Effects of Taste Stimulation on the Behavior of Serum Amino Acid Concentrations and Amylase and Trypsin Activities in Fasting Rats

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Summary The effect of taste stimulation on serum free-amino acid concentrations and amylase and trypsin activities in fasting rats was studied. Following an acclimation period of 5 d, male Sprague-Dawley rats were fasted for 4 d and sacrificed after taste stimulation with a palatable sodium saccharin or unpalatable quinine sulfate flavored diet. Blood was collected from the portal vein and inferior vena cava at 0, 5, 10, 20 and 30 min after taste stimulation. Intestinal contents were also collected at the same time intervals as the blood collections. Total amino acid concentrations in the saccharin stimulated group increased significantly at 5 and 20 min following taste stimulation in comparison with the control of 0 time in the portal vein, and a significant difference between the saccharin and quinine stimulated groups was also observed at 5 min. No difference was found in the inferior vena cava. A high level of alanine and low level of glutamine were depicted in the portal vein as compared to that of the inferior vena cava. The elevation of alanine that is gluconeogenic amino acid was remarkable in the saccharin group at 20 min in the portal vein. Moreover, amylase and trypsin activities in the saccharin group reached peak values promptly and kept constant throughout the experiment as compared to the quinine group. The results suggest that taste stimulation originates changes in the cephalic phase amino acid concentrations in the portal vein and that taste information, overcoming a hunger, plays an important role in amino acid metabolism and digestive enzyme activities. Therefore, eating with gusto is significant for the maintenance of body functions even under starvation conditions.

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Abbreviation: PEP, purified egg protein.
The ingestion of nutrients is well-known to be controlled by cephalic components, taste, smell and/or texture, as well as gastric and intestinal components (1–3). Regarding gestation, remarkable response in pancreatic exocrine secretion in the cephalic phase has been obtained by palatable taste stimuli such as sucrose (4–6). Ohara et al (6) proved that sweet or umami taste, a taste quality represented typically by glutamates and 5'-nucleotides (7), promoted pancreatic secretory response, but sour taste suppressed it. Moreover, it has been demonstrated that the palatability of meals has an effect on the response of hormones (8–14). Among these hormones, cephalic phase insulin release has been well documented in a number of species including the rat (9–11), dog (12), sheep (13) and human (13, 14).

Concerning digestion and absorption of the nutrients, Ramirez (15) reported that fat digestion coupled with tasting was conducted more effectively than that alone. However, few studies have been conducted to determine that protein digestion, absorption and amino acid metabolism are affected by taste stimulation.

On the other hand, the activities of digestive enzymes in the rat small intestine are known to show circadian rhythmic changes when rats are fed ad libitum (16, 17). We also demonstrated, in a previous paper, that changes of gastrointestinal pH values and amylase activities are influenced by feeding conditions and the responses following taste stimulation are disturbed under the presence of daily rhythms (18). Once the enzyme rhythm had been set up, it persisted for at least 2 d when the animals were fasted and gradually disappeared thereafter (16).

This study was conducted to observe the effect of taste stimulation, palatable or unpalatable taste, on the behavior of serum free-amino acid concentrations and intestine-secreted amylase and trypsin activities in fasting rats lacking circadian rhythms.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (Japan SLC, Hamamatsu, Japan) were used. The rats were acclimated to our laboratory for 5 d before the experiments. The animals were housed individually in wire mesh cages (21 × 24 × 20 cm) at a temperature of 22 ± 2°C with a 12:12 h light-dark cycle (dark between 19:00 and 07:00). During the acclimation period, the animals were kept on experimental basal diets and deionized water ad lib. The approval of the Animal Care and Use Committee of Kobe Women’s University was given for our experiments.

Diet. The experimental basal diets contained 10% purified egg protein (PEP), 79.5% α-potato starch, 5% corn oil, 1% cellulose, 3.5% salt mixture, and 1% vitamin mixture. PEP was purchased from Taiyo Kagaku (Mie, Japan), α-potato starch from Matsunami Kagaku Kogyo (Hyogo, Japan), salt mixture, corn oil and...
vitamin mixture from Oriental Yeast (Osaka, Japan) and cellulose powder from Yoneyama Yakuhin Kogyo (Osaka, Japan).

Composition of the salt mixture was as follows (g/kg mix): CaHPO₄·2H₂O, 145.6; KH₂PO₄, 257.2; NaH₂PO₄, 93.5; NaCl, 46.6; Ca-lactate, 350.9; Fe-citrate, 31.8; MgSO₄, 71.7; ZnCO₃, 1.1; MnSO₄·4H₂O, 1.2; CuSO₄·5H₂O, 0.3; and KI, 0.1. Composition of the vitamin mixture was as follows (g/kg mix): retinol acetate, 1.0; cholecalciferol, 0.0025; all-rac-α-tocopheryl acetate, 5.0; menadione, 5.2; thiamine hydrochloride, 1.2; riboflavin, 4.0; pyridoxine hydrochloride, 0.8; cyanocobalamin, 0.0005; ascorbic acid, 30.0; d-biotin, 0.02; folic acid, 0.2; calcium pantothenate, 5.0; p-aminobenzoic acid, 5.0; nicotinic acid, 6.0; inositol, 6.0; and choline chloride, 200.0.

Furthermore, basal diets with 8.29 mM sodium saccharin and 0.64 mM quinine sulfate were used as palatable and unpalatable taste stimulants, respectively.

Taste stimulation was conducted by feeding 1 g of dumpling diet that was composed of 0.5 g basal diet and the same amount of water, with or without taste stimulants. To make a ball of the diet proved to be best way for taste stimulation within a very short time without spillage.

Procedure. Fifty-four rats, 7 wk old and weighing 212 ± 0.9 g were used. During the acclimation period of 5 d, the animals were fed the experimental diet and deionized water freely for 24 h. Subsequently, the rats were fasted for 4 d, from days 6 to 9. The rats were provided deionized water freely while they were fasted. On the day of sacrifice, day 10, they were randomly divided into nine groups (6 animals/group). Final body weights were 206 ± 1.3 g. The rats were fed either sodium saccharin or quinine sulfate dumpling diets following fasting. The time to consume the diets averaged 2.57 ± 0.25 min for the rats fed saccharin and 4.44 ± 0.59 min for those fed quinine.

The rats were anesthetized with ether at 5, 10, 20 and 30 min after taste stimulation and rats before taste stimulation were used as 0 time. Immediately after laparotomy, the portal vein was clamped proximal to liver in order to prevent the contamination of blood in the liver and intestinal-side portal blood was collected. Blood was also collected from the inferior vena cava. Sera obtained by centrifuging the blood at 2,000 × g for 10 min at 4°C were used for the determination of free amino acids. Moreover, the digestive tract was ligated at both sides of the pylorus and at the ileocecal valve. The small intestine was excised free from the mesentery, and the contents were spat out into a beaker with 10 mL saline from a pipette. The small intestine was then slit open and the remaining contents washed out with saline in another beaker. The samples of both beakers were mixed and homogenized with a homogenizer (Nihonseiki Kaisha, Tokyo, Japan). Supernatants obtained by centrifuging the homogenates at 2,000 × g for 10 min at 4°C were used for determining amylase and trypsin activities. Sera and supernatants of the intestinal contents were stored at -20°C until analyzed.

The rats lacking taste stimulation were used as the control (0 time).

Analysis. Free amino acids in sera were analyzed by a Type 835 amino acid
analyzer (Hitachi, Tokyo, Japan). The sera were deproteinized with twice the volume of 4.5% sulfosalicylic acid. Following centrifugation at 10,000 \( \times g \) for 15 min at 4°C, the supernatants were analyzed.

Amylase activity secreted in the small intestine was analyzed spectrophotometrically (Amylase-B TEST Wako; code #438-28201: Wako Pure Chemical Industries, Japan). One unit was equivalent to resolving 10 mg starch into its component element completely per 30 min per 100 mL sample.

Trypsin activity secreted in the small intestine was analyzed spectrophotometrically according to the procedure of Erlanger et al (19). BAPNA (N-benzoyl-DL-arginine-4-nitroanilide) was used as the substrate, and the color intensity which produced p-nitroaniline was measured at 410 nm.

**Statistical analysis.** The results are given as means \( \pm SE \). The statistical comparison between groups was assessed by one-way analysis of variance (ANOVA) (20). A p value less than 0.05 denoted statistical significance.

**RESULTS**

Figure 1 gives the alteration of serum free-amino acid concentrations in the portal vein: A, total; B, essential; and C, non-essential amino acids, respectively. In the saccharin stimulated group, total amino acid concentration increased significantly at 5 and 20 min after taste stimulation in comparison with the rats lacking taste stimulation (0 time, Fig. 1A). Moreover, total free amino acid concentrations in the saccharin stimulated group also increased significantly as compared to those in the quinine group at 5 min. Non-essential amino acids were predominantly responsible for these elevations (Fig. 1, B and C). No difference in total amino acid concentration was, however, observed in the quinine stimulated group at any time following feeding in response to taste stimulation. Regarding individual amino acid concentrations (Table 1), several amino acids, such as threonine and serine, increased in response to taste stimulation. The behavior of alanine, especially at 20 min, was closely related to the changes in total amino acid concentrations.

Figure 2 gives the alteration of serum free-amino acid concentrations in the inferior vena cava: A, total; B, essential; and C, non-essential amino acids, respectively. Differing from the case of the portal vein, no significant differences were observed between the saccharin, quinine and/or control groups at any time following feeding. Regarding individual amino acids (Table 2), alanine in rats fed the saccharin diet was the only amino acid that increased significantly at 20 min after tasting as compared to the control group. Comparing alanine and glutamine concentrations in the portal vein with those in the inferior vena cava, alanine was high in the portal vein and glutamine was high in the inferior vena cava (Tables 1 and 2).

Amylase activity secreted in the small intestine is shown in Fig. 3. The activity increased significantly at 5 min after the feeding in both the saccharin and quinine
Fig. 1. Alteration of serum free-amino acid concentrations in the portal vein after taste stimulation by 8.29 mM saccharin or 0.64 mM quinine. (A) total free amino acids, (B) essential free amino acids, (C) non-essential free amino acids. Each point is the mean value for 6 rats, and vertical bars represent SE. In some instances, error bars are not visible. Different superscript letters represent a significant difference between the two groups at each time and the control of 0 time (p < 0.05).

stimulated groups. However, the response to saccharin was more rapid than that to quinine. The elevation of amylase activity in the saccharin group kept constant throughout the experiment as compared to the alteration in the quinine group, which showed slow and irregular patterns.

The trypsin activity is shown in Fig. 4. The activity in the saccharin stimulated group increased significantly at 5 min after feeding as compared to the control group and kept constant throughout the experiment. Responses in rats fed the quinine flavored diets were delayed as compared to those in the rats fed the saccharin diet.
Table 1. Alteration of individual serum free-amino acid concentrations in the portal vein after taste stimulation by 8.29 mM sodium saccharin or 0.64 mM quinine sulfate (μmol/100 mL).

| Amino acid | Stimulant | Non-stimulation (0 time) | Time after stimulation (min) |
|------------|-----------|--------------------------|------------------------------|
|            |           |                          | 5   | 10  | 20  | 30  |
| Essential  |           |                           |     |     |     |     |
| Arg        | Saccharin | 15.66 ± 0.27a             | 16.62 ± 0.72a | 17.03 ± 0.32a | 17.09 ± 1.38a | 15.50 ± 1.08ab |
|           | Quinine   | 15.66 ± 0.27a             | 17.14 ± 0.67a | 16.77 ± 1.08a | 15.08 ± 0.80b | 15.18 ± 0.68ab |
| Met        | Saccharin | 5.19 ± 0.24ab             | 5.83 ± 0.26ab | 6.11 ± 0.33a  | 6.67 ± 0.22a  | 6.78 ± 0.63a  |
|           | Quinine   | 5.19 ± 0.24ab             | 5.67 ± 0.25ab | 6.00 ± 0.40ab | 5.91 ± 0.31ab | 6.48 ± 0.43b  |
| Phe        | Saccharin | 9.30 ± 0.34b              | 9.38 ± 0.43ab | 9.31 ± 0.47ab | 11.68 ± 0.64a | 10.99 ± 0.62a |
|           | Quinine   | 9.30 ± 0.34b              | 8.74 ± 0.32ab | 10.42 ± 1.36ab| 10.28 ± 0.73ab| 10.27 ± 0.78ab|
| Lys        | Saccharin | 39.98 ± 3.20              | 39.72 ± 2.91 | 40.75 ± 1.82 | 43.38 ± 2.87 | 35.88 ± 4.04 |
|           | Quinine   | 39.98 ± 3.20              | 43.24 ± 3.88 | 39.46 ± 2.41 | 41.25 ± 3.66 | 36.73 ± 2.43 |
| His        | Saccharin | 8.15 ± 0.32b              | 8.23 ± 0.48b | 8.13 ± 0.21ab | 9.83 ± 0.60a  | 8.62 ± 0.50ab |
|           | Quinine   | 8.15 ± 0.32b              | 8.53 ± 0.51ab | 7.92 ± 0.25b | 7.56 ± 1.03ab | 8.63 ± 0.85ab |
| Trp        | Saccharin | 11.79 ± 1.21b             | 15.76 ± 0.93a | 13.46 ± 1.11ab| 15.30 ± 0.65a | 14.63 ± 0.38ab|
|           | Quinine   | 11.79 ± 1.21b             | 15.09 ± 0.55a | 15.25 ± 1.88ab| 12.36 ± 1.39ab| 14.31 ± 1.19ab|
| Ile        | Saccharin | 11.97 ± 0.50a             | 12.01 ± 0.89a | 11.64 ± 0.73a | 12.57 ± 0.49a | 14.14 ± 0.99a |
|           | Quinine   | 11.97 ± 0.50a             | 11.15 ± 0.51a | 10.99 ± 1.18a | 12.14 ± 1.12a | 13.23 ± 1.01a |
| Leu        | Saccharin | 16.79 ± 0.74b             | 18.63 ± 1.10ab| 18.13 ± 1.09ab| 20.18 ± 1.56a | 19.09 ± 1.32ab|
|           | Quinine   | 16.79 ± 0.74b             | 16.81 ± 1.09ab| 16.86 ± 1.27ab| 19.22 ± 1.40ab| 16.54 ± 2.01ab|
| Val        | Saccharin | 23.19 ± 1.24               | 26.01 ± 1.43 | 23.46 ± 1.05 | 27.91 ± 2.17 | 25.93 ± 2.28 |
|           | Quinine   | 23.19 ± 1.24               | 22.89 ± 0.91 | 25.77 ± 2.39 | 26.40 ± 1.91 | 26.18 ± 2.86 |
| Thr        | Saccharin | 22.95 ± 1.19b             | 28.61 ± 0.59a | 24.70 ± 1.31ab| 28.13 ± 1.57a | 21.60 ± 4.36ab|
|           | Quinine   | 22.95 ± 1.19b             | 24.52 ± 1.07b | 24.36 ± 1.89ab| 27.91 ± 1.63a | 25.39 ± 2.72ab|
| Amino acid | Stimulant | Non-stimulation (0 time) | Time after stimulation (min) |
|------------|-----------|--------------------------|-----------------------------|
|            |           |                          | 5   | 10  | 20  | 30  |
| Non-essential |          |                          |     |     |     |     |
| Asp        | Saccharin | 3.24 ± 0.25              | 3.49 ± 0.20                  | 3.44 ± 0.37                  | 3.53 ± 0.31                  | 3.43 ± 0.20                  |
|           | Quinine   | 3.24 ± 0.25              | 3.29 ± 0.30                  | 4.51 ± 1.10                  | 3.64 ± 0.25                  | 3.20 ± 0.18                  |
| Ser        | Saccharin | 26.44 ± 1.37<sup>b</sup> | 32.23 ± 0.89<sup>a</sup>    | 29.58 ± 0.61<sup>ab</sup>   | 32.29 ± 1.79<sup>a</sup>    | 28.17 ± 2.05<sup>ab</sup>   |
|           | Quinine   | 26.44 ± 1.37<sup>b</sup> | 28.14 ± 0.89<sup>ab</sup>   | 28.74 ± 1.66<sup>ab</sup>   | 31.45 ± 1.81<sup>a</sup>    | 28.83 ± 3.12<sup>ab</sup>   |
| Asn        | Saccharin | 9.31 ± 0.73<sup>b</sup>  | 8.15 ± 0.35<sup>ab</sup>    | 15.23 ± 2.58<sup>a</sup>    | 15.59 ± 2.18<sup>a</sup>    | 12.87 ± 2.22<sup>ab</sup>   |
|           | Quinine   | 9.31 ± 0.73<sup>b</sup>  | 8.17 ± 0.73<sup>ab</sup>    | 13.41 ± 3.24<sup>ab</sup>   | 14.85 ± 2.39<sup>a</sup>    | 11.41 ± 2.16<sup>ab</sup>   |
| Glu        | Saccharin | 15.72 ± 1.03             | 15.70 ± 1.03                 | 16.17 ± 0.54                 | 14.54 ± 0.97                 | 13.18 ± 1.13                 |
|           | Quinine   | 15.72 ± 1.03             | 14.75 ± 0.37                 | 16.42 ± 2.33                 | 13.94 ± 1.70                 | 13.41 ± 0.35                 |
| Gln        | Saccharin | 69.03 ± 1.59<sup>ab</sup>| 72.21 ± 4.42<sup>a</sup>    | 67.74 ± 3.91<sup>abc</sup>  | 66.32 ± 4.03<sup>abc</sup>  | 59.04 ± 3.35<sup>c</sup>    |
|           | Quinine   | 69.03 ± 1.59<sup>ab</sup>| 62.78 ± 1.36<sup>bc</sup>   | 72.72 ± 1.72<sup>a</sup>    | 72.53 ± 7.62<sup>a</sup>    | 58.63 ± 1.12<sup>c</sup>    |
| Pro        | Saccharin | 18.38 ± 1.22             | 19.04 ± 2.01                 | 20.94 ± 1.48                 | 22.58 ± 2.00                 | 24.39 ± 2.79                 |
|           | Quinine   | 18.38 ± 1.22             | 17.70 ± 3.01                 | 19.37 ± 2.57                 | 19.88 ± 1.72                 | 24.80 ± 4.28                 |
| Gly        | Saccharin | 46.70 ± 1.53             | 49.89 ± 1.03                 | 45.58 ± 1.50                 | 47.41 ± 2.61                 | 44.56 ± 3.74                 |
|           | Quinine   | 46.70 ± 1.53             | 46.57 ± 2.04                 | 47.18 ± 1.80                 | 47.91 ± 2.39                 | 44.01 ± 4.07                 |
| Ala        | Saccharin | 63.65 ± 5.77<sup>b</sup> | 72.65 ± 8.05<sup>ab</sup>   | 75.26 ± 7.77<sup>abc</sup>  | 103.61 ± 10.33<sup>a</sup>  | 93.41 ± 13.90<sup>a</sup>   |
|           | Quinine   | 63.65 ± 5.77<sup>b</sup> | 66.10 ± 5.50<sup>abc</sup>  | 72.50 ± 6.77<sup>ab</sup>   | 78.23 ± 9.02<sup>ab</sup>   | 86.57 ± 10.89<sup>ab</sup>  |
| Tyr        | Saccharin | 7.98 ± 0.36<sup>b</sup>  | 8.76 ± 0.44<sup>ab</sup>    | 8.72 ± 0.57<sup>a</sup>     | 9.97 ± 0.85<sup>a</sup>     | 8.72 ± 0.34<sup>ab</sup>    |
|           | Quinine   | 7.98 ± 0.36<sup>b</sup>  | 8.55 ± 1.08<sup>ab</sup>    | 10.60 ± 1.87<sup>ab</sup>   | 8.93 ± 0.70<sup>ab</sup>    | 9.37 ± 0.83<sup>ab</sup>    |

Values represent means ± SE (n = 6). Different superscript letters represent a significant difference between groups (p < 0.05).
Table 2. Alteration of individual serum free-amino acid concentrations in the inferior vena cave after taste stimulation by 8.29 mM sodium saccharin or 0.64 mM quinine sulfate (μmol/100 mL).

| Amino acid | Stimulant | Non-stimulation (0 time) | Time after stimulation (min) |
|------------|-----------|--------------------------|-----------------------------|
|            |           |                          | 5          | 10          | 20          | 30          |
| Essential  |           |                          |            |             |             |             |
| Arg        | Saccharin | 18.00 ± 0.73             | 17.85 ± 0.79 | 17.44 ± 0.71 | 17.68 ± 1.45 | 17.18 ± 0.97 |
|            | Quinine   | 18.00 ± 0.73             | 19.24 ± 0.53 | 18.10 ± 0.75 | 16.19 ± 1.11 | 16.62 ± 0.31 |
| Met        | Saccharin | 5.62 ± 0.35<sup>b</sup>  | 5.48 ± 0.43<sup>b</sup> | 5.72 ± 0.34<sup>b</sup> | 6.27 ± 0.44<sup>b</sup> | 6.98 ± 0.13<sup>a</sup> |
|            | Quinine   | 5.62 ± 0.35<sup>b</sup>  | 5.78 ± 0.35<sup>b</sup> | 5.90 ± 0.32<sup>b</sup> | 5.84 ± 0.31<sup>b</sup> | 6.44 ± 0.11<sup>b</sup> |
| Phe        | Saccharin | 9.22 ± 0.34<sup>b</sup>  | 9.62 ± 0.26<sup>b</sup> | 8.61 ± 0.84<sup>b</sup> | 9.83 ± 0.73<sup>b</sup> | 11.37 ± 1.06<sup>a</sup> |
|            | Quinine   | 9.22 ± 0.34<sup>b</sup>  | 8.90 ± 0.21<sup>b</sup> | 9.45 ± 0.69<sup>b</sup> | 9.48 ± 0.68<sup>b</sup> | 9.38 ± 0.23<sup>b</sup> |
| Lys        | Saccharin | 40.65 ± 2.93             | 42.21 ± 3.26 | 41.29 ± 1.71 | 42.41 ± 2.69 | 36.78 ± 2.75 |
|            | Quinine   | 40.65 ± 2.93             | 46.86 ± 3.75 | 44.16 ± 3.77 | 41.40 ± 3.37 | 38.51 ± 2.21 |
| His        | Saccharin | 8.17 ± 0.29              | 7.96 ± 0.34  | 7.84 ± 0.20  | 8.40 ± 0.67  | 8.43 ± 0.51  |
|            | Quinine   | 8.17 ± 0.29              | 8.15 ± 0.13  | 7.52 ± 0.37  | 7.79 ± 0.62  | 8.10 ± 0.47  |
| Trp        | Saccharin | 11.28 ± 0.64<sup>b</sup> | 13.36 ± 0.88<sup>b</sup> | 10.84 ± 0.81<sup>b</sup> | 12.74 ± 0.81<sup>b</sup> | 14.04 ± 0.93<sup>a</sup> |
|            | Quinine   | 11.28 ± 0.64<sup>b</sup> | 13.45 ± 1.23<sup>ab</sup> | 13.55 ± 1.22<sup>ab</sup> | 13.02 ± 1.75<sup>ab</sup> | 13.64 ± 0.85<sup>a</sup> |
| Ile        | Saccharin | 11.83 ± 0.62             | 10.41 ± 0.84 | 10.00 ± 0.93 | 10.34 ± 0.91 | 11.34 ± 0.58 |
|            | Quinine   | 11.83 ± 0.62             | 10.31 ± 0.43 | 9.81 ± 0.68  | 9.95 ± 0.56  | 10.65 ± 0.44 |
| Leu        | Saccharin | 17.11 ± 0.70             | 16.44 ± 1.25 | 15.48 ± 0.96 | 17.59 ± 1.78 | 16.70 ± 0.79 |
|            | Quinine   | 17.11 ± 0.70             | 15.55 ± 0.37 | 16.18 ± 0.75 | 17.95 ± 1.24 | 15.87 ± 0.72 |
| Val        | Saccharin | 23.05 ± 1.34             | 24.59 ± 1.88 | 23.69 ± 1.27 | 25.46 ± 2.63 | 25.23 ± 1.57 |
|            | Quinine   | 23.05 ± 1.34             | 24.11 ± 1.11 | 23.87 ± 0.84 | 24.82 ± 1.58 | 24.59 ± 2.02 |
| Thr        | Saccharin | 23.98 ± 1.08             | 25.61 ± 2.44 | 23.75 ± 1.49 | 25.30 ± 2.88 | 22.08 ± 0.73 |
|            | Quinine   | 23.98 ± 1.08             | 25.36 ± 1.53 | 25.63 ± 2.54 | 26.09 ± 2.63 | 24.31 ± 1.56 |
| Amino acid | Stimulant  | Non-stimulation (0 time) | Time after stimulation (min) |
|------------|------------|--------------------------|----------------------------|
|            |            |                          | 5  | 10 | 20 | 30 |
| Non-essential |           |                          |    |    |    |    |
| Asp        | Saccharin  | 2.00 ± 0.13              | 2.10 ± 0.19 | 1.72 ± 0.20 | 2.16 ± 0.33 | 2.29 ± 0.29 |
|            | Quinine    | 2.00 ± 0.13              | 1.50 ± 0.25 | 2.52 ± 0.62 | 2.48 ± 0.41 | 1.92 ± 0.23 |
| Ser        | Saccharin  | 31.13 ± 1.22             | 32.09 ± 2.10 | 28.45 ± 1.69 | 27.10 ± 2.52 | 28.29 ± 1.88 |
|            | Quinine    | 31.13 ± 1.22             | 31.03 ± 2.18 | 30.23 ± 3.08 | 28.89 ± 2.98 | 28.64 ± 1.78 |
| Asn        | Saccharin  | 10.95 ± 1.75             | 13.63 ± 2.74 | 13.24 ± 1.64 | 9.53 ± 0.97  | 9.17 ± 1.63  |
|            | Quinine    | 10.95 ± 1.75             | 13.01 ± 1.91 | 14.32 ± 2.91 | 10.15 ± 1.71 | 8.16 ± 0.72  |
| Glu        | Saccharin  | 12.05 ± 0.61\textsuperscript{b} | 12.46 ± 0.89\textsuperscript{ab} | 13.17 ± 0.65\textsuperscript{ab} | 13.23 ± 1.30\textsuperscript{ab} | 10.53 ± 0.73\textsuperscript{ab} |
|            | Quinine    | 12.05 ± 0.61\textsuperscript{b} | 12.85 ± 0.66\textsuperscript{ab} | 12.54 ± 0.93\textsuperscript{ab} | 14.19 ± 2.33\textsuperscript{ab} | 9.28 ± 0.31\textsuperscript{a} |
| Gln        | Saccharin  | 95.84 ± 4.17             | 97.86 ± 4.74 | 93.74 ± 4.55 | 99.1 ± 9.12  | 86.56 ± 4.90 |
|            | Quinine    | 95.84 ± 4.17             | 99.88 ± 5.21 | 93.92 ± 3.97 | 95.51 ± 11.94 | 85.65 ± 4.88 |
| Pro        | Saccharin  | 20.08 ± 2.31             | 22.11 ± 1.73 | 22.14 ± 2.53 | 17.48 ± 1.75 | 17.34 ± 0.76 |
|            | Quinine    | 20.08 ± 2.31             | 21.42 ± 2.44 | 23.48 ± 4.67 | 17.37 ± 1.66 | 19.35 ± 1.23 |
| Gly        | Saccharin  | 43.36 ± 1.88\textsuperscript{b} | 44.65 ± 2.34\textsuperscript{ab} | 41.47 ± 1.12\textsuperscript{ab} | 38.47 ± 2.03\textsuperscript{ab} | 40.08 ± 2.26\textsuperscript{ab} |
|            | Quinine    | 43.36 ± 1.88\textsuperscript{b} | 42.81 ± 1.98\textsuperscript{ab} | 43.34 ± 0.59\textsuperscript{ab} | 41.62 ± 3.00\textsuperscript{ab} | 37.11 ± 2.16\textsuperscript{a} |
| Ala        | Saccharin  | 44.64 ± 4.40\textsuperscript{b} | 49.63 ± 4.49\textsuperscript{ab} | 44.72 ± 3.07\textsuperscript{ab} | 62.33 ± 9.24\textsuperscript{a} | 61.04 ± 6.10\textsuperscript{b} |
|            | Quinine    | 44.64 ± 4.40\textsuperscript{b} | 45.74 ± 2.64\textsuperscript{ab} | 49.02 ± 3.70\textsuperscript{ab} | 50.50 ± 2.61\textsuperscript{ab} | 52.68 ± 4.65\textsuperscript{ab} |
| Tyr        | Saccharin  | 9.20 ± 0.48              | 8.94 ± 0.31 | 9.16 ± 0.53 | 9.78 ± 0.36 | 10.33 ± 0.87 |
|            | Quinine    | 9.20 ± 0.48              | 8.72 ± 0.42 | 9.24 ± 0.71 | 9.13 ± 0.70 | 9.99 ± 0.35 |

Values represent means ± SE (n = 6). Different superscript letters represent a significant difference between groups (p < 0.05).
Fig. 2. Alteration of serum free-amino acid concentrations in the inferior vena cava after taste stimulation by 8.29 mM saccharin or 0.64 mM quinine. (A) total free amino acids, (B) essential free amino acids, (C) non-essential free amino acids. Each point is the mean value for 6 rats, and vertical bars represent SE. In some instances, error bars are not visible. Different superscript letters represent a significant difference between the two groups at each time and the control of 0 time (p < 0.05).

DISCUSSION

This study demonstrated that taste stimulation originates changes in cephalic phase amino acid concentrations in the portal vein and taste information influences on amino acid metabolism and digestive enzyme activities in fasting rats.

A significant elevation of total free amino acid concentrations in the portal vein at 5 min after feeding in the saccharin stimulated group, as compared to the quinine stimulated group and group without taste stimulation, was observed (Fig. 1). To the best of our knowledge, this is the only study in which free amino acid concentrations in the serum were observed to change in such a short time following feeding, although studies have reported that cephalic responses for insulin (9–14)
Fig. 3. Amylase activity secreted in the small intestine after taste stimulation by 8.29 mM saccharin or 0.64 mM quinine. Each column is the mean value for 6 rats, and vertical bars represent SE. Different superscript letters represent a significant difference between the two groups at each time and the control of 0 time ($p < 0.05$).

Fig. 4. Trypsin activity secreted in the small intestine after taste stimulation by 8.29 mM saccharin or 0.64 mM quinine. Each column is the mean value for 6 rats, and vertical bars represent SE. Different superscript letters represent a significant difference between the two groups at each time and the control of 0 time ($p < 0.05$).
and digestive enzyme (4–6) secretion appear as soon as the start of ingestion. When the amino acid mixture was administered intraduodenally, amino acids in the portal vein appeared within 10 min after administration in humans (21). However, no report was found that amino acids change rapidly after the oral ingestion of ordinary diets, not an amino acids mixture that is absorbed rapidly. Considering the time lag in the present study, it is correct that the rise of serum free-amino acid concentration at 5 min after taste stimulation with saccharin is not a reflection of ingested protein but changes in endogenous amino acids.

It is well-known that cephalic phase hormone secretions are induced by feeding, to prepare for efficient metabolism before the actual appearance of ingesting nutrients in the gut. Cephalic phase insulin release is one of the typical examples consequent from the taste stimulation of saccharin (11, 13). Insulin generally hastens amino acid uptake into the muscle from blood and protein synthesis as a consequence. Concerning the mechanisms of cephalic phase for insulin secretion, Niijima explains that the stimulation of sweet taste receptors leads a vagal pancreatic efferent to become active as a gustatory reflex, which increases the rate of insulin secretion (22). Moreover, he described that the suppression of efferent activity by strong salty taste stimulation seems to be an inhibitory response of the vagus nerve due to stressful stimulation (23). Therefore, cephalic phase amino acid changes in the portal vein are thought to be precipitated by the change of cephalic phase endocrine secretion mediated by the oral vagus nerve system.

Comparing changes of individual amino acid concentrations in the portal vein together with those in the inferior vena cava, the high levels of alanine and low levels of glutamine were characterized in the portal vein regardless of the time after stimulation and kinds of taste (Tables 1 and 2). A large amount of alanine and glutamine are said to be released from the muscle in long-term fasting conditions because the body is lacking in energy (24). Glutamine is used partly as the nitrogen source for alanine synthesis. The gut is one of the major sites of glutamine uptake that provides the nitrogen source for alanine synthesis (24). Therefore, it is supposed that the low levels of glutamine in the portal vein, as compared to that in the inferior vena cava, were consequent from the utilization of glutamine in the gut. The significant elevation of total free amino acid concentrations in the portal vein at 20 min was also found in the saccharin stimulated group as compared to the group without taste stimulation. Alanine contributed predominantly to this elevation among amino acids (Table 1). Although alanine is said to be used for the material of gluconeogenesis under such a fasting condition, no report in which the amino acid metabolism is changed by taste stimulation has been published before. Further investigation, however, is necessary to elucidate this phenomenon.

On the other hand, total, essential and non-essential free amino acid concentrations in the serum changed little in the inferior vena cava following gustatory stimulation (Fig. 2 and Table 2). These results suggest that the homeostasis of amino acid concentrations in the inferior vena cava is well regulated by the reconstitution of various amino acids.
In addition, amylase and trypsin activities in the saccharin and quinine stimulated groups increased immediately after feeding as compared to the control group, although amylase responded more sharply than trypsin. However, these activities reached peak values more rapidly in the rats fed the saccharin flavored dumpling diet and kept constant throughout the experiment in spite of the animals being fed the same amount and constitution of diets (Figs. 3 and 4). These facts also suggest that taste information has an important role in digestion.

The present study testified that palatable diet furthers amino acid metabolism efficiently even under severe fasting conditions. Animals generally prefer sweet foods over bitter foods. Sweet tastes are said to be an important signal of energy source and bitter tastes are that of toxic substances. The significant difference between saccharin and quinine is a consequence of the phenomenon in which rats recognize that the saccharin flavored diet is nutrient rich and appropriate to eat. Although other attributes such as texture, etc. remain to be examined, taste palatably is a principal factor for maintaining digestion, absorption and metabolism.

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