Amino Acids and Glucose Differentially Increased Extracellular 5-Hydroxyindoleacetic Acid in the Rat Brain

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(Received December 27, 1994)

Summary To reveal the role of serotonergic neurons in the regulation of feeding, the levels of 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of serotonin, in the striatum and the hypothalamus were continuously monitored by an in vivo microdialysis technique. Intake of 20% casein diet did not induce significant changes in the 5-HIAA level in these regions. When rats were fed on 5% casein diet (83.5% carbohydrate diet) for 2 h, the level of 5-HIAA in the striatum gradually increased and reached a maximum (226±44% of basal level, M±SEM, n=7) at 4 h after stopping the diet. In the medial hypothalamus, its level also increased to 183±19% (n=10) at 2 h after starting the diet. On the other hand, a 60% casein diet increased the level of 5-HIAA in the lateral hypothalamus to 138±19% (n=10) at 2 h after starting the diet. The intravenous infusion of each of these nutrients, glucose, amino acid mixture or lipid, produced more rapid elevation of the 5-HIAA level than oral intake of the diets. When rats were infused with glucose, its level in the striatum continued to be elevated. In the medial hypothalamus, glucose infusion increased 5-HIAA to the maximum (189±38%, n=7) at 4 h after starting infusion. In contrast, serotonergic neurons in the lateral hypothalamus seemed to respond only to infusion of the amino acid mixture, and the level of 5-HIAA reached 163±14% (n=5) of the basal level at 1 h after starting the infusion. These results suggest that rapid elevation of glucose or amino acids may independently stimulate serotonin metabolism in these brain areas, participating in the feedback regulation of nutrient intake.

Key Words nutrient intake, serotonin, 5-HIAA, hypothalamus, striatum, in vivo microdialysis

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Feeding is one of the most instinctive behaviors for animals. The regulatory system of feeding has been studied mainly from the viewpoint of energy balance (1). Although various sites of the central nervous system participate in the control of food intake, the feeding and the satiety centers, which are located in the hypothalamus, play a fundamental role in the regulation of feeding behavior (1). The hypothalamus receives and integrates inputs of various signals reflecting the nutritional status of the body, such as blood levels of glucose, amino acids and lipids (2–4). However, the mechanism by which nutritional signals are transmitted to the centers has not been elucidated.

Monoamines including epinephrine, norepinephrine, dopamine and serotonin have been proposed to regulate food ingestion in animals and possibly in humans (5, 6). Serotonin has been reported to suppress feeding (7, 8). In vivo microdialysis makes it possible to continuously monitor extracellular serotonin and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in specific regions of the brain in relation to feeding behavior. Using this technique, Schwartz et al. (9) reported that food intake rapidly increased serotonin levels in the lateral and medial hypothalamus.

Serotonin is synthesized from its amino acid precursor tryptophan (Trp), and its synthesis is known to be directly controlled by the availability of Trp. Brain uptake of Trp is known to be regulated by many factors including the plasma level of Trp, the plasma levels of other large neutral amino acids (LNAA), which compete with Trp for transport into the brain, and the extent of Trp binding to serum albumin (10–13). Rapid alteration of these factors after food ingestion may modify serotonin metabolism in specific brain regions, thereby leading to changes in feeding behavior. However, the differential responses of serotonergic neurons in the various brain areas to food ingestion have not been precisely studied.

To clarify the regulatory mechanism of feeding behavior by serotonin, we studied the nutrient-induced activation of serotonergic neurons in the lateral hypothalamus (LH), medial hypothalamus (MH), and the striatum (ST) by monitoring extracellular 5-HIAA by an in vivo microdialysis technique.

MATERIALS AND METHODS

Animals and surgery. Male Wistar strain rats at 8 weeks of age were purchased from Japan SLC (Hamamatsu, Japan). They were individually housed in wire-mesh cages in a room maintained at 23±1°C on a 12-h light-dark cycle. Rats were allowed free access to standard laboratory chow (Oriental Yeast Co., Ltd., Tokyo, Japan) and water. Rats weighing about 250 g were used in the experiments. They were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg). A guide cannula, made from an intravenous catheter placement unit (Jelco™; I.V. Catheter Placement Unit, 22 G, Johnson & Johnson Medical Co., Tokyo, Japan), was stereotaxically implanted for the placement of a microdialysis probe in the LH, MH, or ST according to the atlas of Paxinos and Watson (14). With the incisor bar 3.3 mm below the interaural line, the stereotaxic coordinates of
the LH were \(-3.3\) mm from the bregma, \(\pm 1.3\) mm lateral from the midsagittal sinus and \(7.7\) mm ventral from the cortical surface; those of the MH were \(-3.3\) mm from the bregma, \(\pm 0.6\) mm lateral from the midsagittal sinus and \(8.0\) mm ventral from the cortical surface; and those of the ST were \(-0.3\) mm from the bregma, \(\pm 3.5\) mm lateral from the midsagittal sinus and \(3.5\) mm ventral from the cortical surface.

**Microdialysis procedures and histology.** Hollow fibers (molecular mass cut-off of \(5,000\)) with an outer diameter of \(208\,\mu\text{m}\) were used for the microdialysis probe. The microdialysis probe had the form of a concentric cannula similar to that described previously (15). The tip of the dialysis probe extended beyond the tip of the guide cannula to reach the dialysis site; \(2.1\) mm in the LH, \(2.4\) mm in the MH and \(4.0\) mm in the ST. The dialysis probe was perfused at a rate of \(1.5\,\mu\text{l/min}\) with Ringer’s solution (\(\text{Na}^+\ 147\), \(\text{K}^+\ 4\), \(\text{Ca}^{2+}\ 4.5\), \(\text{Cl}^-\ 155.5\) mEq/liter; \(\text{pH} 6.4\)) through a polyethylene tube connected to a microinfusion pump (Harvard Apparatus Compact Infusion Pump No. 975, Harvard Apparatus Co., Inc., USA). The *in vitro* recovery of 5-HIAA (i.e., the ratio between the concentrations in the perfusate and outside the dialysis membrane) was \(7\%\) from the probe for the LH or MH and \(10\%\) for the ST. Dialysates for analyses were collected every 30 min during the indicated periods. Microdialyses were repeated for 3 days. Samples were kept at \(-20^\circ\text{C}\) until analysis. The position of the microdialysis probe was histologically confirmed after finishing the experiments, as previously described (16), and data from the dialysates collected from wrong positions were omitted.

**HPLC procedures.** To estimate the activity of serotonergic neurons, 5-HIAA levels in recovered dialysates were measured according to the method of Robinson and Whishaw (17) with modifications. The dialysates were analyzed by reverse-phase high-performance liquid chromatography with an amperometric detector system (E-502: Irica, Kyoto, Japan) with a C-18 packing column (RP-18T, \(4 \times 250\) mm) equilibrated with \(0.1\,\text{m KH}_2\text{PO}_4\) buffer (\(\text{pH} 3.5\)) containing \(400\,\text{mg/liter sodium 1-octanesulfonate, 7\% acetonitrile, 3\% acetone and 0.1\,\text{g/liter EDTA}\). The column was kept at \(30^\circ\text{C}\) and was developed with the same buffer at the flow rate of \(0.5\,\text{ml/min}\). The glassy carbon electrode was set at \(+700\,\text{mV}\) (vs. an Ag/AgCl reference electrode).

**Diets.** After the operation, the animals (\(n=24\)) were divided into 3 groups: high-protein diet (60% casein diet), low-protein diet (5% casein diet), and standard diet (20% casein diet) groups. The dietary compositions are shown in Table 1. Experimental animals were fed between 11:00 and 13:00 (spaced-feeding) for 5 days. Food intake was measured every day at 13:00.

**Infusion of glucose, lipid, or amino acid mixture.** A 0.5-mm-diameter silicone tube (Iuchi, Osaka, Japan) was inserted from the external jugular vein into the superior vena cava under diethyl ether anesthesia on the day before starting dialysis. Twenty-four rats were divided into three groups: One group was intravenously infused with 12.5% glucose for 4 h (11:00–15:00) at the rate of 4 ml/h; the second and third groups were infused at the same rate with a 12% amino
Table 1. Composition of experimental diets.

| Protein level (%) | 5     | 20    | 60    |
|-------------------|-------|-------|-------|
| Carbohydrate (%)  | 83.5  | 68.5  | 28.5  |

| Composition (g/kg diet) |
|-------------------------|
| Casein                  | 50    | 200   | 600   |
| a-Starch                | 557   | 457   | 190   |
| Sucrose                 | 278   | 228   | 95    |
| Corn oil                |       | 50    |       |
| Mineral mixture¹        |       | 35    |       |
| Vitamin mixture²        |       | 10    |       |
| Cellulose               |       | 20    |       |

¹ AIN-76™ mineral mixture obtained from Oriental Yeast Co., Ltd., Tokyo contains as follows (g/100 g mineral mixture): CaHPO₄, 50; NaCl, 7.4; K₂C₅H₇O₂·H₂O, 22; K₂SO₄, 5.2; MgO, 2.4; MnCO₃, 0.35; Fe-citrate (Fe 17%), 0.6; ZnCO₃, 0.16; CuCO₃·Cu(OH)₂·H₂O, 0.03; Na₂SeO₃·5H₂O, 0.001; KIO₃, 0.001; CrK(SO₄)₂·12H₂O, 0.055; cellulose, 11.803. ² Vitamin mixture obtained from Oriental Yeast Co., Ltd., Tokyo contains as follows (mg/100 g vitamin mixture): d-biotin, 2; folic acid, 20; calcium pantothenate, 500; p-aminobenzoic acid, 500; nicotinic acid, 600; inositol, 600; choline chloride, 20,000; retinyl acetate, 100 (50,000 IU); cholecalciferol, 0.25 (10,000 IU); tocopheryl acetate, 500; menadione, 520; thiamin hydrochloride, 120; riboflavin, 400; pyridoxine hydrochloride, 80; cyanocobalamin, 0.05; ascorbic acid, 3,000; cellulose, 73,057.7.

Table 2. Composition of amino acid mixture.

| Amino acid                  | (g/100 ml) |
|-----------------------------|------------|
| L-Isoleucine                | 0.597      |
| L-Leucine                   | 1.138      |
| L-Lysine hydrochloride      | 0.980      |
| L-Methionine                | 0.433      |
| L-Phenylalanine             | 0.974      |
| L-Threonine                 | 0.504      |
| L-Tryptophan                | 0.187      |
| L-Valine                    | 0.690      |
| L-Cysteine                  | 0.023      |
| L-Tyrosine                  | 0.057      |
| L-Arginine hydrochloride    | 1.488      |
| L-Histidine hydrochloride   | 0.706      |
| L-Alanine                   | 0.821      |
| L-Aspartic acid             | 0.202      |
| L-Glutamic acid             | 0.102      |
| Glycine                     | 1.568      |
| L-Proline                   | 1.063      |
| L-Serine                    | 0.467      |

Total amino acids 12,000
acid solution (Table 2) and a 5% lipid solution (Intralipid; Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), respectively. Total infused energy in these three groups was about 8 kcal. The rats were fasted during these infusion days, but they were allowed free access to water.

**Statistical analysis.** The levels of 5-HIAA in the brain dialysate are expressed as a percent of the basal release (the concentration of 5-HIAA before feeding or nutrient infusion). All data are expressed as M±SEM. Statistical analyses were performed using one-way analysis of variance (one-way ANOVA) followed with post hoc Fisher’s PLSD test for determination of significant differences. A p value of less than 0.05 was considered statistically significant.

**RESULTS**

**Food intake**

Figure 1 shows energy intakes from protein, fat and carbohydrate in the three groups during spaced-feeding. There was no significant change in total energy intake.
intake between the 60% casein and 20% casein diet groups. The total energy intake in the 5% casein diet group was lower than that of the 20% casein diet group (Fig. 1a). Fat intakes were proportional to total energy intakes among the three groups (Fig. 1b), because each diet contained the same amounts of fat. Protein energy intakes were in proportion to protein levels of each diet (Fig. 1c), and carbohydrate energy intake in the 60% casein diet group was about half of that in the 5% and 20% casein diet groups (Fig. 1d).

The baseline level of 5-HIAA

The baseline levels of 5-HIAA in recovered dialysates from the ST, LH, and MH were 11,176±747 (M±SEM, n=29), 8,021±723 (n=38), and 2,797±384 pg/20 μl (n=25), respectively. These values are similar to those reported by others (9, 18).

Fig. 2. Feeding-induced changes in 5-HIAA levels in dialysates from ST. Rats were fed on 5% (a, n=7), 20% (b, n=14), and 60% casein diets (c, n=8). Values are M±SEM. *Significantly different from the values of basal, p<0.05; by one-way ANOVA and Fisher PLSD.

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**Effect of feeding on the levels of 5-HIAA in the ST**

When the rats were fed on 5% casein diet for 2 h, the levels of 5-HIAA in the ST were gradually increased after stopping the diet and reached the maximum level (226±44% of the basal level, n=7) at 4 h after starting the diet (Fig. 2a). However, the 60% casein and 20% casein diets did not affect the level in the ST (Fig. 2b and 2c).

**Effect of feeding on the levels of 5-HIAA in the hypothalamus**

When rats were fed on 60% casein diet for 2 h, the levels of 5-HIAA in the LH significantly increased to 138±17% (n=10) of the basal level at 2 h after starting the diet (Fig. 3c) and gradually decreased to the basal level. In contrast, the levels were not significantly changed when rats were fed on the 20% casein or 5% casein diet (Fig. 3a and 3b).

![Figure 3](image-url)

Fig. 3. Feeding-induced changes in 5-HIAA levels in dialysates from LH. Rats were fed on 5% (a, n=14), 20% (b, n=14), and 60% casein diets (c, n=10). Values are M±SEM. *Significantly different from the values of basal, p<0.05; by one-way ANOVA and Fisher PLSD.

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The responses to the diets in the MH were different from those in the LH. The 5-HIAA levels in the MH were affected by the low-protein diet (high-carbohydrate diet). After rats started eating the 5% casein diet, the levels began to increase and reached the maximum (183±19% of the basal level, n=10) at 2 h after feeding (Fig. 4a). However, feeding of the 60% and 20% casein diets did not alter the levels in the MH (Fig. 4b and 4c). Thus, only the low-protein diet induced the elevation of 5-HIAA levels in the MH, while the level in the LH was increased by the high-protein diet. These results cannot be explained solely by dietary protein levels because dietary carbohydrate levels also changed with the protein levels. Therefore, the next experiments were designed to reveal which nutrient promoted these changes.

![Fig. 4. Feeding-induced changes in 5-HIAA levels in dialysates from MH. Rats were fed on 5% (a, n=10), 20% (b, n=11), and 60% casein diets (c, n=4). Values are M±SEM. *Significantly different from the values of basal, p<0.05; by one-way ANOVA and Fisher PLSD.](image)

*J. Nutr. Sci. Vitaminol.*
Effect of nutrient infusion on 5-HIAA levels in the ST

The responses of 5-HIAA to nutrient infusion in the ST are summarized in Fig. 5. Infusion of the amino acid solution did not significantly change the 5-HIAA level (Fig. 5a), while the serotonergic neurons of the ST seemed to respond to glucose infusion. Glucose infusion increased the level more rapidly than oral intake of the high-carbohydrate diet, and the increase became significant at 3–4 h after the infusion. The levels continued to elevate during the experimental period (Fig. 5b). Lipid infusion also induced a small but significant increase in the 5-HIAA level after finishing the infusion (Fig. 5c).

Effect of nutrient infusion on the 5-HIAA levels in the hypothalamus

Lipid infusion did not change the level of 5-HIAA in both the LH (Fig. 6c)
Fig. 6. Changes in 5-HIAA levels in dialysates from LH caused by single nutrient infusion. Rats were intravenously infused with 12% amino acids (a, n=7), 12.5% glucose (b, n=5) or 5% lipid (c, n=8) solution at a rate of 4 ml/h for 4 h. Values are M±SEM. *Significantly different from the values of basal, p< 0.05; by one-way ANOVA and Fisher PLSD.

and the MH (Fig. 7c). Infusion of the amino acid solution rapidly increased the levels in the LH immediately after starting the infusion, and the level became maximum (163±14% of the basal level, n=5) at 1 h after the infusion. The levels remained elevated during the experimental period (Fig. 6a). In contrast, glucose infusion did not affect the 5-HIAA level in the LH (Fig. 6b).

Compared with the LH, the MH showed opposite responses to infusion of glucose and the amino acid solution. Glucose infusion caused rapid elevation (189±38% of the basal level, n=7) of the 5-HIAA level in the MH (Fig. 7a), while infusion of the amino acid solution did not alter the 5-HIAA level (Fig. 7b).

Infusion of amino acid mixture produced more rapid elevation than ingestion of 60% casein diet in the LH, and the MH also showed a more rapid response to glucose infusion than to ingestion of the 5% casein diet (high-carbohydrate diet).
Fig. 7. Changes in 5-HIAA levels in dialysates from MH caused by single nutrient infusion. Rats were intravenously infused with 12% amino acids (a, n=7), 12.5% glucose (b, n=4) or 5% lipid (c, n=5) solution at a rate of 4 ml/h for 4 h. Values are M±SEM. * Significantly different from the values of basal, p<0.05; by one-way ANOVA and Fisher PLSD.

DISCUSSION

In this study, the nutrient-induced activation of serotonergic neurons was estimated by monitoring extracellular 5-HIAA levels in efflux of microdialysis tubes inserted into the hypothalamus and the striatum. Measurement of neurotransmitter levels in brain homogenate does not prove whether they are of intracellular or extracellular origin, but in vivo microdialysis makes it possible to monitor the extracellular levels of neurotransmitters released from nerve endings into the synaptic space in free-moving animals. Serotonin mainly undergoes oxidation to 5-HIAA by monoamine oxidase. The level of 5-HIAA in the extracellular space reflects serotonin reuptake (19) as well as release (9, 11). In fact, Auerbach et al.
showed that inhibition of serotonin release decreased extracellular 5-HIAA levels (19). Furthermore, it has been suggested that released and recaptured serotonin is the major source of extracellular 5-HIAA (20, 21). Thus, extracellular 5-HIAA may be a good indicator for the release and turnover of serotonin.

Our results show that rapid elevation of blood levels of amino acids and glucose independently increased 5-HIAA levels in different brain regions which participate in the regulation of feeding behavior. The high-protein diet increased the extracellular 5-HIAA levels in the LH, as did infusion of the amino acid solution but with a shorter lag time than the high-protein diet. The levels of 5-HIAA in the LH were not affected by the low-protein diet, glucose infusion or lipid infusion. These results suggest that serotonergic neurons in the LH may be sensitive to rapid elevation of the blood level of amino acids. Schwartz et al. (11), also reported that intraperitoneal injection of Trp increased serotonin and 5-HIAA levels in the LH. Kai et al. (22) reported that stimulation of the serotonergic neurons which arise from the dorsal raphe suppressed glucose-sensitive neurons in the LH, and suggested that this inhibitory action of serotonin may be associated with suppression of feeding. However, the mechanism by which the serotonergic neuron is stimulated has not been elucidated.

In contrast to the high-protein diet, the high-carbohydrate diet increased the extracellular 5-HIAA levels in the MH. Infusion of glucose, but not the amino acid solution or lipids, more rapidly induced the elevation of 5-HIAA levels than the high-carbohydrate diet, suggesting that serotonergic neurons in the MH may respond to rapid elevation of blood glucose. The MH has been suggested to have glucoreceptor neurons that are directly activated by glucose (23, 24). Leibowitz et al. (25) reported that serotonin injection into the MH resulted in a decrease in carbohydrate intake. In addition, MH lesions are known to induce hyperphagia and to increase carbohydrate intake (1, 16). These reports and our findings support the hypothesis that the stimulation of serotonergic neurons in the MH by glucose may be a satiety signal for carbohydrate intake. It was reported that MH lesions did not affect protein intake while it increased energy intake, suggesting that the MH may not be involved in the regulation of protein intake (16, 26). In the present study, serotonin metabolism in the MH was not affected by the high-protein diet or by the infusion of amino acids, supporting the foregoing suggestion with regard to serotonergic neurons.

Schwartz et al. (9) already showed that feeding stimulated serotonin release both in the LH and the MH as measured by in vivo microdialysis. However, nutritional and regional differences were not studied in their report. Our study shows that amino acids stimulate serotonergic neurons in the LH, while glucose in the MH does not, suggesting that serotonergic neurons might be involved in nutrient-dependent regulation of feeding.

In this study, we also found that the high-carbohydrate diet and infusion of glucose or lipids induced a significant increase in 5-HIAA levels in the ST, but the elevation started after stopping food intake or infusion. At present, we do not know
whether these elevations are related to the regulation of feeding or not. Although nigro-striatal dopaminergic neurons have been suggested to be involved in the regulation of feeding behavior (27), the role of serotonergic neurons in the ST has not been well documented. The administration of serotonin or serotonin agonists to the ST is an important next step to reveal the role of serotonergic neurons in the regulation of macronutrient intake.

The mechanism of nutrient-induced activation of serotonergic neurons seems to be complex. One possible explanation is that rapid elevation of plasma amino acids or glucose may increase the level of the serotonin precursor Trp in the brain. Transport of Trp into the brain is influenced by many factors. Rapid elevation of serum Trp is reported to elevate serotonin levels in the brain (10). The relative ratio of Trp to LNAA (Trp/LNAA) in the serum is suggested to determine the availability of Trp to the brain. Thus, rapid changes in serum amino acid levels may stimulate serotonin neurons through the elevation of brain Trp levels. Rapid elevation of blood glucose causes insulin release which stimulates the transport of Trp into the brain by elevating the Trp/LNAA ratio (12). Increasing the level of serum nonesterified fatty acids (NEFA) has been known to increase the serum free Trp level, because of the competitive effect of the serum NEFA on Trp-albumin binding (13). It has been reported that increasing the NEFA level did not elevate the brain Trp level in spite of the elevation of free Trp in the plasma (28). However, the many findings that free Trp influences the brain Trp level can not be disregarded (29-31). Further, our results showed the lipid-stimulated serotonin metabolism in the ST. Above reports together with our findings suggest that nutrient dependent activation of serotonergic neuron may exist in the brain.

Yokogoshi and Wurtman (32) reported that the protein-free diet-induced elevation of plasma Trp/LNAA ratio was inhibited by addition of 5% protein to the diet. However, Fernstrom et al. (33) reported that 6% and 12% protein increased the plasma Trp/LNAA ratio and Trp levels in the cortex and the hypothalamus. In our results, the levels of 5-HIAA in the ST and MH were elevated by the intake of the 5% casein diet, while the level of 5-HIAA in the LH was not altered. It was reported that there are regional differences in serotonin metabolism in the brain (30,34). Our results and the above reports suggest that the feeding-stimulated serotonin metabolism may not be similar in all areas of the brain. At present, the mechanism by which the regional differences in serotonin metabolism were produced is unknown.

We provide evidence of the selective activation of serotonergic neurons in different brain regions, but the effects of the serotonergic neurons in these brain areas on macronutrient intake remain to be elucidated. The administration of serotonin or serotonergic agents to these brain areas is needed to clarify the regulatory mechanism of nutrient intake by serotonergic neurons in the central nervous system.
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