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Comparison of Hypothalamic Monoamine Contents of Broiler- and Layer- Type Chickens at Prehatch and Posthatch

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Domestic chickens, which are precocial, have relatively well developed mechanisms of food intake regulation at hatch. Although their body weight is similar at hatch, broiler- and layer-type chickens have different growth rates and food intake following hatch. The purpose of the present study was to compare the hypothalamic content of the monoamines norepinephrine (NE), epinephrine (E), dopamine (DA) and serotonin (5-HT), and their metabolites dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylacetic acid (HVA) and 5-dihydroxyindolacetic acid (5-HIAA) between these strains on day 18 of incubation, and at 0 day-of-age. In both strains, 5-HT and 5-HIAA levels increased with age. On day 18 of incubation, the amounts of NE and E were almost the same between the two strains. Thereafter, the content of both monoamines rapidly increased at hatch in both strains, and layer-type chicken embryos had significantly higher E levels compared to broiler-type chicken embryos. The levels of DOPAC and HVA on day 18 of incubation were higher in broiler-type chicken embryos than in layer-type chicken embryos, but these differences were reduced at hatch. These changes at hatch may partly explain the difference in the performance between the two strains.

Key words : monoamines, monoamine metabolites, hypothalamus, broiler-type chicken, layer-type chicken

Introduction

Modern broiler-type chickens have been intensively selected for rapid body weight gain and high meat yield, whereas layer-type chickens have been selected for higher egg production. As a result, broiler-type chickens grow more rapidly than layer-type chickens so that at any given age broiler-type chickens have greater muscle mass than layer-type chickens (Aberle and Stewart, 1983). This is especially evident in breast muscle in which broiler-type chickens can have up to 8 times the mass of layer-type chickens (Bulfield *et al.,* 1988).

The differences in the growth rate could be partly explained by the differences in food intake between the two strains. In fact, food intake and growth rate are 2- to 3-

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fold greater in broiler- than in layer-type chickens (Masic *et al.,* 1974 ; Mahagna and Nir, 1996), as is the rate of consumption (Hocking *et al.,* 1997). Broiler- and layer-type chickens tend to spend a similar proportion of time feeding but have very different daily food intakes associated with their different body weight gains (Masic *et al.,* 1974 ; Savory, 1974 ; Hocking *et al.,* 1993)

It is well known that feeding behavior is regulated by the central nervous system. Especially, the hypothalamus plays an important role in the feeding regulation in chickens because electric lesions in the hypothalamic regions altered food intake (Lepkovsky and Yasuda, 1966). Several factors are involved in the regulation of feeding, among them, the central nervous system, catecholaminergic and serotonergic systems, have received considerable attention. For instance, intracerebroventricular (ICV) injection of six-hydroxydopamine, which destroys catecholaminergic systems, induces the reduction in food intake (Kuenzel *et al.,* 1987). Furthermore, ICV injection of fusaric (5-butylpicolinic) acid, an inhibitor of rate-limiting enzyme of norepinephrine (NE) synthesis, increases food intake in chicks (Bungo *et al.,* 1999). In addition, microinjections of NE into the specific hypothalamic nuclei stimulated food intake of chicks (Denbow and Sheppard, 1993). Tachibana *et al.* (2001) investigated that NE and 5-HT levels increased within the hypothalamus of chick during feeding.

For the reasons mentioned above, it was suggested that the differences of feeding behavior betweens the two strains might be induced by the differences of catecholaminergic and serotonergic system of the hypothalamus. If this may be true, the hypothalamic monoamines contents vary in an early developmental stage between the two strains. Therefore, the difference in development of the catecholaminergic and serotonergic systems of the brain were examined in both strains. This experiment was conducted to investigate whether the monoamine contents of the hypothalamus differ between the two strains before and at hatch.

Materials and Methods

Eggs

Fertilized eggs were obtained from Mori hatchery, Fukuoka, Japan (broiler-type : Cobb) and Murata hatchery, Fukuoka, Japan (layer-type : Julia), and were incubated at 37.6℃ and a relative humidity of 58 to 68%. They were candled at day 10 of incubation to remove those that were infertile or contained dead embryos.

Analysis

Chick embryos on day 18 of incubation and chicks at 0 day-of-age were sacrificed with an overdose of urethane, and decapitated. Thereafter, the brains were rapidly removed within a minute, frozen on powdered dry ice, weighed, and stored at −85℃ for further analysis. The hypothalamus was dissected referring to a stereotaxic atlas drawn by Kuenzel and Masson (1988) and weighed. Briefly, the rostral part of the frozen brain was removed with a scalpel up to the level including anterior commissure. The whole hypothalamus was dissected in size of 2.0 mm×2.0 mm×2.0 mm, as follows : 2.0 mm posterior to anterior commissure, 1.0 mm bilateral to the midline, 2.0 mm dorsal to the chiasma opticum, and then, weighted. The levels of NE, E, dopamine

(DA), 5-HT and the amine metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 4-hydroxy-3-methoxyphenylacetic acid (HVA) and 5-dihydroxyindolacetic acid (5-HIAA) were determined by high-performance liquid chromatography (HPLC) system. The monoamines were extracted according to the method described by Sugahara *et al.* (1999). Briefly, the tissue was homogenized in 0.05 M ice-cold perchloric acid containing isoproterenol as an internal standard with low temperature (0–4℃). The homogenate was centrifuged at 10,000×g, 4℃ for 4 min, and the supernatant was centrifuged with a centrifuge-filtration unit (Ultra Free C3-GV, Millipore, Bedford, MA, USA) at 10,000×g, 4℃ for 4 min. The 30 μl filtrate was injected into a HPLC system (Eicom, Kyoto, Japan) with a 150×2.1 mm ODS column (MA-5ODS, Eicom, Kyoto, Japan) for measurement of monoamine contents. The mobile phase consisted of 1.0 M aceto-citric acid buffer, 2.3 mM sodium 1-octane sulfonate, 1.0 mM disodium ethylenediaminetetraacetic acid and 17% methanol. The pH was adjusted to 3.5. Monoamines were detected using an electrochemical detector (ECD-300, Eicom, Kyoto, Japan) at an applied potential of +0.70 V. The external standard was used to identify peaks eluting in the chromatogram according to retention time and conformation. The monoamine and metabolite contents were determined by integration of peak areas. The detection limits of the system for all monoamines were 0.1 pg/sample.

The number of embryos and chicks used for this analysis were as follows: broiler-type chicken embryos 12 and layer-type chicken embryos 8 on 18 days of incubation, broiler-type chicks 10 and layer-type chick embryo 8 at 0 day-of-age. The results were analyzed by a two-way analysis of variance using a commercial package, Stat View (version 5, SAS Institute, Cary, USA, 1998). To compare the group mean at each developmental stage, t-test was also done.

Experimental procedure followed the guidance for Animal Experiments in Faculty of Agriculture and in the Graduate Course of Kyushu University and the Law (No. 105) and Notification (No. 6) of the Government.

Results

Embryo and body weights, and whole brain weights are shown in Table 1. A significant ($P<0.01$) interaction between strain and age was observed in which body weight at hatch was comparable between the two strains, but broiler-type chicken embryos were heavier than layer-type chicken embryos on day 18 of incubation. No significant differences in whole brain weight between the two strains were observed, but brain weight significantly ($P<0.0001$) increased in both strains at hatch compared to day 18 of incubation. The interaction in brain weight between strain and age approached significance ($P=0.058$). This was due to a large change in the brain weight of layer-type chicken embryos.

The contents of hypothalamic monoamines and their metabolites are shown in Figures 1 and 2. In hypothalamic catecholaminergic monoamines, broiler- contained more DA compared to layer-type chicken embryos while there were no differences in NE and E contents. At 0 day-of-age, the hypothalamic E level of layer-type chicks was higher than in broiler-type chicks. The level of DOPAC was higher in broiler-type

chicken embryos than in layer-type chicken embryos at 18 days of incubation (Fig. 1). There were no significant differences in 5-HT or 5-HIAA contents between the two strains at either age (Fig. 2). Both catecholaminergic and serotonergic monoamine contents rapidly increased at hatch in both strains.

The ratios of DOPAC/DA and 5-HIAA/5-HT were calculated (Table 2). The value for DOPAC/DA significantly decreased toward hatch ($P<0.0001$), while there

Table 1. Body and brain weights of broiler and layer

	Day 18 of incubation		0 day-of-age	
	Broiler-type	Layer-type	Broiler-type	Layer-type
Number	12	8	10	8
Body weight (g)	27.0±0.5	19.1±1.2*	47.8±0.7	45.5±1.3
Brain weight (g)	0.793±0.070	0.732±0.021	0.898±0.026	0.919±0.016

Asterisk indicates a significant difference between broiler- and layer-type chicks at the same age at $P<0.05$. Values are means±S.E.M.

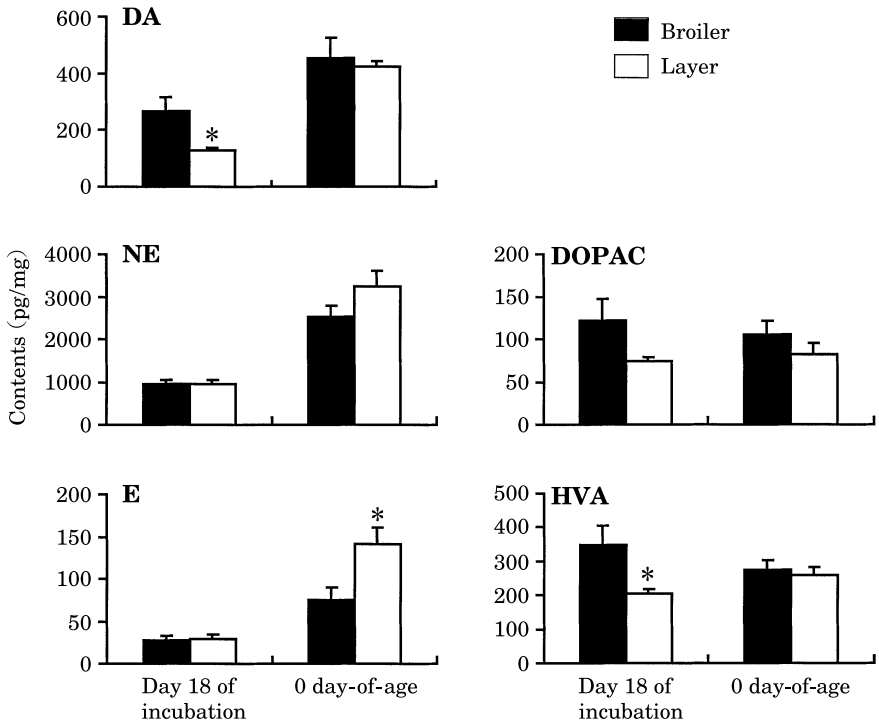

Fig. 1. Catecholaminergic monoamines and their metabolites in layer- and broiler-type chickens at pre- and post-hatch. Asterisk indicates a significant difference between broilers and layers at the same age at $P<0.05$. Values are means±S.E.M. Numbers of samples were : broiler-type chicken embryos 12 and layer-type chicken embryos 8 on 18 days of incubation, broiler-type chicks 10 and layer-type chick 8 at 0 day-of-age.

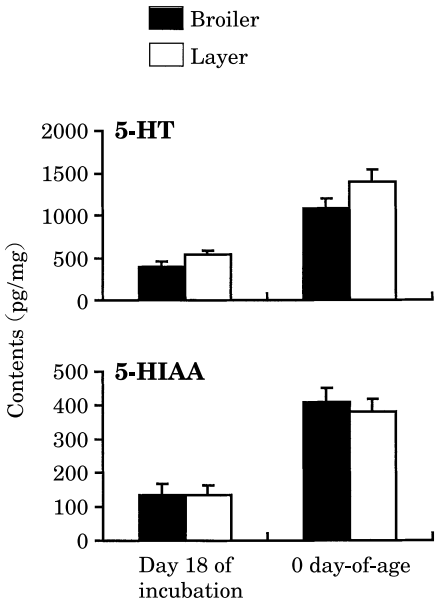

Fig. 2. Serotonergic monoamines and their metabolites in layer- and broiler-type chickens at pre- and post-hatch. Values are means±S.E.M. Numbers of samples were : broiler-type chicken embryos 12 and layer-type chicken embryos 8 on 18 days of incubation, broiler-type chicks 10 and layer-type chick 8 at 0 day-of-age.

Table 2. The ratio of DOPAC/DA and 5-HIAA/5-HT

Ratio	Strain			
	Broiler-type		Layer-type	
	Day 18 of incubation	0 day-of-age	Day 18 of incubation	0 day-of-age
DOPAC/DA	0.622±0.188	0.244±0.061	0.644±0.243	0.202±0.076
5-HIAA/5-HT	0.440±0.110	0.431±0.073	0.269±0.069	0.308±0.068

Values are means±S.E.M.

were no significant differences between the two strains and significant interaction between strain and age. In the ratio of 5-HIAA/5-HT, no significant differences and interaction were detected.

Discussion

There are two main pathways in the metabolism from DA : one advances to NE and E while the other involves conversion to DOPAC and HVA. Results of the present study suggest that most DA is converted to NE and E in both strains, since NE and E levels increased (Fig. 1) and the turnover rate of DA to DOPAC significantly decreased

with age. Therefore, it is suggested that both Figure legends
strains have higher activities of dopamine β-hydroxylase (DBH) and phenyl-ethanolamine N-methyltransferase (PNMT), which are enzymes which biosynthesize NE and E, rather than those of monoamine oxidese and chatecholmetyltransferase, the enzymes in the biosynthesis of DOPAC and HVA. Particularly in layer-type chicks, the activities of DBH and PNMT might be higher compared to broiler-type chicks since both NE and E levels were higher in layer-type chicks.

At 18 days of incubation, most of the γ-amino butyric acid systems have developed (Rogers, 1995). Revilla *et al.* (2001) investigated that NE, E and methoxy-hydroxy-phenyl-glycol, a metabolite of NE, levels in dissected chick telencephalon, diencephalon/mesencephalon and cerebellum in a number of stages from the late embryonic period and post-hatching period. They reported that NE levels increase dramatically after hatching in all brain structures studied. Taken together, it is suggested that the development of the catecholaminergic and serotonergic systems has not reached a maximum on day 18 of incubation.

The contents of NE and E also increased with the developmental stage in the both strains. At day 18 of incubation, there were no significant differences in the contents between the strains. However, the content of the hypothalamic E in layer-type chicks was significantly greater than that in broiler-type chicks at hatch. Similar to this, that of the hypothalamic NE in layer-type chicks tended to be greater than that in broiler-type chicks. It is suggested that development of noradrenergic and adrenergic systems may be different between strains.

On the other hand, it is well-known that monoamines are involved in food intake regulation. ICV injections of NE and E increased food intake in broiler-type chicks while having no effect in layer-type chicks (Denbow, 1994, 1999 ; Denbow *et al.,* 1981, 1983), also suggesting that these catecholaminergic systems in broiler-type chicks are different from layer-type chicks.

It was also suggested that these differences in catecholaminergic systems observed here might be related to the difference in some behaviors between the strains, including feeding behavior. In fact, it was reported that noradrenergic system was involved in the difference in feeding behavior between genetically selected high- and low-weight lines of chickens (Denbow *et al.,* 1984). According to this report, central administration of methoxamine, α_1-adrenergic receptor agonist, significantly increased food intake in the high-weight line of chickens but had no effect in the low-weight line. In addition, there were some studies comparing the activity of two inbred strains of rats (Skolnick *et al.,* 1974 ; Segal *et al.,* 1975 ; Perry *et al.,* 1983). These strains differed in spontaneous motor activity, cortical and midbrain tyrosine hydroxylase activity, and behavioral responsiveness to NE and amphetamine, central nervous system stimulants. From these, it is possible that genetic selection might change hypothalamic noradrenergic and adrenergic systems and then might modify food intake and growth rate in chickens. In conclusion, it is suggested that the hypothalamic catecholaminergic system develops at an increased rate during late incubation, since NE and E levels greatly increased toward hatch in the present study and Revilla *et al.* (2001). Futhermore, the development is

modified by genetic selection.

The present study demonstrated that increase patterns in the contents of the hypothalamic monoamines at early developmental stage was different between broiler- and layer-type chicks. However, since we compared the hypothalamic catecholaminergic and serotonergic systems by evaluating monoamine contents, the difference in the number of monoaminergic neurons, in monoamine release, and in the development of monoaminergic receptors are not known yet. Further investigations are needed to clarify these problems in future.

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