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ABSTRACT—While brain development during embryogenesis has been extensively studied in precocial birds, there is no information available on altricial birds. Thus, the concentrations of the catecholamines norepinephrine (NE), epinephrine (E), and dopamine (DA), and the dopaminergic metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) were determined at several stages during the late embryonic period (E13, E14, E15, E16, E17 and E18) and the day-of-hatch (P0) in the pigeon telencephalon, cerebellum, optic lobe, and brainstem. The concentrations of all catecholamines were higher than those reported in chicken embryos. During embryogenesis, NE, E, DOPAC and HVA concentrations in the various brain parts increased throughout embryonic development until shortly before hatching at which time they decreased. DA, however, continued to increase through hatching in the brainstem, and the changes in DA concentrations varied in several brain parts.

In conclusion, catecholamine concentrations in the various brain parts tended to increase with embryonic age, and the concentrations were higher than those in chickens. Furthermore, brain catcholamine metabolism changed at hatch in pigeons.

Key words: pigeons, altricial, embryonic period, catecholamines, dopaminergic metabolites

INTRODUCTION

Domestic chickens are precocial and therefore have relatively well-developed processes at hatch including the ability to feed alone, walk, open their eyes, and thermoregulate. Previous reports describe the brain concentrations and distribution of one or more monoamines and their metabolites in chickens at various embryonic and/or post-hatching periods (Pscheidt and Himwich, 1965; Anton and Sayre, 1964; Jurio and Vogt, 1967; Kobayashi and Eiduson, 1970; Gallingham and Sharman, 1970; Sparber and Shideman, 1970; Johnson *et al.* 1981; Davies *et al.*, 1983; Siuciak *et al.*, 1992; Pendleton *et al.*, 1998; Revilla *et al.*, 2001). According to these studies, DA, NE and E concentrations increased after hatching in all brain structures. During the late embryonic periods, the concentrations of NE, E and methoxy-

hydroxy-phenyl-glycol in chick telencephalon, diencephalon/mesencephalon and cerebellum gradually increased (Revilla *et al.*, 2001). Tyrosine hydroxylase, one of enzymes involved in the synthesis of catecholamines, was first detected at E4 in whole brain homogenates, and its activity increased throughout incubation (Kentroti and Vernadakis, 1989). Additionally, dopamine-β-hydroxylase, which catalyzes the synthesis of NE, was present after E6 (von Bartheld and Bothwell, 1992). Thus, it is suggested that catecholamines begin to be biosynthesized at early embryonic ages, and increase thereafter.

In contrast, pigeons are typical of altricial birds that have undeveloped processes at hatch including closed eyes and an inability to walk or feed themselves (Baicich and Harrison, 1997; Starck and Ricklefs, 1998). To the authors' knowledge, there are no reports on brain neurotransmitter levels during embryonic development in the pigeon since only adult pigeons (body weights; 300–550g) have been studied.

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The purpose of present study was to determine the concentrations of DA, NE, E, and the dopaminergic metabolites DOPAC and HVA during embryonic development. In addition, the changes in the concentrations of these compounds at the time of hatch were investigated.

MATERIAL AND METHODS

Egg and Husbandry

Fertilized eggs of homing pigeons (*Columba livia*) were obtained from a local pigeon shop (Fukuoka, Japan) and incubated at 37.6°C with a relative humidity of 58 to 68%. The eggs were candled at day 10 of incubation to remove those that were infertile or contained dead embryos.

Analysis

Pigeon embryos at days E13, E14, E15, E16, E17 and E18 (just before hatch) of incubation, and pigeons at day-of hatch (P0) were sacrificed with an overdose of sodium pentobarbital and decapitated. Experimental procedures 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. The brains were rapidly removed, weighed, and divided into four parts including the telencephalon, cerebellum, optic lobe, and brainstem according to the atlas of Karten and Hodos (1967). They were frozen on dry ice, weighed, and stored at −85°C prior to analysis. The contents of the catecholamines dopamine (DA), norepinephrine (NE), and epinephrine (E), and the dopaminergic metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (HVA) were determined by high-performance liquid chromatography (HPLC), and their concentrations (contents/g wet tissue) were calculated. All brain samplings were done at 1:00 p.m. to remove circadian variations in concentrations (Siuciak *et al.*, 1992). The catecholamines and metabolites were extracted according to the method described elsewhere (Sugahara *et al.*, 1999). Briefly, the tissue was homogenized in 0.05 M ice-cold perchloric acid containing isoproterenol as an internal standard. The homogenate was centrifuged at 10,000×g for 5 min. Then the supernatant was centrifuged with a centrifuge-filtration unit (Ultra Free C3-GV, Millipore, Bedford, MA, USA) at 10,000×g for 4 min. The 30 μl filtrate was injected into a HPLC system (Eicom, Kyoto, Japan) with a 150×2.1 mm ODS column (SC-5ODS, Eicom, Kyoto, Japan) for measurement of catecholamine and dopaminergic metabolite content. The mobile phase was 1.0 M aceto-citric acid buffer, 2.3 mM sodium 1-octane sulfonate, 1.0 mM disodium ethylenediaminetetraacetic acid, 17% methanol, at pH 3.5. The catecholamines and metabolites 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 detection limits of the system for all catecholamines and metabolites were 0.1 pg/sample. The numbers of embryos and pigeons used were as follows: 5, 6, 7, 6, 7, 5, and 6 on E13, E14, E15, E16, E17, E18 and P0, respectively. The catecholamine and metabolite contents were determined by integration of peak areas and their concentrations expressed as ng/g wet tissue.

Statistical analysis

Data for catecholamine and metabolite concentrations were analyzed by one-way ANOVA, and NE/DA and DOPAC/DA ratios were analyzed by two-way ANOVA using a commercially available package (StatView, Version 5, SAS Institute, Cary, USA, 1998). The results are presented as means±S.E.M.
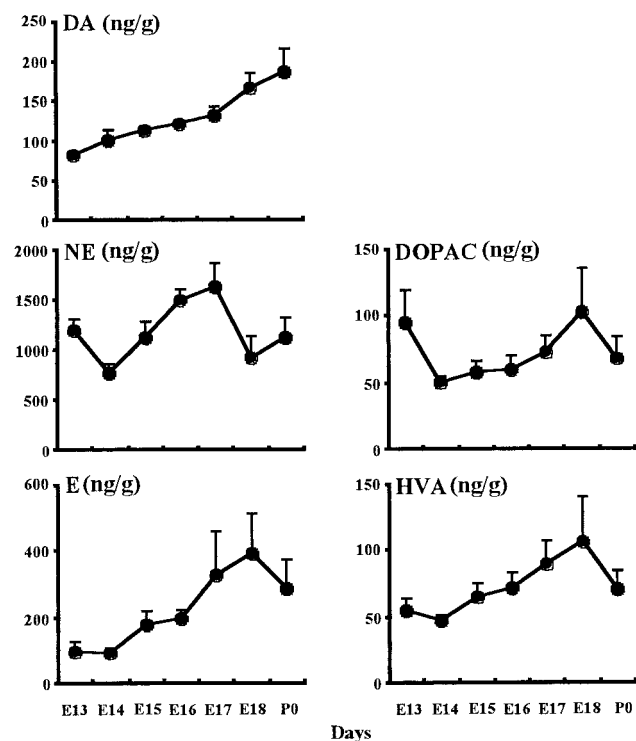

Fig. 1. The concentrations of catecholamines and dopaminergic metabolites in dissected pigeon brainstem. Values are means ±S.E.M.
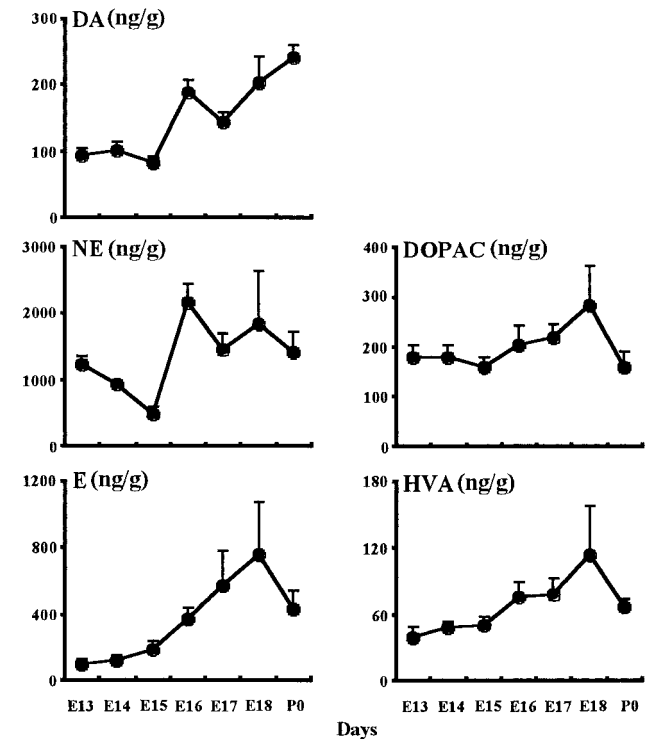
Fig. 2. The concentrations of catecholamines and dopaminergic metabolites in dissected pigeon telencephalon. Values are means± S.E.M.
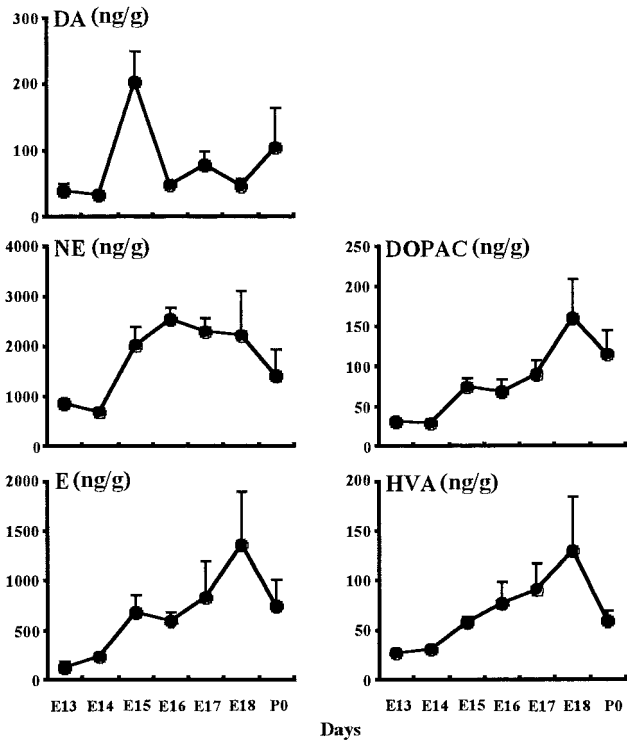
Fig. 3. The concentrations of catecholamines and dopaminergic metabolites in dissected pigeon cerebellum. Values are means± S.E.M.
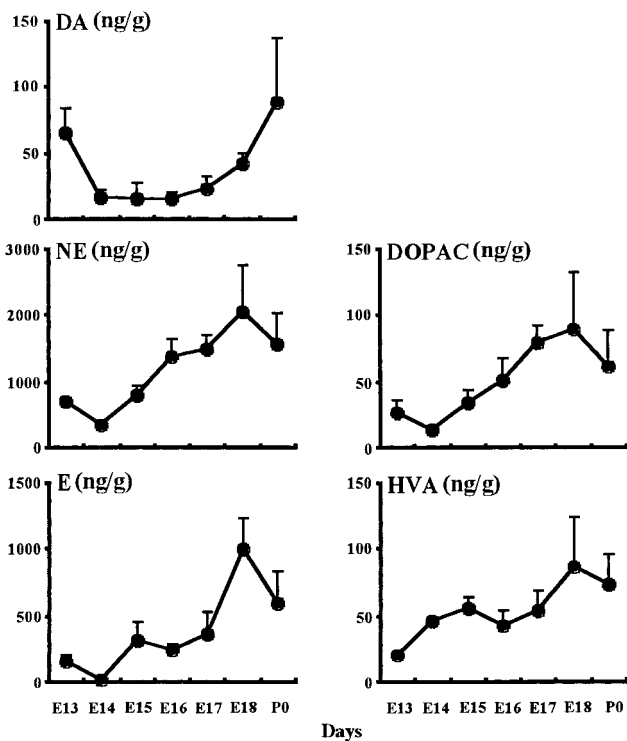

Fig. 4. The concentrations of catecholamines and dopaminergic metabolites in dissected pigeon optic lobe. Values are means± S.E.M.

RESULTS

The concentration of catecholamines and dopaminergic metabolites in the brainstem, telencephalon, cerebellum and optic lobe are shown in Figs. 1, 2, 3 and 4, respectively. During embryonic development, the changes in DA varied in each part. DA concentration in the brainstem increased through hatching. In the cerebellum, DA concentration remained relatively constant except for a peak on day E15, whereas in the optic lobe increased from E16 to hatch (Figs. 1, 3 and 4). In the brainstem, the concentrations of NE, E, DOPAC and HVA decreased from E13 to E14, then gradually increased, followed by a decrease at hatching (Fig. 1). A similar trend was observed in the cerebellum and the optic lobe (Figs. 3, 4). In the telencephalon, E, DOPAC and HVA concentrations increased to E18 and then decreased at hatching whereas the NE concentration suddenly increased at E16 and remained relatively steady through hatching (Fig. 2).

The NE/DA ratio was higher than the DOPAC/DA ratio in each brain part (Fig. 5). For the NE/DA ratio, the effects
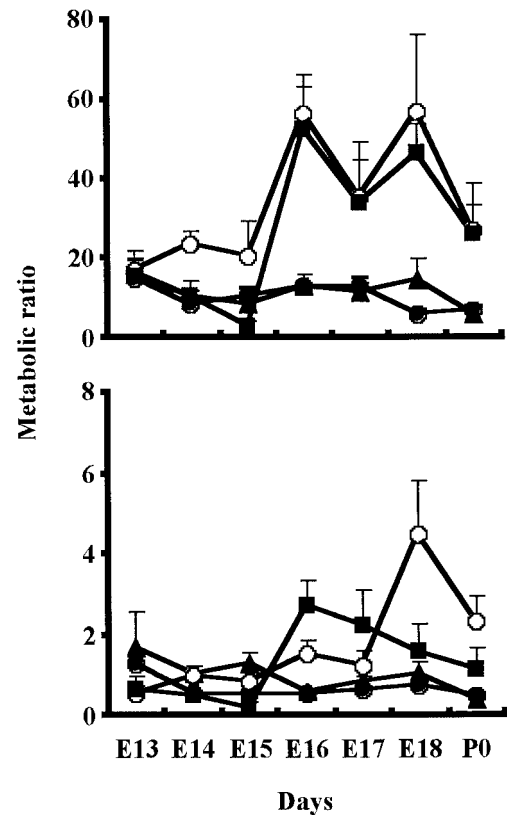

Fig. 5. NE/DA (upper panel) and DOPAC/DA (lower panel) ratios at each part of pigeon brain from E13 to P0. Values are means± S.E.M.

of brain parts and days were significant (P<0.001), and there was a significant (P<0.05) interaction between brain part and day. In the brainstem and telencephalon, the NE/DA ratio was almost constant over embryonic period whereas in the cerebellum and optic lobes, the ratio increased to E18 and then decreased at hatching. For the DOPAC/DA ratio, the effects of brain parts and days were significant (P<0.01), and there was a significant (P<0.0001) interaction between brain part and day. The DOPAC/DA ratio in the brainstem and telencephalon remained constant during the embryonic periods. In the cerebellum and optic lobe, the DOPAC/DA ratio increased to E18 and E16, respectively, and then decreased through hatching.

DISCUSSION

The results of the present study were similar to those obtained in the domestic chicken in that the concentration of catecholamines increased with embryonic age. However, the catecholamine concentrations in the brain of pigeon embryos were different from the domestic chicken. For instance, NE concentration in the pigeon telencephalon was 1211±147, 914±62, 466±110 and 2149±282 ng/g wet tissue at embryonic periods of E13, E14, E15 and E16, respectively. Revilla *et al.* (2001) reported that NE concentrations in the chick telencephalon at E16, E17, E18 and E19 were 130±11, 145±21, 206±19 and 283±12 ng/g wet tissue, respectively. NE concentration in the telencephalon and cerebellum of embryonic pigeons was also higher than that of embryonic chicks. Similarly, E concentrations were higher in the pigeon. In contrast, NE concentrations in adult pigeon and chicken brains were comparable, i.e., 1.5, 1.4 μg/g wet tissue in the hypothalamus, 527, 496 ng/g tissue in telencephalon, respectively (Jurio and Vogt, 1967; Lane *et al.*, 1976; Revilla *et al.*, 2001). Additionally, NE and E concentrations decreased immediately after hatching followed by a dramatic increase (Revilla *et al.*, 2001).

NE and E levels decreased during the late embryonic period or immediately post-hatch. The reason for this decrease is unknown. The locus ceruleus within brainstem, which is the nucleus containing 80% of brain noradrenergic neurons, receives a wide range of inputs and can thus, integrate information from a variety of sources. For example, this area can process information from the external environment including auditory, visual and somatic senses as well as information from the internal environment including hypoglycemia, hypoxia, blood loss and hypercapnia. Such information can activate noradrenergic neurons in this nucleus, which then project to multiple brain areas including the limbic system, cortex, hypothalamus, cerebellum and spinal cord (Foote *et al.*, 1983; Siegel *et al.*, 1998). During incubation, chick embryos receive auditory stimuli including clicking noises, vocalizations, bill tapping, breathing, limb movements, head-lifting, heart beats, and beak-clapping (Vince, 1969). Additionally, immediately before hatch, clicking and vocalizing commence and beak-clapping increases.

As their auditory abilities improve, embryos can respond to maternal calls. In addition, if the nares are free of obstructing materials, olfactory discrimination is precise (Rogers, 1995). Thus, it is suggested that chick embryos may receive auditory and olfactory stimuli, and noradrenergic neurons are then activated send signals to multiple brain areas. No information about pigeons, however, has been published, and it is uncertain whether pigeons have auditory and olfactory ability during the late embryonic period. Therefore, further work is necessary to determine if such sensory information is received in precocial birds during late embryonic periods.

Prehatching behavior begins on E16 or E17 in the domestic chicken (Rogers, 1995). The cerebellum is an important brain site controlling motor function. The NE/DA and DOPAC/DA rations in the cerebellum in the present study increased just before hatching (Fig. 5). The contribution of these changes to prehatching behavior is unclear and remains to be elucidated.

In conclusion, catecholamine concentrations in the brain of pigeon embryos increased with embryonic age and then decreased at hatch. Furthermore, these concentrations in pigeons were much higher than those in the domestic chicken.

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