Effects of Ornithine and Some Amino Acids on the Growth Depression by Excess Glycine in Young Rats

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The previous experiments demonstrated that the growth depression effect of excess glycine was eliminated by the addition of arginine and methionine to a diet containing excess glycine. Then, the urinary creatine increased excessively by adding glycine and arginine to a 10% casein diet rather than by adding glycine or arginine alone to the diet, but an amount of the increased creatine was very small compared with that of added glycine and arginine. When urinary creatine rose, the activity of kidney transamidinase fell. The supplements of methionine alone, glycine plus methionine or arginine plus methionine did not almost influence the excretion of urinary creatine. The clear correlation between body weight and urinary creatine was not observed. Therefore, the creatine metabolic system may not be useful of metabolizing excess glycine smoothly. In the prevention of the growth depressive effect of excess glycine by the supplement of arginine and methionine, methionine may be required because it is the limiting amino acid in casein, but the effect of arginine cannot be explained. It is of interest to determine whether there are amino acids effective in the prevention of growth depression like arginine.

In the present experiments, growth and creatine formation were investigated in rats fed on the 10% casein diets containing excess glycine supplemented with some amino acids; ornithine, threonine, tryptophan, glutamic acid, proline and aspartic acid, with or without methionine.

Animals and diets. Male weanling rats of the Donryu strain were fed on a stock diet (25% casein) for 3 to 4 days prior to starting the experiments. Rats weighing about 65 g were divided into two series.

Experiment I. Rats were separated into two groups fed on the 10% casein diets with or without 10% glycine and nine groups fed on the 10% casein diets containing 10% glycine supplemented with 1.4% arginine, 1.2% threonine, 0.4% tryptophan or 6.5% glutamic acid with or without 0.9% methionine.

Experiment II. Rats were divided into two groups fed on the 10% casein diets with or without 10% glycine and six groups given the 10% casein diets containing 10% glycine supplemented with 1.4% arginine, 1.2% threonine, 3.4% proline or 1.8% aspartic acid with or without 0.9% methionine.

The added amounts of amino acids except glycine and ornithine in each experiment corresponded to the levels normally present in a 30% casein diet. The compositions of the 10% casein diet (control), feeding conditions and the treatment of animals were as described previously. Owing to neutralizing HCl in arginine HCl and ornithine HCl salts, each equimolar NaHCO₃ was added at

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### TABLE I. LAST BODY WEIGHS, FOOD INTAKES, LIVER AND KIDNEY WEIGHS, URINARY CREATININE, AND CREATINE IN PLASM AND MUSCLE OF RATS FED ON THE 10% CASEIN DIETS SUPPLEMENTED WITH AMINO ACIDS FOR 14 DAYS

<table>
<thead>
<tr>
<th>Dietary group</th>
<th>Last body weight g</th>
<th>Food intake g</th>
<th>Liver weight g</th>
<th>Kidney weight g</th>
<th>Urinary total creatinine&lt;sup&gt;a&lt;/sup&gt; mg/day</th>
<th>Urinary creatinine mg/day</th>
<th>Plasma creatine µg/ml plasma</th>
<th>Muscle creatine mg/g muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>10% Casein (control)</td>
<td>101 ± 4</td>
<td>144 ± 9</td>
<td>3.66 ± 0.10</td>
<td>0.84 ± 0.04</td>
<td>0.97 ± 0.12</td>
<td>1.08 ± 0.09</td>
<td>41 ± 4</td>
<td>2.75 ± 0.19</td>
</tr>
<tr>
<td>+10% Gly</td>
<td>66 ± 3</td>
<td>80 ± 5</td>
<td>2.90 ± 0.29</td>
<td>0.88 ± 0.05</td>
<td>1.31 ± 0.10</td>
<td>1.22 ± 0.39</td>
<td>78 ± 5</td>
<td>2.36 ± 0.05</td>
</tr>
<tr>
<td>+10% Gly + 1.4% Arg&lt;sup&gt;b&lt;/sup&gt;</td>
<td>69 ± 5</td>
<td>94 ± 11</td>
<td>3.82 ± 0.21</td>
<td>0.97 ± 0.07</td>
<td>2.22 ± 0.24</td>
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</tr>
<tr>
<td>+10% Gly + 0.9% Met&lt;sup&gt;b&lt;/sup&gt;</td>
<td>67 ± 4</td>
<td>74 ± 5</td>
<td>2.85 ± 0.24</td>
<td>0.96 ± 0.04</td>
<td>1.31 ± 0.10</td>
<td>0.97 ± 0.15</td>
<td>72 ± 9</td>
<td>2.76 ± 0.19</td>
</tr>
<tr>
<td>+10% Gly + 1.4% Arg + 0.9% Met</td>
<td>99 ± 6</td>
<td>101 ± 9</td>
<td>4.10 ± 0.29</td>
<td>1.26 ± 0.05</td>
<td>1.93 ± 0.31</td>
<td>1.17 ± 0.20</td>
<td>52 ± 3</td>
<td>2.63 ± 0.38</td>
</tr>
<tr>
<td>+10% Gly + 1.2% Thr&lt;sup&gt;b&lt;/sup&gt;</td>
<td>65 ± 5</td>
<td>78 ± 1</td>
<td>3.18 ± 0.16</td>
<td>0.84 ± 0.05</td>
<td>1.62 ± 0.24</td>
<td>1.04 ± 0.13</td>
<td>88 ± 2</td>
<td>2.58 ± 0.10</td>
</tr>
<tr>
<td>+10% Gly + 1.2% Thr + 0.9% Met</td>
<td>69 ± 6</td>
<td>81 ± 6</td>
<td>3.87 ± 0.39</td>
<td>1.01 ± 0.11</td>
<td>1.60 ± 0.47</td>
<td>0.94 ± 0.07</td>
<td>68 ± 6</td>
<td>2.67 ± 0.07</td>
</tr>
<tr>
<td>+10% Gly + 0.4% Trp&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68 ± 3</td>
<td>76 ± 4</td>
<td>2.96 ± 0.09</td>
<td>0.91 ± 0.03</td>
<td>1.41 ± 0.04</td>
<td>0.92 ± 0.16</td>
<td>70 ± 3</td>
<td>2.54 ± 0.05</td>
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<tr>
<td>+10% Gly + 0.4% Trp + 0.9% Met</td>
<td>72 ± 2</td>
<td>73 ± 1</td>
<td>4.02 ± 0.14</td>
<td>0.90 ± 0.05</td>
<td>1.18 ± 0.04</td>
<td>0.82 ± 0.15</td>
<td>61 ± 3</td>
<td>2.70 ± 0.09</td>
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<tr>
<td>+10% Gly + 6.5% Glu&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68 ± 2</td>
<td>80 ± 4</td>
<td>3.07 ± 0.13</td>
<td>0.92 ± 0.04</td>
<td>1.32 ± 0.45</td>
<td>0.67 ± 0.07</td>
<td>70 ± 2</td>
<td>2.53 ± 0.12</td>
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<tr>
<td>+10% Gly + 6.5% Glu + 0.9% Met</td>
<td>51 ± 4</td>
<td>58 ± 1</td>
<td>3.25 ± 0.28</td>
<td>0.74 ± 0.03</td>
<td>2.21 ± 0.34</td>
<td>0.74 ± 0.15</td>
<td>62</td>
<td>2.90 ± 0.14</td>
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<tr>
<td><strong>Experiment II</strong></td>
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<tr>
<td>10% Casein (control)</td>
<td>107 ± 4</td>
<td>139 ± 7</td>
<td>3.98 ± 0.20</td>
<td>0.82 ± 0.05</td>
<td>0.80 ± 0.13</td>
<td>0.93 ± 0.14</td>
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</tr>
<tr>
<td>+10% Gly</td>
<td>65 ± 3</td>
<td>75 ± 4</td>
<td>3.04 ± 0.12</td>
<td>0.88 ± 0.05</td>
<td>1.22 ± 0.40</td>
<td>1.05 ± 0.19</td>
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</tr>
<tr>
<td>+10% Gly + 1% Orn</td>
<td>66 ± 1</td>
<td>78 ± 4</td>
<td>2.57 ± 0.14</td>
<td>0.84 ± 0.03</td>
<td>1.58 ± 0.17</td>
<td>1.08 ± 0.11</td>
<td></td>
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</tr>
<tr>
<td>+10% Gly + 1% Orn + 0.9% Met</td>
<td>98 ± 2</td>
<td>118 ± 7</td>
<td>4.44 ± 0.25</td>
<td>1.17 ± 0.03</td>
<td>1.33 ± 0.11</td>
<td>1.34 ± 0.10</td>
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</tr>
<tr>
<td>+10% Gly + 3.4% Pro&lt;sup&gt;b&lt;/sup&gt; + 0.9% Met</td>
<td>71 ± 5</td>
<td>76 ± 8</td>
<td>3.92 ± 0.26</td>
<td>0.93 ± 0.06</td>
<td>1.17 ± 0.09</td>
<td>0.92 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+10% Gly + 1.8% Asp&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68 ± 1</td>
<td>81 ± 4</td>
<td>2.92 ± 0.14</td>
<td>0.85 ± 0.06</td>
<td>1.31 ± 0.70</td>
<td>0.99 ± 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+10% Gly + 1.8% Asp + 0.9% Met</td>
<td>47</td>
<td>50</td>
<td>2.78</td>
<td>0.74</td>
<td>1.87</td>
<td>0.93</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+10% Gly + 1.4% Arg + 0.9% Met</td>
<td>90 ± 9</td>
<td>107 ± 6</td>
<td>3.81 ± 0.26</td>
<td>1.19 ± 0.14</td>
<td>2.19 ± 0.30</td>
<td>1.54 ± 0.31</td>
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</table>

<sup>a</sup> Creatine plus creatinine in urine.  
<sup>b</sup> The amounts of added amino acids corresponded to the levels normally present in a 30% casein diet.  
<sup>*</sup> Mean ± standard error.
1300 H. TAKEUCHI and K. MURAMATSU

FIG. 1. The Changes in Body Weight Gain of Rats Fed the Various Diets for 14 Days.

The amino acids except glycine were added at the levels which would be normally found in a 30% casein diet. The dotted line indicates the weight gain level of rats fed the control.

Table I and Figs. 1 to 4 show the results of each experiment.

Experiment I. The body weight gains of the 10% casein groups containing excess glycine (CG group), excess glycine plus arginine (CGArg group) and excess glycine plus methionine (CGMet group) were about 20% of the 10% casein group (control). The 10% casein groups containing excess glycine supplemented with threonine (CGThr group), tryptophan (CGTrp group), glutamic acid (CGGlu group), threonine plus methionine (CGThr Met group) or tryptophan plus methionine (CGTrp Met group) maintained the initial body weights during 2 weeks. The addition of glutamic acid and methionine to the 10% casein diet containing excess glycine (CGGlu Met group) decreased extremely the body weights.

The food intakes of each group were in correlation with the body weight gains.

The liver weights of groups containing methionine were almost equal to the control, but those of other groups were less than the control. The kidney weights of each group were almost constant.

The urinary total-creatinine of the CGArg...
Effects of Ornithine and Some Amino Acids

FIG. 2. The Changes in Kidney Transamidinase Activity of Rats Fed the Various Diets.

The amino acids except glycine were added at the levels which would be normally found in a 30% casein diet. Total units meant μmoles of product per wet kidney per hour.

The dotted line indicates the enzyme activity of rats fed the control.

FIG. 3. The Changes in Body Weight Gain of Rats Fed the Different Diets for 14 Days.

The amino acids except glycine and ornithine were added at the levels which would be normally found in a 30% casein diet.

The dotted line indicates the weight gain level of rats fed the control.

FIG. 4. The Changes in Kidney Transamidinase Activity of Rats Fed the Various Diets.

The amino acids except glycine were added at the levels which would be normally found in a 30% casein diet. Total units meant μmoles of product per wet kidney per hour.

The dotted line indicates the enzyme activity of rats fed the control.

and CGGluMet groups was about 2 times of the control. That of other groups was 1 to 1.5 times of the control. The urinary creatinine of each group was almost constant.

The plasma creatine of groups supplemented with different amino acids was generally increased as compared with the control.
muscle creatine of each group was constant.

The kidney transamidinase activities of the CG, CGArg and CGMet groups of which body weights increased a little were about 70% of the control. The enzyme activities of the CGThr, CGThrMet, CGTrp, CGTrpMet and CGGlu groups of which body weights were maintained were about 50% of the control. The enzyme activity of the CGGluMet group of which body weight decreased was approximately 20% of the control.

Experiment II. The body weight gain of the 10% casein group containing excess glycine supplemented with ornithine plus methionine (CGOrnMet group) was compared to those of the 10% casein group (control) and 10% casein group containing excess glycine with arginine plus methionine (CGArgMet group). The 10% casein groups containing excess glycine (CG group), excess glycine plus ornithine (CGOrn group) or excess glycine plus aspartic acid (CGAsp group) maintained almost the initial body weights during 2 weeks. The body weight of the 10% casein group containing excess glycine supplemented with aspartic acid and methionine (CGAspMet group) decreased extremely.

The food intakes of each group were in correlation with the body weight gains.

The liver weights of groups containing methionine were almost equal to the control, but those of other groups were less than the control. The kidney weights of each group were constant.

The urinary total-creatinine of the CGAspMet group was about 170% of the control.

The growth depression by excess glycine was prevented by the addition of ornithine plus methionine to the 10% casein diet containing excess glycine as likely as the addition of arginine plus methionine. Namely, the useful effect of ornithine was essentially the same as that of arginine. It is suggested that the urea cycle system may play a role for the prevention of the growth depressive effect as both ornithine and arginine are the members of the urea cycle.

The 10% casein groups containing excess glycine supplemented with threonine or tryptophan with methionine (CGThrMet or CGTrpMet groups) could maintain the initial body weight, while the body weight was extremely decreased when glutamic acid or aspartic acid was added to the 10% casein diet containing excess glycine with methionine (CGGluMet or CGAspMet groups). Muramatsu et al. have shown that the addition of 5% glutamic acid or aspartic acid to a 10% casein diet did not produce severe growth depression in rats. In the present experiment, the levels of supplemented glutamic acid and aspartic acid were 6.5 and 1.8%, respectively. Therefore, it seems possible that the growth depression effect by the addition of glutamic acid or aspartic acid is accelerated by excess glycine.

In general, the urinary total-creatinine of the amino acids supplemented groups tended to increase as compared with the 10% casein group. With glycine plus arginine added groups, the supplemented glycine and arginine may promote the formation of creatine, and thereby result in the elevation of urinary creatine as in the previous reports. In the CGGluMet and CGAspMet groups, the decrease of body weight may result in the catabolization of muscle, and released creatine from muscle may be excreted in urine. However, creatine synthesis system may not
Effects of Ornithine and Some Amino Acids  

be useful in metabolizing excess glycine smoothly, because the amount of creatine formed per day is very small as compared with glycine intake per day.\(^1\),\(^3\),\(^5\)

The activity of kidney transamidinase was not related to the amounts of urinary excreted creatine, but with the gain in body weight of young rats. The 10% casein groups containing excess glycine supplemented with ornithine plus methionine or arginine plus methionine (CGOrnMet or CGArgMet groups), of which growth was normal, gave the high enzyme activity. In the previous experiment,\(^9\) the enzyme activity of rats fed on the 10% casein diet supplemented with methionine was higher than that of rats fed on the 10% casein diet. It has been observed that the enzyme activity was in correlation with the quality of protein.\(^6\) Pilsum and Ungar\(^7\),\(^8\) have also reported that the injection of growth hormone to hypophysectomized rats increased the activity of rat kidney transamidinase. These results indicate that the enzyme activity is high in the feeding conditions which rats grow normally. The enzyme activity of the CGOrnMet group was high rather than the control and CGArgMet groups. Given \(e_t\)\(^9\) have also indicated the addition of ornithine alone to the 10% casein diet increased the activity of kidney transamidinase. However, the effect of ornithine cannot be explained at present.

Walker\(^10\) has reported that the activity of liver transamidinase might be related to tryptophan, because the enzyme activity in chicks fed on the acid-hydrolyzed casein diet supplemented with tryptophan has been almost equal to that of chicks fed on the equimolar casein. In the present experiment, however, the change in the kidney transamidinase activity seems to have nothing to do with dietary tryptophan.