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Changes of Dopamine-Beta-Hydroxylase Activity During Ontogenesis in Healthy Subjects and in an Experimental Model (Rats)

I. PACLT[1], J. KOUDELOVÁ[2]

[1]Department of Psychiatry, First Faculty of Medicine, Charles University Prague and General Teaching Hospital and [2]Department of Physiology, First Faculty of Medicine, Charles University, Prague, Czech Republic

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Summary

In children and adolescents (250 healthy subjects) serum dopamine-beta-hydroxylase (DBH) activity (23.9±5.2 to 57.1±17.5 μmol/min/ml) increases with the age between 3-10 years, later it decreases approximately by the age of 10-14 years. At the age of 21 to 60 years DBH level is stable. Our study described decreasing DBH activity in adolescents at the age of 10-14 years in the studied sample of healthy persons. Experimental animals (200 Wistar rats, 5-120 days old) show the same trend of enzymatic activity, similarly as in humans. DBH activity in rats is between 0.85±0.1 to 2.8±0.05 μmol/min/ml. This activity is highest in 5-day-old rats; it decreases till the age of 14 days and increases mainly in 14- to 35-day-old animals. Decrease of DBH activity in rats between 35 to 40 days is significant and corresponds to the reduction of DBH activity in adolescent humans (10-14 years). Adult rats (aged 90-120 days) show a stable DBH activity. DBH activity intermediately decreases in 10- to 14-year-old children. This decrease corresponds to the intermediate developmental changes of electrophysiological parameters (decreasing EEG activity in healthy adolescents occurs in 10-14 years old children). Puberty is coupled with intermediate decreasing of DBH activity in man and also in experimental animals in the period of prominent psychological and physiological changes.

Key words

Dopamine-beta-hydroxylase (DBH) • Rats • Human • Serum • Developmental changes • Noradrenergic system

Introduction

Enzymatic functions of dopamine-beta-hydroxylase (DBH) concern the catalysis of 3, 4-dihydroxyphenylethylamine (dopamine) conversion to the neurotransmitter norepinephrine. The enzyme is localized in the brain specifically in the catecholamine vesicules of noradrenegic neurons of the gray matter in nerve terminals (Lewis *et al.* 1992), in sympathetic nerves and in the adrenal medulla. The DBH activity in the serum was described in humans and also in experimental animals. It is biochemically and immuno-chemically identical with the activity that was found in sympathetic nerves and also in the adrenal medulla (Weinshilboum *et al.* 1973a). Sympathetic nerve endings do not secrete only neurotransmitters, since peripheral

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

sympathetic nerves of mammals, including the adrenal medulla, also release specific proteins together with catecholamines (Planz and Palm 1973). One of these proteins is the enzyme dopamine-beta-hydroxylase. A positive correlation between the DBH level and the plasma level of noradrenaline as well as a similar positive correlation between plasma DBH activity and the urinary clearance of noradrenaline were described (Planz and Palm 1973, Geffen *et al.* 1973). These findings suggest that plasma DBH activity could be regarded as an indicator of sympathetic nerve activity.

Dopamine-beta-hydroxylase in the serum originates from sympathetic nerves and is also present in the cerebrospinal fluid into which is released only from independent noradrenergic neurons of the brain (Lewis *et al.* 1992). Fujita *et al.* (1982) and Suzuki *et al.* (1990) found increasing DBH activity in the cerebrospinal fluid in humans depending on the age and corresponding to changes in the plasma. Lewis *et al.* (1992) described noradrenergic DBH neurons in the cortex of primates. Dopaminergic neurons do not contain DBH. The level of DBH in the plasma and CSF is a stable, inherited trait, and the DBH gene has been shown to be the major locus influencing this level (Cubells *et al.* 2000, Wigg *et al.* 2002). The alleles of several polymorphisms identified for the DBH locus have been found to be associated with serum DBH levels, suggesting that these alleles are in linkage disequilibrium with a DNA variant controlling the function or expression of this gene. Genetic determination of DBH activity is one of the main factors influencing its activity. Acquired neurobiological or psychosocial risk factors could cause the same or a similar abnormality (Gerring *et al.* 1998). In response to the stimulation of sympathetic nerves and the adrenal gland, DBH is released together with catecholamines. This enzyme activity increases in the blood of experimental animals and in humans under stress (Weinshilboum *et al.* 1973a). Changes of DBH activity (reduction) were found in several psychiatric diseases such as infantile autisms, attention hyperactivity disorders and psychotic depression (Paclt and Koudelová 1986, Robinson *et al.* 2001, Wood *et al.* 2002, Cubells *et al.* 2002, Wigg *et al.* 2002).

Dopaminergic and noradrenergic transmission was found to be dependent on age, while the serotoninergic system probably does not change too much in the course of development (Gottfries *et al.* 1975, Leckman *et al.* 1980).

Research on the development of dopaminergic transmission performed by means of PET shows that the density of D_1 as well as D_2 receptors in the striatum increases at the age from 2 to 3 years and then decreases (Antonini and Leenders 1993, Seeman *et al.* 1987). This decrease is characteristic for adolescents and young adults but it continues slowly throughout the life with a quotient of 3.2 % per decade with regard to the D_1 receptors. The reduction of the number of the D_2 receptors is slower and thus the D_1/D_2 quotient increases with age (Seeman *et al.* 1987). The decline of activity in the dopaminergic system was also proved by an age-dependent decline of the main dopamine metabolite, i.e. homovanillic acid, in the cerebrospinal fluid (Cohen *et al.* 1974).

Developmental studies of noradrenergic transmission are conflicting. According to some investigations the activity of noradrenergic system increases with age. Ziegler *et al.* (1976) described a rise of noradrenaline in the serum in direct relation to age, but the investigation of serum noradrenaline is very complicated for methodological reasons especially in children. Many authors have not mentioned developmental aspects of serum noradrenaline in children. The study of Freedman *et al.* (1972) dealing with DBH serum activity in relation to age did not show convincing results due to methodological shortcomings (small patient groups, absence of some important age groups etc.). These authors found an age-related rise of DBH activity. According to other authors DBH activity does not change after the sixth year of age. (Weinshilboum *et al.* 1973a, Rappaport *et al.* 1974, Weishilboum 1979, Ciaranello and Boehme 1981). Weinshilboum and Axelrod (1971) and Ogihara *et al.* (1974) did not find any differences in plasma DBH activity in male and female subjects, including children. Fujita *et al.* (1982) and Suzuki *et al.* (1990) have shown evidence of the age-dependent rise of DBH activity in the cerebrospinal fluid.

Developmental changes of DBH activity in Sprague-Dawley rats were described by Behrens and Depocas (1975) and Lamprecht and Wooten (1976). The activity was very high in the earlypostnatal period, reaching its peak at about 16 days of age and approached adult activity at the age of around seven weeks. Nagatsu and Udenfriend (1972), Nagatsu *et al.* (1974) and Kato *et al.* (1975) described developmental changes in rats with peak activity in the interval from 2 to 3 weeks and adult levels at about 10 weeks.

The purpose of the present study was to

implement a more complete evaluation of the dynamics of DBH plasma activities in relation to age in humans and to compare the results to a similarly conceived experimental study in animals. The rat was selected as a model of developmental changes of DBH activity because these results in rats compared to other experimental animals (pig, cat, monkey) were conflicting and different.

Methods

Examined subjects

Check-up, sampling of healthy subjects was performed during the periodic preventive investigations of general practitioners for children and adolescents. A total of 250 healthy children aged from 3 to 16 years (group a) were investigated. Out of these half were boys, and half girls. The sample was divided into two age groups. The first group was from 3 to 5 years old (five boys and five girls) and the second group was from 6 to 16 years (ten boys and ten girls in each group).

Eighty healthy adult volunteers (group b) were investigated at the age of 21, 35, 50 and 60 years. In each age group there were 10 men and 10 women (Table 1)

Informed consent in a written form was confirmed by parents as well as the children above 7 years (group a) and adult healthy volunteers (group b).

Experimental animals

In the experiments juvenile Wistar rats were used. The age of animals was: 5, 10, 14, 21, 25, 30, 35, 40 days and the age of adult rats was 60, 90 and 120 days. The examined animals were divided into two groups according to gender. Every age group consisted of 20 animals (10 males and 10 females). All animals were from one breeding colony.

Assessment of dopamine-beta-hydroxylase activity

Samples of human blood were collected and investigated according to the method described by Nagatsu and Udenfried (1972) and Nagatsu *et al.* (1974).

The DBH activity was measured in the serum of rats (after the decapitation). Samples were refrigerated for a period of 10 h and than centrifuged at 10 000 x *g* for 10 min. DBH activity of rat serum was estimated by an assay modified by Mellod because rat blood DBH activity depends much more on the presence of endogenous inhibitors.

Statistical processing

For statistical processing the Mann Whitney U-test, non-parametric analysis of variance testing was applied.

Results

In children and adolescents (250 healthy subjects), serum dopamine-beta-hydroxylase (DBH) activity (23.9±5.2 to 57.1±17.5 μmol/min/ml) increases with the between 3-10 years and later decreases approximately at the age of 10-14 years. At the age of 21 to 60 years the DBH level is stabile.

Table 1. DBH activities - changes during ontogenesis in experimental persons (children, adolescents and adults)

Age (years)	DBH activity
3	23.9±5.2
4	39.1±12.5
5	39.9±15.3
6	59.3±23.0
7	50.9±20.8
8	53.7±10.7
9	57.1±17.5
10	55.8±23.4
11	45.5±18.4
12	45.7±13.8
13	40.3±14.8
14	52.0±7.6
15	41.5±5.4
16	40.9±9.8
21	46.6±12.2
35	43.2±6.2
50	48.5±8.1
60	40.1±10.5

Data are means ± SEM.

Our study found a decreasing DBH activity in adolescents at the age of 10-14 years in the studied sample of healthy persons. In the statistical evaluation of children, adolescents values of DBH activity in children aged 10-15 years were compared (Fig. 1) Significant differences were found between groups aged 10 and 13 years and between groups aged 10 and 15 years ($p<0.05$).

Experimental animals (200 Wistar rats, 5-120 days old) show the same trend of enzymatic activity,

similarly as in humans. DBH activity in rats is between 0.85±0.1 to 2.8±0.05 μmol/min/ml. This activity is highest in 5-day-old rats, it decreases till the age of 14 days and increases mainly in 14- to 35-day-old animals. Reduction of DBH activity in rats between 35 to 40 days of age is significant and corresponds to the decrease of DBH activity in adolescent humans (10-14 years). At the adult age (90-120 days) DBH activity was stable.

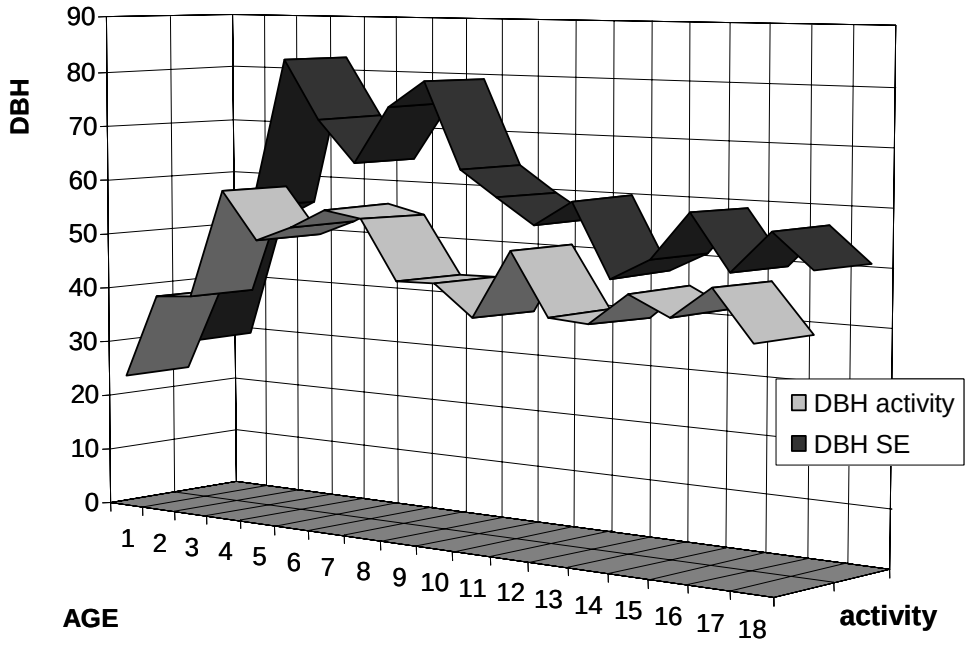

Fig. 1. Serum dopamine-beta-hydroxylase (DBH) activity in healthy children and adolescents (enzyme activity is expressed in μmol/min/ml, ± S.E.M., age in years)

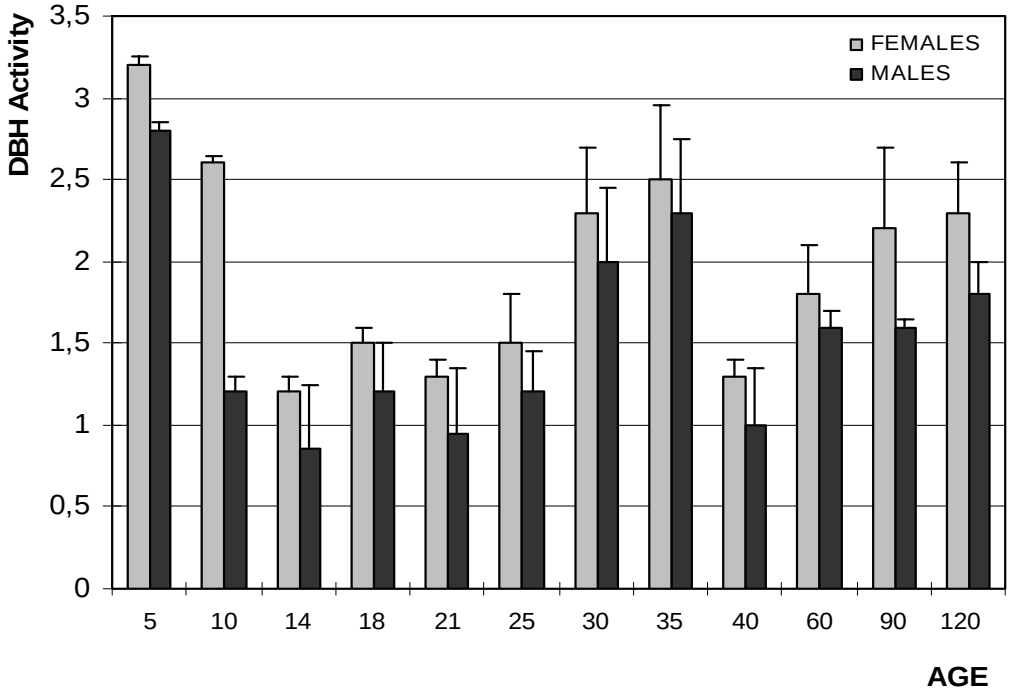

Fig. 2. Developmental changes of serum dopamine-beta-hydroxylase (DBH) activity in the female and male rats (enzyme activity is expressed in μmol/min/ml ± S.E.M., age in days)

Statistical significance of differences of DBH activity in animals aged 30, 35 and 40 days was evaluated (Fig. 2) separately for males and females. In females, there was no significant difference between animals aged 30 and 35 days. A significant difference between 30- and 40-day-old animals was found. In males there is also a significant difference but only between 30- and 40-day-old animals ($p<0.05$).

Discussion

The activity of the noradrenergic system evaluated according to the changes of DBH activity differs between children, adolescents and the adult age. The DBH activity rises continually with the exception of the pubertal period, when it suddenly decreases in the population of healthy children and adolescents and similarly in the experimental animals. Ogihara *et al.* (1974) also described a decreasing DBH activity in a sample of 42 children at the age of 10-14 years. The dependence of DBH in adults in the study of Ogihara *et al.* (1974) is very similar to our results. In our study, a decrease was not found because 40-year-old patients were not investigated (we investigated only 35-year-old subjects). Weinshilboum and Axelrod (1971), and Ogihara *et al.* (1974) found no differences in plasma DBH activity in male and female subjects.

Compared to the reports of Freedman *et al.* (1972) and Young *et al.* (1980), the numbers of subjects in different age groups of our children were significantly higher. This enables the construction of some standards for investigation of the effects of pathological changes (e.g. genetic, perinatal etc.) of the DBH activity in different age periods (age 3-20 years). These results are different from other publications that report constant values of the DBH plasmatic activity in children above 6 years (Weinshilboum *et al.* 1973a,b, Rappaport *et al.* 1974, Weinshilboum 1979, Ciaranello and Boehme 1981). Some previous data from the literature with respect to the similarity of ontogenesis of DBH activity in the rat and other experimental animals (pig, cat, monkey) were confirmed contrary to several other studies (Behrens and Depocas 1975, Lamprecht and Wooten 1976, Nagatsu and Udenfried 1972, Nagatsu *et al.*1974, Kato *et al.* 1975). In both species decreasing DBH activity was found during the pubertal period (40 days in rats and 11-13 years in man).

Our data obtained in human plasma correspond partly to the study of Suzuki *et al.* (1990). These authors investigated the DBH values in the cerebrospinal fluid of children without psychiatric or neurological diagnosis. They found continual rise of the DBH activity with the exception of children from 10 to 11 years. The transient decrease of DBH activity was coupled with increasing DBH activity at the later age from 12 to 13 years. Fujita *et al.* (1982) studied DBH activity in human cerebrospinal fluid of various age groups. The authors described increasing DBH activity in a group of 10 to 19-year-old subjects and a decreasing activity in the 60-69 and 70-79 years old subjects. For the other age groups the DBH activity was not changed.

Suzuki *et al.* (1990) described developmental changes of DBH activity in cerebrospinal fluid of children, adolescents and adults. DBH activity represents a noradrenergic developmental marker. Serum DBH activity is mainly genetically determined and is connected to abnormal regulation of hypothalamo-pituitary-adrenal functions which modify the expression of DBH protein, thus altering the ratio of dopamine and norepinephrine in noradrenergic neurons (Cubells *et al.* 2002).

We assume that observed changes of DBH activity may help in explaining some problems in the pathophysiology of psychiatric disorders in children and adult, including the different reactivity of children and adolescents, opposite to adults, to stimulants and antidepressants (Paclt and Koudelová 1997, Paclt and Florian 1998, Drtílková 1999).

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Reprint requests

I. Paclt, Department of Psychiatry, First Faculty of Medicine, Charles University Prague and General Teaching Hospital, Prague, Czech Republic. E-mail: pacltovi@email.cz