Effect of High- and Low-Protein Diets on the Regulation of Rat Liver Tyrosine Aminotransferase and Tryptophan Oxygenase Activities by L-Tyrosine and L-Tryptophan

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Induction of rat liver tyrosine aminotransferase by L-tyrosine and tryptophan oxygenase by L-tryptophan was studied in groups of rats fed on diets containing 18 or 5% protein. The basal activity of hepatic tyrosine aminotransferase of rats receiving 5% protein gradually increased with the age of the animals but that of rats receiving 18% protein did not. L-Tyrosine induced hepatic tyrosine aminotransferase in rats receiving 18% protein when tested at ages from 4 to 20 weeks. When induction by L-tyrosine was carried out in rats receiving the 5% protein diet, significant induction of tyrosine aminotransferase occurred only in 4- or 6-week-old rats. Induction by L-tryptophan of tryptophan oxygenase in liver or the basal activity of this enzyme in liver did not differ between the groups fed on 5 and 18% protein. On changing the diet from 0 to 18% protein, the above-mentioned effects on the induction of hepatic tyrosine aminotransferase were reversed.

In adult rats, depletion of available protein by feeding a 5% lactalbumin diet for 7 days leads to decreased rates of oxidation of tyrosine and *p*-hydroxyphenylpyruvate but not that of homogentisic acid (Al-Nejjar & Litwack, 1961). These authors found that decreased rates of oxidation of L-tyrosine and *p*-hydroxyphenylpyruvate correlated with a decrease in the activities of tyrosine aminotransferase and *p*-hydroxyphenylpyruvate oxidase. Also, the induction of tyrosine aminotransferase by L-tyrosine or by cortisol depends on the amount of protein ingested by the animal. By contrast, starvation for 48 h causes the hepatic tyrosine aminotransferase activity to rise 100% above the basal value in 4-week-old intact and adrenalectomized rats fed on a 7% protein diet (Goswami & Chatagner, 1966). The basal activities of hepatic tyrosine aminotransferase in male adrenalectomized or hypophysectomized rats fed for 5 days on diets containing various concentrations of protein are proportional to the amount of protein in the diet (Mavrides & Lane, 1969). All these observations indicate a correlation between protein intake and the activity of hepatic tyrosine aminotransferase.

The effect of long-term protein malnutrition on the status of hepatic tyrosine aminotransferase or its inducibility by tyrosine is not known. An earlier report from this laboratory presented evidence that hepatic tyrosine aminotransferase is induced by pharmacologically unrelated drugs (Mukhtar & Krishna Murti, 1971). We therefore investigated whether inducibility of tyrosine aminotransferase by drugs depends on the protein status of the animal and whether the basal activity of tyrosine amino-

transferase or its response to L-tyrosine could be restored on increasing the amount of protein in the diet.

Materials and Methods

Animals and feeding

Colony-bred male weanling rats (20–25 g, 28 days old and bred in the Animal House of the Central Drug Research Institute) were divided into two batches. One batch received a diet of the following composition (%): casein (24), starch (36), glucose (10), cane sugar (15), groundnut oil (10), salt mixture (Hubbell *et al.*, 1937) (4) and vitamin mixture (Hayashida & Portman, 1959) (1). The other batch received the same diet but with 7% casein and 53% starch. The protein contents of the two diets were 18 and 5% respectively. Animals on 18% protein diet gained 15–20 g body weight per week whereas those receiving the 5% protein diet gained very little weight and did not survive beyond 10 weeks. For short-term feeding the diet did not contain any casein at all.

Enzyme assays

Tyrosine aminotransferase (L-tyrosine-2-oxoglutarate aminotransferase, EC 2.6.1.5) was assayed by the method of Kenney (1959), the *p*-hydroxyphenylpyruvic acid formed being estimated by the method of Canellakis & Cohen (1956). Tryptophan oxygenase [L-tryptophan oxygen (acceptor) oxidoreductase, EC 1.13.1.12] was assayed essentially by the method of Knox *et al.* (1966) with adaptations as described by Azhar & Krishna Murti (1971).

Protein

Protein estimation was carried out colorimetrically by the Folin procedure of Lowry *et al.* (1951) with bovine plasma albumin as standard.

Results

Effect of amount of dietary protein on induction of hepatic tyrosine aminotransferase by L-tyrosine and tryptophan oxygenase by L-tryptophan

The effects of 5 and 18% dietary protein on the basal activity of tyrosine aminotransferase and tryptophan oxygenase and also on the induction of tyrosine aminotransferase by L-tyrosine and trypto-

phan oxygenase by L-tryptophan are shown in Table 1 and Fig. 1.

The animals maintained on a standard diet did not show any significant change in the tyrosine aminotransferase activity with age. However, the animals maintained on low-protein diet showed a considerable increase with age in the basal activity of tyrosine aminotransferase.

Animals receiving the standard diet showed a continuous stimulation in tyrosine aminotransferase activity which ranged from 3.2- to 4.1-fold on administration of L-tyrosine. This increase in activity of the enzyme was independent of the age of the animals. On the other hand the animals receiving low-protein diet, when treated with L-tyrosine, showed an

Table 1. *Effect of amount of dietary protein on induction of hepatic tryptophan oxygenase by L-tryptophan*

Values are expressed as means $\pm$ S.E.M. for three animals and are units/mg of protein. One unit is the amount of enzyme needed to produce 1 nmol of kynurenine/min at 37°C. L-Tryptophan ($368 \mu\text{mol}/100 \text{g}$ body weight) in 1 ml of 150 mM-NaCl was administered intraperitoneally to the experimental group. Control groups received 1 ml of 150 mM-NaCl and animals were killed 4 h later.

Weeks on diet	Low-protein diet		Standard protein diet	
	Control	Experimental	Control	Experimental
4	25.0 ± 2.1	156 ± 4.0	24.6 ± 1.0	168 ± 6.8
6	23.1 ± 1.2	155 ± 4.1	23.2 ± 1.9	154 ± 4.0

Fig. 1. Activity of tyrosine aminotransferase in animals maintained on (a) the standard protein diet and (b) the low-protein diet

●, 150 mM-NaCl injected; ○, induced with $414 \mu\text{mol}$ of L-tyrosine/100 g body wt. and killed 4 h later. Each point on the curve represents values from four animals with ranges of values shown as vertical bars.

Table 2. *Induction of hepatic tyrosine aminotransferase by L-tyrosine and drugs in protein-deficient rats*

Values are means from two animals in each set. One enzyme unit is 1 nmol of *p*-hydroxyphenylpyruvate formed/min. Animals received 414 μ mol of L-tyrosine, 14.6 μ mol of chloroquine phosphate, 18.6 μ mol of tetracycline or 33 μ mol of phenacetin/100 g body weight, dispersed in 1 ml of 150mM-NaCl, and were killed 4 h later.

Diet	Body weight of animals (g)	Enzyme activity (units/mg of protein) on treatment with				
		NaCl	L-Tyrosine	Chloroquine phosphate	Tetracycline	Phenacetin
Standard protein diet	180	6.3	22.3	19.1	26.4	23.8
Low-protein diet	100	9.5	24.7	—	—	19.8
	80	12.5	23.8	18.2	25.9	19.9
	60	17.8	23.1	22.5	31.5	25.8
	50	24.2	24.8	—	—	—

increase in tyrosine aminotransferase activity over the basal value only up to the age of 8 weeks.

In contrast, no appreciable change in the basal activity of tryptophan oxygenase (Table 1) in either of the groups was observed. Also the magnitude of enzyme induction by L-tryptophan was unaffected. Since the alteration of the amount of dietary protein caused changes only in tyrosine aminotransferase further experiments were confined to the induction of this enzyme.

Induction of hepatic tyrosine aminotransferase by L-tyrosine and drugs in protein-deficient rats

From the stock of rats maintained on standard and protein-deficient diets, animals were drawn at the age of 9 weeks. Induction by L-tyrosine and drugs was studied in both the groups and the results are summarized in Table 2. With rats on the low-protein diet there was an inverse relationship between body weight and hepatic tyrosine aminotransferase activity and as activity increased in response to dietary protein deprivation the extent to which it could be induced by L-tyrosine or the drugs tested diminished.

Induction of hepatic tyrosine aminotransferase by L-tyrosine and drugs in protein-depleted and -replenished rats

Male albino rats (8 weeks old, 125–150 g body wt.) were divided into two groups; one was maintained on the standard diet and the other on protein-free diet. After a 10 day feeding period induction by L-tyrosine or drugs in both groups of animals was carried out. Animals that received a protein-free diet were then given the standard diet. After 10 days of feeding, induction by L-tyrosine or drugs was carried out in rats of the protein-replenished group and in those on the standard diet. The results are shown in Table 3. Protein starvation caused a 50% rise in the basal tyrosine aminotransferase activity but the extent of inducibility of the enzyme with L-tyrosine, 6-

mercaptapurine and tetracycline was significantly decreased as compared with the animals maintained on standard diet. L-Tyrosine administration to the animals on the standard and protein-free diet resulted in a 3.5- and 2-fold stimulation of the enzyme respectively over the appropriate saline controls. Tetracycline and 6-mercaptapurine administration to the protein-deficient animals resulted in 1.8- and 1.4-fold stimulation in enzyme activity over the appropriate saline controls.

The increase in the basal activity caused by protein starvation was reversed by protein replenishment. The 50% rise in the basal activity of tyrosine aminotransferase registered by protein-starved animals was decreased to 23%. The extent of inducibility of the enzyme by L-tyrosine, tetracycline and 6-mercaptapurine was also reversibly affected. L-Tyrosine administration to the animals on standard diet and to protein-replenished groups caused 3.5- and 2.5-fold increases in enzyme activity over their appropriate saline controls. Tetracycline and 6-mercaptapurine administration to protein-replenished animals resulted in 2.4- and 1.6-fold stimulation respectively in the enzyme activity over the saline controls.

Discussion

The present results reveal that the feeding of a protein-deficient diet to animals resulted in a more than threefold increase in the basal activity of liver tyrosine aminotransferase. In control animals, receiving adequate amounts of protein, such a rise in enzyme activity required induction by L-tyrosine or stabilization by drugs (Mukhtar *et al.*, 1973). No such effects were observed in the activity of hepatic tryptophan oxygenase. The depletion of body reserve protein as a consequence of protein malnutrition results in disturbances in protein metabolism and as a consequence excess of amino acids are excreted in urine. The basal activities of xanthine oxidase (Rowe & Wyngaarden, 1966), histidase and urocanase (Sahib

Table 3. Induction of hepatic tyrosine aminotransferase by L-tyrosine and drugs in protein-depleted and protein-replenished rats

Diet	Body weight (g)	Liver wet weight (g)	Enzyme activity (units/mg of protein) on treatment with			
			NaCl	L-Tyrosine	Tetracycline	6-Mercapto- purine
Maintained on 18% protein diet for 10 days	150 ± 3.3 (6)	5.43 ± 0.12 (6)	6.6 ± 0.5 (4)	22.8 ± 0.6 (4)	19.9 ± 0.4 (6)	11.5 ± 0.2 (6)
Maintained on protein-free diet for 10 days	102 ± 2.5 (12)	3.78 ± 0.09 (12)	9.6 ± 0.5 (4)	19.0 ± 0.6 (4)	16.8 ± 0.3 (4)	13.4 ± 0.4 (4)
Maintained on 18% protein diet for 20 days	159 ± 1.2 (6)	5.66 ± 0.13 (6)	6.1 ± 0.1 (6)	21.8 ± 0.7 (4)	19.9 ± 0.4 (6)	11.5 ± 0.2 (6)
Maintained on protein-free diet for 10 days and then on 18% protein diet for 10 days	127 ± 2.4 (12)	4.69 ± 0.11 (12)	7.5 ± 0.3 (4)	19.0 ± 0.2 (4)	18.1 ± 0.5 (4)	11.9 ± 0.5 (4)

injections were ($\mu\text{mol}/100\text{ g body wt.}$): L-tyrosine (414), tetracycline (8.4) and 6-mercaptopurine (6.0). Animals were killed 4 h after administration of drugs or L-tyrosine in 1 ml of 150 mM-NaCl. Values are expressed as means $\pm$ S.E.M. for the numbers of animals in parentheses.

& Krishna Murti, 1969; Schirmer & Harper, 1970), arginase (Schimke, 1964), alanine aminotransferase (Rosen *et al.*, 1959) and other functional enzymes are known to be significantly decreased in protein deficiency. In contrast, the basal activity of tyrosine aminotransferase appears to increase significantly. It is possible that, because of readjustment of overall protein synthesis, the activity of tyrosine aminotransferase increases, preventing accumulation of excess of tyrosine formed because of the degradation of tissue proteins. The increased activity of tyrosine aminotransferase in protein-malnourished animals may, therefore, be caused by synthesis of the enzyme *de novo*. Another possible explanation is that more insulin, or other hormones that cause induction of tyrosine aminotransferase, is released. Tyrosine aminotransferase could not be stimulated in protein-deficient animals by L-tyrosine or drugs over the high amount already reached as a consequence of protein malnourishment.

Short-term feeding of a protein-free diet to rats caused a 50% increase in the activity of hepatic tyrosine aminotransferase. Also, in these animals, the inducibility by L-tyrosine or drugs was less. Refeeding protein to the animals reversed this effect. Stabilization of tyrosine aminotransferase by drugs occurs in animals receiving an adequate protein supply (Mukhtar *et al.*, 1973). The present results indicate that the extent of stabilization by drugs depends on the amount of protein in the diet. The reasons for the changes in tyrosine aminotransferase activity in protein-malnourished animals are not very clear but the physiological relevance of the changes is quite understandable. Thus the nutritional status of an animal may alter its ability to metabolize drugs. Conventional studies on drug toxicity have been restricted to healthy and well-fed animals. The question arises as to whether the response elicited by a drug in a healthy animal would be the same as in a protein-deficient animal. Because experimentally induced protein malnutrition leads to disturbances in the overall enzyme physiology of the hepatic system, as demonstrated in the present study, it would seem logical to extend drug-toxicity studies to protein-deficient animals, to obtain toxicological information that can be translated to the actual situation in the human population.

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