Effect of Treatment with Testosterone Derivatives on Morphometric Characteristics and Free Radical Oxidation in Rat Cerebral Cortex

B. Ya. Ryzhavskii, O. V. Zadvornaya, and O. A. Lebed'ko*

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Morphometric characteristics and free radical oxidation in neurons of the neocortex and hippocampus of male and female rats were studied 1 month after administration of Sustanon-250 in a dose of 8 mg/kg during the pubertal period of ontogeny. The weight of the brain was shown to decrease in males. In both female and male rats, the width of the anterior parietal lobe and the numerical density of neocortical and hippocampal neurons decreased. Sex differences were found in free radical oxidation in the cerebral cortex. The intensity of this process increased significantly in females, but decreased in males.

Key Words: *brain; morphometry; testosterone; free radical oxidation*

The concentration of sex hormones, including androgens, in the body varies significantly during ontogeny, sex cycle, pregnancy, and endocrine dysfunction. Endocrine disorders include the suppressed or increased production of specific sex hormones, as well as the reduced or elevated concentration of opposite sex hormones [15]. For instance, hyperandrogenemia is a typical symptom of polycystic ovary syndrome (one of the common gynecological diseases) in women of various ages, juvenile females, and girls [1]. By contrast, blood testosterone level decreases in men of various ages and juvenile males with hypogonadism [3,5]. Androgen deficiency requires long-term replacement therapy with hormones. Hence, endogenous hyperandrogenemia can be observed in women with endocrine dysfunction. Male patients are characterized by the increase in androgen level due to long-term consumption of testosterone derivatives. These changes can occur after the completion of brain maturation (prepubertal and pubertal periods). It should be emphasized that male sex hormones produce a strong effect on the development, metabolism, and function of the brain. Treatment with these hormones in the critical period of organogenesis has a modulatory effect on sex differentiation and morphometric characteristics of the organ during the following periods of ontogeny [6]. Castration of male rats violates their behavior and content of neuroactive steroids in various areas of the brain [7]. One of the effects of male sex hormones is their influence on free radical oxidation (FRO). Administration of androgens is followed by a decrease in the intensity of FRO (less significantly than estrogens) [8]. The degree of oxidative stress and stimulation of apoptosis in rat brain cells due to ovariectomy were shown to be less significant after treatment with testosterone [14].

Much attention was paid to clinical, physiological, and biochemical changes in the brain under the influence of androgens in high concentrations. However, the effect of these substances on morphological characteristics and FRO in the brain areas that are not directly related to the regulation of reproduction in

Department of Histology, Far-Eastern State Medical University; *Clinical and Diagnostic Laboratory, Institute of Maternity and Child Welfare, Siberian Division of the Russian Academy of Medical Sciences, Khabarovsk. ***Address for correspondence:*** zadvornaya.87@mail.ru. O. V. Zadvornaya

male and female animals remains unclear. This work was designed to answer this question.

MATERIALS AND METHODS

Experiments were performed on outbred albino rats. At the age of 60 days, male and female animals from 4 litters were divided into two groups (treatment and control). The treatment group consisted of 10 males and 7 females. The control group consisted of 9 males and 7 females. No between-group differences existed in the initial body weight of rats (Table 1). Males and females of the treatment group received an intramuscular injection of Sustanon-250 in a dose of 8 mg/kg [4]. This agent has a prolonged effect and provides the supraphysiological concentration of testosterone in the blood plasma of males [8,9]. Control animals received an equivalent volume of the solvent (peach oil). Rats of the control and treatment groups were maintained in the same vivarium and received water and food *ad libitum*. The animals were decapitated at the age of 90 days. We measured the body weight, weight of the brain, and weight of the sex glands. Cortical segment of the right cerebral hemisphere were sampled to estimate the intensity of FRO. The left hemisphere was fixed in Carnoy's fluid. The anterior parietal (APL) and parietal lobes (PL) were embedded into paraffin. Sections (7 μ) were prepared and stained with Einarson's gallocyanin for a morphometric study and measurement of nucleic acid concentration. The width of the cortex in APL and PL, average diameter of the seminiferous tubule, and average diameter of the largest ovarian follicle were evaluated with a MOV-15 ocular micrometer. The numerical density of neurons was measured in 5 standard fields of view (neocortical layers II and V; and hippocampal area I). The size of neurons and RNA concentration in the cytoplasm were estimated by means of computerized morphometry on a MECOS device. FRO in the brain was studied by the method of chemiluminescence (CML). CML in homogenates of the right cerebral cortex from rats was measured on a LS 50B luminescence spectrometer (Perkin Elmer). Spontaneous and Fe^{2+}-induced CML was recorded as described elsewhere [1]. The following parameters were estimated: total luminescence over 1 min of spontaneous CML (Ssp); maximum flash amplitude (h) of induced CML; and total luminescence over 4 min of the post-flash period (Sind1). Kinetic parameters of H_2O_2-induced luminol-dependent CML [12] were evaluated from the maximum luminescence (H) and total luminescence over 2 min of CML (Sind2). The intensity of CML was calculated per 1 g wet tissue (sampled immediately after decapitation) and expressed in arbitrary units.

The results were analyzed by Statistica 6.0 software (descriptive statistics).

RESULTS

Administration of Sustanon to 2-month-old rats did not affect the body weight gain in animals. No between-group differences were revealed in body weight of 3-month-old rats. However, the weight of the gonads in treated males was much lower than in control specimens (1203±29 and 1385±36 mg, respectively; $p<0.05$). These data reflect delayed growth of the gonads in Sustanon-receiving males. The diameter of the seminiferous tubule practically did not differ in males of these groups (354±12 and 338±8.9 μ, respectively). No significant differences were found in the weight of the ovaries in females of the treatment and control groups (45±5.7 and 35±4 mg, respectively). However, the size of the follicles in treated females was lower than in control specimens (586±37 and 789±64 μ, respectively; $p<0.05$). Hence, Sustanon treatment had a modulatory effect on the development of the gonads in male and female animals.

The weight of the brain in treated males was much lower than in control specimens. However, Sustanon injection was followed by the retardation of brain growth in animals from the 2nd to the 3rd month of life. No between-group differences were revealed in the weight of the brain in females (Table 1). These data illustrate the existence of sex differences in the effect of testosterone in high concentration on the growth of the brain in young and sexually mature rats. Therefore, sex differences in the weight of the brain that are observed in control animals become statistically insignificant (Table 1).

As differentiated from sex differences in variations in the weight of the brain, male and female animals of the treatment group were characterized by similar changes in some morphometric parameters. The width of the APL cortex in treated males and females was much lower than in control specimens. However, no between-group differences were found in the width of PL (Table 1). The numerical density of neurons in layers II and V of APL and PL in treated males and females was much lower than in control animals. The numerical density of hippocampal neurons decreased significantly in males (Table 1) and tended to decrease in females ($p<0.1$). Hence, these changes in the cortex of males and females had the same directionality and localization. Together with the data on brain weight (reduced brain weight in males) and width of the cortex (decrease in the width of the APL cortex in males and females), these results suggest that Sustanon treatment is followed by a decrease in the total number of neocortical neurons in rats (particularly in males) as compared to the control. The observed effect of testosterone derivatives on morphometric characteristics of brain development, neocortex, and hippocampus agree

TABLE 1. Effect of Sustanon-250 on Various Indexes of Brain Development and FRO in Rats

Parameter			Males		Females	
			treatment (n=10)	control (n=9)	treatment (n=7)	control (n=7)
Body weight, g		2 months	145.0±5.7	151.0±6.9	117.0±3.7	116±3
		3 months	207.0±5.7	211.0±7.8	164.0±7.8	153±5
Weight of the brain, mg			1722±15*	1790±20	1685±38	1632±23
Width of the cortex, μ		APL	1589±30*	1739±34	1604±17*	1694±36
		PL	1124±36	1141±26	1177±44	1194±34
Number of neurons in the standard field of view						
APL		layer II	16.3±0.6*	19.70±0.56	14.50±1.03*	19.90±0.55
		layer V	8.00±0.43*	10.00±0.43	7.40±0.51*	10.80±0.31
PL		layer II	17.80±0.72*	21.8±0.8	16.40±0.83*	21.90±1.12
		layer V	9.0±0.3*	11.3±0.4	8.60±0.58*	11.90±0.49
		hippocampus	20.50±0.47*	23.1±0.4	21.30±0.62	23.40±0.79
Sectional area, μ^2						
APL	Layer II	nucleoli	2.28±0.04	2.40±0.07	2.700±0.068*	3.10±0.16
		nuclei	54.00±0.89	57.0±0.7	55.00±3.62	56.00±1.06
		cytoplasm	46.0±1.3	48.00±1.43	54.00±2.41	55.00±1.07
	Layer V	nucleoli	6.60±0.24	6.60±0.25	6.3±0.2*	7.20±0.25
		nuclei	109.0±3.8	113.00±2.43	99.00±4.07	102.00±3.38
		cytoplasm	142.0±7.8	148.00±3.95	143.00±8.23	152.00±7.13
PL	Layer II	nucleoli	2.20±0.06*	2.40±0.07	2.40±1.14	2.700±0.105
		nuclei	51.00±1.05*	55.00±0.9	54.00±0.79	52.00±0.74
		cytoplasm	44.00±1.87	46.00±0.95	48.00±2.16	48.00±0.41
	Layer V	nucleoli	5.10±0.27*	5.80±0.15	5.60±0.29	5.80±0.26
		nuclei	85.0±4.2*	96.00±2.7	86.00±3.73	92.00±3.59
		cytoplasm	105±7.6	118±5.6	113±7.48	129±7.57
	Hippocampus	nucleoli	2.70±0.08*	3.00±0.12	2.800±0.079*	3.50±0.14
		nuclei	65.00±1.34	68.00±0.75	67.00±1.88	67.00±1.17
		cytoplasm	46.0±0.6	47.00±1.14	46.60±1.97	51.00±0.85
RNA concentration in the cell cytoplasm, arb. units						
APL		layer II	0.640±0.017	0.620±0.019	0.566±0.022	0.571±0.011
		layer V	0.60±0.03	0.600±0.027	0.545±0.026	0.535±0.024
PL		layer II	0.64±0.02	0.600±0.015	0.607±0.025	0.550±0.021
		layer V	0.620±0.023	0.590±0.016	0.580±0.027	0.540±0.031
		hippocampus	0.690±0.035	0.710±0.027	0.682±0.034	0.618±0.033
CML, arb. units		Scp	0.0910±0.0023*	0.125±0.008	0.152±0.003	0.119±0.005
		h	0.5560±0.0227*	0.734±0.035	1.116±0.034*	0.794±0.031
		Sind1	0.7520±0.0223*	1.109±0.072	1.601±0.064*	1.109±0.026
		H	1.154±0.043*	1.756±0.059	2.223±0.061*	1.627±0.039
		Sind2	2.680±0.078*	3.194±0.093	3.732±0.177*	2.963±0.059

Note. *p<0.05 in comparison with the control.

well with published data. It was reported that salivary testosterone level in students from gifted child schools is lower than in those from common schools [14]. Previous studies revealed some specific features of cerebral cortex growth in children with extraordinary intellectual capacity [16].

A histological examination of samples from animals of the treatment group did not reveal destructive changes in study areas of the brain. A morphometric study showed that treated males are characterized by a significant decrease in the size of neuronal nuclei (layer II of APL; and layers II and V of PL) and nucleoli (layers II and V of PL; and hippocampus). Variations in the size of cells in females were less pronounced than in males. They were manifested in a decrease in the sectional area of neuronal nucleoli in layers II and V of APL and hippocampus. The observed differences between males and females of the treatment and control groups were not very significant (Table 1). RNA concentration in the cytoplasm of neocortical and hippocampal neurons in treated males and females practically did not differ from the control.

A study of FRO in the cerebral cortex showed that CML practically does not differ in males and females of the control group. We showed that morphometric characteristics of the cortex in males and females change similarly after Sustanon administration. However, this agent had the opposite effects on FRO in the brain of animals. All parameters of CML were reduced in Sustanon-receiving males (as compared to the control). We revealed a decrease in the intensity of free radical processes (Ssp), concentration of lipid hydroperoxides (h), and formation of peroxide radicals (Sind1). The resistance to peroxidation was elevated under these conditions (H and Sind2). Ssp, h, and Sind1 significantly increased, while H and Sind2 decreased in females after Sustanon treatment.

Our results indicate that the intensity of FRO decreases in males, but increases in females after administration of testosterone derivatives. The inhibition of FRO in males can be related to the ability of androgens to suppress these processes [10]. It should be emphasized that the intensity of FRO in rats decreases in the period between the 10th and 90th day of life [2]. Administration of Sustanon to 2-month-old males probably accelerates the age-related decrease in FRO. This explanation agrees well with the data that brain weight gain, which occurs in maturing animals under normal conditions [6], is less pronounced in males of the treatment group. When considering the increase of FRO in females, it is necessary to take into account that estrogens (*e.g.*, estradiol) have very high antiradical or antioxidant activity [13]. It can be suggested that administration of testosterone derivatives impairs the synthesis of estrogens in the ovaries, which contributes to activation of FRO in the brain. A decrease in the size of abdominal follicles (main source of estrogens) in the ovaries of treated females (Table 1) illustrates the influence of Sustanon on histophysiological characteristics of the ovaries. We conclude that the effect of Sustanon on FRO in the brain of treated females can be mediated by this mechanism.

Our results indicate that Sustanon affects the major morphological parameters of brain development in young and sexually mature animals (width of the cortex and numerical density of cortical neurons). We revealed that this hormone has various effects on morphometric characteristics of functionally different zones of the neocortex (APL and PL). Sex differences were found in the brain response to administration of testosterone derivatives. It should be emphasized that treatment of males and females with testosterone agents of prolonged action during the pubertal period did not cause necrotic or atrophic changes in the neocortex and hippocampus. Cytophysiological characteristics of neurons (size of the cytoplasm and cytoplasmic RNA level) remained unchanged under these conditions. These data illustrate the ability of cortical neurons to develop and function at a varying concentration of sex hormones in the blood (*i.e.*, significant changes in the content of these hormones) due to the age, functional state of steroid-producing organs, and pathological processes.

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