NUTRITIONAL ANEMIA INDUCED BY EXCESS METHIONINE IN RAT AND THE ALLEVIATIVE EFFECTS OF GLYCINE ON IT

Fumi YOKOTA, Takatoshi ESASHI, and Ryokuero SUZUE

The National Institute of Nutrition,
1, Toyama-cho, Shinjuku-ku, Tokyo 162, Japan

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Summary Experiments were performed to investigate the mechanisms of the nutritional anemia caused by the excess methionine in rat, and the alleviative effects of glycine on it. After two months of feeding with 2.5% L-methionine on 9% casein diet, a moderate degree of anemia was manifested, but, the addition of glycine to the excess methionine diet prevented this phenomenon. The activity of heme-α-methenyl oxygenase, the key enzyme in the metabolic pathway of hemoglobin degradation, in the liver was not altered by the supplementation of either methionine or glycine to the diet. In order to investigate the mechanisms of the alleviation of the toxic effects of excess methionine by glycine, and also, whether glycine is the only amino acid which has the alleviative effect on the toxicities of excess methionine, two amino acids other than glycine, L-leucine and L-alanine had been chosen as the supplemental amino acid to the excess methionine diet. Deposition of iron in the spleen was greatly increased in the rat fed excess methionine diet, but not in the rat fed methionine + glycine diet. Activity of δ-aminolevulinic acid synthetase (ALAS), the key enzyme in the hemoglobin biosynthesis, in the bone marrow was remarkably elevated by the addition of methionine, and only glycine could prevent this phenomenon. According to the results obtained, it is considered that the excess methionine would lower the biosynthesis of globin because of the amino acid imbalance. But the addition of glycine enhanced the metabolism of excess methionine, and this helps the acceleration of globin synthesis in the rat to promote the biosynthesis of hemoglobin.

The toxicities of the excess methionine in rats and alleviative effects of glycine on this toxicities had previously been reported by many workers (1–9, 12–14). When rats were fed the low protein diet supplemented with excess methionine, such as 9% casein diet + 2.5% L-methionine, moderate degree of anemia was manifested with in
two months. In order to investigate the possible mechanisms of the anemia caused by the excess methionine and the effect of glycine on it, two experiments were performed using albino rats of the Wistar strain.

MATERIALS AND METHODS

In experiment 1, four groups were made. These were as follows: 9% casein basal diet, basal +2.5% L-methionine, basal +2.5% glycine and basal +2.5% L-methionine +2.5% glycine. 24 weanling albino rats of the Wistar strain were used as the experimental animals. At an average body weight of 70 g, they were fed 9% casein basal diet. At an average body weight of 90 g, animals were divided into 4 groups of 6 rats each and fed the respective diet for 2 months. The composition of basal diet is shown in Table 1. Amino acids were added to the individual diet at the expense of starch. Food and water were given ad libitum. At the end of two months, animals were sacrificed by decapitation and liver was taken out immediately for the determination of heme-α-methenyl oxygenase activity.

Experiment 2 was performed in the same manner as that described in experiment 1, except, 4 groups other were added. These were, basal +2.5% L-leucine, basal +2.5% L-leucine +2.5% L-methionine, basal +2.5% L-alanine and basal +2.5% L-alanine +2.5% L-methionine. Therefore, 48 weanling rats of the same strain were used. Food intake was measured daily and body weight of the animals were weighed twice a week. At the end of two months, iron deposition in the spleen, nature of blood and δ-aminolevulinic acid synthetase activity in the bone marrow were determined.

Heme-α-methenyl oxygenase in the liver was determined by the method of Nakajima et al. (10). Blood specific gravity, hemoglobin concentration, hematocrit value, and the red blood cell count of peripheral blood were determined by the conventional procedures. ALAS activity in the bone marrow was determined by the method described by Urata and Granick (11). Iron was determined by atomic absorption spectrometry after wet ashing of spleen by acid digestion.

| Table 1. Composition of basal diet. |
|------------------------------------|
| **Component** | **g/100 g** |
| Casein<sup>a</sup> | 9 |
| Corn starch<sup>b</sup> | 81 |
| Vitamin mix<sup>c</sup> | 1 |
| Mineral mix<sup>d</sup> | 4 |
| Soybean oil<sup>e</sup> | 5 |

<sup>a</sup> Junsei Chemical Co., Tokyo.
<sup>b</sup> Tanabe Pharmaceutical Co., Osaka.
<sup>c</sup> Vitamin mixture is composed of; Panvitan 20%, choline chloride 10%, dextrin 70%.
<sup>d</sup> Tanabe Pharmaceutical Co., Osaka.
<sup>e</sup> Japan Pharmacopoeia.
RESULTS AND DISCUSSION

In the experiment 1, key enzyme in the hemoglobin degradation, heme-α-methenyl oxygenase in the liver was determined. As shown in Table 2, significant difference was not observed in this enzyme activity among individual groups.

The results of the experiment 1, which are shown in Table 3, have clearly demonstrated that the addition of excess methionine to the low protein diet caused a moderate degree of anemia in rat, and supplementation of glycine to the excess methionine diet could partially alleviate this toxic effect of excess methionine. In order to investigate the mechanisms of the nutritional anemia caused by the excess methionine, and to study whether glycine is the only amino acid which has a special effect on the toxicities of the excess methionine, experiment 2 was performed.

Final body weight and average food intake of the animals in the individual group and ratio of liver, kidney, spleen weight to final body weight are shown in Table 4. Final body weight of the animals in each methionine-supplemented group was lower than that in the control, except the methionine + glycine-supplemented group. Average daily food intake of the animals did not vary remarkably among the individual groups except for the excess-methionine-added group. As for the organ

Table 2. Heme-α-methenyl oxygenase activity in the liver (units).*

| Group | Activity (units) |
|-------|-----------------|
| 9% casein basal | 10.1 ± 0.86** |
| Basal + 2.5% L-methionine | 8.6 ± 0.96 |
| Basal + 2.5% glycine | 8.1 ± 0.66 |
| Basal + 2.5% L-methionine and 2.5% glycine | 10.1 ± 0.60 |

* The absorption of 0.01 at 656 nm of formylbiliverdin is defined as the one unit of enzyme activity.
** Mean ± S.E.

Table 3. The effect of supplementation of amino acids on hematological changes in rats.

| Group | Specific gravity | R.B.C. number (10⁶/mm³) | Hematocrit (%) | Hemoglobin (g/dl) |
|-------|-----------------|--------------------------|----------------|------------------|
| 9% casein basal | 1.050** | 668 ± 35** | 49.7 ± 0.6 | 14.9 ± 0.6 |
| Basal + 2.5% L-methionine | 1.048b | 508 ± 41 | 43.0 ± 1.8 | 12.1 ± 0.1 |
| Basal + 2.5% glycine | 1.052a | 622 ± 12 | 51.2 ± 1.1 | 14.9 ± 0.2 |
| Basal + 2.5% L-methionine and 2.5% glycine | 1.053a | 646 ± 66 | 48.3 ± 1.5 | 15.1 ± 0.4 |
| Basal + 2.5% L-leucine | 1.051a | 670 ± 46 | 49.2 ± 0.5 | 14.0 ± 0.3 |
| Basal + 2.5% L-leucine and 2.5% L-methionine | 1.048b | 470 ± 17 | 46.0 ± 1.1 | 12.6 ± 0.3 |
| Basal + 2.5% L-alanine | 1.051a | 617 ± 20 | 48.8 ± 0.4 | 14.1 ± 0.2 |
| Basal + 2.5% L-methionine and 2.5% L-alanine | 1.050a | 521 ± 20 | 43.9 ± 2.3 | 13.0 ± 0.3 |

* Means having same superscripts are not significantly different (p < 0.05).
** Mean ± S.E.
Table 4. The effect of supplemental amino acids upon rat growth, food intake and ratio of liver, kidney, spleen weight to final body weight.

| Diet                     | Final body weight (g) | Food intake (g/day)* | Liver (%) | Kidney (%) | Spleen (%) |
|--------------------------|-----------------------|----------------------|-----------|------------|------------|
| 9% casein basal          | 235* ± 12***          | 13.6* ± 1.2          | 3.17* ± 0.21 | 0.63* ± 0.01 | 0.18* ± 0.02 |
| Basal + 2.5% L-methionine| 175* ± 14             | 9.0* ± 0.8           | 3.71* ± 0.07 | 1.21* ± 0.04 | 0.37* ± 0.05 |
| Basal + 2.5% glycine     | 234* ± 8              | 14.9* ± 1.3          | 3.25* ± 0.09 | 0.71* ± 0.03 | 0.17* ± 0.02 |
| Basal + 2.5% L-methionine|                       |                      |           |            |            |
| and 2.5% glycine         | 255* ± 11             | 15.5* ± 0.8          | 3.70* ± 0.11 | 0.86* ± 0.04 | 0.21* ± 0.02 |
| Basal + 2.5% L-leucine    | 220* ± 11             | 15.7* ± 0.8          | 3.05* ± 0.09 | 0.65* ± 0.03 | 0.18* ± 0.00 |
| Basal + 2.5% L-leucine    |                       |                      |           |            |            |
| and 2.5% L-methionine    | 171* ± 4              | 11.4* ± 0.7          | 3.99* ± 0.22 | 1.21* ± 0.06 | 0.37* ± 0.05 |
| Basal + 2.5% L-alanine    | 250* ± 16             | 14.5* ± 1.6          | 3.30* ± 0.15 | 0.62* ± 0.02 | 0.17* ± 0.02 |
| Basal + 2.5% L-alanine    |                       |                      |           |            |            |
| and 2.5% L-methionine    | 199* ± 5              | 12.4* ± 0.6          | 3.97* ± 0.17 | 1.18* ± 0.11 | 0.35* ± 0.08 |

* Group average food intake g/day through the experimental period.
** Means having same superscripts are not significantly different (p<0.05).
*** Mean ± S.E.

weight, ratio of liver weight to final body weight was elevated by the supplementation of methionine in the diet. Kidney and spleen weight was also high in the methionine-supplemented group but not in the methionine + glycine group.

As shown in Table 3, significant decrease in red blood cell counts, hematocrit values and hemoglobin concentrations was observed in rats fed excess methionine diet. On the other hand, no anemia was detected in rats fed the methionine + glycine-supplemented diet.

Since the color of spleen in the rats fed excess methionine group was remarkably darkened in the experiment 1, suggesting the presence of the iron deposition, in experiment 2, iron contents in the spleen was examined. As shown in Table 5, iron deposition in the spleen was remarkably increased in the methionine-supplemented, as well as in the L-leucine + L-methionine and L-alanine + L-
methionine groups, but not in the L-methionine + glycine group. It was speculated that the deposited iron in the spleen had not been utilized because of the lowered hemoglobin biosynthesis.

In the experiment 2, key enzyme in the hemoglobin biosynthesis, δ-aminolevulinic acid synthetase (ALAS) in the bone marrow was determined. As shown in Table 6, this enzyme activities in all methionine supplemented groups were remarkably elevated except in the methionine + glycine group. In the latter group, the enzyme activity stayed in the normal range. The elevation of the enzyme activity in this case could be considered as the compensatory activation of the enzyme caused by the excess methionine.

The toxicities of the excess methionine had been reported by many investigators, but the mechanisms of this anemia as well as of the alleviative effect of glycine had not been thoroughly investigated. BENEVENGA and HARPER suggested in their reports (6, 9, 12, 13) that the glycine accelerates the methionine oxidation resulting in a low levels of blood methionine and promotes the appetite of rat. In addition to these observations, as we reported in the previous paper (14), the metabolism of methionine to cysteine via cystathionine is considered one of the important metabolic pathway of excess methionine in rats, the activities of the limiting enzymes in this pathway, L-serine dehydratase and homoserine dehydratase were accelerated by the addition of excess methionine to the diets, and by the supplementation of glycine to the methionine excess diet, these enzyme activities were further elevated. It was considered that the addition of glycine to the excess methionine diet would accelerate this metabolic pathway, then the part of excess methionine would be metabolized via this way. This could be one of the detoxificative effects of glycine on the excess methionine in rats. From the results obtained in this experiment, the biosynthesis of hemoglobin seemed to have been altered by the addition of excess methionine in rat, and the supplementation of glycine could partially prevent this toxic effect of excess methionine. In this experiment, enough iron for the hemoglobin biosynthesis was supplied in the diet,
therefore, anemia observed in this experiment is not due to the iron deficiency. It was considered that the excess methionine would lower the globin biosynthesis in rat, and the addition of glycine accelerate the metabolism of excess methionine resulting in the enhanced hemoglobin biosynthesis. In order to ensure these speculations, further studies using $^{14}$C-labeled amino acid are in progress in our laboratory. There are numerous other ways which could be considered as a cause of anemia by the excess methionine, such as, the acceleration of the breakdown of red blood cell (15–17), or, the inhibition of ferrochelatase activity by the methyl of the excess methionine, or unknown effects of the intermediate metabolite of the excess methionine. In order to confirm these observations, further studies are also in progress in our laboratory.

And also, as for the elevation of ALAS activity in this experiment, although lowered globin biosynthesis due to the amino acid imbalance could be considered one of the important cause of the elevation of this enzyme activity, consideration should be given to the unknown other factors, such as, direct or indirect effects of the intermediate metabolites or the end products of methionine on this enzyme activity.

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