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Effect of Prenatal and Postnatal Exposure to Low Doses of DDT on Catecholamine Secretion in Rats in Different Period of Ontogeny

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We studied the effects of prenatal and postnatal exposure to low doses of DDT on secretion of basic catecholamines epinephrine and norepinephrine in pubertal and adult rats. It was found that the endocrine-disrupting chemical under study led to a progressive decrease in the content of epinephrine and especially norepinephrine in systemic circulation, which indicated their disturbed secretion by the adrenal medulla and sympathetic nervous system cells. In animals exposed to low doses of DDT in both pre- and postnatal periods, the decrease in catecholamine secretion after puberty was less pronounced than in animals exposed only during the postnatal period, which can indicate the development of compensatory processes.

Key Words: *endocrine disruptors; DDT; catecholamines; epinephrine; norepinephrine*

Permanent organic pollutant DDT belongs to the most widespread disruptors and its influence on the developing organism is now actively studied [4,8]. Most studies are related to the disruptor effect of DDT on the development of the reproductive system organs and thyroid gland [2,3]. The development of the neuroendocrine system and regulation of body functions under the influence of the disruptor remain little studied. The least explored aspect is the effect of low DDT doses on the production of catecholamines, bioactive substances that participate in both nervous and humoral regulation of diverse physiological processes.

We studied the effect of prenatal and postnatal exposure to DDT in low doses on epinephrine and norepinephrine secretion in pubertal and adult rats.

MATERIALS AND METHODS

The study was carried out on male Wistar rats (n=36). The effect of low doses of DDT on the developing organism was simulated: prenatal and postnatal effects on the body (experimental group 1, n=12) and only postnatal exposure starting from the first day of life (experimental group 2, n=12). To this end, female rats after mating were given aqueous solution of DDT (20 μg/liter; Sigma) instead of water. Group 1 pups received low doses of DDT with mother's milk during the suckling period; after weaning (at the age of 3 weeks), they received the same DDT solution instead of water. Rat pups of experimental group 2 received DDT only during postnatal period from the 1st day of life, first with the mother's milk, and then with drinking water. The average daily intake of DDT was 3.71±0.15 μg/kg. The consumed dose of DDT was calculated according to definition of the National Toxicology Program (USA) [9] and using Russian sanitary standards for allowable DDT content in food [1]. Controls (n=12) received tap water. The absence of DDT, its metabolites, and related organochlorine contaminants in water and food was confirmed by gas-liquid chromatography. The animals were sacrificed by zoletil overdose at the age of 1.5 (pubertal period) or 2.5 months (post-pubertal period). In the plasma separated from EDTA-stabilized blood, the content of epinephrine and norepinephrine was measured by ELISA using highly sensitive CatCombi kits (IBL International) with detection limits of 0.01 and 0.02 ng/ml

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for epinephrine and norepinephrine, respectively. The experiments were conducted in accordance with the Order No. 577 of the Ministry of Health of the USSR (August 12, 1977).

Statistical processing of the results was conducted with Statistica 7.0 software. The differences were considered significant at $p<0.01$.

RESULTS

In pubertal male rats of the control group, plasma concentration of norepinephrine was slightly higher than epinephrine (epinephrine/norepinephrine ratio <1; Table 1).

In male rats exposed to low doses of DDT during the prenatal and postnatal periods of ontogeny, plasma content of catecholamines was significantly lower by one third than in animals in the control group and epinephrine concentration was lower by ~20%. The decrease in norepinephrine concentration was more pronounced (by 45%). The epinephrine/norepinephrine ratio in the plasma of experimental rats was >1 and surpassed the control values by 40% (Table 1).

In males exposed to low doses of DDT only during the postnatal period, the decrease in catecholamine concentration was also observed during puberty, but it was less pronounced (Table 1). The epinephrine concentration was lower by ~10%, and norepinephrine — by 30% in comparison with the control. Thus, the decrease in norepinephrine concentration in systemic circulation was more pronounced, similar to animals subjected to DDT during pre- and postnatal periods. The epinephrine/norepinephrine ratio in rat plasma increased by one third.

In control adult rats, the total content of epinephrine and norepinephrine decreased by 13% (Table 1), primarily at the expense of a decrease in plasma concentration of epinephrine by ~20%. The level of norepinephrine did not significantly differ. The ratio of epinephrine/norepinephrine was decreased by 15% in control animals.

In mature rats exposed to DDT in low doses during the pre- and postnatal periods, the total content of epinephrine and norepinephrine decreased by one third in comparison with the previous period and was by 2 times below the corresponding value in age-matched controls (Table 1). Epinephrine content in the blood plasma decreased by 30% and was significantly lower (by one-third) than in the control group. The concentration of norepinephrine in adult rats decreased by 35% and was below the control by 60%. The epinephrine/norepinephrine ratio differed significantly from that during the previous period. It was >1 and exceeded the control values by 80%.

In adult rats exposed to low doses of DDT only during the postnatal period, the total plasma content of epinephrine and norepinephrine significantly decreased (by more than 60%; Table 1). The content of epinephrine and norepinephrine attained minimum. As during the previous period, norepinephrine concentration decreased more drastically (by ~76%). The epinephrine/norepinephrine ratio exceeded the control values by more than 2 times.

It is known that plasma content of norepinephrine reflects activity of the peripheral sympathetic nervous system, while the content of epinephrine reflects activity of the adrenal medulla, because more than 90% epinephrine in the systemic circulation is released

TABLE 1. Plasma Levels of Epinephrine and Norepinephrine in Pubertal and Adult Rats Exposed to Low Doses of DDT during the Postnatal and Prenatal+Postnatal Periods ($M\pm m$)

Parameter	Control	Prenatal and postnatal exposure to DDT	Postnatal exposure to DDT
Pubertal rats			
Epinephrine+norepinephrine, ng/ml	19.76±0.98	13.38±1.09*	15.65±1.02*
Epinephrine, ng/ml	9.09±0.15	7.35±0.61*	8.28±0.58
Norepinephrine, ng/ml	10.67±0.88	6.03±0.49*	7.37±0.54*
Epinephrine/norepinephrine ratio	0.85±0.07	1.21±0.07*	1.12±0.07*
Adult rats			
Epinephrine+norepinephrine, ng/ml	17.19±1.29	9.19±0.35*°	6.41±0.42*+°
Epinephrine, ng/ml	7.26±0.58°	5.22±0.13*°	4.03±0.27*+°
Norepinephrine, ng/ml	9.93±0.70	3.97±0.21*°	2.38±0.18*+°
Epinephrine/norepinephrine ratio	0.73±0.06	1.32±0.03*°	1.69±0.11*+°

Note. $p<0.01$ in comparison with *controls, +prenatal and postnatal exposure, °previous period.

from the adrenals [6]. In sympathetic nerve endings, norepinephrine is stored in synaptic vesicles and is in fact the final product of catecholamine synthesis, as its conversion into epinephrine is insignificant. In the adrenal medulla, norepinephrine is intensively methylated and its content is by 4-6 times lower than the content of epinephrine [7]. The blood—brain barrier is impermeable for catecholamines. Thus, circulating epinephrine and norepinephrine are typical hormones that are eliminated from the circulation via methylation and oxidation in various organs, mainly in the liver.

We found reduced plasma content of both catecholamines in rats exposed to DDT. Norepinephrine showed the highest sensitivity to the effects of low doses of the endocrine disruptor. This induces a shift of catecholamine balance towards the stronger adrenoceptor stimulator epinephrine probably aimed at the compensation of catecholamines insufficiency. After puberty, the decrease in the content of epinephrine and norepinephrine becomes even more pronounced. The leading role in the decrease in epinephrine level in systemic circulation is played by reduction of its production by the adrenal gland, rather than enhanced inactivation [10]. The degree of the drop of catecholamine content in both experimental groups suggests that reduced secretion is the leading mechanism underlying their reduced content in systemic circulation. Changes in plasma levels of norepinephrine and epinephrine in adult rats exposed to DDT during the postnatal and during both prenatal and postnatal periods were similar, but in rats treated with DDT in the postnatal period, reduction of catecholamines, especially of norepinephrine, were more pronounced after puberty.

Normally, the total content of epinephrine and norepinephrine in intact rats little changes with age, but the epinephrine/norepinephrine ratio decreases due to the decrease in epinephrine secretion. In rats subjected to prolonged action of DDT, the dynamics of the plasma content of epinephrine and norepinephrine is similar, but the drop in norepinephrine concentration is more expressed. Age-related increase in the epinephrine/norepinephrine ratio attests to significant imbalance in hormonal regulation, because norepinephrine produces less pronounced effect on carbohydrate and lipid metabolism than epinephrine; secretion of the latter, in addition to neuronal regulation, is also stimulated by insulin and glucocorticoids. The revealed changes in catecholamine production suggest that exposure to low doses of DDT is a potential risk factor that disrupts the function of the nervous system. The relationship between prenatal exposure to DDT and subsequent disorders of higher nervous activity, mental retardation, increased incidence of attention deficit disorder, and behavioral abnormalities was previously reported [5,8]. Disorders of mental development are traditionally attributed to the disruptor effect of DDT on the thyroid gland and gonads [11,12]. However, the data obtained by us indicate the possibility of a direct influence of DDT on the nervous system due to a decrease in the level of catecholamines and their imbalance, which may to become the reason of disturbance of protective reactions of an organism in response to stress.

Thus, low doses of DDT can modulate secretion of not only the sex and thyroid hormones, but also catecholamines. Secretion of norepinephrine is more sensitive to the influence of the disruptor. Exposure to low doses of DDT during the pre- and postnatal periods leads to progressive decrease in the content of epinephrine and norepinephrine in systemic circulation, which attests to disturbances in their secretion by both adrenal medulla chromaffin cells and sympathetic nervous system neurons.

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