Suppression of Methionine-Induced Hyperhomocysteinemia by Glycine and Serine in Rats

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The hyperhomocysteinemia induced by a dietary addition of 1% methionine was significantly suppressed by the concurrent addition of 1% glycine or 1.4% serine to the same degree. The methionine-induced increase in the hepatic concentration of methionine metabolites was significantly suppressed by glycine and serine, but the hepatic cystathionine β-synthase activity was not enhanced by these amino acids. When the methionine-supplemented diet was changed to the methionine plus glycine or serine diet, the plasma homocysteine concentration rapidly decreased during and after the first day. The hyperhomocysteinemia induced by an intraperitoneal injection with methionine was also suppressed by concurrent injection with glycine or serine, although the effect of serine was significantly greater than that of glycine. These results indicate that glycine and serine were effective for suppressing methionine-induced hyperhomocysteinemia: serine and its precursor glycine are considered to have elicited their effects mainly by stimulating cystathionine synthesis by supplying serine, another substrate for cystathionine synthesis.

Key words: homocysteine; methionine; glycine; serine; rat

Homocysteine is an intermediate in the metabolism of methionine, a sulfur-containing essential amino acid in mammals (Fig. 1), but an elevated plasma homocysteine concentration is recognized as an independent risk factor for cardiovascular disease. It has been shown that the plasma homocysteine concentration was affected by genetic factors, physiological and lifestyle determinants, nutritional and clinical conditions, and drugs. Of these factors, genetic and nutritional factors are thought to have the greatest influence on the plasma homocysteine concentration. Since the major part of plasma homocysteine is derived from the liver, the hepatic homocysteine concentration is thought to reflect the plasma homocysteine concentration. The homocysteine concentration in the liver is affected by the rates of the following processes: (i) production of homocysteine from S-adenosylhomocysteine (SAH) and its precursor S-adenosylmethionine (SAM), (ii) remethylation of homocysteine to methionine, (iii) formation of cystathionine, and (iv) export of homocysteine into blood plasma. Glycine and serine participate in the metabolism of methionine as a methyl-group acceptor and as a substrate for cystathionine synthesis, respectively. Based on these metabolic features, glycine and serine have been shown to accelerate the metabolism of methionine and thereby alleviate the adverse effects of excess methionine, although the effect of glycine was somewhat greater than that of serine. Some reports have recently shown that serine had a hypohomocysteinemic effect. However, little information is available about the effect of glycine on the plasma homocysteine concentration.

Fig. 1. Participation of Glycine and Serine in the Metabolism of Methionine.

DMG, N,N-dimethylglycine; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; Sar, sarcosine; THF, tetrahydrofolate. Enzymes: (1) glycine N-methyltransferase [EC 2.1.1.20], (2) cystathionine β-synthase [EC 4.2.1.22], (3) methionine synthase [EC 2.1.1.13], (4) betaine:homocysteine S-methyltransferase [EC 2.1.1.5], (5) serine hydroxymethyltransferase [EC 2.1.2.1], (6) 5,10-methylenetetrahydrofolate reductase [EC 1.1.99.15].
To investigate regulation of the plasma homocysteine concentration, appropriate animal models are useful. One of the experimental hyperhomocysteinemia models in rodents is the methionine-loading model; e.g., force-feeding\(^1\) or an intraperitoneal injection\(^2\) of methionine causes a transient increase in the plasma homocysteine concentration. It has been shown that dietary supplementation with methionine could also induce persistent hyperhomocysteinemia,\(^3\) although the experimental conditions for this model have not yet been fully established.

We conducted a series of experiments in the present study (1) to establish a model for dietary methionine-induced hyperhomocysteinemia and (2) to assess the comparative effects of glycine and serine on the plasma homocysteine concentration in hyperhomocysteinemic rats induced by both dietary supplementation and intraperitoneal injection with methionine.

Materials and Methods

**Animals and diets.** Male six-week-old rats (120–140 g) of the Wistar strain were obtained from Japan SLC (Hamamatsu, Japan). They were individually housed in hanging stainless-steel wire cages kept in an isolated room at a controlled temperature (23–25 °C) and humidity (40–60%). Lighting was maintained on a 12-h cycle (lights on from 0700 to 1900 h). Before starting the experiments, the rats were acclimatized to the facility for 4 or 5 d. The control diet (25C) consisted of the following ingredients (g/100 g): casein, 25; corn starch, 43.25; sucrose, 20; corn oil, 5; mineral mixture (AIN-93), 3.5; vitamin mixture (AIN-93), 1; choline bitartrate, 0.25; and cellulose, 2. Amino acids were added to the diet at the expense of starch. Four separate experiments were conducted in this study. In experiment 1-A, the rats were divided into four groups and fed on the control diet or on a diet supplemented with l-methionine at the level of 0.5, 1, or 2% for 10 d. In experiment 1-B, the rats were fed on the control diet for 7 d and then injected intraperitoneally with saline alone or with saline containing l-methionine at the level of 100, 200, 300, or 500 mg/kg of body wt. In experiment 2, the rats were divided into four groups and fed on the control diet or on a diet supplemented with 1% l-methionine with or without additional 1% glycine or 1.4% l-serine for 10 d. In experiment 3, the rats were fed on the diet supplemented with 1% l-methionine for 7 d, and seven of them were killed on the final day of the 7-d period. The remaining rats were divided into three groups; one group continued to be fed on the methionine-supplemented diet, and the other groups of rats were fed on a diet supplemented with 1% l-methionine + 2.5% glycine or on a diet supplemented with 1% l-methionine + 2.5% l-serine. In experiment 4, the rats were divided into four groups after feeding the control diet for 7 d and were then injected with saline alone or with saline containing l-methionine (300 mg/kg) together with or without glycine (300 mg/kg) or serine (420 mg/kg). The dose levels of glycine and serine were adjusted on a molar basis, except for experiment 3. In experiments 1-A, 2 and 3, the rats were killed by decapitation between 1000 and 1030 h without prior starvation. In experiments 1-B and 4, the rats were injected with saline alone or with saline containing amino acid(s) between 1100 and 1130 h, before being killed 2 h after the injection. The experimental plan was approved by the Laboratory Animal Care Committee of the Faculty of Agriculture at Shizuoka University.

**Biochemical analysis.** Blood plasma was separated from the heparinized whole blood by centrifugation at 2000 × g for 15 min at 4 °C and stored at −30 °C until needed for analyses. After collecting the blood, the whole liver was quickly removed, rinsed in ice-cold saline, blotted on filter paper, cut into two portions, weighed, quickly frozen in liquid nitrogen and stored at −80 °C until needed for analyses. One portion of the liver was homogenized in 4 volumes (vol/wt) of an ice-cold 25% perchloric acid solution and then centrifuged at 10,000 × g for 10 min at 4 °C. The supernatant was subjected to an assay of methionine metabolites. The other portion of the liver was homogenized in 4 volumes (vol/wt) of a 100 mM Tris–HCl buffer (pH 7.4) containing 150 mM KCl and the homogenate was centrifuged at 14,000 × g for 10 min at 4 °C. The supernatant was subjected to an enzyme assay. The concentrations of total homocysteine and cysteine in the plasma and liver were measured by HPLC according to the method of Durand et al.\(^14\) The concentrations of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in the liver were measured by HPLC essentially according to the method of Cook et al.\(^15\) with slight modifications as described previously.\(^16\) The activity of cystathionine β-synthase (CBS) in the liver was measured according to the method of Mudd et al.,\(^17\) but using HPLC for the assay of the reaction product, cystathionine, according to the method of Einarsson et al.\(^18\) Protein was measured according to the method of Lowry et al.,\(^19\) using bovine serum albumin as the standard.

**Statistical analysis.** Each value is expressed as the mean ± SEM. Data were analyzed by a one-way analysis of variance, and the difference between mean values was tested by the Tukey test when the F value was significant. A statistical analysis was performed with Mac Toukei-Kaiseki ver. 1.5 software (Esumi, Tokyo, Japan). A P value of 0.05 or less is considered significant.

**Results**

**Experiment 1**

The body weight gain, food intake and relative liver weight of the rats were not affected by methionine when added to the control diet at levels of 0.5% and 1%, but
pressed by the concurrent addition of glycine or serine.

The methionine-induced increase in plasma growth, food intake or relative liver weight of the rats on the methionine-supplemented control diet did not affect the in an addition level of 2% significantly depressed the growth and food intake (Table 1). Dietary supplementation with methionine increased the plasma homocysteine concentration in a dose-dependent manner, although the effect of 0.5% methionine was not statistically significant (Fig. 2A). The intraperitoneal injection with methionine also increased the plasma homocysteine concentration in response to the amount of methionine injected and nearly reached a plateau at a dose level of 300 mg/kg of body weight (Fig. 2B). Based on these data, we used dose levels of methionine with 1% addition and 300 mg/kg injection in experiments 2 to 4 to make the effects of glycine and serine clear.

**Experiment 2**

Addition of 1% glycine or 1.4% serine to the 1% methionine-supplemented control diet did not affect the growth, food intake or relative liver weight of the rats (Table 1). The methionine-induced increase in plasma homocysteine concentration was significantly suppressed by the concurrent addition of glycine or serine to the same degree (Fig. 3A). The plasma cysteine concentration, which was measured for comparison with homocysteine, was slightly decreased by glycine and serine (Fig. 3B). A dietary addition of 1% methionine markedly increased the hepatic concentrations of SAM, SAH and homocysteine, and these increases being significantly suppressed by glycine and serine to the same degree (Figs. 3C, D and E). The activity of CBS in the liver tended to be increased by methionine addition, and this increase was significantly suppressed by serine (Fig. 3F).

**Experiment 3**

When the diet was changed from one with 1% methionine added to one with 1% methionine + 2.5% glycine or 1% methionine + 2.5% serine added on d 0, the plasma homocysteine concentration decreased significantly on and after d 1 to the same degree and reached a nadir (Fig. 4). The hepatic concentrations of SAM, SAH and homocysteine were also affected in a similar manner by the diet change. The activity of CBS in the liver tended to be decreased by the dietary addition of serine and glycine, although no statistical significance could be detected.

**Experiment 4**

Methionine (300 mg/kg) injected intraperitoneally in combination with glycine (300 mg/kg) or serine (420 mg/kg) to rats that were killed 2 h after the injection resulted in the enhancement of plasma homocysteine concentration being significantly suppressed by both glycine and serine, although the effect of serine was significantly greater than that of glycine (Fig. 5).

**Discussion**

The present study demonstrated that methionine in the range of 0.5–1% could induce hyperhomocysteinemia in rats without growth retardation when added to a 25% casein diet. This dietary methionine-induced hyperhomocysteinemia might be useful as a convenient

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**Table 1.** Body Weight Gain, Food Intake and Relative Liver Weight of Rats Fed on the Experimental Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body wt. gain (g/10 d)</th>
<th>Food intake (g/10 d)</th>
<th>Liver wt. (% of body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1-A:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25C (6)</td>
<td>44 ± 1^a</td>
<td>115 ± 4^a</td>
<td>4.67 ± 0.12^a</td>
</tr>
<tr>
<td>25C + 0.5% l-Met (6)</td>
<td>46 ± 2^b</td>
<td>115 ± 3^c</td>
<td>4.63 ± 0.10^b</td>
</tr>
<tr>
<td>25C + 1.0% l-Met (6)</td>
<td>43 ± 2^b</td>
<td>114 ± 3^c</td>
<td>4.94 ± 0.06^e</td>
</tr>
<tr>
<td>25C + 2.0% l-Met (6)</td>
<td>19 ± 1^b</td>
<td>83 ± 1^b</td>
<td>4.47 ± 0.04^b</td>
</tr>
<tr>
<td>Experiment 2:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25C (8)</td>
<td>46 ± 2</td>
<td>125 ± 2</td>
<td>4.77 ± 0.03</td>
</tr>
<tr>
<td>25C + 1.0% l-Met (25CM) (7)</td>
<td>42 ± 2</td>
<td>123 ± 3</td>
<td>4.68 ± 0.10</td>
</tr>
<tr>
<td>25CM + 1.0% Gly (6)</td>
<td>42 ± 1</td>
<td>123 ± 4</td>
<td>4.76 ± 0.08</td>
</tr>
<tr>
<td>25CM + 1.4% l-Ser (6)</td>
<td>43 ± 2</td>
<td>123 ± 7</td>
<td>4.93 ± 0.08</td>
</tr>
</tbody>
</table>

^a Values with different letters are significantly different at p < 0.05. 25C, 25% casein diet.

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1Each value is the mean ± SEM for the number of rats indicated in the parentheses; values in each experiment with different superscripts are significantly different at p < 0.05.
and chronic hyperhomocysteinemia model. Hyperhomocysteinemia in humans is a measure of the plasma total homocysteine concentration above 15 \( \mu \text{M} \) and is defined as being moderate (15–30 \( \mu \text{M} \)), intermediate (30–100 \( \mu \text{M} \)) or severe (> 100 \( \mu \text{M} \)).

Therefore, the hyperhomocysteinemia model used in the present study to assess the effects of glycine and serine was at the intermediate level. The results of experiment 2 clearly demonstrate that methionine-induced hyperhomocysteinemia could be effectively suppressed by the concurrent addition of a relatively low level of glycine (1%) or serine (1.4%). It was also shown that the methionine-induced enhancement of plasma homocysteine concentration could be rapidly decreased by a dietary addition of glycine or serine, even with only one day of feeding, although glycine or serine was added to the diet at a relatively high (2.5%) level (experiment 3). The hypohomocysteinemic effect of serine observed here is consistent with that in some previous reports.

**Fig. 3.** Effects of Dietary Addition of 1% Methionine with or without 1% Glycine or 1.4% Serine on the Plasma Concentrations of Homocysteine (A) and Cysteine (B), the Hepatic Concentrations of \( \text{S} \)-Adenosylmethionine (C), \( \text{S} \)-Adenosylhomocysteine (D) and Homocysteine (E), and the Activity of Hepatic Cystathionine \( \beta \)-Synthase (F) in Rats (Experiment 2).

Each value is the mean ± SEM for 6 to 8 rats. **Values with different letters are significantly different at \( p < 0.05 \). 25C, 25% casein diet; CBS, cystathionine \( \beta \)-synthase; SAH, \( \text{S} \)-adenosylhomocysteine; SAM, \( \text{S} \)-adenosylmethionine.**

**Fig. 4.** Effects of Dietary Glycine and Serine on the Plasma Concentrations of Homocysteine (A) and Cysteine (B), the Hepatic Concentrations of \( \text{S} \)-Adenosylmethionine (C), \( \text{S} \)-Adenosylhomocysteine (D) and Homocysteine (E), and the Activity of Cystathionine \( \beta \)-Synthase (F) in Rats (Experiment 3).

Each value is the mean ± SEM for 6 or 7 rats. **Values with different letters are significantly different at \( p < 0.05 \). CBS, cystathionine \( \beta \)-synthase; SAH, \( \text{S} \)-adenosylhomocysteine; SAM, \( \text{S} \)-adenosylmethionine.**
suppressed by dietary supplementation with 3% serine. Verhoef et al. have also demonstrated that serine supplementation suppressed the increase in plasma homocysteine concentration due to one-day ingestion of a methionine-fortified meal in humans. On the other hand, to our knowledge, this is the first demonstration of the hypohomocysteinemic effect of glycine. An experiment in another study of ours demonstrated that dietary supplementation with 1% glycine or 1.4% serine did not decrease the plasma homocysteine concentration when added to the control (25% casein) diet (unpublished data), suggesting that these amino acids might act to normalize hyperhomocysteinemia, rather than to decrease the normal range of plasma homocysteine concentration.

The rate-limiting step of methionine metabolism in animals loaded with an excess of methionine is thought to be the demethylation of SAM, since methionine loading decreases the hepatic concentration of glycine, an exclusively major methyl-group acceptor, thereby hampering the metabolism of SAM. Glycine and serine can be mutually converted in a reaction catalyzed by serine hydroxymethyltransferase, but the reaction is known to favor the synthesis of serine. This might be the reason why the alleviating effect of glycine on methionine toxicity is somewhat greater than that of serine. In contrast, cystathionine synthesis appears to be more critical than the demethylation of SAM for maintaining the hepatic homocysteine concentration at a low level, since cystathionine synthesis is directly linked to the removal of homocysteine. This warrants the potential efficacy of serine in reducing the homocysteine concentration, since serine participates in cystathionine formation as a substrate. In fact, the hypohomocysteinemic effect of serine was significantly greater than that of glycine in a short-term (2 h) experiment (Fig. 5).

The most important finding of this study is that the potency of the hypohomocysteinemic effect of glycine was equivalent to that of serine when these amino acids were separately added to the diet. It has been reported that direct or indirect acceptors of the methyl group of SAM, such as guanidinoacetic acid and nicotinic acid, brought about an increase in the plasma homocysteine concentration, probably by accelerating the conversion of SAM to SAH. Glycine also stimulates the metabolism of SAM to SAH by the glycine N-methyltransferase system. Therefore, it should be noted that glycine exhibited a hypohomocysteinemic effect, despite being a methyl-group acceptor. The exclusively different feature of glycine from other methyl-group acceptors is that glycine can be converted to serine, suggesting that glycine might elicit its homocysteine-reducing effect mainly after being converted to serine.

The result that the methionine-induced increase in the hepatic concentration of such methionine metabolites as SAM, SAH and homocysteine was significantly suppressed by both glycine and serine indicates that the metabolism of methionine proceeded smoothly when glycine or serine was exogenously provided (Figs. 3 and 4). Although homocysteine has two major fates in the liver, i.e., cystathionine formation and remethylation, cystathionine formation is thought to predominate under the condition of methionine loading. It seems reasonable to consider that serine stimulated cystathionine synthesis as a substrate for CBS and thereby increased homocysteine removal, although the dietary addition of serine did not increase the enzyme activity, but rather decreased it (Fig. 3). In support of this, we have previously demonstrated that the alleviating effect of dietary glycine on methionine toxicity was primarily elicited by restoration of the hepatic glycine and serine concentrations, rather than by any increase in the activities of methionine-metabolizing enzymes. It is thus likely that serine and its precursor, glycine, elicit their effects mainly by stimulating cystathionine synthesis by supplying serine, a substrate for cystathionine synthesis.

Both glycine and serine can provide a one-carbon unit which is finally converted to 5-methyltetrahydrofolate (Fig. 1). This raises the possibility that glycine and serine may increase the formation of 5-methyltetrahydrofolate and thereby stimulate the remethylation of homocysteine catalyzed by methionine synthase. It has been shown that the activity of methionine synthase declined with increasing dietary methionine from 0.3% to 1.0%. Furthermore, it is known that SAM, which increases in response to the amount of methionine ingested, inhibits 5,10-methylenetetrahydrofolate reductase, a key enzyme in the formation of 5-methyltetrahydrofolate. These facts suggest that the 5-methyltetrahydrofolate-dependent remethylation of homocysteine is depressed under the condition of methionine loading. Since glycine and serine both suppressed the methionine-induced increase in hepatic SAM concentration, the
possibility that these amino acids may stimulate the formation of 5-methyltetrahydrofolate and thereby enhance the removal of homocysteine cannot be completely excluded. However, it remains to be elucidated whether glycine and serine actually affect the remethylation of homocysteine by methionine synthase. On the other hand, there is currently no information about the effects of glycine and serine on the remethylation of homocysteine catalyzed by betaine-homocysteine S-methyltransferase.

It has been shown that the addition of methionine increased the plasma cholesterol concentration\(^\text{27-29}\) and that this increase could be effectively suppressed by the concurrent addition of glycine or serine, although the effect of glycine was greater than that of serine.\(^\text{30}\) In addition, the present study has provided data which indicate that methionine-induced hyperhomocysteinemia could also be effectively suppressed by dietary glycine and serine to the same degree. Since increased concentrations of plasma cholesterol and homocysteine are both risk factors for cardiovascular disease, it is likely that both glycine and serine might be effective in suppressing the pathogenesis of cardiovascular disease by counteracting such deleterious effects of methionine as methionine-induced hypercholesterolemia and hyperhomocysteinemia.

References


