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Norepinephrine, epinephrine and MHPG levels in chick brain development

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Abstract

In this study we determined the norepinephrine (NE), epinephrine and methoxy-hydroxy-phenyl-glycol (MHPG) levels in dissected chick telencephalon, diencephalon/mesencephalon and cerebellum in a number of stages from the late embryonic period (E16, E17, E18 and E19) and post-hatching period (P1, P2, P3, P4, P5, P15 and P30) using HPLC coupled with a coulometric detection system. A mobile phase which permits the detection of NE, epinephrine and MHPG simultaneously is also described. During development, NE levels increase dramatically after hatching in all brain structures studied and are not correlated in the same period with an increase in the MHPG/NE ratio. The values obtained for epinephrine and MHPG were significantly lower than the NE values in all the structures and stages studied. Our results support the notion of a specific role for NE during the first days after hatching.

Keywords: Epinephrine; Norepinephrine; Chick brain; Development; HPLC

1. Introduction

Neurotransmitters are not only involved in synaptic communication, but also act as important trophic factors. Norepinephrine (NE) is one of the first neurotransmitters that can be detected in the fetal brain and is considered to play a trophic role in brain maturation (Felten et al., 1982). In chick embryos, tyrosine hydroxylase, dopamine, NE, and epinephrine are expressed as from the first days of incubation (Pendleton et al., 1998).

The chick has often been used as a model for studying development and has provided a good paradigm of the brain and its behavioural course. The chick hatches with its brain ready to undergo learning, especially the visual characteristics of the hen, quickly and with strength; this is known as filial imprinting (Rogers, 1995). The biochemical events of memory formation are regulated by GABAergic, cholinergic and noradrenergic systems early on after training. The late modulation relies on dopamine D1, beta-noradrenergic, and $5HT_{1A}$ receptors in the hippocampus and the dopaminergic, noradrenergic and serotonergic pathways (Izquierdo and Medina, 1997; Izquierdo et al., 1998).

A large body of research has explored the distribution and levels of catecholamines in the mammalian brain, including mammalian development (Ribary et al., 1986; Herregodts et al., 1990; Erdtsieck-Ernste et al., 1991; Tomasini et al., 1997). However, reports on catecholamines in the chick brain are very scarce. Some of them describe the distribution and levels of one or more of these neurotransmitters in P15 or P30 chick brain in specific areas (Pscheidt and Himwich, 1963; Jurio and Vogt, 1967; Callingham and Sharman, 1970; Johnson et al., 1981; Kruzelock and Barbato, 1991; Siuciak et al., 1992), but we have not found references to catecholamines in which the different stages of pre- and post-hatching development are compared.

The relative contents of NE, epinephrine and MHPG indicate the changes occurring in the catecholaminergic system along the most critical period in the chick's life;

Abbreviations: NE: Norepinephrine; MHPG: 4-hydroxy-3-methoxy-phenyl-glycol; CNS: central nervous system.

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i.e. hatching and imprinting. Here we report for the first time a comparative study of NE, epinephrine and their metabolite MHPG levels in the chick brain during late embryonic development and during the hatching and post-hatching period and describe a mobile phase able to detect these three compounds both easily and simultaneously.

2. Methods

Embryonic stages from E16 to E19 (E referring to embryonic day), as well as post-hatching stages P1, P2, P3, P4, P5, P15 and P30 (P referring to post-hatching day) were studied. The brains of eight chicks from each stage were removed, and the telencephalon, diencephalon/mesencephalon and cerebellum were rapidly dissected out at 4°C, frozen in liquid nitrogen, and stored at −80°C until used.

Tissue was homogenised in 0.4 M $HClO_4$ and 0.1% $Na_2S_2O_5$ and centrifuged at 20 000***g*** for 15 min at 4°C. The resulting supernatant was diluted 1:1 (v/v) in the mobile phase.

The detection of NE, epinephrine and MHPG was modified from a previous method for the analysis of catecholamines (Gil-Martin et al., 1995). Chromatographic detection was carried out with an ESA Coulochem II Multi-Electrode Detector (Model 5200, Esa, USA) dual potentiostat electrochemical detector equipped with an RS232 interface. Separation of analytes was achieved on a reversed-phase column (C-18, 5 μm, 3.9 mm×15 cm, Waters, USA). Analytes were detected on a dual electrode analytical cell (Model 5011) with the first electrode set to oxidize at 100 mV and the second one (E2) set to oxidize at −300 mV. A guard cell (Model 5021) was placed between the pump (Beckman System Gold Programmable Solvent Module 116, Beckman Instruments) and the injector to oxidize contaminants at a potential of 800 mV (vs. Pd). The mobile phase consisted of 0.1 M Na_2HPO_4, 0.1 M citric acid, 1.8 mM octane sulphonic acid and 5% (v/v) methanol. All chemicals were of the highest purity commercially available. Elution was performed at a flow rate of 1 ml/min at room temperature. The procedure was monitored using a PC-based data station and the Gold Chromatography Data System 1.7 program (Beckman Instruments).

Standard mixtures were prepared from norepinephrine bitartrate, epinephrine hydrochloride and 4-hydroxy-3-methoxyphenyl-glycol (MHPG) hemipiperazinium salt (all compounds from Sigma). These standards were routinely injected at the beginning and end of each determination, and the coefficients of variation were always less than 10%.

3. Results

The NE, epinephrine and MHPG values as well as the MHPG/NE ×100 ratios obtained from the telencephalon, diencephalon/mesencephalon and cerebellum from the different stages examined are shown in Table 1.

3.1. Telencephalon

The P30 stage showed the highest levels of NE, MHPG and epinephrine. The values obtained for epinephrine and MHPG were significantly lower than those of NE at all the stages studied. Furthermore, the values of NE, MHPG, epinephrine and the MHPG/NE ratio underwent changes along the development, exhibiting a pattern characterized by three peaks (Figs. 1A and 2A). The highest epinephrine, MHPG and MHPG/NE ratio values corresponded to the E19, P4 and P30 stages,

Table 1
NE, MHPG and epinephrine values (ng/g tissue) and MHPG/NE x 100 ratio in telencephalon, diencephalon/mesencephalon and cerebellum (mean ± S.E.M.)

	NE	MHPG	Epinephrine	MHPG/NE
Telencephalon				
E16	130±11	3±1	7±2	2.8
E17	145±21	6±1	7±3	4.1
E18	206±19	9±2	9±3	4.4
E19	283±12	14±1	14±3	4.8
P1	297±31	6±2	8±1	2.1
P2	319±24	7±2	6±2	2.3
P3	251±19	5±2	9±1	1.9
P4	253±21	10±2	14±3	3.5
P5	302±17	8±4	12±2	2.8
P15	284±16	10±4	10±3	3.3
P30	527±29	25±4	14±4	5.0
Diencephalon/mesencephalon				
E16	246±3	12±1	10±2	4.7
E17	263±31	14±1	16±4	4.5
E18	414±20	16±1	19±1	3.9
E19	458±32	21±3	16±2	4.2
P1	452±7	11±1	17±4	2.3
P2	666±36	11±2	23±5	1.6
P3	436±44	9±1	10±1	2.1
P4	444±39	9±2	13±1	2.0
P5	314±27	7±1	16±3	2.1
P15	228±29	8±2	20±6	3.6
P30	439±22	21±5	20±2	4.8
Cerebellum				
E16	136±23	7±1	6±1	4.8
E17	157±13	6±1	5±1	3.8
E18	187±24	6±1	5±2	3.3
E19	257±12	8±2	6±1	3.1
P1	154±9	6±1	10±1	3.9
P2	140±24	7±2	10±2	4.7
P3	114±11	5±1	5±2	4.0
P4	124±7	5±1	4±0	3.7
P5	210±10	7±1	3±1	3.3
P15	179±32	7±1	10±2	4.2
P30	285±52	11±2	17±2	4.1

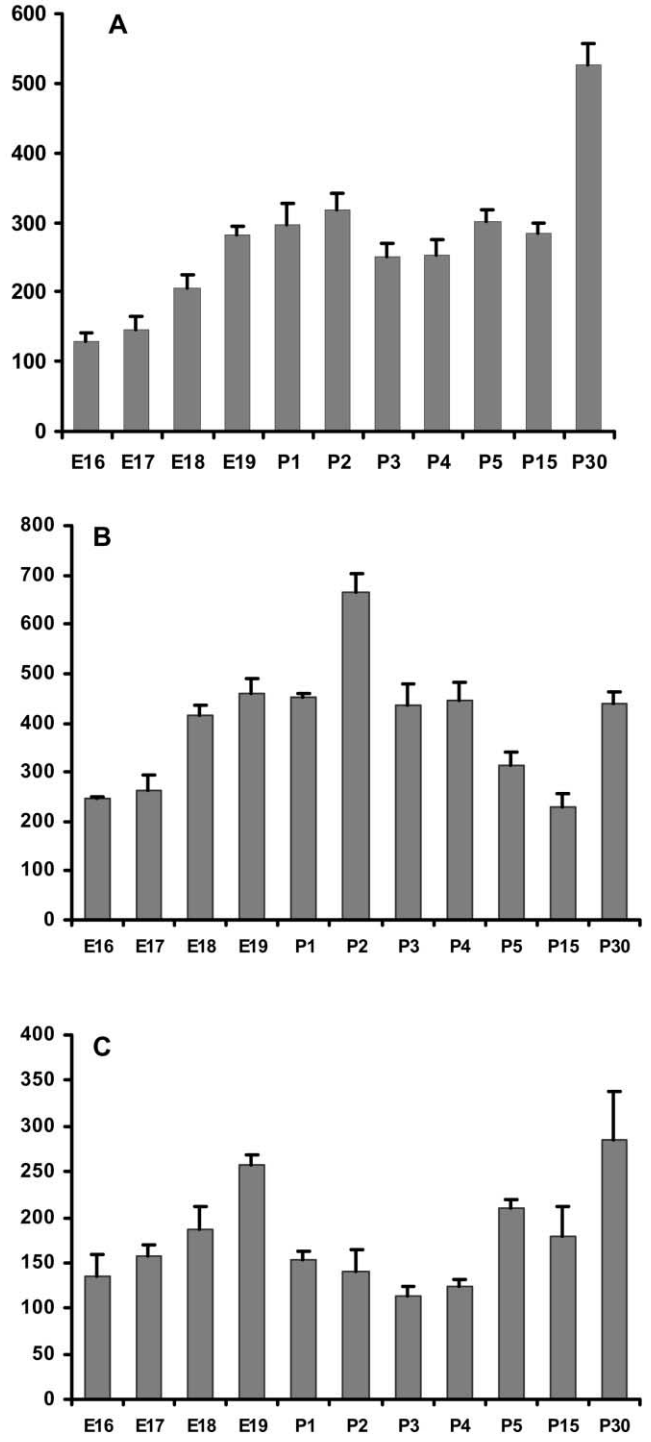

Fig. 1. Patterns of NE levels during development in telencephalon (A), mesencephalon/diencephalon (B) and cerebellum (C).

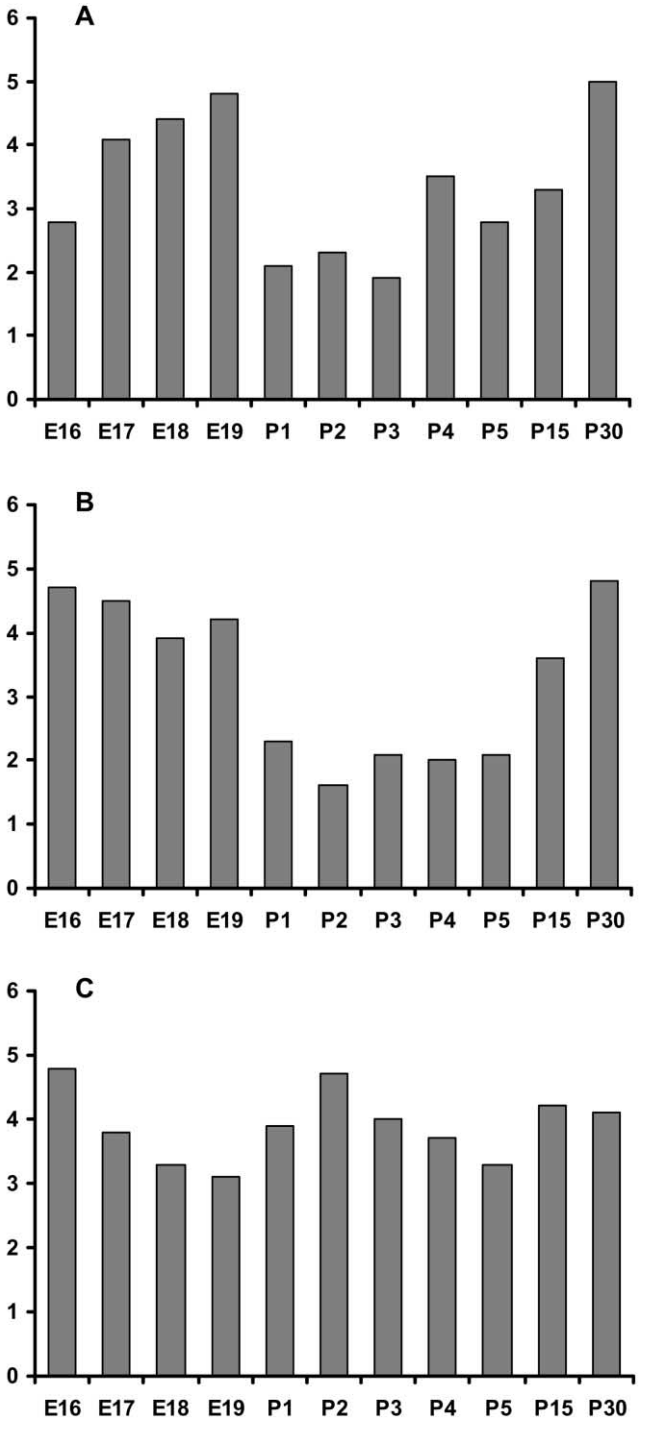

Fig. 2. Patterns of HMPG/NE ratios during development in telencephalon (A), mesencephalon/diencephalon (B) and cerebellum (C).

while NE showed the highest values in stages P2, P5 and P30.

3.2. Diencephalon/mesencephalon

In our study, the diencephalon/mesencephalon was the structure seen to have the highest levels of NE, MHPG and epinephrine. As in the telencephalon, NE levels were significantly higher than those of MHPG and epinephrine. Regarding the developmental stages, the highest levels of NE and epinephrine in the diencephalon/mesencephalon were observed in stage P2, while the highest concentrations of MHPG were detected in stages E19 and P30.

Epinephrine levels showed three peaks (E18, P2 and

P15-P30) while only two peaks were detected for NE and MHPG levels (stages P2 and P30, and stages E19 and P30, respectively, see Table 1). As a result the MHPG/NE ratio showed a different pattern to that observed in the telencephalon (Fig. 2B).

3.3. Cerebellum

The values of NE, MHPG and epinephrine were similar to those observed in the telencephalon, all of them were lower in the post-hatching stages. Maximum levels of all these molecules were found in P30.

MHPG and epinephrine levels followed a similar pattern to those observed in the diencephalon/mesencephalon, although the first stage of maximum epinephrine values in cerebellum appeared later (P1-P2). NE values followed a pattern with three peaks corresponding to the highest values (E19, P5 and P30), as in the telencephalon, although the first stage was different (Table 1).

4. Discussion

Here we report the results obtained with a mobile phase that allows one to detect epinephrine and NE together with their common metabolite MHPG simultaneously. As far as we are aware, this has not been reported previously in the literature. We checked a number of mobile phases (Baker et al., 1988; Tapie et al., 1988; Kruzelock and Barbato, 1991; Gil-Martin et al., 1995) in which either MHPG, epinephrine or NE are not measured simultaneously, and in our hands they showed co-elution of MHPG with either epinephrine or NE. By contrast, the mobile phase used here is simpler than those used previously and the chromatograms obtained presented more stable basal lines as well as peaks lacking the shoulders that we observed in some of the assays using some of the above reported mobile phases. An important point to be considered when using this technique is that the sample is diluted 1:1 in the mobile phase just prior to injection (see methods section), which in our hands improved the chromatogram considerably.

The present data for NE and MHPG are, in general, consistent with previous data reported for areas of P15 and P30 chick brains. Reports on epinephrine levels in some areas of the chick brain are controversial, the values differing more than 10-fold among the different authors (Jurio and Vogt, 1967; Siuciak et al., 1992). Our values are in agreement with the lower epinephrine levels observed by Siuciak et al. (1992).

Previous studies in later developing stages of chick brains have reported the highest levels of NE in thalamus and hypothalamus (Kruzelock and Barbato, 1991; Siuciak et al., 1992), in agreement with our data for NE during the different developmental stages. However, our data for P30 reveal the telencephalon to be the structure with the highest levels of NE, suggesting an increase in the relevance of NE in the telencephalon of adult chick brains.

NE, epinephrine and MHPG levels remain low in the rat brain during fetal life and start to increase around birth. This continues up to the fourth week of life, when steady-state levels appear (Pares-Herbute et al., 1989; Herregodts et al., 1990), although transient fluctuations around birth are also observed (Ribary et al., 1986). Our data in the chick also indicate that the catecholaminergic system may start to be functional around the hatching time, and that must play a crucial role during the first early post-hatching period, similar to what has been described for post-natal development in rat (Ribary et al., 1986; Pares-Herbute et al., 1989; Herregodts et al., 1990). This suggests an important role for catecholamines in the early post-natal (post-hatching) period in birds and mammals. In this regard, in birds the activity of tyrosine hydroxylase, one of the enzymes involved in the synthesis of catecholamines, is detectable after only 4 days of incubation (Kentroti and Vernadakis, 1989). Dopamine-beta-hydroxylase, which catalyses the synthesis of NE, detected by in situ hybridisation, is present after six days of incubation (von Bartheld and Bothwell, 1992). Our data confirm that NE is already present by E16, before the presence of beta-adrenoceptors, which are detected as from the E17-E18 stage (Revilla et al., 1998). All these data indicate that during early development NE could act on adrenoceptors other than beta-adrenoceptors although the increase in NE observed around the hatching period may be mainly mediated by beta-adrenoceptors.

NE levels are approximately one order higher than those of epinephrine and MHPG in all the structures studied, confirming that at least in the chick NE is the most important adrenergic neurotransmitter. Also, the low levels of its metabolite, MHPG, suggest that it is mainly re-uptaken.

MHPG levels are usually measured in urine to estimate the degree of NE turnover in the CNS (Joseph et al., 1976; Roy et al., 1988), but although urinary MHPG is considered to be a poor indicator of NE turnover in the CNS because although the exact proportion of urinary MHPG deriving from the brain is still debated, about 20% appears to come from central NE pools (Potter et al., 1984; Schatzberg and Schildkraut, 1995). Thus, the mobile phase used in this work allowing the determination of MHPG together with NE and epinephrine offers a more accurate way of measuring catecholaminegic turnover.

An outstanding result of this study is the observation of specific patterns in the levels of NE, epinephrine and MHPG related to the period of hatching. Thus, NE levels showed maximum peaks in stages P2 in both diencephalon/mesencephalon and telencephalon and in

E19 in cerebellum. A common pattern is seen in the decrease in NE levels during the first week after hatching, followed by new peaks in the P30 stage. This indicates that NE exerts a special regulation in the first week, in agreement with a pattern of an increase in beta-adrenoceptor levels up to P2 in the chick, followed by a decrease in the first week (Revilla et al., 1998). The similar pattern observed in MHPG confirms the notion that the noradrenergic system is strongly involved in hatching and early post-hatching life.

In the diencephalon/mesencephalon and telencephalon, but not in cerebellum, the progressive increase in NE and MHPG levels up to hatching matches a progressive increase in the MHPG/NE ratio. However, the ensuing decrease in the MHPG/NE ratio and MHPG levels during the early post-hatching period contrasts with the increase in NE up to P2. This suggests a hypothetical regulation in the activity in the enzymes responsible for metabolising NE that would lead to a decrease in NE degradation and to the high levels of NE observed in P1 and P2. These high levels, in turn, would drive a decrease in NE synthesis during the first week, probably as a consequence of the well-known mechanism of inhibition of an enzyme (tyrosine hidroxylase) by its end-product inhibitor. This regulation would be different from that found in cerebellum, in which MHPG and NE display a similar pattern of increases and decreases to those described for adult mammals, in which peaks in NE are followed by peaks in MHPG on the same day (Beckmann and Goodwin, 1975).

In the chick diencephalon/mesencephalon and telencephalon, the sequence of a peak of MHPG levels (E19) followed by a peak in NE and beta-adrenoceptor levels (P2), and low amounts of all of them during the first week, indicates that the NE system plays a crucial role in hatching and early post-hatching. Since the NE system has been reported to play a role in habituation to external stimuli (Sara et al., 1994; Clayton and Williams, 2000) and since the first week seems to be a critical learning period for the chick (for imprinting in P2 stage, and for the no-fear behaviour seen over the following days (Rogers and Astiningsih, 1991; Fluck et al., 1996), it seems quite possible that the peak seen in the P2 stage and the ensuing decrease in the activity of NE system would be strongly related to the specific behaviour observed in the chick during this period.

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