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Centrally Administered Tryptophan Suppresses Food Intake in Free Fed Chicks through the Serotonergic System

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The effects of intracerebroventricular injection of L-tryptophan on feeding behavior and the levels of brain neurotransmitters (amino acids or monoamines) were investigated in *ad libitum* chicks. The tryptophan treatment (3 or 6 μmol) significantly inhibited food intake in chicks at 30 min postinjection. The levels of serotonin (5-HT) and its metabolite, 5-dihydroxyindolacetic acid, in chicks treated with tryptophan were significantly higher than those with saline at 15 min postinjection. However, there were no differences in the levels of catecholamines (adrenaline, noradrenaline and dopamine) and amino acid neurotransmitters (e.g., γ-aminobutyric acid, glycine and glutamic acid). The tryptophan-induced anorexia tended to be attenuated by the 5-HT_{2A} receptor antagonist ketanserin (10 μg). These results suggest that the administration of tryptophan into the chick brain produces the anorexic effect, and that the change in brain 5-HT content may be involved in this anorexia.

Key words: central nervous system, chick, food intake, tryptophan, serotonin

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Introduction

Several studies have shown that amino acid deficiencies or imbalances adversely affect food intake (e.g., Noble *et al.,* 1993; Choi *et al.,* 1996; Blair *et al.,* 2007). Anorexia after ingestion of an amino acid imbalanced diet has been postulated to be metabolic in origin, involving the changes in plasma amino acid pattern (Leung and Rogers, 1969). Neurotransmitter (catecholamines or serotonin) levels may also be affected by changes in plasma amino acids. Differences in these transmitters have been observed in relation to changes in plasma amino acid profiles (Fernstrom, 1983). It is thought that the changes are one of the causes of the anorexic effect of an amino acid imbalanced diet.

Tryptophan, the amino acid precursor of serotonin (5-hydroxytryptamine, 5-HT), has been shown to decrease feeding in the chicken (Lacy *et al.,* 1982). It has been assumed that the mechanism by which tryptophan affects food intake involves changes in central 5-HT concentrations because 5-HT levels in the brain can be modified with dietary manipulation of the intake of tryptophan (Pinchasov *et al.,* 1989). However, it is not known whether central administration of tryptophan induces anorexia in neonatal chicks, and comparable data is controversial and limited. Therefore, the purpose of this study is to examine the acute effect of central injection of L-tryptophan on feeding behavior, and to investigate the levels of brain amino acids or monoamines in chicks.

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Materials and Methods

Animals

Day old male layer chicks (Kudoh-sha Hatchery, Ehime, Japan; Akita Co. Ltd., Hiroshima, Japan) were kept in a windowless temperature-controlled room with 24-h lighting and maintained at a temperature of 30℃. They were given free access to a commercial starter diet (Nihon Nosan Kogyo, Yokohama, Japan; Nichiwa Sangyo Co. Ltd., Kobe, Japan) and water during the pre-experimental period. They were distributed into experimental groups based on their body weight so that the average body weight was as uniform as possible for each treatment. The birds were reared individually in experimental cages and had *ad libitum* access to food up to the time of experiments. The handling of birds was performed in accordance with the regulations of the Animal Experiment Committee of Hiroshima University, and the recom-

mendations of the National Research Council (1985).

Drugs and Intracerebroventricular (ICV) Infusion Protocols

Intracerebroventricular (ICV) injection was done according to Davis *et al.* (1979) and the solutions (10μL) were administered using a microsyringe. L-Tryptophan methyl ester and ketanserin (KTS: 5-HT_{2A} receptor antagonist) were purchased from Sigma (St. Louis, MO, USA). These drugs were dissolved in 0.85% saline, which contain 0.1% Evans Blue solution. The control group was given saline containing Evans Blue solution. Each chick was injected once only with saline or drug(s) solution. At the end of the experiments, birds were sacrificed by decapitation, after which the location of the injection site was confirmed. Data from individuals that were not verified by the presence of Evans Blue dye in the lateral ventricle were deleted. The number of birds used for data analysis is shown in each figure and table.

Effect of ICV Tryptophan on Food Intake

Chicks (3-day-old) were injected ICV with saline or tryptophan (3 or 6μmol) under *ad libitum* condition. Food intake was measured at 30 and 60 min after injection. The weight of feeders was measured using an electric digital balance of precision$\pm$1 mg.

Effect of ICV Tryptophan on the Concentrations of Brain Amino Acids

In this experiment, *ad libitum* birds (5-day-old) were administered by the ICV route with saline or tryptophan (3μmol). At 15 min after the injection, chicks were decapitated and had their brain rapidly removed. Brain diencephalon specimens were weighed, frozen on dry ice, and stored at -80℃ prior to analysis. The diencephalon that are involved in the regulation of feeding behavior, including the ventromedial, paraventricular (PVN) and arcuate hypothalamic nuclei and the lateral hypothalamic area (Kuenzel *et al.,* 1999), was dissected according to the brain atlas of chicks (Kuenzel and Masson, 1988). The tissue was homogenized with 2.0% w/v sulfosalicylic acid solution. The homogenate was centrifuged at 10,000 rpm for 60 min. Supernatants were filtered through a 0.22μm filter. Then, 50μL of the filtrate was applied to an Amino Acid Analyzer (Aminolyzer 21, SIC, Tokyo, Japan).

Effect of ICV Tryptophan on the Concentrations of Brain Monoamines

Brain samples for monoamine analysis were collected using the same procedure as in the aforementioned experiment. The tissue was homogenized with 0.1 N $HClO_4$ solution. The homogenate was centrifuged at 13,000 rpm for 15 min. Supernatants were filtered through a 0.22μm filter. The extractions were injected into a high performance liquid chromatography system (Tosoh, Tokyo, Japan) with a 150$\times$2.1 mm ODS column (CA-5ODS, EICOM, Kyoto, Japan) for the measurement of monoamines and their metabolites. Column temperature was kept at 40℃ by a thermocontroller (TSK CO-8000; Tosoh, Tokyo, Japan). The solvent delivery system (TSK CCPD; Tosoh, Tokyo, Japan) contained 2.5 mM 1-octanesulfonic acid sodium salt (SOS), 20μM Na_2 EDTA and 15% methanol in a 0.1 M phosphate buffer solution (0.1 M NaH_2PO_4: 0.1 M Na_2HPO_4=1000: 85). The pH of the buffer was adjusted to 3.0 with H_3PO_4. The buffer was filtered and degassed by degasser (TSK SD-8022; Tosoh, Tokyo, Japan) and then the flow rate adjusted to 180μL/min. The electrochemical detector (TSK EC-8020; Tosoh, Tokyo, Japan) was set at 750 mV and peak heights were measured using a computer integrator. All values were corrected for actual recovery based on the extraction rate of the internal standard isoproterenol.

Effect of ICV 5-HT_{2A} Receptor Antagonist on Tryptophan-Induced Anorexia

Birds (6-day-old) were injected by the ICV route with saline, tryptophan (3μmol) or tryptophan co-injected with KTS (5 or 10μg). The doses of antagonist applied here were decided according to the preliminary trials, in which they did not affect feeding behavior in *ad libitum* chicks. Food intake was measured at 30 min postinjection.

Statistical Analysis

ANOVA was used to determine overall statistical significance due to treatment. When a treatment effect was significant, the Turkey-Kramer test was used to compare the significance among means. Statistical significance was set at $p<0.05$. Data were expressed as means$\pm$SEM.

Results

Effect of ICV Tryptophan on Food Intake

The effect of ICV administration of tryptophan (3.0 and 6.0μmol) on food consumption in chicks fed *ad libitum* over a 60-min period is shown in Fig. 1. Both levels of tryptophan suppressed feeding behavior in chicks at 30 min postinjection when compared with control ($p<0.05$), but the differences were not significant at 60 min.

Effect of ICV Tryptophan on the Concentrations of Brain Amino Acids

After 15 min of treatment, the levels (ng/mg wet tissue) of amino acid neurotransmitters in saline-treated and tryptophan-treated chicks did not differ significantly (γ-aminobutyric acid: 4.16$\pm$0.43 [n=6] vs. 4.35$\pm$0.54 [n=5]; glycine: 1.88$\pm$0.19 vs. 2.14$\pm$0.28; glutamic acids: 5.78$\pm$0.39 vs. 5.90$\pm$0.69). Also, the significant differences between treatments were not detected in 16 other amino acids (alanine, arginine, aspartic acid, cysteine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, taurine, threonine, tyrosine and valine) (data not shown).

Effect of ICV Tryptophan on the Concentrations of Brain Monoamines

Table 1 shows the result of the effect of ICV injection of tryptophan on the concentrations of monoamines and their metabolites in brain tissue at 15 min after the injection. The concentrations of L-DOPA, noradrenalin (NA), adrenalin (A), dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC) were not significantly different between the control and the tryptophan treatment groups. However, 5-HT, 5-hydroxyindole, 3-acetic acid

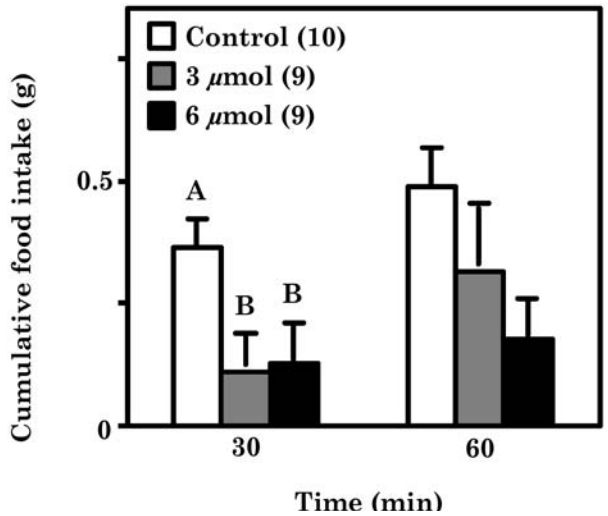

Fig. 1. **Cumulative food intake of the *ad libitum* chick injected intracerebroventricularly with saline or one of two doses of tryptophan (3 or 6 μmol).** Values are means ±SEM of the number of chicks in parentheses. Means with different letters are significantly different at $p < 0.05$.

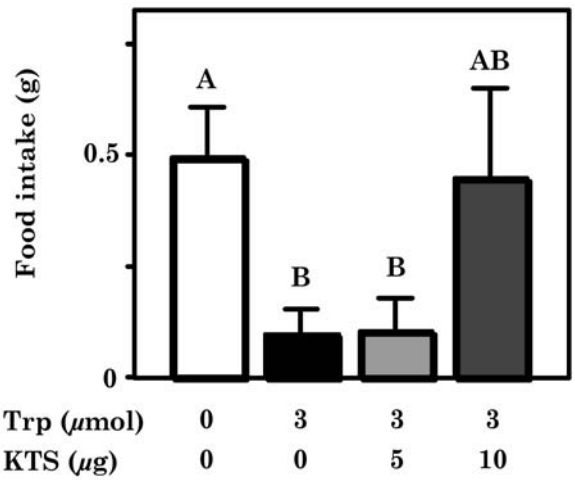

Fig. 2. **Food intake during the 30-min period postinjection of the *ad libitum* chicks intracerebroventricularly injected with saline, tryptophan (Trp; 3 μmol) or tryptophan co-injected with two doses of ketanserin (KTS), 5-HT_{2A} receptor antagonist (5 or 10 μg).** The number of chicks in each group was as follows: control, 7; Trp alone, 5; Trp+KTS (5 μg), 5; Trp+KTS (10 μg), 4. Data are expressed as means±SEM. Means with different letters are significantly different at $p < 0.05$.

(5-HIAA) and 4-hydroxy-3methoxyphenylacetic acid (HVA) significantly increased in chicks treated with tryptophan ($p < 0.01$, $p < 0.001$ and $p < 0.05$, respectively). The values for HVA/DA and 5-HIAA/5-HT were also higher in the tryptophan treated group than in the control group ($p < 0.001$ and $p < 0.01$, respectively), while there was no significant difference in the ratio of DOPAC/DA between the groups.

Effect of ICV 5-HT_{2A} Receptor Antagonist on Tryptophan-Induced Anorexia

The effect of ICV injection of 5-HT_{2A} antagonist on tryptophan-induced hypophagia for 30 min is shown in Fig. 2. Although the differences were not significant when compared with the use of tryptophan alone, co-injection of KTS (10 μg) showed a tendency to recover food intake from tryptophan-induced hypophagia in chicks.

Table 1. **Effect of intracerebroventricular injection of tryptophan on brain monoamines and their metabolite concentrations in chicks**

	Control	Tryptophan
L-DOPA (pg/mg)	60.82± 4.21	63.79± 3.49
NA (pg/mg)	511.88±34.51	502.39±35.95
A (pg/mg)	198.80± 8.57	184.21± 8.46
DA (pg/mg)	171.45±10.75	149.10± 6.65
DOPAC (pg/mg)	9.80± 0.94	10.23± 1.84
HVA (pg/mg)	46.31± 1.46	54.32± 2.39*
5-HT (pg/mg)	1517.30±46.31	1922.32±93.33**
5-HIAA (pg/mg)	62.14± 4.20	131.61±11.52***
DOPAC/DA	0.059±0.009	0.068±0.011
HVA/DA	0.273±0.014	0.364±0.006***
5-HIAA/5-HT	0.041±0.004	0.068±0.004**

NA, noradrenalin; A, adrenalin; DA, dopamine; DOPAC; 3,4-dihydroxyphenylacetic acid; HVA, 4-hydroxy-3methoxyphenylacetic acid; 5-HT, serotonin; 5-HIAA, 5-hydroxyindole, 3-acetic acid.
Data were presented as means±SEM of 5 chicks per groups.
*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with control group.

Discussion

The present results show that central injection of tryptophan suppressed feeding behavior (Fig. 1), and increased the hypothalamic concentration of 5-HT in neonatal chicks (Table 1). It is well known that central administration of 5-HT induces anorexia in chicks (Sashihara *et al.,* 2002) and chickens (Denbow *et al.,* 1986). In rats, it was suggested that 5-HT_{2A} receptors were involved in feeding-suppressant effect of 5-HT (Hewson *et al.,* 1988). We also found that the attenuation of tryptophan-induced anorexia by the 5-HT_{2A} receptor antagonist (Fig. 2). Thus, it seems that tryptophan injected into the brain of chicks was promptly converted to 5-HT, and induced hypophagia via 5-HT_{2A} receptors. The increased level of 5-HIAA (Table 1) is also good evidence to support the postulation mentioned above.

Among a number of neurotransmitters, hypothalamic 5-HT contributes to satiety (Leibowitz and Alexander, 1998). The synthesis of brain 5-HT depends on the brain availability of its precursor, tryptophan (Schaechter and Wurtman, 1990), which parallels plasma free tryptophan concentrations (Fernstrom and Wurtman, 1972; Landel *et*

al., 1987). Increased plasma as well as brain tryptophan concentrations and brain serotonergic activity occur in experimental animal models with anorexia (Kurzer *et al.,* 1988; Meguid *et al.,* 1992). In the early studies, Lacy *et al.* (1986a, 1986b) found that systemic injections of tryptophan inhibited food intake in the domestic fowl. Additionally, 5-HT levels in the brain can be modified with dietary manipulation of the intake of tryptophan (Pinchasov *et al.,* 1989). Collectively, central tryptophan, which is a precursor for synthesis of 5-HT, might be involved in appetite impairment after ingestion of an excessive tryptophan diet in chicks.

Although the action site of 5-HT in central nervous system did not identified in this study, it is possible that serotonin acts at the PVN. It has been reported that 5-HT infusion in the PVN causes changes in meal patterns suggesting the introduction of satiety (Shor-Posner *et al.,* 1986), and there are some serotonin-containing fibers in the PVN that have been shown to overlap with neurons of corticotropin-releasing factor (CRF), which is a potent anorexigenic factor (Liposits *et al.,* 1987). Recently, it was implied that serotonin might facilitate CRF release in the brain of chicks (Zhang *et al.,* 2004). They also indicated that central injection of serotonin induced sedation in chicks. Thus, the possibility exists that sedative effect of 5-HT may partially involve in the anorexia. The relationship between feeding behavior and sedation in chicks remains to be studied.

Increased levels of some amino acids in the brain, such as that resulting from central injection, could influence amino acid metabolism and the related enzymes, and change the levels of other amino acids (e.g., Hashimoto, 2002; Asechi *et al.,* 2006). However, the present study reveals that tryptophan treatment did not affect the levels of brain amino acids in chicks. From these results, it is possible that any amino acids acting as neurotransmitters or neuromodulators may be not contributed to short term hypophagia due to tryptophan in chicks.

Besides 5-HT and its metabolite, the concentration of HVA, but not DOPAC, was increased by ICV injection of tryptophan (Table 1). The HVA levels reflect the overall metabolism of DA (both intraneuronal by monoamine oxidase and extraneuronal by catechol-O-methyl transferase) while the DOPAC level is believed to reflect primarily the intraneuronal DA metabolism (Guldberg and Marsden, 1975; Roffler-Tarlov *et al.,* 1971). Thus, our result implies that tryptophan treatment might stimulate the release of DA. Because the hypothalamic DAergic neurons express tryptophan hydroxylase, the serotonin-synthesizing enzyme in these neurons (Vanhatalo and Soinila, 1999), it is likely that stimulated expression of tryptophan hydroxylase induces the release of DA. In any case, it is assumed that the anorexic effect of tryptophan might not be due to the releasing DA because central injection of DA had no effect on feeding behavior in chicks (Bungo *et al.,* 2001).

Tryptophan is also converted into kynurenines in astrocytes and neurons, and acts as an endogenous excitatory amino acid receptor ligand in brain regions with neuronal damage and concomitant *N*-methyl-D-aspartate receptor hyperexcitability (Ganong and Cotman, 1986; Giles *et al.,* 2003; Hilmas *et al.,* 2001). These metabolites may possibly be related to long term hypophagia due to high dietary levels of tryptophan in chicks. Further research on the metabolism of tryptophan in chick brains is needed to understand the role of tryptophan in central regulation of appetite in avian species. However, the results described here suggest that central tryptophan influences the activity of the serotonergic system in the brain of chicks. In addition, these changes in brain monoamine content may be involved in tryptophan-induced hypophagia in neonatal chicks.

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