Effect of Testosterone Derivatives Administered to Pregnant Rats on the Brain of Their Offspring (Delayed Consequences)

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We examined the offspring of rats receiving sustanon with testosterone derivatives on day 19 of pregnancy. At the age of 1 month, these rats differed from control animals in the indexes of higher nervous activity. The relative weight of the brain increased in 54-day-old rats receiving the hormone. Female rats were characterized by increased sudanophilia of the white matter in cerebellar hemispheres and high RNA content in layer V neurons of the parietal lobe, increased volume of nuclei in hippocampal neurons, weight of the ovaries (tendency), size of the largest follicles, and high activity of 3β-ol-steroid dehydrogenase. The weight of the adrenal glands and 3β-ol-steroid dehydrogenase activity in adrenocortical cells of the fascicular zone increased in male rats.

Key Words: *brain; development; testosterone; gonads; adrenal glands*

Various disturbances accompanied by hyperandrogenia and associated with disorders of the adrenal cortex and ovaries in pregnant women and adrenogenital syndrome in fetuses and newborn infants determine considerable attention of scientists to the effect of androgens on brain development [2,4,6]. Published data show that male and female children with congenital hyperplasia of the adrenal glands have low volume of the amygdaloid complex [8]. Magnetic resonance studies showed that congenital hyperplasia of the adrenal glands is accompanied by disturbances in the formation of brain asymmetry and difficulties in learning [10]. It was hypothesized that testosterone acting during embryogenesis increases the capacity for spatial analysis [7]. Saliva testosterone concentration in male and female pupils trained at schools for talented children was lower than in pupils of normal schools [9]. Signs of accelerated brain development were revealed in 1-day-old offspring of rats receiving sustanon, a preparation containing testosterone derivatives, during pregnancy [5]. Here we studied the delayed effect of sustanon administered to pregnant rats.

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MATERIALS AND METHODS

We examined the offspring of albino rats receiving the suspension of sustanon-250 (0.05 ml) on day 19 of pregnancy. The preparation contains 1.5 mg testosterone propionate, 3 mg testosterone phenyl propionate, 3 mg testosterone isocaproate, and 5 testosterone decanoate. The effect of sustanon-250 rapidly develops and persists for a long time. The experimental and control groups included offspring of 3 treated (n=31) and 3 intact females (n=24), respectively. The animals of both groups were kept in a vivarium under standard conditions and received food and water *ad libitum*. The weight of female rats on day 19 of the first pregnancy was 250-270 g. Total exploratory activity of 1-month-old offspring was studied in an elevated plus-maze (EPM) for 3 min. The duration (% of the total time spent in EPM) and number of various

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movements, including nuzzling, hanging down, vertical rearing postures, grooming, ambulations, and entries into open and closed arms, were recorded on a computer using special software. The treated and control rats were decapitated on day 54 of life, body weight and weights of the brain, cerebral hemispheres, cerebellum, adrenal glands, and sex glands were determined. Paraffin sections of the brain (7 μ) from the left anteroparietal (APL) and parietal lobes (PL) were stained with gallocyanin for nucleic acids (method of Einarson) and analyzed by morphometry. RNA concentration was measured in the cytoplasm of layer V cortical neurons in APL, PL, and hippocampus. The test indexes were determined on a MECOS device (Medical Computer Systems) with Morfodensitometriya software. We examined 25 neurons in each brain area. The width of the cortex and layer I in both zones of the neocortex was measured using a MOB-15 ocular micrometer. Cryostat sections (25 μ) were prepared from the right hemisphere and cerebellum of 40-day-old rats. The sections were stained with Sudan black to determine the degree myelinization of the white matter. It was determined by optical density of the end product on a MECOS device. The reaction for 3β-olsteroid dehydrogenase (StDH) was performed on cryostat sections of the adrenal glands and ovaries [1]. Enzyme activity was measured in 25 adrenocortical cells of the zona glomerulosa, zona fasciculata, and zona reticularis of the adrenal cortex and in 25 thecal cells of cavity follicles on a MECOS device. The width of the adrenal cortex and diameter of the largest follicle were measured using an ocular micrometer. The mean diameter of the twisted seminal tubule was estimated on cryostat sections of the testes (10 measurements). The results were analyzed using Statistica software.

RESULTS

Body weight was similar in the offspring of treated and control females. The absolute weight of the brain

TABLE 1. Effect of Sustanon Administered to Pregnant Rats on Brain Development in the Offspring (*M*±*m*)

Index		Males		Females	
		control	treatment	control	treatment
Brain weight	abs., mg	1711±17	1763±15*	1666±12	1696±17
	rel., mg/g	10.6±0.2	11.5±0.2*	12.4±0.2	13.2±0.2*
Hemisphere weight, mg		609±14	620±12	596±22	588±11
Cerebellum weight, mg		224±3	232±3*	206±9	224±3
Width, μ	APL cortex	1572±38	1548±40	1545±45	1498±29
	APL layer I	152.0±7.7	150±12	140±14	132±9
	PL cortex	1165±36	1195±41	1126±20	1186±55
	PL layer I	147±6	135±6	117±6	132±9
Area of neuron cytoplasm, μ^2					
	APL layer V	119.0±11.2	103.0±12.3	93.0±8.6	95.0±8.8
	PL layer V	73.0±3.8	77.0±2.8	67.0±1.2	67.0±2.6
	hippocampus	41.0±4.5	43.0±2.8	39.0±2.2	44±3
Area of neuron nuclei, μ^2					
	APL layer V	81.0±4.3	82.0±3.8	71.0±2.8	77.0±4.9
	PL layer V	67.0±1.9	73.0±1.8	71.0±2.2	67.0±2.5
	hippocampus	57.0±5.6	65.0±2.7	55.0±2.8	65.0±1.9*
Cytoplasmic RNA concentration in neurons, arb. units					
	APL layer V	420±25	394±24	445±20	438±27
	PL layer V	429±14	437±18	438±19	493±18*
	hippocampus	606±23	594±4	540±27	534±24
Lipid concentration (white matter), arb. units					
	cerebral hemispheres	544±48	675±57	571±81	714±47
	cerebellar hemispheres	929±45	906±32	635±32	853±26*

Note. Here and in Table 2: *statistically significant differences compared to the control.

in male offspring, relative weight of the brain in male and female offspring, and weight of the cerebellum in male offspring of treated rats were significantly higher than in control animals (Tables 1 and 2). Lipid content in the white matter of cerebral hemispheres tended to increase in male and female offspring of treated rats (statistically insignificant). The reaction for lipids in the white matter of cerebral hemispheres increased in prenatally treated females, but remained practically unchanged in males. Sex differences in this index were observed in the control, but not in treated animals (Table 1).

Morphometric indexes of the brain cortex and neurons of the hippocampus and layer V of APL and PL did not differ in prenatally treated and control males. Female offspring of treated rats was characterized by an increased size of nuclei in hippocampal neurons and tendency toward a rise in the volume of their cytoplasm. Cytoplasmic RNA concentration increased in layer V neurons of PL in females (Table 1).

Sex differences in the EPM behavior were revealed in animals of the control group. Male rats exhibited long-lasting vertical rearing postures (6.2±0.9 *vs*. 3.7±1.0% in females) and greater number of vertical rearing postures (9.6±1.2 *vs*. 5.8±1.2% in females) and entries into the open (3.8±0.5 *vs*. 1.9±0.2% in females) and closed arms (4.0±0.4 *vs*. 2.2±0.2% in females). Prenatally treated animals were characterized by a decrease in the number of movements (15.8±0.9 *vs*. 20.4±1.2 in the control) and entries into the open (2.2± 0.4 *vs*. 3.8±0.5 in the control) and closed arms (2.5± 0.3 *vs*. 4.0±0.4 in the control). Female offspring of treated rats exhibited an increase in the duration (9.8± 2.9 *vs*. 3.7±1.0 in the control) and number of vertical rearing postures (12.0±1.7 *vs*. 5.8±1.2 in the control). Significant sex differences in the indexes of higher nervous activity were observed in the control, but not in treated animals.

The weight of the adrenal glands increased in prenatally treated males. Sexual dimorphism manifesting in a greater size of the adrenal glands in control females was not typical of treated rats. StDH activity significantly increased in the zona fasciculata and tended to increase in the zona glomerulosa and zona reticularis in male offspring of treated animals. The weight of the testes and mean diameter of tubules practically did not differ in treated and control rats (Table 2). No between-group differences were revealed in histophysiological indexes of the adrenal glands in females. The weight of the ovaries, diameter of the largest secondary follicle, and StDH activity in the inner follicular theca tended to increase in female rats ($p>0.05$, Table 2).

Signs for accelerated development of the brain were revealed in 1-day-old offspring of females receiving sustanon [5]. They were less pronounced in the brain of 54-day-old animals and included an increase in the weight of the brain and cerebellum and high lipid concentration in the white matter of the brain and cerebellum. The reaction to prenatal treatment with sustanon depended on the sex of rats. As distinct from control rats, sex differences in the behavior and lipid concentration in the white matter of the cerebellum that depends on the degree of myelinization were insignificant in the offspring of treated females. A major cause for the reduction of sexual

TABLE 2. Effect of Sustanon Administration to Pregnant Rats on the Development of the Adrenal Glands and Gonads in the Offspring (*M*±*m*)

Index		Males		Females	
		control	treatment	control	treatment
Body weight, g		162.0±3.2	156.0±2.6	135.0±2.3	129.0±2.5
Weight, mg	adrenal glands	19±1	22.0±1.1*	21.0±1.1	22±1
	testes	1054±23	1025±20	—	—
	ovaries	—	—	43.0±3.1	49.0±2.3
Width of adrenal cortex, μ		959±33	996±24	1058±77	1099±36
Diameter, μ					
	ovarian follicles	—	—	760±48	864±44
	seminal tubules	328±10	325±7	—	—
StDH activity, arb. units					
	zona glomerulosa	483±62	625±58	517±86	549±40
	zona fasciculata	916±55	1130±66*	876±105	990±79
	zona reticularis	823±48	902±64	816±41	798±45
	follicular theca	—	—	586±70	704±58

dimorphism was masculinization of the test indexes in females (Tables 1 and 2). The increased weight of the adrenal glands did not differ in prenatally treated male and female animals. We revealed no intergroup differences in morphometric indexes of neocortical neurons in male rats (Table 1). By contrast, female offspring of sustanon-treated animals was characterized by a significant increase in the size of nuclei in hippocampal neurons and cytoplasmic RNA concentration in layer V neurons of PL. These morphometric indexes of the brain in treated rats can be considered as a morphological equivalent to modified characteristics of higher nervous activity.

It should be emphasized that sustanon contains testosterone derivatives with long-lasting effects (up to 1 month). These data suggest that the offspring was exposed to the influence of sustanon not only during the prenatal period (transplacental passage), but also after birth (mother's milk). Testosterone derivatives can modulate organogenesis of the brain, which is determined by their anabolic effect, reduction of the response to stress factors [3,4], and receptor-mediated influence on neurons [2-4]. The effect of these compounds can be modified due to transformation of androgens into estrogens in the placenta [3]. Androgens produce a programming effect on brain development [2-4]. Morphofunctional manifestations of this influence can be revealed in the prepubertal period.

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