EFFECT OF INDIVIDUAL AMINO ACID SUPPLEMENTS ON THE TOXICITY OF EXCESS TYROSINE IN RATS

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Summary The object of this study was to determine the effects of individual amino acid supplements on the development of tyrosine toxicity in growing rats fed 10% casein containing 5% tyrosine. Each amino acid was added at levels equivalent to its content in 20% casein. Supplement of methionine to the high tyrosine diet partially alleviated both growth depression and pathological lesions. Threonine and cystine had a somewhat beneficial effect, but the single addition of other amino acids was not effective. Besides, some amino acids enhanced the severity of the toxicity even more. The effects of methionine supplementation were highest at 0.66 to 1.32% levels (equivalent to the methionine content in 20 to 40% casein). By the supplement of both 0.66% methionine and 0.90% threonine to the high tyrosine diet, growth was significantly improved and toxic lesions were completely prevented. It was confirmed that the counteracting effects to the toxicity, caused by the extra addition of protein (casein) to rats fed a high tyrosine-low protein diet, were mainly attributed to the effectiveness of the methionine and threonine, i.e., first- and second-limiting amino acids, respectively, contained in it.

It is well recognized (1, 2) that the growth rate and food intake of rats fed a low protein diet containing high tyrosine are depressed, and the animals develop a specific toxicity syndrome which is characterized by eye and paw lesions. These adverse effects can be overcome by an addition of dietary protein (3–7). In previous work, we have observed (7) that the tyrosine toxicity which occurs in rats fed 10% casein containing 5% tyrosine is entirely counteracted by the supplementation of 15%, or more, of casein, wheat gluten or corn gluten. The beneficial effect of extra addition of the proteins on tyrosine toxicity can probably be attributed to the contained amino acids.

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Actually, the effects of some amino acid supplements on tyrosine toxicity have been reported (8–13). Sullivan et al. (8) observed that a high level of cystine alleviated the toxicity. Glycine and cystine (9), threonine (10), and glycine, methionine, tryptophan or a mixture of branched-chain amino acids (13) were also found to diminish the toxic signs. Alam et al. (11–13) reported that the addition of threonine to a high tyrosine diet alleviated growth retardation and the severity of symptoms, and lowered the tyrosine level in plasma, liver and muscle. However, more detailed studies on the individual amino acids have not as yet been done.

The present study was undertaken mainly, therefore, to examine the effect of supplementation of individual amino acids, which correspond to their contents in 20% casein, on tyrosine toxicity. Since methionine was most effective in all amino acids examined the influence of graded levels of the amino acid was also investigated.

EXPERIMENTAL

Male weanling albino rats of the Donryu strain were used in these experiments. After a prefeeding of 3 to 4 days on a diet containing 25% casein, the animals, from 60 to 65 g, were separated into groups. They were kept in individual suspended cages in a room with 12 hr of light (between 8:00 a.m. to 8:00 p.m.) and maintained at 24°C.

The composition (in %) of the basal diet was: casein, 10.0; soybean oil, 5.0; salt mixture, 5.0; vitamin mixture, 1.0; choline chloride, 0.1; and α-potato starch to make up to 100. Vitamin A (2,000 IU) and D₃ (200 IU) were also added (7). Experimental diets were prepared by adding L-tyrosine, other L-amino acids or extra casein to the basal diet at the expense of starch.

All diets were made in paste form by mixing with an appropriate amount of water daily. Diets and water were given ad libitum for a 2-week period. Body weights were recorded daily and observations were made on the toxicity syndrome, mainly the development of cataracts, and the mortality in rats fed high tyrosine diets.

Experiment 1 was done to test the effects of individual amino acids on tyrosine toxicity. The additional amount of each amino acid to the 10% casein plus 5% L-tyrosine diet was as follows: (% of the diet) L-Arg·HCl, 1.02; L-His·HCl·H₂O, 0.81; L-Lys·HCl, 2.05; L-Trp, 0.30; L-Phe, 1.16; L-Cys, 0.08; L-Met, 0.66; L-Thr, 0.90; L-Leu, 2.02; L-Ile, 1.32; L-Val, 1.48; L-Ser, 1.26; L-Glu, 4.72; L-Asp, 1.30; Gly, 0.42; L-Ala, 0.62; L-Pro, 2.46. The amounts were selected to simulate amino acid contents in 20% casein.
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acids distribution in 20% casein, respectively.

Experiment 2 was done to determine the effect of graded levels of L-methionine, which was simulated to the amino acid content in 10 to 60% casein, on tyrosine toxicity.

Experiment 3 was done to test the effects of supplementation of extra casein, essential-, non-essential-, and complete-amino acid mixtures, L-methionine, and L-methionine plus L-threonine on tyrosine toxicity. Amino acids were simulated to their contents in 20% casein and the complete amino acid mixture consisted of all amino acids listed in Experiment 1.

RESULTS

Experiment 1

The effects of supplementation of single amino acids on the growth and susceptibility of rats fed the 10% casein containing 5% tyrosine (10C5T) diet are summarized in Table 1. Amino acids were ranked in decreasing order of the mortality.

Rats consuming the 10C5T diet showed a progressive loss of body weight, which was accompanied by the development of eye lesions and high mortality. All animals in this group lost their eye sight due to cataract formation within one week, and half the animals died within 9 to 11 days. These results were consistent with a previous report (7). The addition of phenylalanine, arginine, or lysine to the high tyrosine diet enhanced the adverse effects even more. Phenylalanine and arginine groups caused a more pronounced loss of body weight than that of the 10C5T group. Lysine supplement also caused death of all the animals in this group although the body weight was not substantially decreased. All animals of the phenylalanine, arginine and lysine groups developed severe eye lesions within one week and failed to survive the experimental period.

The addition of glycine, aspartic acid, alanine or isoleucine did not improve the growth and failed to decrease the mortality of animals fed the high tyrosine diet. Proline, histidine, leucine, and valine also were not able to promote a weight gain, but the mortality in these groups was decreased, only one out of 4 rats died, respectively. Serine, tryptophan and glutamic acid were ineffective in increasing the body weight, yet all of the animals survived. The single addition of glycine, aspartic acid, isoleucine, proline, leucine, valine, serine and glutamic acid were ineffective in alleviating the pathological lesions, and one out of 4 rats of the alanine and histidine groups developed eye lesions.

The only single amino acids that had a growth promoting effect were cystine, threonine and methionine. The highest weight gain was obtained with methionine, followed by cystine and threonine. In these groups all rats survived except one animal which died with a cystine supplement. In addition, methionine supplement delayed the onset of eye lesions, and 3 out of 6 rats of the methionine group and one
Table 1. Effects of supplementation of individual amino acids on survival, eye lesions (cataracts) and growth of rats fed 10% casein containing 5% L-tyrosine.

| 10% casein +5%L-Tyr (10CST)* | Survival | Death day | Eye Lesions | Weight gain |
|-----------------------------|----------|-----------|-------------|-------------|
|                             |          |           | No. of cataracts | Day of appearance | (g/2 weeks) |
| Non                         | 6/12     | 9, 10, 10, 11, 11 | 12/12 | 4, 4, 5, 5, 5, 5, 5, 5, 6, 6, 6, 7, 7, 7, 9 | -10.5 ± 1.9 |
| +1.16% L-Phe                | 0/4      | 6, 7, 11, 11 | 4/4 | 4, 5, 5, 7 | - |
| +1.02% L-Arg·HCl           | 0/4      | 9, 11, 12, 13 | 4/4 | 3, 4, 4, 5 | - |
| +2.05% L-Lys·HCl           | 0/4      | 11, 12, 13, 14 | 4/4 | 5, 5, 6, 7 | - |
| +0.42% Gly                 | 1/4      | 10, 14, 14 | 4/4 | 6, 6, 6, 7 | -6.0 |
| +1.30% L-Asp               | 2/4      | 10, 13 | 4/4 | 6, 6, 6, 8 | -5.5 |
| +0.62% L-Ala               | 2/4      | 10, 11 | 3/4 | 6, 6, 7 | -3.5 |
| +1.32% L-Ile               | 2/4      | 9, 11 | 4/4 | 6, 6, 6, 6 | -3.0 |
| +2.46% L-Pro               | 3/4      | 10 | 4/4 | 6, 6, 6, 7 | -6.7 ± 4.3 |
| +0.81% L-His·HCl·H₂O       | 3/4      | 11 | 3/4 | 6, 6, 6 | -4.3 ± 2.6 |
| +2.02% L-Leu               | 3/4      | 13 | 4/4 | 6, 6, 6, 7 | -2.9 ± 0.7 |
| +1.48% L-Val               | 3/4      | 10 | 4/4 | 6, 8, 8, 14 | -2.8 ± 1.3 |
| +0.08% L-Cys               | 3/4      | 10 | 3/4 | 7, 9, 10 | 11.8 ± 2.2 |
| +1.26% L-Ser               | 4/4      | 3/4 | 6, 6, 7, 7 | -10.2 ± 3.4 |
| +0.30% L-Trp               | 4/4      | 4/4 | 4, 6, 8, 10 | -5.8 ± 1.5 |
| +4.72% L-Glu               | 4/4      | 4/4 | 5, 6, 8, 8 | -1.1 ± 1.4 |
| +0.90% L-Thr               | 4/4      | 3/4 | 10, 10, 11 | 4.9 ± 1.7 |
| +0.66% L-Met               | 6/6      | 3/6 | 10, 12, 13 | 31.2 ± 1.2 |
| 10% casein (10C)            | 12/12    | 0/12 | 33.2 ± 3.5 |

* Individual amino acids were added at levels equivalent to their contents in a 20% casein.

out of 4 animals of the cystine and threonine groups did not develop the pathological lesions.

Experiment 2

The effects of graded levels of methionine supplement on the growth of rats fed the 10CST diet are presented in Fig. 1.

As the supplemental level of methionine was elevated, the weight gain was greatly increased. The highest weight gains were obtained with rats fed the high tyrosine diet containing 0.66 to 1.32% methionine (equivalent to the amino acid content in a 20 to 40% casein diet), and these gains were comparable to that of animals fed the 10% casein diet, whereas with the supplement of 1.98% methionine (equivalent to the amino acid content in a 60% casein diet) growth fell to about half of the maximal gains. All of the rats supplemented 0.33 to 1.98% methionine were able to survive for the 2-week experimental period, and half the animals of the 0.33 and 0.66% methionine groups and one-fourth of the 0.99% methionine group
Fig. 1. Effects of graded levels of L-methionine on the growth and toxic lesions of rats fed high tyrosine diet.

developed eye lesions, but the 1.32 and 1.98% methionine groups did not develop either eye or paw lesions.

Experiment 3

The effects of supplementation of extra protein (20% casein), essential-, non-essential-, or complete-amino acid mixtures, 0.66% methionine, 0.66% methionine plus 0.90% threonine on the growth and pathological lesions of rats consuming the 10C5T diet are shown in Table 2. The weight gains of the extra protein (20%}

Table 2. Effects of supplementation of amino acid mixtures, L-methionine, and L-methionine plus L-threonine on the toxicity of rats fed high tyrosine diet.

| Dietary groups | Survival | Eye lesions No. of cataracts | Weight gain (g/2 weeks) |
|----------------|----------|-----------------------------|-------------------------|
| 10% casein (10C) | 6/6 | 0/6 | 31.0 ± 6.3* |
| 30% casein (30C) | 6/6 | 0/6 | 81.9 ± 9.3** |
| 10% casein +5% l-Tyr (10C5T) | 3/6b | 6/6c | 7.2 ± 2.0 |
| 30% casein +5% l-Tyr (30C5T) | 6/6 | 0/6 | 83.0 ± 5.0** |
| 10C5T + essential amino acid mix | 6/6 | 0/6 | 33.8 ± 3.1* |
| 10C5T + non-essential amino acid mix | 6/6 | 4/6d | 5.3 ± 2.6 |
| 10C5T + complete amino acid mix | 6/6 | 0/6 | 86.6 ± 3.7** |
| 10C5T + 0.66% l-Met | 6/6 | 2/6e | 30.8 ± 0.9* |
| 10C5T + 0.66% l-Met + 0.90% l-Thr | 6/6 | 0/6 | 68.8 ± 4.6*** |

* Composition of the amino acid mixture shown in text.
\(^*^ Three rats died at 9, 11, and 12 days.
\(^*^ All rats lost their eye sight during 5 to 7 days.
\(^*^ Eye lesions of 4 rats developed during 6 to 13 days.
\(^*^ Eye lesions of 2 rats developed at 11 and 12 days.
\(^*^ Mean ± SE. Values with different asterisk mark are significantly different at P < 0.05.
casein) and the complete-amino acid mixture supplement groups were essentially the same as that of the 30% casein group. The supplementation of the non-essential amino acid mixture caused only a slight increase in weight gain, and eye lesions developed. The effectiveness of the essential amino acid mixture supplement for growth was comparable to that of the 0.66% methionine supplement, and the growth of the 0.66% methionine plus 0.90% threonine supplement group was approximately 84% of that of the 30% casein group. All of the animals of these supplemental groups survived and did not develop eye lesions except the non-essential amino acid mixture group in which the eye lesions occurred in 4 out of 6 animals.

DISCUSSION

The beneficial effects of some amino acid supplements against toxicity in rats fed a high tyrosine-low protein diet have been demonstrated extensively. Previously, Sullivan et al. (8) had reported that the toxic symptoms in rats fed a 5 or 10% tyrosine containing diet could be counteracted to a considerable degree by the addition of 5% cystine. Martin (9) showed that the toxicity of tyrosine was reduced by addition of 1% cystine or 5% glycine. Benton et al. (10) observed that a small supplement of threonine (0.3% DL-Thr) to a 9% casein diet containing 3% tyrosine stimulated growth without alleviating the severity of the pathological lesions. Subsequently, Alam et al. (11) found that the addition of large amounts of threonine (1.25% L-Thr) to a 3% tyrosine containing diet improved growth and prevented the development of external lesions. In addition, the supplements of glycine, methionine, tryptophan or a mixture of leucine, isoleucine and valine prevented the development of eye and paw lesions, but were not associated with increased growth (13). They concluded (13) that threonine was the only amino acid which alleviated both the growth depression and the pathological lesions.

In the present study, we confirmed that the supplementation of methionine, threonine or cystine, in amounts corresponding to their content in 20% casein, to the 10% casein diet containing 5% tyrosine (10CST) improved growth and survival, and partially prevented the appearance of external lesions, but the single addition of other amino acids did not appreciably alleviate the tyrosine toxicity, in fact, some amino acids even enhanced the severity. Methionine was the most effective amino acid, and both supplements of methionine and threonine significantly promoted growth while completely preventing the development of the pathological lesions. The major part of the counteracting effect caused by the extra casein supplement could be attributed to the action of the two amino acids.

In the basal diet (10% casein) used in this study, methionine is the first limiting amino acid, and threonine is the second limiting one (14). Hence it is suggested that the beneficial effects of methionine or methionine plus threonine supplements to a high tyrosine diet are probably due to the improvements of the amino acid balance and the nutritional quality of the protein, thus stimulating growth which
in turn enhances the tolerance to excess tyrosine. In this connection, it has been demonstrated (6) that the extent of the adverse effects caused by ingestion of excess amino acid was usually dependent upon the protein quality of the diet.

It should be noticed, however, that larger amounts of methionine (0.66 to 1.32%) and threonine (0.9%) than the requirements for growth, i.e., methionine 0.5 or 0.6% and threonine 0.5% (15, 16), were needed to improve both the growth and the external lesions caused by the ingestion of high tyrosine. Similar observations for threonine have been made by Alam et al. (11), who reported that large amounts of threonine (0.8 to 1.25%), 3 to 4 times the requirement, were more effective than small amounts (0.2%) in improving both growth and the pathological lesions. Therefore, it is expected that both methionine and threonine might also be related to a particular action other than the role of limiting amino acid. Further study is necessary to elucidate the relationship between the limiting amino acids and amino acid toxicity.

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