

INTRACEREBROVENTRICULARLY ADMINISTERED PHENYLALANINE AND TYROSINE: EFFECTS ON FEEDING BEHAVIOR AND NOREPINEPHRINE CONCENTRATIONS OF SPECIFIC BRAIN SITES IN THE CHICKEN

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Summary

A study was carried out to investigate the action of central L-phenylalanine (Phe) and L-tyrosine (Tyr) on food intake of the chicken. In the first trial, Phe (200 $\mu\text{g}/10 \mu\text{l}$) or saline was acutely administered into the right lateral ventricle (i.c.v.) of chickens (5 birds per each group). Birds (4 birds per each group) were administered with the i.c.v. Tyr (200 $\mu\text{g}/10 \mu\text{l}$) or saline in the second trial. The brains of the birds were removed for catecholamine assay 30 min postadministration. Catecholamine concentrations were measured at specific sites of the brain (LH: lateral hypothalamus, PVN: paraventricular nucleus, and VMH: ventromedial hypothalamus). No significant effect of amino acids on the concentration of norepinephrine of brain sites investigated was detected. Food intake and rectal body temperature were also monitored for 6 h after central administrations of Phe, Tyr or saline (5 birds per each group). Both Phe and Tyr, up to 1 mg/10 μl , failed to modulate food intake or rectal body temperature.

(Key Words : Phenylalanine, Tyrosine, Food Intake, Chicken, I.C.V. Injection)

Introduction

Animals tend to decrease food intake when given a diet containing a large amount of single essential amino acid (EAA) (Harper et al., 1970; Li and Anderson, 1983; Longton, 1978; Okumura et al., 1980; Ueda et al., 1981). Ingestion of a diet excessive in an EAA increases the plasma concentration of the amino acid and alters the profile of amino acids in the brain because various amino acids share common carrier transport systems for uptake across the blood-brain barrier (Anderson, 1979; Li and Anderson, 1983; Pardridge, 1977).

An L-phenylalanine Phe-excess diet not only inhibits feeding behavior expressed as food intake (Okumura et al., 1980; Okumura and Yanaka, 1980), but also elevates concentrations of Phe and L-tyrosine (Tyr) in the plasma (Kerr and Waisman, 1967) and brain (Elkin and Rogler, 1983). Since Tyr, the precursor of catecholamines, can be

biosynthesized from Phe by hepatic Phe hydroxylase, and both Phe and Tyr in high concentrations act as competitive substrates for Tyr hydroxylase (Badawy and Williams, 1982; Wurtman et al., 1974), a Phe-excess diet may influence monoamine concentrations in the brain.

Denbow et al. (1981) reported that intracerebroventricular (i.c.v.) injections of epinephrine significantly increased food intake whereas dopamine had no effect. The i.c.v. injection of norepinephrine (NE) caused a narcoleptic response, but the NE stimulated food intake in birds who did not show a narcoleptic response. More recently, the circumscribed brain sites at which NE either stimulates or inhibits food intake was clarified in the chicken (Denbow and Sheppard, 1993). We found that central injection of clonidine, an α_2 -agonist, increased food intake in chickens (Choi et al., 1995). However, whether the i.c.v. administration of the catecholamine precursors (i.e., Phe and Tyr) alters feeding behavior has not been investigated in chickens. It was hypothesized that Phe-excess diets may suppress food intake in the chicken by rapidly elevating Phe levels in the brain and influencing neurohumoral systems involved in food-intake control.

Materials and Methods

Birds and operation

Day-old broiler chicks of mixed sexes were purchased

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from a local hatchery (Fusoen, Aichi, Japan). Birds were maintained in a room with 24 h light and 20°C, and given free access to water and a commercial broiler starter (Nihon Nosan Co. Ltd., Tokyo, Japan) *ad libitum*, unless otherwise mentioned.

At four weeks of age, the birds were anesthetized with sodium pentobarbital (25 mg/kg BW, i.v.), placed in a stereotaxic apparatus and chronically implanted with stainless steel guide cannulae (23 G) at the coordinates lateral 0.7 mm, anterior 6.7 mm and the dura below 3.5–4.0 mm. The cannulae were secured to the skull with four stainless steel machine screws and dental cement. A stylet (27 G) was then inserted into the guide cannula to occlude between experiments (Choi et al., 1994; Denbow et al., 1981). The birds were allowed at least 1 week to recover from the operation and to become accustomed to the experimental procedure. Location of the cannula was verified by overflowing the cerebrospinal fluid, and injecting Evans Blue (Wako Pure Chemicals, Osaka, Japan) into the lateral ventricle and anatomically slicing the brain tissue after the experiments were finished.

Brain sampling and catecholamine assay

Phe and Tyr were administered i.c.v. in order to study whether i.c.v. Phe or Tyr affect catecholamine concentrations at the specific brain sites (lateral hypothalamus (LH), paraventricular nucleus (PVN) and ventromedial hypothalamus (VMH)). In the first trial, Phe (200 µg/10 µl; n = 5) or 0.85% NaCl (n = 5) was injected into the lateral ventricle of chickens fasted for 3 h. The birds were killed by slitting their necks 30 min postadministration, and the brains were removed within 5 min, frozen in dry ice and stored in -80°C until assay. The second trial was performed under similar conditions to the previous trial except for Tyr (n = 5 per each group), which was prepared equimolar to Phe (200 µg/10 µl). The PVN, VMH and LH were dissected immediately before analysis. Frozen brain samples were placed on slides over dry ice. The rostral part was cut away with a microtome blade until the face corresponding to A 7.6 of the brain atlas of Kuenzel and Masson (1988) appeared. Three nuclei were dissected bilateral in the thick of 2 mm with a scalpel. The optic chiasm, optic tract, third ventricle, lateral ventricle, and tractus occipitomesencephalicus were used as a landmarks for identification of nuclei. After the nuclei were dissected, they were put into a Potter-Elvehjem type micro glass homogenizer and weighed. Nuclei were homogenized in the cold bath and 100 µl of 0.05 M cold perchloric acid (HClO₄) solution with cysteine as an antioxidant and dihydroxybenzylamine as an internal standard was added to the sample twice.

The extraction was placed in a micro-test tube and centrifuged at 8000 × g for 5 min. The supernatant was then placed in a centrifuge-filtration unit (Millipore, Ultra Free C3-GV) and centrifuged at 8000 × g for 1 min. The filtrate of 100 µl was injected into the HPLC.

The mobile phase consisted of 0.1 M NaH₂PO₄ buffer containing 1.0 × 10⁻⁴ M EDTA, 2.75 × 10⁻³ M octane sulfonic acid, 2.5 × 10⁻⁴ M triethylamine and 50 g/l methanol. The pH was adjusted to 3.10 with H₃PO₄ (3 M). The mobile phase was filtered through a 0.45 µm filter. The flow rate was 1 ml/min. The HPLC system used consisted of a model PU-980 solvent delivery pump, DG-980-50 degasser, Chathecolpak (150 × 4.6 mm i.d.) column and a model 840-EC electrochemical detector from Nihon Bunko Co. (Tokyo, Japan). NE was oxidized with an applied potential of 0.7 V against platinum-silver chloride reference cell. Chromatograms were monitored, recorded and analyzed with a model 807-IT integrator from Nihon Bunko. Peaks were measured by area and compared with daily calibrations. All chemicals were either HPLC or analytical grade. Stock solution with 1–2 mg/100 ml of NE was prepared in 0.05 M HClO₄ containing 8.3 × 10⁻³ M cysteine as an antioxidant. The standard solution was obtained by diluting the stock solution 50 times with 0.05 M HClO₄ with cysteine.

Feeding trials

Several pilot experiments were carried out to study feeding response to graded doses (including 200 µg/bird) of i.c.v. Phe. It was found that food intake was not significantly affected by a level of Phe as low as 400 µg/bird. Therefore, a higher level (1 mg/bird) of Phe or Tyr was administered into the brain of chickens, and food intake and rectal body temperature were monitored for 6 h. Phe (Sigma) and Tyr (Aldrich) solutions were prepared as their methyl ester HCl in 0.85% NaCl saline. Before i.c.v. injection, birds were fasted for 1 h. All injections were done between 10:00 and 12:30. Solutions were injected in a volume of 10 µl over 30 seconds and the injection cannula was in place for an additional 1 min before removal. The birds were returned to their cages and given a diet to monitor food intake.

Statistical analysis

Data were subjected to two way ANOVA (NE assay) and a split plot design (feeding trials) by taking bird as a main plot and time as a subplot by General Linear Model procedure using the procedure of SAS (1985), and comparisons between means were made. The results are indicated as means ± SEM.

Results

Figure 1 shows NE concentrations at specific brain sites 30 min after i.c.v. administration of Phe at a level of

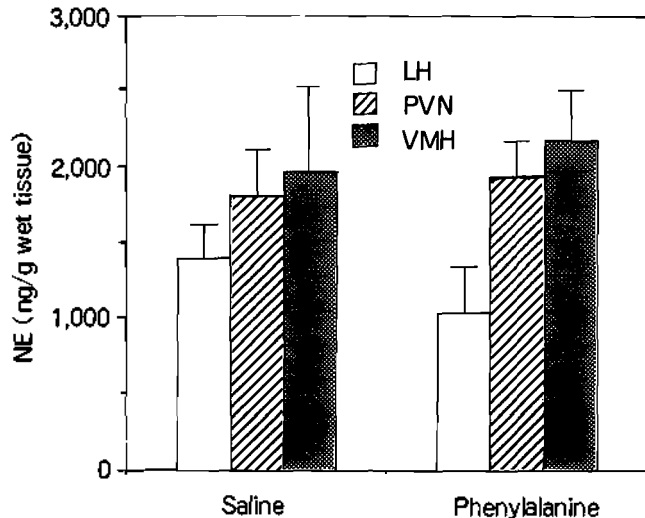


Figure 1. Norepinephrine (NE) concentrations in the specific brain sites 30 min after intracerebroventricular administrations of phenylalanine at a level of 200 $\mu\text{g}/10 \mu\text{l}$. Data indicate the means \pm SEM of 5 birds. LH: lateral hypothalamus, PVN: paraventricular nucleus, VMH: ventromedial hypothalamus. The effect of phenylalanine was not significant. VMH vs. LH $p < 0.02$.

administration of Tyr at a level of 200 $\mu\text{g}/10 \mu\text{l}$. There were no effects of Tyr on NE levels within brain sites.

The results from feeding trials are shown in figure 3. Neither Phe nor Tyr, up to 1 mg/10 μl , significantly affected food intake. Rectal body temperature was not significantly altered by i.c.v. administration of Phe or Tyr (figure 4).

Discussion

The present results showed that the i.c.v. administration of Phe and Tyr had no effect on feeding behavior and NE concentrations of specific brain sites in the chicken (figure 3). The reason why i.c.v. Phe or Tyr failed to influence these responses is unclear, but some hypothesis may be considered. Since a major route of Phe metabolism in animals is to be hydroxylated to Tyr by Phe hydroxylase in the liver, which is not present in the brain, Phe may not be converted to Tyr in the brain.

200 $\mu\text{g}/10 \mu\text{l}$. There were no significant effects of Phe within a brain site, although there were significant differences between brain sites ($p < 0.02$). Figure 2 shows NE concentrations at specific brain sites 30 min after i.c.v.

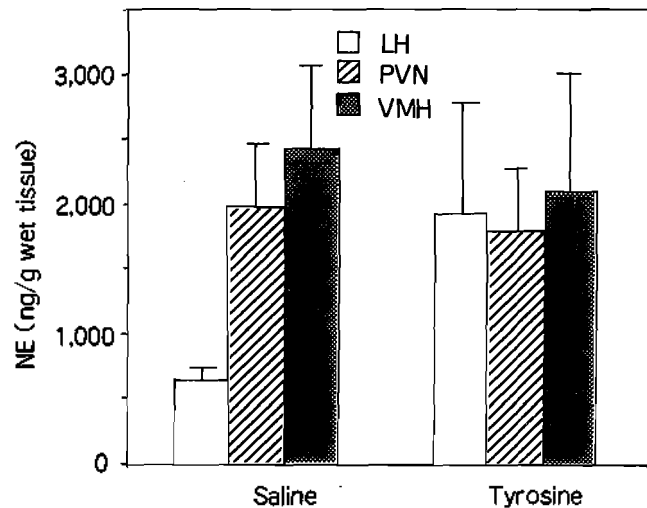


Figure 2. Norepinephrine (NE) concentrations in the specific brain sites 30 min after intracerebroventricular administrations of tyrosine at a level of 200 $\mu\text{g}/10 \mu\text{l}$. Data indicate the means \pm SEM of 4 birds. LH: lateral hypothalamus, PVN: paraventricular nucleus, VMH: ventromedial hypothalamus. No significant effects were observed.

However, Tyr hydroxylase and tryptophan hydroxylase can catalyze Phe to Tyr in the brain to a minor extent. Therefore, it is possible that i.c.v. Phe may be either absorbed into the brain tissues or metabolized by way of some minor pathways of degradation (Mosnaim et al., 1980). However, the latter is not the case since the pathways will not be activated in normal animals given a normal diet. Anyway, Phe or Tyr in the lateral ventricle hardly affected on the NE contents of the important nuclei investigated.

There may be different effects between Phe-excess diet and i.c.v. Phe on amino acid concentrations in the brain. In the former, Phe levels in the plasma as well as brain increase with ingestion. Increased Phe in plasma competes with other large neutral amino acids (LNAAs) across the blood-brain barrier for uptake into the brain (Pardridge, 1977). Therefore, there may be a deranged amino acid profile with increased plasma Phe causing a decrease in other LNAAs (e.g., valine, leucine, isoleucine) in the brain, which decrease brain protein synthesis (Binek-Singer and Johnson, 1982), being important in controlling

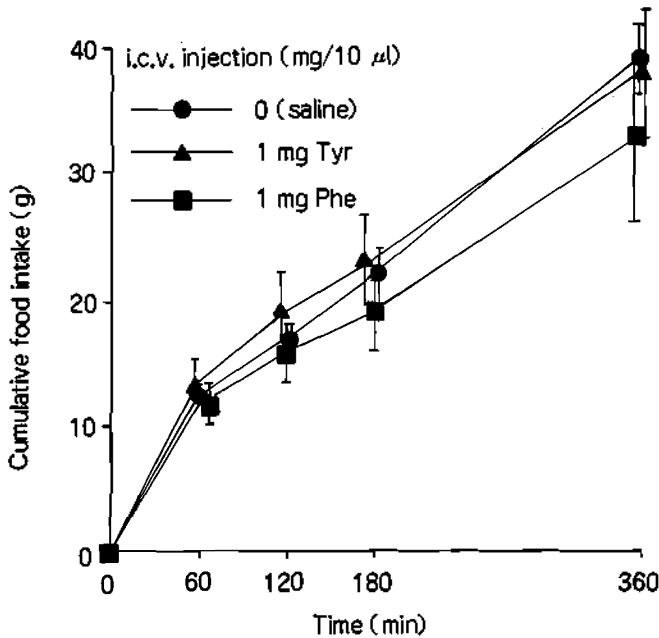


Figure 3. Food intake in broiler chickens intracerebroventricularly (i.c.v.) injected with L-phenylalanine (Phe) or L-tyrosine (Tyr) at a level of 1 mg/10 μ l. Data indicate the means \pm SEM of 5 birds. No significant effects were observed.

food intake. In case of the latter, while i.c.v. injection of Phe may increase Phe level in the cerebrospinal fluid after injection, Phe levels gradually decreases, probably with absorption into the brain tissues as previously mentioned. In fact, even on a Phe excess diet, the depressed food intake was not observed until at least a few hours after feeding (Y. H. Choi, M. Furuse, K. Asakura and J. Okumura, unpublished data). It was implied that accumulation of large amount of Phe in the brain was required for the depressed food intake.

Since birds were fasted for 1 h and the food ingested before fasting may be present in the digestive tract of the birds, it is not possibly expected that the 1 h-food deprivation had an influence on amino acid profiles in plasma. The effect that decreases food intake is higher in low protein-than in high protein-diets with a large amount of a single amino acid (Muramatsu et al., 1971; Okumura et al., 1980). Because the chickens were given a commercially available diet (21% protein diet), it is likely that LNAA levels in plasma are not affected, and they entered the brain at a constant rate without influencing the amino acid profiles in the brain. Therefore, the concentrations of i.c.v. Phe in a condition where animals ingest a normal diet, would be low to affect short-term food intake in the chicken.

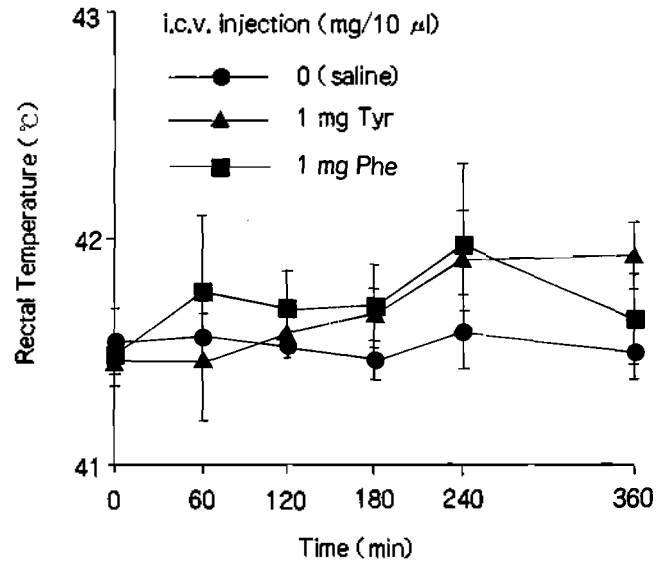


Figure 4. Rectal body temperature after i.c.v. administration of L-phenylalanine (Phe) or L-tyrosine (Tyr) at a level of 1 mg/10 μ l. Data indicate the means \pm SEM of 5 birds. No significant effects were observed.

Although there are some reports that catecholamine levels in the brain can be controlled by brain Tyr concentrations (Gibson and Wurtman, 1977, 1978; Fernstrom and Fernstrom, 1994; Wurtman et al., 1974) and by brain Phe levels (Gibson and Wurtman, 1977; McKean, 1972; Wurtman et al., 1974), it is not surprising that i.c.v. Phe or Tyr at a level of 1 mg had no effect on both food intake and body temperature in the present study, since Tyr levels and catecholamine synthesis in the central nervous system appear to be at an upper plateau when protein intake is at or above requirement levels (Fernstrom and Fernstrom, 1994), and since i.c.v. injections of some catecholamines failed to change food intake and body temperature in both broiler and Leghorn chickens (Denbow et al., 1981, 1983).

In conclusion, it was suggested that centrally acute loading (up to 1 mg) of Phe or Tyr did not affect feeding behavior in the chicken.

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