Functional Activity of Catecholaminergic System in Human Fetal Midbrain and Diencephalon

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The development of catecholaminergic system of the midbrain and diencephalon was studied in human embryos and fetuses aged 6, 8, 10, and 12 weeks by specific capture and K^+-stimulated release of ^{3}H-dopamine *in vitro*. Specific capture of ^{3}H-dopamine was first detected in the midbrain of 6-week embryos and in the diencephalon of 8-week fetuses. The time course of the capture points to on-going differentiation of catecholaminergic neurons and fiber growth and the presence of the caudorostral gradient in the development of brain catecholaminergic system. The release of catecholamines was not stimulated in response to membrane depolarization in the midbrain and diencephalon at any of the studied stages of development. The difference in the time of capture and K^+-stimulated release of catecholamines is related to specific features of differentiation of these neurons in human fetuses.

Key Words: *catecholamines; midbrain; diencephalon; embryo; human*

The interest in the development of brain catecholaminergic (CA) system is explained by the participation of CA in the regulation of neuroendocrine functions, behavior, motor activity, etc. [15]. Special attention was paid to human embryonal and fetal dopaminergic (DA) neurons [11,16] because they are used for compensation of local DA deficiency in patients with Parkinson's disease [1,2,4]. For effective grafting, the neurons should be taken from embryos or fetuses immediately after their formation, i.e., after the last mitotic division of precursor cells. The direct method for assessing the time of neuronal birth *in vivo* — ^{3}H-thymidine autoradiography in combination with specific markers of CA neurons — is not fit for investigating human embryonal brain.

The aim of this study was to find out any relationship between immunomorphological data of indicators of CA neurons and fibers in human embryonal and fetal midbrain and diencephalon and their functional activity.

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MATERIALS AND METHODS

Human embryos and fetuses aged 6 weeks (4 embryos), 8, 10, and 12 weeks (2-3 fetuses per term) were used. The fetuses were removed from the uterus in the course of medical abortion by sparing aspiration monitored by ultrasonography (Toshiba Sal-38). The age of embryos and fetuses was determined by the parietococcygeal length [5]. Being members of the European Association of Neurotransplantation and Repair of the Central Nervous System, we adhered to the ethical standards of this organization in the utilization of human fetal nervous tissue [3]. Ventral parts of diencephalon (hypothalamus rudiment) and midbrain (substantia nigra rudiment) were isolated under magnifying glass and divided into 5-7 small fragments (Fig. 1).

Specific capture and release of ^{3}H-DA was assessed as described previously [4]. Brain fragments

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were preincubated in Krebs—Ringer bicarbonate buffer (pH 7.4) and carbogen-rich environment at 37°C with slight shaking. Incubation was carried out in the presence of 2.5×10^{-9} M ^{3}H-DA (specific activity 17-20 Ci/mM, Amersham). For assessing nonspecific binding, preincubation was carried out with nomifansine, an inhibitor of inverse neuronal capture (10^{-5} M, Hoechst). The duration of each incubation was 10 min. Radioactivity was expressed in cpm/mg tissue in the presence and absence of the neuronal capture inhibitor.

For assessing dopamine release, brain fragments washed after incubation with ^{3}H-DA were transferred in perfusion chambers, and four 2-min fractions of perfusate were collected for estimating spontaneous dopamine release. Then the medium was replaced with a fresh one with increased K^+ concentration (60 mM KCl), and four more fractions were collected (stimulated release in response to depolarization). K^+-depolarization was carried out for 8 min. Radioactivity was assessed by liquid scintillation spectrometry and expressed in cpm per 400 μl fraction and 1 mg tissue. Stimulated release was estimated as the difference in the fraction radioactivities before and after stimulation.

Experimental data on specific capture were processed using Student's *t* test. ANOVA test was used to test the relationship between the differences in specific capture and fetal age. Data on ^{3}H-DA release were not statistically processed because of small amounts of embryonal material.

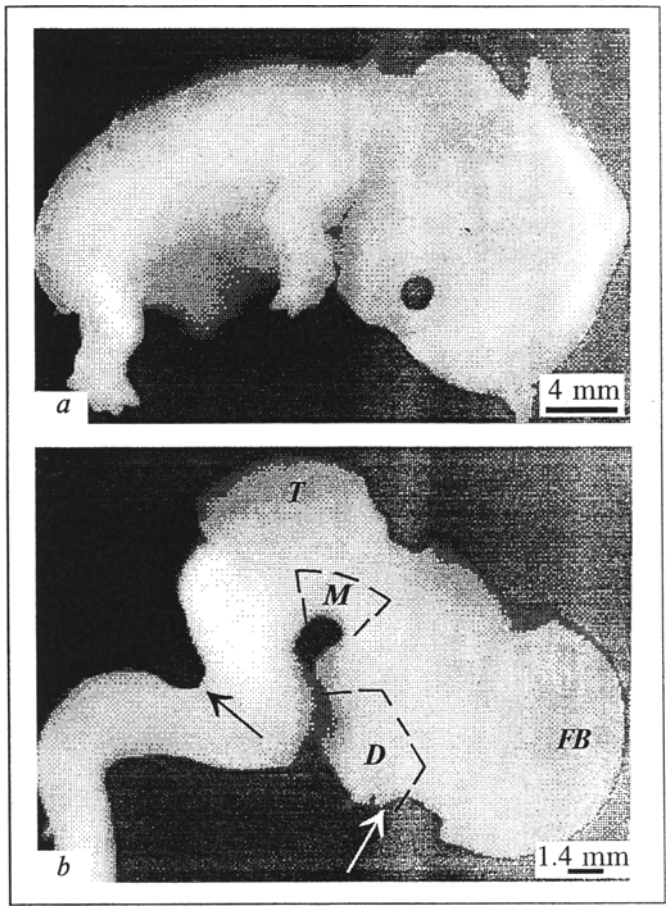

Fig. 1. Eight-week-old human embryo (*a*) and human embryonal brain (*b*). *D*) ventral portion of the diencephalon (hypothalamus rudiment); *M*) ventral portion of the midbrain (substantia nigra rudiment); *FB*) forebrain; *T*) tectum; black arrow shows the bridge flexure, white arrow shows the optic inlet.

RESULTS

Specific capture of ^{3}H-DA depends on the binding capacity (affinity) and density of binding sites. Affinity remains virtually constant during brain development [9]. By contrast, the density of binding sites correlating with the concentration of transport molecules is highly variable [13]. The level of specific capture of ^{3}H-monoamines is usually regarded as the "index" of monoaminergic innervation in the central nervous system [14], proportionate to the total surface of neuronal membrane and, hence, to the number and size of neuronal corpuscles and fibers [4,13].

Development of catecholaminergic system of the diencephalon. Specific capture of ^{3}H-DA at a relatively high level was for the first time detected in rudimental hypothalamus of an 8-week human fetus (Fig. 2, *a*), which indicates the appearance of CA neurons and/or fibers in this brain area between the 6th and the 8th week of gestation. The majority of histofluorescent and immunohistochemical studies revealed the first CA- and tyrosine hydroxylase (TH)-containing neurons and fibers in human hypothalamus on weeks 10-12 [10] and 14-15 [11] of intrauterine life. Comparison with our findings shows that maturation of the membrane mechanism of specific CA capturing precedes the expression of specific enzyme and production of CA. Only C. Verney *et al.* [16] observed TH-immunopositive neurons in the hypothalamus of 5-week human embryos, which *a priori* cannot be considered true CA neurons [8]. A decrease in specific capture of ^{3}H-DA on the 10th week followed by an increase in 12-week fetuses was unexpected for us (Fig. 2, *a*). Since the level of specific capture is directly proportionate to the total membrane surface of CA neurons and fibers and inversely proportionate to the nervous tissue weight, it is probable that from week 8 to week 10 of intrauterine development the bulk of nervous tissue of the diencephalon increases more rapidly than the number of specific binding sites.

The level of spontaneous release of ^{3}H-DA in the diencephalon was 59.7, 30.6, and 29.0 cpm/mg tissue in a fraction at weeks 6, 10, and 12, respectively. However, there was no reaction to K^+ increase

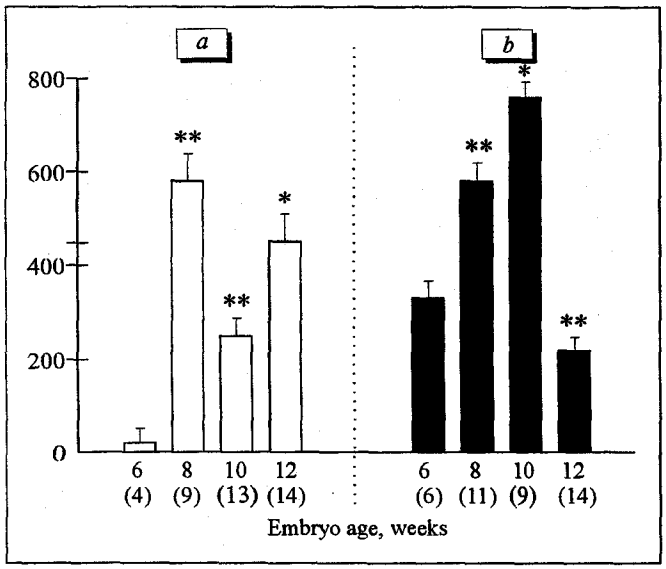

Fig. 2. Specific capture of ^{3}H-dopamine by fragments of ventral portion of diencephalon (*a*) and midbrain (*b*). Ordinate: specific capture of ^{3}H-dopamine, cpm/mg tissue. *$p<0.05$, **$p<0.01$ *vs.* previous stage of development. The number of measurements is given in parentheses.

in Ca^{2+}-containing medium during all periods. These data indicate that time dissociation in the formation of mechanisms of specific capture and release of CA in response to membrane depolarization take place during differentiation of CA neurons. By contrast, in mice [12] and rats [4] both mechanisms are expressed almost simultaneously in the ontogenesis.

Development of catecholaminergic system in the midbrain. In the midbrain (rudimental substantia nigra), specific capture of ^{3}H-DA was first detected at 6 weeks (Fig. 2, *b*), i.e., CA neurons and/or fibers in the substantia nigra rudiment appear earlier than was demonstrated in the majority of histofluorescent and immunocytochemical studies [10,11]. This agrees with reports on isolation of TH-immunoreactive neurons from human embryonal midbrain at 5-6.5 weeks [7,16]. Anyway, CA neurons/fibers were detected in the midbrain earlier than in the diencephalon, which confirms the presence of caudorostral gradient in the development of CA systems of the brain [6]. Specific capture of ^{3}H-DA gradually increases from week 6 to week 10, which indicates an on-going increase in the number and size of CA neurons and/or fibers. Intense growth of fibers of the midbrain CA neurons on the 8th week of gestation was demonstrated by others [7]. On the 12th week, the specific capture drops to a level lower than that on the 6th week. This may indicate that the bulk of nervous tissue at this age increases much more rapidly than CA neurons/fibers.

Spontaneous release of ^{3}H-DA from the midbrain amounts to 128.8, 40.7, and 28.8 cpm/mg tissue in fractions of 6-, 10-, and 12-week-old fetuses, respectively. Similar to the diencephalon, the rate of labeled amine release did not increase in response to K^+-depolarization of membranes at any of the studies stages, which confirms the hypothesis on the time dissociation between maturation of mechanisms of specific capture and K^+-stimulated release of CA.

Our observations confirm the optimal age of the donor (6-10 weeks), which was determined by grafting human fetal midbrain to the rat brain [5]. Our data show that at this age human fetal midbrain has the maximum count of poorly differentiated CA neurons capable of specific capture of CA. Maturation of the mechanism of CA release in response to membrane depolarization lags behind the development of the specific capture mechanism during differentiation of these cells.

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