Self-Selection of Phenylalanine in the Rat

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Summary The regulation of phenylalanine intake in rats was investigated by means of a self-selection feeding method. The relationship between phenylalanine intake and the alteration of phenylalanine and tyrosine concentrations in plasma and brain was also studied. Weanling rats were offered a choice of two diets differing only in phenylalanine content for 2 weeks. Weight gain and food consumption of all the self-selecting rats were not significantly different from each other and were of the same levels as those in rats fed on a 10% casein or 10% casein plus 1.0% phenylalanine diet as a fixed ratio. Phenylalanine intake of the self-selecting rats ranged from 0.31 to 2.29% of the food consumed. The phenylalanine and tyrosine concentrations in plasma and brain of self-selecting rats were within normal levels. It became clear that rats have an ability to regulate phenylalanine intake, and that they select phenylalanine to meet their requirement for the amino acid.

Key Words phenylalanine intake, self-selection, intake regulation

The self-selection technique has been used to demonstrate the existence of appetites for specific nutrients in deficient animals and the regulation of nutrient intake in normal animals. Rats deficient in certain specific nutrients such as thiamin (1–3), riboflavin (2, 3), pyridoxine (2, 3) and calcium (4, 5) were able to select food containing these nutrients, and the intake of energy (6–8), protein (9–11) and phosphorus (12) by rats not deficient any nutrients was shown to be under physiological regulation. Ashley and Anderson (10) have reported that there are two mechanisms operating independently for protein intake and energy intake, and Anderson et al. (13, 14) suggested that the ratio of tryptophan to neutral amino acids (phenylalanine, tyrosine, leucine, isoleucine and valine) in plasma is important for the regulation of protein intake. On the other hand, a number of studies have described the relationship between food intake depression in rats fed on a high protein, amino acid-deficient, amino acid-imbalanced or amino acid-toxicity diet...
and altered blood amino acid patterns (15–18). However no obvious results have been reported (19–22). Muramatsu et al. (23, 24) observed that, when weanling rats were offered a choice of pairs of diets varying in lysine or methionine by a self-selection feeding method, the amounts of both lysine and methionine intakes were closely regulated, and that they consumed enough to satisfy these requirements. But the mechanism responsible for the intake of a single amino acid remains to be clarified.

The present investigation was undertaken to determine the regulation of phenylalanine intake in rats offered a choice of two diets differing only in phenylalanine content. The relationship between phenylalanine intake and the changes in phenylalanine and tyrosine concentrations in plasma and brain was also studied.

METHODS

Male weanling rats of the Wistar strain (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Hamamatsu) were used. They were fed on a stock diet containing 25% casein for 3 to 4 days before the experiments, and then rats weighing 55 to 60g were separated into groups. Animals were housed in

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>10AA-P</th>
<th>10AA0.11P</th>
<th>10AA0.22P</th>
<th>10C</th>
<th>10C1P</th>
<th>10C2P</th>
<th>10C3P</th>
<th>10C5P</th>
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<tbody>
<tr>
<td>Casein</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>10.0</td>
<td>10.0</td>
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<tr>
<td>Phenylalanine</td>
<td>—</td>
<td>0.11</td>
<td>0.22</td>
<td>—</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
<td>5.0</td>
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<td>Amino acid mixture¹</td>
<td>8.99</td>
<td>8.88</td>
<td>8.77</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<td>—</td>
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<tr>
<td>Potato α-starch</td>
<td>79.91</td>
<td>79.91</td>
<td>79.91</td>
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<td>77.9</td>
<td>76.9</td>
<td>75.9</td>
<td>73.9</td>
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<td>5.0</td>
<td>5.0</td>
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<td>Salt mixture²</td>
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<td>5.0</td>
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<tr>
<td>Vitamin mixture²</td>
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<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
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<tr>
<td>Choline chloride</td>
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<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
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</tr>
</tbody>
</table>

Table 1. Composition of experimental diets.

The diet also contained vitamin A (2,000 IU), vitamin D<sub>2</sub> (200 IU) and α-tocopherol (0.01 g) per 100 g of diet. The amino acid mixture in the 10AA-P group was prepared on the basis of an amino acid mixture simulating casein, and contained the following amino acids (in g): L-arginine·HCl, 0.38; L-histidine, 0.23; L-lysine·HCl, 0.76; L-tyrosine, 0.48; L-tryptophan, 0.11; L-cystine, 0.03; L-methionine, 0.25; L-serine, 0.48; L-threonine, 0.35; L-leucine, 0.77; L-isoleucine, 0.50; L-valine, 0.57; L-glutamic acid, 2.24; L-aspartic acid, 0.50; L-glycine, 0.16; L-alanine, 0.24; L-proline, 0.94. The amino acid mixture in 10AA0.11P and 10AA0.22P groups had L-phenylalanine substituted for L-glutamic acid in the 10AA-P and these diets were kept isonitrogenous. Muramatsu, K. et al. (1976): J. Nutr. Sci. Vitaminol., 22, 397 (25).
individual suspended cages with screen bottoms, at 24 ± 1°C in an air-conditioned
room with a light period from 6:00 to 18:00.

The composition of experimental diets is given in Table 1. The experimental
diets and water were provided ad libitum and the feeding period was 2 weeks.
Animals were weighed every morning. Daily food consumption was recorded on a
dry matter basis, and amounts of phenylalanine ingested were calculated from the
food consumed. Two food cups were kept in opposite corners of the cages and were
changed daily to prevent any position effects, as previously reported (23, 24).

Fixed feeding experiment. Rats were fed on a single diet containing various
amounts of phenylalanine. The dietary groups were the amino acid mixture diet
lacking L-phenylalanine (10AA-P), the amino acid mixture diet with 0.11 or 0.22%
L-phenylalanine (10AA0.11P, 10AA0.22P), the 10% casein diet (10C), and the
10% casein diet supplemented with 1.0, 2.0, 3.0 and 5.0% L-phenylalanine (10C1P,
10C2P, 10C3P, 10C5P).

Self-selection feeding experiment. Rats were given a choice between the 10C and
the 10AA-P, 10AA0.11P, 10AA0.22P, 10C1P, 10C3P or 10C5P diets, and a choice
between the 10AA-P and the 10C3P diets, or the 10AA0.11P and the 10C1P diets.

Amino acid determination. At the end of the experimental period, blood was
obtained in a heparinized tube by heart puncture after anesthetizing the animals
with ether. Plasma was prepared by centrifugation and stored at −20°C for
analysis. Brain was removed as soon as possible after decapitation, and homoge-
nized in 2 volumes of 0.14 M KCl with a glass homogenizer. A portion of the plasma
or brain homogenate was used for the determination of free phenylalanine
concentration using the spectrophotometric method of Udenfriend and
Cooper (26), and the other portion was used for the determination of free tyrosine
with the method of Udenfriend and Cooper (27).

RESULTS

The effects of fixed-ratio and self-selection feeding methods on body weight
gain, food consumption and phenylalanine intake are shown in Table 2. When rats
were fed on the fixed-ratio diet, the maximum weight gain was obtained in the
animals consuming the 10% casein diet (10C) or the 10% casein diet supplemented
with 1.0% phenylalanine (10C1P). The rats fed on the 10C diet supplemented with
2.0 or 3.0% phenylalanine (10C2P, 10C3P) or amino acid mixture diet containing
0.11 or 0.22% phenylalanine (10AA0.11P, 10AA0.22P) gained significantly less. The
weight loss was observed in animals fed on the 10C diet supplemented with 5.0%
phenylalanine (10C5P) or the diet lacking phenylalanine (10AA-P). Food con-
sumption of these groups was proportional to the weight change.

When rats were offered an ad libitum choice of two diets differing in
phenylalanine content, there was no significant difference in weight gain and total
food consumption among all these groups, and these values were of the same levels
as those in rats fed on the 10C or the 10C1P diet as a fixed ratio. The preference

Vol. 30, No. 3, 1984
<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight gain (g, 2 weeks)</th>
<th>Total intake</th>
<th>Food preference</th>
<th>Phenylalanine intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fixed feeding</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10AA-P</td>
<td>-11.8 ± 0.24*</td>
<td>63.1 ± 1.7**</td>
<td>3.9 ± 0.6*</td>
<td>3.8 ± 1.4*</td>
</tr>
<tr>
<td>10AA0.11P</td>
<td>28.9 ± 0.16</td>
<td>135.6 ± 1.5*</td>
<td>153.8 ± 1.9*</td>
<td>153.0 ± 2.0*</td>
</tr>
<tr>
<td>10C</td>
<td>38.0 ± 1.0**</td>
<td>134.4 ± 3.8*</td>
<td>144.0 ± 1.3*</td>
<td>103.9 ± 2.7*</td>
</tr>
<tr>
<td>10C1P</td>
<td>31.4 ± 2.7</td>
<td>128.1 ± 3.9</td>
<td>52.4 ± 6.5</td>
<td>48.2 ± 0.9</td>
</tr>
<tr>
<td>10C2P</td>
<td>14.0 ± 1.3</td>
<td>121.5 ± 1.5</td>
<td>21.5 ± 6.6</td>
<td>21.5 ± 6.6</td>
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<tr>
<td>10C3P</td>
<td>1.2 ± 0.9</td>
<td>128.4 ± 1.5</td>
<td>21.4 ± 0.9</td>
<td>21.4 ± 0.9</td>
</tr>
<tr>
<td>10CSP</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Self-selection feeding</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(1)10AA-A-P vs. (2)10C</td>
<td>36.8 ± 1.4**</td>
<td>156.5 ± 7.4</td>
<td>52.4 ± 6.5</td>
<td>48.2 ± 0.9</td>
</tr>
<tr>
<td>(1)10AA0.11P vs. (2)10C</td>
<td>37.2 ± 2.0**</td>
<td>154.4 ± 5.8</td>
<td>52.4 ± 6.5</td>
<td>48.2 ± 0.9</td>
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<td>(1)10AA0.22P vs. (2)10C</td>
<td>39.2 ± 2.3**</td>
<td>155.6 ± 4.2</td>
<td>52.4 ± 6.5</td>
<td>48.2 ± 0.9</td>
</tr>
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<td>(1)10AA0.11P vs. (2)10C1P</td>
<td>35.4 ± 2.1*</td>
<td>151.5 ± 1.5</td>
<td>52.4 ± 6.5</td>
<td>48.2 ± 0.9</td>
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<tr>
<td>(1)10AA0.22P vs. (2)10C1P</td>
<td>36.4 ± 1.4**</td>
<td>155.9 ± 1.7</td>
<td>52.4 ± 6.5</td>
<td>48.2 ± 0.9</td>
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<td>(1)10C vs. (2)10C2P</td>
<td>35.3 ± 0.6</td>
<td>153.8 ± 1.9</td>
<td>52.4 ± 6.5</td>
<td>48.2 ± 0.9</td>
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<td>(1)10C vs. (2)10C3P</td>
<td>35.3 ± 0.6</td>
<td>153.8 ± 1.9</td>
<td>52.4 ± 6.5</td>
<td>48.2 ± 0.9</td>
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<td>(1)10C vs. (2)10CSP</td>
<td>149.0 ± 3.5**</td>
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</table>

*Mean ± SE (n = 4 for fixed feeding rats, n = 5 for self-selection feeding rats). Values in the same column without common superscripts are significantly different (p < 0.05).

**J. Nutr. Sci. Vitaminol.**
Fig. 1. Daily food preference in rats offered a choice of two diets differing in phenylalanine content.

Fig. 2. The relationship between body weight gain and phenylalanine intake. Symbols in the graph used are: -P, 10AA-P; 0.11P, 10AA0.11P; 0.22P, 10AA0.22P; 1P, 10C1P; 2P, 10C2P; 3P, 10C3P; 5P, 10C5P; 1, 10AA-P + 10C; 2, 10AA-P + 10C3P; 3, 10AA0.11P + 10C; 4, 10AA0.11P + 10C1P; 5, 10AA0.22P + 10C; 6, 10C + 10C1P; 7, 10C + 10C3P; 8, 10C + 10C5P.
between two diets depended upon the phenylalanine content. The rats offered a choice between the 10C and the 10AA-P, 10AA0.11P, 10C3P or 10C5P diets ate more of the 10C diet than the latter diets, and the rats given a choice between the 10C and the 10AA0.22P or 10C1P diet consumed about the same amount of both diets. The choice between the 10AA-P and 10C3P, or 10AA0.11P and 10C1P showed a preference for the latter diet.

Figure 1 shows the daily food preference in rats offered a choice of two diets differing in phenylalanine content. Rats had the ability to distinguish between diets containing various amounts of phenylalanine from the beginning of the self-selection experiment.

Figure 2 presents the relationship between the weight gain and the phenylalanine intake in rats which were given diets by the fixed-ratio and the self-selection feeding methods. Phenylalanine intake of self-selecting rats ranged from 0.31 to 2.29% of food consumed.

The relationship between the phenylalanine intake and the phenylalanine concentration in plasma and brain is shown in Fig. 3. Phenylalanine intake was

![Diagram](image-url)

**Fig. 3.** Relationship between phenylalanine in food consumed and the phenylalanine concentrations in plasma and brain. Symbols in the graph are the same as in the footnotes to Fig. 2.

Phenylalanine concentration in plasma of rats fed the 10AA0.11P, 10AA0.22P, 10C or the 10C1P diet was kept between 0.10 and 0.16 μmol/ml, and the concentration in plasma for self-selecting rats was kept within the same level. Phenylalanine concentration in plasma was lowered significantly in rats fed on the 10AA-P diet, and elevated remarkably in rats fed on the 10C2P, 10C3P or the 10C5P diet. Phenylalanine concentration in brain of rats fed on the 10AA-P, 10AA0.11P, 10AA0.22P, 10C, 10C1P or the 10C2P diet was kept between 0.11 and 0.14 μmol/g, and the concentration for self-selecting rats was kept within that level.

Figure 4 shows the relationship between the phenylalanine intake and the tyrosine concentration in plasma and brain. Tyrosine concentration in plasma of rats fed on the 10AA-P, 10AA0.11P, 10AA0.22P, 10C and the 10C1P ranged from 0.13 to 0.46 μmol/ml, and the concentration in brain of rats fed on the 10AA-P, 10AA0.11P, 10AA0.22P and the 10C ranged from 0.34 to 0.48 μmol/g. The tyrosine concentration in plasma and brain of all the self-selecting rats was within that area, with the exception of rats self-selecting the 10AA-P and the 10C3P diets.
DISCUSSION

These results extend previous observations in our laboratory (23, 24) that rats have the ability to regulate lysine and methionine intakes when offered a choice of two diets varying in methionine or lysine content. In the present experiment, it is suggested that the phenylalanine intake in rats offered an ad libitum choice of two diets differing only in phenylalanine content is regulated, and rats gained maximum growth by a selective intake of phenylalanine ranging from 0.31 to 2.29% of the food consumed (Fig. 2). The highest phenylalanine intake corresponded to about 7 times the minimum requirement. The results suggest that rats have the ability to select phenylalanine excessively above physiological needs, which resembles the case with lysine (24).

The lowest phenylalanine intake of the self-selecting rats (0.31%) with the diet containing 0.44% tyrosine was in close agreement with the values of phenylalanine requirement reported by NRC (phenylalanine plus tyrosine, 0.80%) (28) and other investigators (phenylalanine plus tyrosine, 0.69–0.86%) (29–31), but was lower to some extent than the values reported by Womack and Rose and others (0.4–0.6%) with excess tyrosine (32–34).

As reported previously by ourselves (35) and other investigators (36, 37), in rats fed on a single diet containing excess phenylalanine, both phenylalanine and tyrosine concentrations of plasma and brain were elevated markedly and the elevation of tyrosine concentration greatly exceeded that of phenylalanine concentration (Figs. 3 and 4). On the other hand, in rats given diets by the self-selection feeding method, both phenylalanine and tyrosine concentrations in plasma and brain ranged entirely within the same levels as those of rats fixed-fed the 10C or 10C1P diet, which maintains maximum growth. It then, became clear that rats have the ability to maintain phenylalanine and tyrosine concentrations in plasma and brain within normal levels when they are fed on their diets using the self-selection feeding method.

Many studies have reported the mechanism by which rats control intake of individual amino acids. Some studies showed that, when dietary amino acid patterns were unbalanced such as in the case of an amino acid imbalance, a deficiency or an excess, plasma amino acid levels were altered, with the depression of food intake, and suggested that the changes in amino acid pattern in plasma or brain may control the intake of a single amino acid (15–18). But other studies (19–22) have shown that there is not complete correlation between the amount of food intake and the degree of alteration of amino acid patterns in plasma.

Ashley and Anderson (13) examined the relationship between the change in plasma amino acid and protein intake, and observed no linear relationship between food intake and the accumulation of the added amino acids in plasma. They concluded that the ratio in plasma of tryptophan to neutral amino acids (phenylalanine, tyrosine, valine, leucine, isoleucine), which are known to share a common transport system in the brain (38), is important for the mechanism regulating
protein intake.

The present study clearly demonstrated that weanling rats have a capacity to regulate the intake of phenylalanine to meet maximum growth, and to maintain the phenylalanine and tyrosine concentrations in plasma and brain within normal levels. It will be important for the study of the mechanism which controls amino acid intake to determine not only the concentration of an added amino acid but also the patterns of amino acids in plasma and brain.

REFERENCES


Vol. 30, No. 3, 1984


