Self-Selection of Histidine and Arginine Intake and the Requirements for These Amino Acids in Growing Rats

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Summary The regulation of histidine and arginine intake in rats was investigated using the self-selection feeding method. The usefulness of the self-selection feeding technique for the determination of amino acid requirements is also discussed. When weanling rats were offered a choice of two diets differing only in histidine or arginine content for 2 weeks, they chose diets to support maximal growth, with the exception of some groups that did not grow to the maximum level. Histidine and arginine intake of the self-selecting rats ranged from 0.25% to 2.22% and from 0.43% to 2.43% of the diets ingested. These results demonstrate clearly that rats have the ability to consume a minimum amount that satisfies histidine and arginine needs for maximal growth. Previous and the present results suggest that the self-selection feeding method is a useful technique for the determination of amino acid requirements in growing rats.

Key Words histidine and arginine intake, self-selection, intake regulation

In previous studies (1–6), Muramatsu and co-workers demonstrated that when rats were given a choice of two diets containing different amounts of lysine, methionine, phenylalanine, tryptophan, threonine, valine, leucine, or isoleucine, they could maintain maximum growth. These studies also indicated that the minimum intake of self-selected amino acids agrees well with the requirements for amino acids as determined by other investigators. From these results, we suggested that the self-selection feeding method may be useful for the estimation of the requirements for essential amino acids. The estimation of amino acid requirements is one of the most important problems in nutritional science, and the growth and N-balance methods have been used as criteria (7, 8).
This study was undertaken to determine whether growing rats have the ability to regulate histidine and arginine intake by the self-selection feeding method, and whether the feeding method is useful for the estimation of the requirements for these amino acids.

**MATERIALS AND METHODS**

*Animals and diets.* Male Wistar strain rats (Shizuoka Agricultural Cooperation Association for Laboratory Animals, Hamamatsu, Japan) initially weighing about 40 g each were used in all experiments. They were offered a 25% casein diet for 3 to 4 days, and were divided into groups of 5 rats for fixed feeding groups and 6 rats for self-selection feeding groups. Rats were individually housed in suspended wire cages in a room maintained at 24 ± 1°C and operated on a 12-h cycle of light (06:00 to 18:00) and dark. Test diets and water were supplied ad libitum for 2 weeks. For the self-selection feeding experiment, two diets were placed in opposite corners of the cages and were changed daily to prevent the positional effect. Growth and food intake were recorded daily, and the amounts of ingested histidine and arginine were monitored.

| Diet | Casein | L-His | L-Arg·HCl | L-Glu | Amino acid mixture (%) |
|------|--------|-------|-----------|-------|------------------------|
| Histidine selection experiment |        |       |           |       |                        |
| 10AA0H | —      | —     | 0.72      | 4.52  | 7.51¹                  |
| 10AA0.15H | —   | 0.15  | 0.72      | 4.37  | 7.51¹                  |
| 10C0.07H | 10.0  | 0.07  | 0.34      | 2.42  | 0.93²                  |
| 10C1H  | 10.0  | 1.0   | 0.34      | 2.42  | 0.93²                  |
| 10C3H  | 10.0  | 3.0   | 0.34      | 2.42  | 0.93²                  |
| Arginine selection experiment |        |       |           |       |                        |
| 10AA0A | 0.3    | —     | —         | 4.94  | 7.51¹                  |
| 10AA0.36A | —  | 0.3   | 0.36      | 4.58  | 7.51¹                  |
| 10C0.34A | 10.0  | 0.07  | 0.34      | 2.42  | 0.93²                  |
| 10C1A  | 10.0  | 0.07  | 1.0       | 2.42  | 0.93²                  |
| 10C3A  | 10.0  | 0.07  | 3.0       | 2.42  | 0.93²                  |

¹ The amino acid mixture for the 10AA0H, 10AA0.15H, 10AA0A, and 10AA0.36A diets contained the essential amino acids according to the NRC requirements (L-Thr, 0.5; L-Lys·HCl, 1.1; L-Phe, 0.44; L-Trp, 0.15; L-Met, 0.57; L-Ile, 0.55; L-Leu, 0.77; L-Val, 0.6) and the nonessential amino acids simulated by 10% casein (L-Tyr, 0.48; L-Cys, 0.03; L-Ser, 0.48; L-Asp, 0.50; L-Gly, 0.16; L-Ala, 0.24; L-Pro, 0.94). ² The amino acid mixture for the 10C0.07H, 10C1H, 10C3H, 10C0.34A, 10C1A, and 10C3A diets contained deficient essential amino acids compared to the NRC requirements (L-Thr, 0.15; L-Lys·HCl, 0.34; L-Trp, 0.04; L-Met, 0.32; L-Ile, 0.05; L-Val, 0.03).

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arginine were calculated from the food consumed.

The composition of the experimental diets was as follows (%): corn oil, 5.0; salt mixture (9), 5.0; vitamin mixture (9), 1.0; choline chloride, 0.1; casein and amino acid mixture as shown in Table 1; and potato α-starch to yield 100%. The protein source in these diets was based on a 10% casein diet supplemented with some essential amino acids to simulate the essential amino acid pattern of the National Research Council (NRC) requirement (10).

For the histidine selection experiment, rats were divided into 5 fixed feeding groups and 8 self-selection feeding groups. Rats in the fixed feeding groups were fed on a single diet containing various amounts of histidine: an amino acid mixture diet devoid of L-histidine (10AA0H), an amino acid mixture diet with 0.15% L-histidine (10AA0.15H), and a 10% casein plus amino acid mixture diet with 0.07, 1.0, or 3.0% L-histidine added (10C0.07H, 10C1H, 10C3H). Rats in self-selection feeding groups were offered a choice between the 10AA0H or 10AA0.15H diet and the 10C0.07H, 10C1H, or 10C3H diet, and between the 10C0.07H diet and the 10C1H or 10C3H diet.

For the arginine selection experiment, rats were divided into 5 fixed groups and 8 self-selection feeding groups. Rats in fixed feeding groups were given one of the following diets: an amino acid mixture diet devoid of L-arginine (10AA0A), an amino acid mixture diet with 0.36% L-arginine (10AA0.36A) and 10% casein plus an amino acid mixture diet with 0.34, 1.0, or 3.0% L-arginine added (10C0.34A, 10C1A, 10C3A). Rats in self-selection feeding groups were offered a choice between the 10AA0A or 10AA0.36A diet and the 10C0.34A, 10C1A, or 10C3A diet, and between the 10C0.34A diet and 10C1A or 10C3A diet.

RESULTS

The body weight gains, the amount of food consumption, and the histidine intake of rats used for the histidine selection experiment are shown in Table 2. In fixed feeding groups, rats fed the 10C0.07H or 10C1H diet grew to the maximum level, and the growth of rats fed the 10AA0.15H or 10C3H diet was significantly smaller. Rats not given histidine (10AA0H) lost weight. In rats fed by the self-selection feeding method, there was no difference between groups in body weight gains and the amount of food consumed, which were the same as observed in rats fed the 10C0.07H or 10C1H diet by the fixed feeding method, with the exception that the 10AA0H vs. 10C3H and 10AA0.15H vs. 10C3H groups failed to achieve maximal growth.

The relationship between histidine intake and body weight gain in rats fed by fixed feeding and self-selection feeding is shown in Fig. 1. The histidine intake of self-selecting groups was from 0.25% to 2.22% of the food consumed.

The body weight gains, food consumption, and arginine intake of rats used for the arginine selection experiment are listed in Table 3. In the fixed feeding groups, rats fed the 10C0.34A and 10C1A diets grew to the maximum level, and the growth
Table 2. The effects of fixed-ratio and self-selection feeding methods on body weight gains, food consumption, and histidine intake in rats offered diets differing in histidine content.

| Groups | Body weight gain (g/2 weeks) | Food intake (1) | Food intake (2) | Total | Histidine intake |
|--------|------------------------------|-----------------|-----------------|-------|-----------------|
| Fixed feeding | | | | | |
| 10AA0H | $-11.5 \pm 0.6^{a,*}$ | | | $48.6 \pm 3.1^{a}$ | $0$ |
| 10AA0.15H | $38.8 \pm 2.1^{b}$ | | | $149.8 \pm 2.9^{b}$ | $0.22 \pm 0.01$ |
| 10C0.07H | $60.3 \pm 1.5^{c}$ | | | $158.9 \pm 5.0^{bc}$ | $0.48 \pm 0.02$ |
| 10C1H | $64.5 \pm 2.4^{c}$ | | | $170.1 \pm 7.8^{c}$ | $2.09 \pm 0.10$ |
| 10C3H | $51.0 \pm 4.2^{d}$ | | | $138.8 \pm 6.8^{bd}$ | $4.48 \pm 0.22$ |
| Self-selection feeding | | | | | |
| (1) 10AA0H vs. (2) 10C0.07H | $66.7 \pm 1.1^{a}$ | $29.1 \pm 3.4$ | $140.8 \pm 1.6$ | | $170.0 \pm 2.6^{c}$ | $0.42 \pm 0.004$ |
| (1) 10AA0H vs. (2) 10C1H | $63.7 \pm 1.6^{c}$ | $29.3 \pm 6.9$ | $131.4 \pm 6.4$ | | $160.7 \pm 1.5^{bc}$ | $1.62 \pm 0.08$ |
| (1) 10AA0H vs. (2) 10C3H | $50.3 \pm 2.6^{d}$ | $44.9 \pm 4.1$ | $99.1 \pm 4.3$ | | $144.0 \pm 3.1^{bd}$ | $3.20 \pm 0.14$ |
| (1) 10AA0.15H vs. (2) 10C0.07H | $65.6 \pm 1.6^{c}$ | $50.8 \pm 8.0$ | $120.5 \pm 8.0$ | | $171.3 \pm 2.7^{c}$ | $0.44 \pm 0.01$ |
| (1) 10AA0.15H vs. (2) 10C1H | $61.5 \pm 2.6^{c}$ | $51.0 \pm 11.3$ | $116.7 \pm 13.8$ | | $167.7 \pm 4.1^{ce}$ | $1.51 \pm 0.15$ |
| (1) 10AA0.15H vs. (2) 10C3H | $48.7 \pm 3.7^{d}$ | $49.8 \pm 15.5$ | $90.9 \pm 9.9$ | | $140.7 \pm 6.9^{bd}$ | $3.01 \pm 0.30$ |
| (1) 10C0.07H vs. (2) 10C1H | $65.8 \pm 1.3^{e}$ | $76.2 \pm 6.9$ | $80.8 \pm 2.9$ | | $157.0 \pm 4.4^{bc}$ | $0.23 \pm 0.01$ |
| (1) 10C0.07H vs. (2) 10C3H | $57.6 \pm 2.0^{ed}$ | $71.0 \pm 5.0$ | $85.7 \pm 6.2$ | | $156.7 \pm 4.1^{bc}$ | $2.98 \pm 0.20$ |

* Means ± SEM. Values in the same column without common superscripts are significantly different ($p<0.05$).
Table 3. The effects of fixed-ratio and self-selection feeding methods on body weight gains, food consumption, and arginine intake in rats offered diets differing in arginine content.

| Groups          | Body weight gain (g/2 weeks) | Food intake (g/2 weeks) | Arginine intake (g) |
|-----------------|------------------------------|-------------------------|---------------------|
|                 |                              | (1)                     | (2)                | Total              |
| Fixed feeding   |                              |                         |                     |                    |
| 10AA0A          | 19.0 ± 1.1a,*                | 111.2 ± 4.6c            | 0                   |
| 10AA0.36A       | 48.6 ± 1.7b                  | 157.3 ± 4.1bc           | 0.48 ± 0.01         |
| 10C0.34A        | 60.4 ± 1.3c                  | 155.7 ± 2.6bc           | 0.94 ± 0.02         |
| 10C1A           | 61.1 ± 2.0c                  | 162.5 ± 5.5bcd          | 1.87 ± 0.07         |
| 10C3A           | 53.1 ± 1.3bd                 | 146.3 ± 2.2seed         | 4.13 ± 0.06         |
| Self-selection feeding |                  |                         |                     |                    |
| (1) 10AA0A vs. (2) 10C0.34A | 52.9 ± 1.0bde              | 43.1 ± 7.0             | 106.2 ± 6.8         | 149.3 ± 3.3bde     | 0.64 ± 0.04         |
| (1) 10AA0A vs. (2) 10C1A       | 64.7 ± 2.7c                 | 34.8 ± 3.3             | 134.2 ± 1.7         | 169.0 ± 3.6d       | 1.55 ± 0.02         |
| (1) 10AA0A vs. (2) 10C3A       | 60.3 ± 3.0c                 | 39.1 ± 9.8             | 118.8 ± 9.4         | 158.0 ± 3.4bde     | 3.36 ± 0.27         |
| (1) 10AA0.36A vs. (2) 10C0.34A | 55.4 ± 2.1d                 | 61.2 ± 12.6            | 97.9 ± 15.7         | 159.1 ± 3.7bde     | 0.77 ± 0.06         |
| (1) 10AA0.36A vs. (2) 10C1A    | 61.6 ± 3.4c                 | 41.8 ± 14.2            | 125.5 ± 10.3        | 167.3 ± 4.7bd      | 1.57 ± 0.75         |
| (1) 10AA0.36A vs. (2) 10C3A    | 63.5 ± 1.9c                 | 26.8 ± 5.9             | 142.1 ± 5.1         | 168.8 ± 1.5d       | 4.10 ± 0.13         |
| (1) 10C0.34A vs. (2) 10C1A     | 60.6 ± 2.1c                 | 59.6 ± 7.2             | 103.6 ± 11.0        | 163.2 ± 5.4bde     | 1.56 ± 0.09         |
| (1) 10C0.34A vs. (2) 10C3A     | 60.7 ± 1.9c                 | 69.5 ± 5.5             | 90.7 ± 5.7          | 160.3 ± 4.4bde     | 2.99 ± 0.14         |

* Means ± SEM. Values in the same column without common superscripts are significantly different (p < 0.05).
Fig. 1. The relationship between body weight gain and histidine intake. 
a, 10AA0H; b, 10AA0.15H; c, 10C0.07H; d, 10C1H; e, 10C3H; 1, 10AA0H vs. 10C0.07H; 2, 10AA0H vs. 10C1H; 3, 10AA0H vs. 10C3H; 4, 10AA0.15H vs. 10C0.07H; 5, 10AA0.15H vs. 10C1H; 6, 10AA0.15H vs. 10C3H; 7, 10C0.07H vs. 10C1H; 8, 10C0.07H vs. 10C3H.

Fig. 2. The relationship between body weight gain and arginine intake. 
a, 10AA0A; b, 10AA0.36A; c, 10C0.34A; d, 10C1A; e, 10C3A; 1, 10AA0A vs. 10C0.34A; 2, 10AA0A vs. 10C1A; 3, 10AA0A vs. 10C3A; 4, 10AA0.36A vs. 10C0.34A; 5, 10AA0.36A vs. 10C1A; 6, 10AA0.36A vs. 10C3A; 7, 10C0.34A vs. 10C1A; 8, 10C0.34A vs. 10C3A.

of rats given the 10AA0A, 10AA0.36A, or 10C3A diet was less significant. When rats were fed by the self-selection feeding method, they grew to the same levels as rats fed the 10C0.34A or 10C1A diet by the fixed feeding method. But the 10AA0A vs. 10C0.34A and the 10AA0.36A vs. 10C0.34A groups did not achieve maximum
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growth.

Figure 2 shows the relationship between the amount of arginine ingested and the body weight gains of rats used for the arginine selection experiment. The arginine intake of self-selecting rats was from 0.43% to 2.43% of the food consumed.

**DISCUSSION**

We extended our previous observations (1-6) that rats have the ability to regulate individually their intake of methionine, lysine, tryptophan, phenylalanine, threonine, valine, leucine, and isoleucine when offered a choice of two diets differing only in each amino acid content. The present study showed that histidine and arginine intake can also be controlled by rats fed by the self-selection feeding method, and that the rats achieved maximum growth, with some exceptions. In the histidine selection experiment, the 10AA0H vs. 10C3H and 10AA0.15H vs. 10C3H groups, which were combinations of a diet containing little or no histidine and a diet containing surplus histidine, did not consume sufficient amounts and failed to achieve maximal growth. In the arginine selection experiment, the 10AA0A vs. 10C0.34A and 10AA0.36A vs. 10C0.34A groups could not select their diets to consume sufficient amounts of arginine and could not achieve maximum growth. This fact shows that the arginine requirement cannot be as strictly determined as those the requirements for other essential amino acid by this method.

It has been generally believed that histidine is essential for both growing and adult rats, and that arginine is essential for growing rats but nonessential for adult rats. Recent studies have questioned the classic criteria for amino acid essentiality.

| Amino acid | Amounts consumed by self-selection feeding method (%) | Requirements determined by |
|------------|-----------------------------------------------|-----------------------------|
| l-Lys      | 0.81-3.02                                     | 0.70                        | 0.90                        |
| l-Met + l-Cys | 0.57-1.18                                   | 0.60                        | 0.49                        |
| l-Phe + l-Tyr | 0.79-2.77                                   | 0.80                        | 0.72                        |
| l-Trp      | 0.14-0.75                                     | 0.15                        | 0.11                        |
| l-Thr      | 0.43-1.90                                     | 0.50                        | 0.51                        |
| l-Val      | 0.53-2.07                                     | 0.60                        | 0.56                        |
| l-Leu      | 0.74-3.58                                     | 0.75                        | 0.69                        |
| l-Ile      | 0.50-2.96                                     | 0.50                        | 0.55                        |
| l-His      | 0.25-2.22                                     | 0.30                        | 0.21                        |
| l-Arg      | 0.43-2.43                                     | 0.60                        | 0.21                        |

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and nonessentiality. Histidine and arginine have been the focus of many investigations because data indicate they are essential for adult humans (10–12). The requirements for all essential amino acids in growing rats as determined in previous reports and in the present report are summarized in Table 4, and compared with the requirements reported by the NRC (13) and Rama Rao et al. (14, 15). This table shows that rats offered a choice of two diets varying only in amino acid content can select from diets differing widely in each amino acid content, and the minimum requirements for all essential amino acids as determined by the self-selection feeding method agree well with the values listed by the NRC and Rama Rao et al.

The evaluation of amino acid requirements has been studied for a long time and several methods have been devised. Rose (7, 8) used the growing method for growing rats and the N-balance method for adult humans and rats. The changes in plasma amino acid concentrations (16, 17) and the rate of $^{14}$C labeled amino acid recovered as expired $^{14}$CO$_2$ (18, 19) have also been used. The self-selection feeding method as used in this experiment was also found to be useful for this purpose.

From the present study we can conclude that rats have the ability to regulate histidine and arginine intake although not perfectly, and that they select the minimum amounts of these amino acids that meet essential requirements. This and previous studies in our laboratory suggest that the self-selection feeding method is a useful method to determine the requirements for essential amino acids.

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