Morphological and Cytophysiological Changes in the Adult Rat Adrenal Medulla after Prenatal and Postnatal Exposure to Endocrine-Disrupting DDT

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The aim of the investigation was to study morphological and cytophysiological changes in the adult rat adrenal medulla after prenatal and postnatal exposure to endocrine-disrupting chemical dichlorodiphenyltrichloroethane (DDT).

Materials and Methods. The experiment was carried out on male Wistar rats (n=30). The rats were exposed to low doses of DDT during prenatal and postnatal development (n=10) and during postnatal development (n=10) only. The average daily intake of DDT by rats was 3.30±0.14 μg/kg. The control (n=10) and experimental animals were sacrificed at the age of 10 weeks (post-puberty), when rat adrenal glands reached their maximum development. Histological samples of the equatorial sections of the adrenal glands were prepared. The adrenal medulla was studied using light microscopy and computer morphometry. Immunohistochemistry was performed to evaluate tyrosine hydroxylase expression in chromaffin cells. Plasma epinephrine content was measured using enzyme-linked immunosorbent assay.

Results. Postnatal exposure of rats to low doses of the endocrine disruptor was found to interfere with adrenal medulla development and cytophysiology of chromaffin cells. Prenatal and postnatal exposure resulted in more profound delay in adrenal medulla development of adult rats as compared to the values of rats exposed postnatally, which was manifested by reduced area of the adrenal medulla and reduced total area of chromaffin cells in its section. Postnatal exposure is manifested by more significant functional disorders including decreased synthesis of tyrosine hydroxylase enzyme in the cytoplasm of chromaffin cells and elevated number of tyrosine-hydroxylase-negative cells in the medulla section, leading to a decrease in plasma epinephrine concentration.

Conclusion. Exposure to low doses of DDT in the early stages of development leads to morphological and cytophysiological changes in the adult rat adrenal medulla and, therefore, reduced functional activity of chromaffin cells, which allows considering the endocrine disruptor DDT a potential risk factor disrupting the function of the neuroendocrine system.

Key words: endocrine disruptors; DDT; adrenal gland; chromaffin cells; the function of the neuroendocrine system; catecholamines; epinephrine; tyrosine hydroxylase.

Introduction

The term “endocrine disruptors” was introduced into science in 1993 to describe chemical compounds disrupting the function of the neuroendocrine system [1, 2]. Dichlorodiphenyltrichloroethane (DDT) is one of the most common neuroendocrine disruptors. Its metabolites are found in living organisms around the globe [3, 4]. Currently, the ability of low doses of DDT to disrupt the production of biogenic amines remains an unexplored aspect of the problem. Studies devoted to it are of particular importance due to increasing number of behavioral disorders and development of behavioral reactions in children and adults, which is attributed by some authors to constant low-dose exposure of the body to various chemicals of anthropogenic origin [2, 5, 6].

We earlier found [7, 8] that prenatal and postnatal exposure of rats to low doses of DDT leads to a decrease in norepinephrine and epinephrine concentration in the systemic circulation. In rats, epinephrine produced by the adrenal glands accounts for more than 80% of its total content in the systemic circulation, and the major proportion of norepinephrine enters the blood from the sympathetic part of the autonomic nervous system [7]. However, the mechanism of biogenic amine synthesis disruption remains unclear.

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Materials and Methods

The study was carried out on male Wistar rats (n=30). The rats were exposed to low doses of DDT during prenatal and postnatal development (experimental group 1, n=10) and during postnatal development only (experimental group 2, n=10).

Immediately after mating with males, female rats of experimental group 1 started to consume aqueous solution of o,p-DDT with the concentration of 20 μg/L instead of water (Sigma, USA). During the pre-weaning period, rat offspring of this group received low doses of DDT with mother’s milk. Upon reaching the age of three weeks, these offspring were transferred to independent feeding and their drinking water was replaced with similar DDT solution. Experimental group 2 included rats that consumed DDT only in the postnatal period from postnatal day 1: first with mother’s milk, and then independently.

The average daily independent consumption of DDT by rats was 3.30±0.14 μg/kg body weight. The consumed dose of DDT was calculated according to the requirements for defining low doses for DDT and the Russian standards for this disruptor content in food [9].

Animals of the control group (n=10) were given tap water. The absence of DDT, its metabolites, and related organic chlorine compounds in water and food was confirmed by gas-liquid chromatography. Rats in the control and experimental groups were sacrificed after the experiment with an overdose of Zoletil at the age of 10 weeks (post-puberty) when the adrenal glands reached their maximum development [10].

The adenoglandular structures were fixed in Buen’s solution; after histologic diagnosis, equatorial paraffin-embedded sections were prepared and stained with hematoxylin and eosin. The area of the medulla, the total area of chromaffin cells in it and their sizes were measured. The presence of tyrosine hydroxylase (TH) was detected by the immunohistochemical method using rabbit polyclonal antibodies (Abcam, UK). The reaction was visualized using a commercial test system (Abcam, UK). Sections were stained with Mayer’s hematoxylin. Most chromaffin cells exhibited intense immunopositive reaction to TH with diffuse distribution in the cytoplasm. TH-positive cells with local distribution of the enzyme in the cytoplasm were much rarer. Also, single TH-negative cells were found (Figure 1, Figure 2 (a)).

Results

Rats in the control group had adrenal glands with a typical structure. The cortex and medulla were clearly distinguished on histological sections. Accumulations of chromaffin cells were detected in the medulla, their area was more than two thirds of the total area of the medulla (see the Table). Chromaffin cells had large round nuclei and moderately basophilic cytoplasm. Most chromaffin cells exhibited intense immunohistochemical reaction to TH with diffuse distribution of the enzyme in the cytoplasm. TH-positive cells with local distribution of the enzyme in the cytoplasm were much rarer. Also, single TH-negative cells were found (Figure 1, Figure 2 (a)).

The adrenal glands of rats exposed to low doses of DDT in the prenatal and postnatal periods of ontogenesis also had a typical structure. However, there was a decrease in the medulla area compared to the control group. The area of chromaffin cell accumulations in the medulla section was statistically significantly less than the control values. Comparison of chromaffin cell sizes in this group and the control revealed no differences (see the Table). The number of TH-positive cells was noted examined by light-optical microscopy and computer morphometry using ImageScope software and Leica DM2500 light microscope (Leica Microsystems, Germany).

Blood plasma was obtained by adding ethylenediaminetetraacetic acid (EDTA). Plasma epinephrine concentration was assessed by enzyme immunoassay using a highly sensitive CatCombi reagent kit (IBL International, Germany) with the minimum detectable epinephrine content of 0.03 nmol/L.

Care and use of experimental animals complied with the international guidelines set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and the ethical principles established by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 2006). The work was approved by the Ethics Committee of the Research Institute of Human Morphology.

Statistical data processing was performed using the Statistica v. 7.0 software package (Statsoft Inc., USA). For quantitative parameters, the correspondence of parameter distribution type to normal distribution law was analyzed using Lilliefors test, Shapiro–Wilk test. Central trends and dispersion of quantitative parameters with approximately normal distribution were presented as the mean value and standard error of the mean (M±m). Independent samples were compared based on quantitative parameters using Student’s t-test allowing for the values of Levene’s test for equality of variance. To compare the samples based on qualitative parameters, χ² was used. The differences were considered statistically significant at p<0.01.
Morphological parameters of the adrenal glands of mature rats in the control group and rats exposed to low doses of DDT at various stages of ontogenesis (M±m)

| Parameters                                | Control group                  | Prenatal and postnatal exposure to DDT | Postnatal exposure to DDT |
|-------------------------------------------|---------------------------------|------------------------------------------|----------------------------|
| The area of the medulla (μm²)             | 812,000.00±8315.22              | 598,200.00±45,313.12*                    | 757,000.00±41,000.00*     |
| The total area of chromaffin cells in the medulla section (μm²) | 578,850.00±21,767.91           | 394,938.00±46,496.07*                    | 544,700.00±19,100.00*     |
| The area of the chromaffin cell (μm²)     | 108.39±1.97                     | 112.32±4.40                              | 99.99±3.25*               |

* Statistically significant differences from the values of the control group; # differences between the group with "postnatal exposure to DDT" and the group with "prenatal and postnatal exposure to DDT".

Figure 1. The number of TH-positive and TH-negative cells in the adrenal medulla of rats under study

Figure 2. Immunohistochemical evaluation of TH in chromaffin cells of rat adrenal medulla:
(a) in the control group (most chromaffin cells have high TH content in the cytoplasm); (b) in rats exposed to low doses of DDT in the prenatal and postnatal periods (decrease in TH content in chromaffin cell cytoplasm); (c) exposure only in the postnatal period (most chromaffin cells do not contain TH); Mayer’s hematoxylin staining; ×400
Adrenal Changes in Rats Induced by Exposure to DDT Disruptor

Discussion

The rat adrenal medulla is known to continue developing after the birth of the animal, reaching its maximum development by post-pubertal age [10, 11]. The obtained results show that prenatal and postnatal exposure to low doses of DDT negatively affects the development and functional activity of the adrenal medulla in the post-pubertal period.

In rats exposed to low doses of DDT in the prenatal and postnatal periods of ontogenesis, a decrease in medulla size and the total area of chromaffin cells in the medulla section was revealed after reaching puberty (at the age when adrenal growth normally comes to end [10]), the size of these cells remaining unchanged. This fact implies a decrease in the number of chromaffin cells and delay in the development of the medulla as a whole. The decrease in the development of chromaffin tissue in the adrenal glands of rats exposed to a disruptor was proportional to the decrease in epinephrine production. To determine the mechanism of catecholamine secretion and function of the neuroendocrine system.

In rats exposed to low doses of DDT only in the postnatal stage of ontogenesis, a decrease in the size of chromaffinocytes set against the unchanged area occupied by these cells in the medulla indicates a compensatory increase in their number in this experimental group. However, a decrease in catecholamine secretion and increase in the number of TH-negative cells were significantly more pronounced than in the animals exposed to low doses of DDT in the prenatal and postnatal periods. This may indicate the developing compensatory processes of reactivation of chromaffin cell secretion due to shorter DDT consumption, manifesting as increased number of functionally immature cells.

The obtained results show that prenatal and postnatal exposure to low doses of DDT negatively affects the development and functional activity of the adrenal medulla in the post-pubertal period. This may indicate the developing compensatory processes of reactivation of chromaffin cell secretion due to shorter DDT consumption, manifesting as increased number of functionally immature cells.

Conclusion

Developmental exposure of rats to low doses of endocrine-disrupting DDT impairs the development of adrenal medulla and cytophysiology of chromaffin cells. Exposure initiated in the prenatal period is manifested mainly by morphological changes in the medulla after puberty, while exposure in the postnatal period reveals itself in more pronounced impairment of chromaffin cell functioning. Therefore, exposure to low doses of DDT can be considered a potential risk factor disrupting the function of the neuroendocrine system.

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Conflict of interests. The authors have no conflict of interests related to the present study.

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