitative composition of the mixture and to changes in the dose.

Time Course of Induction at Two Different Levels of Inducing Mixture—When the time course of the induction of both ornithine transaminase and threonine dehydrase was measured after feeding 0.2 g or 0.6 g of equimolar mix per dose (Fig. 2), it is seen that during the first 12 hours of the induction period the induction was the same at both levels for both enzymes. However, after 12 hours little or no further increase occurred with the 0.2-g level of amino acids while the 0.6-g level stimulated a continued high rate of induction.

Effects of Variations in Qualitative Composition of Amino Acid Mixtures—When amino acids were deleted in various combinations from the equimolar mixture and the induction produced by each of the remaining incomplete mixtures was compared with that produced by the complete equimolar mixture, the results shown in Fig. 3 were obtained. The left side of the figure shows the effect on enzyme induction of omitting lysine, histidine, and arginine, singly, and in combinations of two. It is seen that in general the two enzymes responded quite differently to these deletions. The induction of ornithine transaminase decreased progressively with the omission of lysine, histidine, or arginine,
respectively, and reached its lowest level with the omission of lysine and arginine; the omission of histidine and arginine showed the same level of induction as the omission of histidine alone. The induction of threonine dehydrase was markedly reduced by the omission of lysine or lysine and histidine, while the omission of histidine and arginine singly or together produced only a slight decrease in induction. However, the omission of lysine and arginine together had no adverse effect on threonine dehydrase induction, in contrast to the pronounced lowering effect which this deletion had on the induction of ornithine transaminase.

The right side of Fig. 3 shows the effects of deleting methionine, phenylalanine, and threonine singly or in combinations of two. It is apparent that these omissions had no adverse effect on the induction of ornithine transaminase, i.e. in all cases the level of induction was similar to that produced by the complete equimolar mixture. However, the induction of threonine dehydrase was moderately reduced by the omission of phenylalanine, threonine, or threonine and methionine but not by the omission of methionine, or phenylalanine and methionine. The greatest lowering effect was produced by the omission of threonine and phenylalanine.

Fig. 4 shows the time curves for the induction of ornithine transaminase and threonine dehydrase during the feeding of the complete equimolar mixture or the mixture lacking lysine and arginine or threonine and phenylalanine. Ornithine transaminase showed identical induction patterns for the feeding of the complete mixture and the mixture lacking threonine and phenylalanine; however, the omission of lysine and arginine produced a marked change in the shape of the curve, i.e. a lower slope between 6 and 12 hours and a complete cessation of induction between 12 and 18 hours. The induction curve for threonine dehydrase was altered both when lysine and arginine or threonine and phenylalanine were omitted. However, the effect of omitting threonine and phenylalanine was considerably more pronounced since the primary effect of the omission of lysine and arginine appeared to be a lengthening of the initial lag period. It is interesting that, in contrast to ornithine transaminase induction, the poorest inducing mixture for threonine dehydrase did not result in complete cessation of induction of this enzyme between 12 and 18 hours.

Tryptophan Effect—When the equimolar mixture lacking tryptophan was fed to rats which had been deprived of food for 12 hours prior to the first feeding, the induction of ornithine transaminase was equivalent to that produced by the complete equimolar mixture, while threonine dehydrase was induced to approximately half of the control level (Fig. 2) by the tryptophan-free mixture (Table II). This indicates that under these conditions threonine dehydrase required an exogenous source of tryptophan for maximal induction while ornithine transaminase did not. When the rats were not subjected to the 12-hour fasting period prior to the feeding of the experimental diets, it was seen (Table II) that the induction of ornithine transaminase after the feeding of the tryptophan-free diet was reduced to one-half the control value, while the induction of threonine dehydrase was very low. The readjustment of increasing quantities of tryptophan to the tryptophan-free mixture produced proportional increases in the induction of both enzymes; the control level of induction was reached at a level of 40 mg of tryptophan per feeding.

These data indicate that under the proper conditions the induction of both ornithine transaminase and threonine dehydrase requires tryptophan. The more pronounced tryptophan effect observed for both enzymes in rats that were not deprived of food suggests the possibility that fasting caused the mobilization of endogenous tryptophan, which in turn caused the mobilization of endogenous tryptophan, which in part made up the deficit in the tryptophan-free diet and permitted some induction to occur. It is possible that the mobilization of endogenous tryptophan was induced by the fasting and that this was a necessary condition for the induction of these enzymes. When tryptophan was fed alone at the same level at which it occurred in the 0.6-g dose of the equimolar mixture, an induction of both ornithine transaminase and threonine dehydrase occurred (Fig. 5). The induction of both enzymes under these conditions showed a pattern which, during the first 12 hours, was similar to
that obtained with the complete equimolar mixture (Fig. 2). However, after the 12-hour interval the induction of both enzymes was curtailed in the animals fed tryptophan alone. This cessation of induction (Fig. 5) resembles that observed when the equimolar mixture was fed at a level of 0.2 g per dose (Fig. 2). It is interesting that, although the same quantities of tryptophan were fed in Fig. 2 (0.6-g level) and Fig. 5, induction continued from the 12 to 18 hour interval in rats receiving the complete equimolar mixture (Fig. 2), while the rats receiving tryptophan alone showed a cessation of induction after 12 hours. These results indicate that, although tryptophan alone was able to cause limited induction of both enzymes, the other amino acids present in the equimolar mixture were necessary for sustained induction.

Table III shows the lack of induction of either enzyme when each of the remaining nine amino acids was administered singly. None of the other amino acids produced induction comparable to tryptophan under these conditions.

**DISCUSSION**

The results of this investigation have established the complex nature of the relationship between dietary enzyme induction and the composition of the inducing amino acid mixture. The data in Fig. 1 show that variations in the quantitative composition of the mixture modified the response of both ornithine transaminase and threonine dehydrase; certain mixtures produced similar induction responses for both enzymes while other mixtures caused the two enzymes to respond differently. The nature of the response depended not only on the quantitative composition of the mixture modified the response of both ornithine transaminase and threonine dehydrase; certain mixtures produced similar induction responses for both enzymes while other mixtures caused the two enzymes to respond differently. The nature of the response depended not only on the quantitative composition of the mixture (Table I and Fig. 1), but also on the amount fed (Fig. 1).

Holding the quantitative composition of the inducing mixture constant (equimolar mixture, Table I) and omitting various amino acids markedly affected the induction of both enzymes (Fig. 3). Again, it is noteworthy that the two enzymes responded similarly to certain changes in the inducing mixture and differently to other changes; the most striking effects are the low induction of ornithine transaminase and the concomitant high induction of threonine dehydrase produced by the omission of lysine and arginine, as well as the low induction of threonine dehydrase and the high induction of ornithine transaminase produced by the omission of threonine and phenylalanine from the equimolar mixture.

The effect of the omission of tryptophan from the diet on the induction of both enzymes (Table II) clearly indicates that tryptophan plays a central role in the induction process for both enzymes, which is interesting in view of the fact that neither enzyme

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**Table II**

**Tryptophan requirement for induction of ornithine transaminase and threonine dehydrase by equimolar mixture of essential amino acids**

Each rat received an amount of diet equivalent to 0.6 g of the complete equimolar mixture at 0, 6, and 12 hours. All rats were killed at 18 hours after zero time. Tryptophan was given as part of the diet. Each value is the average ± standard error of the mean from a group of three rats. The enzyme units are defined as in Fig. 1. Zero time values are the same as those seen in Figs. 2 and 4.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>Ornithine transaminase (units)</th>
<th>Threonine dehydrase (units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-hr fast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete equimolar mixture</td>
<td>6</td>
<td>85 ± 7</td>
<td>2685 ± 350</td>
</tr>
<tr>
<td>- Tryptophan</td>
<td>3</td>
<td>90 ± 10</td>
<td>1290 ± 300</td>
</tr>
<tr>
<td>No fast</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complete equimolar mixture</td>
<td>6</td>
<td>88 ± 6</td>
<td>3200 ± 400</td>
</tr>
<tr>
<td>- Tryptophan</td>
<td>3</td>
<td>43 ± 5</td>
<td>420 ± 150</td>
</tr>
<tr>
<td>With 49 μmoles of tryptophan</td>
<td>3</td>
<td>49 ± 5</td>
<td>1132 ± 300</td>
</tr>
<tr>
<td>per dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With 98 μmoles of tryptophan</td>
<td>3</td>
<td>60 ± 7</td>
<td>1295 ± 275</td>
</tr>
<tr>
<td>per dose</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With 196 μmoles of tryptophan</td>
<td>3</td>
<td>90 ± 9</td>
<td>2597 ± 524</td>
</tr>
</tbody>
</table>

a Rats were deprived of food for 12 hours prior to the first feeding and did not receive the 1-g dose of protein-free diet as described in "Materials and Methods."
is involved in the metabolism of tryptophan. The importance of tryptophan in the induction of these enzymes is further substantiated by the observation that the feeding of this amino acid alone could produce induction of both enzymes (Fig. 5).

The induction of tryptophan pyrrolase by the administration of tryptophan is well known (12–17), and the mechanism of this phenomenon has been partially elucidated (17). In view of the present results it would appear that tryptophan may play a more general role in the regulation of enzyme levels in mammalian liver than had hitherto been realized. It is essential to note, however, that the administration of tryptophan alone did not produce maximal (i.e., control level) induction of either enzyme. Although the other amino acids when given singly did not cause induction of either enzyme (Table III), their presence in conjunction with tryptophan produced a greater effect than did tryptophan alone (Fig. 2 as opposed to Fig. 5). Thus, tryptophan appears to be a necessary but not completely sufficient component of the diet for the dietary induction of ornithine transaminase and threonine dehydrase.

When the present results as well as those of our earlier papers are considered as a whole, it becomes apparent that the factors that control the levels of ornithine transaminase and threonine dehydrase are independent of the general nitrogen balance of the animal. Thus, the administration of glucagon (11) or tryptophan (present paper) to protein-depleted rats produced enzyme induction in spite of the fact that these rats were obviously not restored to nitrogen equilibrium. Conversely, it was shown that under conditions in which nitrogen repletion did occur induction of these enzymes could be completely prevented (11). The results of the present paper further indicate that the dietary nitrogen source can be modified to control the degree of induction of certain amino acid-catabolizing enzymes. The concept that a uniform tide of protein synthesis occurs as a result of nitrogen repletion is therefore invalid, and it is apparent that it is necessary to consider repletion in terms of restoration of individual enzymes and other proteins.

**SUMMARY**

Both ornithine transaminase and threonine dehydrase were induced in the livers of protein-depleted rats which had been forcibly fed free amino acids. Variations in the quantitative and qualitative compositions of the amino acid mixtures fed affected the induction of each of the enzymes as follows.

1. A mixture consisting of the 10 essential amino acids in equimolar proportion produced the best induction of the four mixtures tested, while a mixture consisting of these amino acids in proportions similar to their relative concentration in mammalian liver produced the poorest induction. These effects were somewhat dependent on the quantity of mixture fed.

2. Feeding the equimolar mixture lacking lysine and arginine caused good induction of threonine dehydrase but poor induction of ornithine transaminase, while the equimolar mixture lacking threonine and phenylalanine had the opposite effect.

3. Feeding the equimolar mixture lacking tryptophan produced little or no induction of threonine dehydrase or ornithine transaminase in rats which had not been deprived of food previously. Prior fasting lessened the tryptophan requirement for threonine dehydrase induction and abolished the tryptophan requirement in the case of ornithine transaminase induction.

4. The feeding of tryptophan alone produced a significant but submaximal induction of both enzymes. None of the other amino acids produced induction of either enzyme when administered singly.

**REFERENCES**

Studies on the Induction and Repression of Enzymes in Rat Liver: III.
INDUCTION OF ORNITHINE δ-TRANSAMINASE AND THREONINE
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