Effect of Injection of Testosterone Derivatives to Pregnant Rats on the Brain of Their One-Day Offspring

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Intramuscular injection of Sustanon-250, a drug with testosterone derivatives of various action rate and duration, to rats on day 19 of gestation affected brain development in their offspring. This effect manifested in greater brain weight and neocortex thickness, smaller density of neurons in the developing cortical layer V, and larger size of these neurons and their nuclei and cytoplasm in comparison with control neonatal rats. These data attest to accelerated cerebral development in the offspring of experimental rats in comparison offspring of control rats.

Key Words: *brain; development; androgens; pregnancy; morphometry*

There is a great variety of factors, which delay and disturb brain development [2,3,6,8]. By contrast, some factors applied during prenatal and early postnatal ontogeny accelerate brain development. This effect was observed after adrenalectomy at the age of 11 days [14], injection of desoxycorticosterone acetate during pregnancy, unilateral ovariectomy performed 1-1.5 months before coupling, and postpartum decrease in litter size [6,7,9]. In some cases the signs of advanced development of the brain observed during early postnatal period persisted in 1- and 2-month-old rats, when the rat brain is similar to that of mature rats by many important indices [6].

Steroid hormones produce a pronounced effect on brain development. There is evidence that this process is delayed by glucocorticoids [6] and accelerated by desoxycorticosterone, an analog of mineralocorticoids [7,9]. In addition, the programming effect of male sex hormones on the brain is described in details [3,4]. At the same time, the effect of androgens on many features of embryonic brain development is not known, although in addition to unequivocal theoretical value, this problem is also of practical importance in the cases with pathology in the cortex of adrenal glands and ovaries, which can be accompanied by hyperandrogenism in women [12,13,15].

MATERIALS AND METHODS

We examined the brain of 1-day-old rats (n=24), whose mothers (n=4) were injected with Sustanon-250 (0.05 ml) on day 19 of gestation. The drug (the Netherlands) contains propionate (1.5 mg), phenylpropionate (3 mg), isocaproate (3 mg), and decanoate (5 mg) forms of testosterone, and it is characterized by rapid onset and large duration of the produced effect. The offspring (n=27) of intact female rats (n=5) and the offspring (n=24) of control placebo-treated female rats (n=3) injected with drug vehicle (peach oil) were also examined. The rats of all groups were kept under vivarium conditions on an unrestricted food and water diet. All female rats were primagravid, their body weight was 250-270 g. Rat pups were sacrificed by decapitation; their body and brain were weighed on a torsion balance. The sections (7 μ) were prepared from the brains of 2 male rats and 2 female rats of each litter fixed in Carnoy fluid and embedded in paraffin. The thickness of cortex and cortical layer I was measured with a MOB-15 ocular micrometer in preparations stained with gallocyanine by the method of Einarson. The number of neurons was calculated in a standard

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TABLE 1. Effect of Sustanon-250 Injections to Pregnant Rats on the Brain of Their One-Day-Old Offspring

Index	Intact		Control		Sustanon-250	
	M±*m*	extremes	*M*±*m*	extremes	*M*±*m*	extremes
Body weight, mg	5599±144	4000-6700	5780±71	4700-6600	5913±213	4100-7500
Brain weight						
mg	222.0±3.3	181-255	232.0±4.3	202-258	264.0±5.8*+	209-306
mg/g	40.1±0.8	33.6-50.9	40.30±0.41	33.4-46	45.6±1.0*+	35.4-55.2
Thickness, μ						
cerebral cortex	572±20	462-715	571±17	460-680	645±19*	550-781
layer I	58.0±3.5	44-88	50.0±2.9	53-66	70.0±4.4*+	44-94
Section area, μ^2						
neurons	70.0±2.8	58-85	69.0±3.1	56-79	83.0±5.3*+	64-124
nuclei	48.0±1.8	42-58	48.0±2.3	40-57	56.0±2.6*+	43-71
cytoplasm	22.0±1.4	16-28	22.0±1.9	14-30	28.0±2.9	18-53
RNA concentration in cytoplasm, rel. unit	300±12	217-340	280.0±9.2	231-313	301±12	246-392
Number of neurons in standard vision field	59.0±2.6	41-69	58±1	52-66	49.0±3.3*+	30-67

Note. The differences are significant compared to *intact and +control rat pups.

vision field of the developing layer V. A MEKOC complex was used to determine the concentration of cytoplasmic RNA and cross-section area of nuclei and cytoplasm in layer V using Morphodensitometry software. In each case, 25 cells in several vision fields were examined.

The data were analyzed statistically using Statistica software. Significant sex-depending differences were not revealed in the control and experimental groups, so indices of males and females were combined.

RESULTS

In comparison with intact and control groups, the offspring of experimental female rats had somewhat larger body weight ($p>0.1$) and significantly greater weight of the brain (by 18.9%, Table 1). The difference in brain weight results not only from the difference in body weight, because the relative brain weight decreases when the body weight grows [1,6], and in experimental rat pups the relative brain weight was significantly greater than in controls (Table 1). Experimental pups had significantly higher brain weight in litters with both minimum (234±6 *vs.* 212±6 mg) and maximum (289.0±6.5 *vs.* 232.0±3.8 mg) brain weight. In the pups of experimental rats, The maximum individual brain weight was also higher in offspring of experimental rat (306 *vs.* 255 mg in rat pup of intact female). These data suggest that the drug containing testosterone derivatives stimulates brain growth during embryogeny and/or early postnatal period (postpartum day 1).

The morphometric parameters of the neocortex in experimental rat differed from those in control and intact pups: the neurons were larger due to increased volume of their nuclei and cytoplasm, the density of these neurons in the developing layer V was lower, the thickness of the cortex and cortical layer I were greater than in intact and control rats (Table 1). Taken together, these features attest to activation of synthesis in neurons and accelerated development of these cells and neuropile in cerebral cortex. On the whole, these peculiarities of brains development in the offspring of female rats treated with Sustanon attest to acceleration of brain growth and development of its structures in these pups. This effect did not result from the procedure of drug injection or from injection of drug vehicle, because the pups of control rats injected with oil had did not significantly differ from the pups of intact rats (Table 1). In addition, Sustanon increased both minimal and maximal indices within the groups (Table 1). These data suggest that Sustanon accelerates brain development in rats, in which this process was initially delayed or accelerated.

Analysis of the mechanisms of this effect of testosterone derivatives should take into account various properties of androgens: their anabolic effect, ability to moderate the reaction induced by stress (e.g. labor stress) [5,10], and receptor-mediated effect on neurons in the developing brain [3,4,12]. In addition, the effect of androgens can be modified, because placenta can

transform them into estrogens, which prevents or moderates the possible masculinizing effect of male sex hormones on the fetus [4,13]. In addition, the reported effects can be caused by the inhibitory effect of sex hormones on apoptosis of cerebral cells [12].

Our findings are important, because the mechanisms accelerating the growth and development of the brain are more intricate than the opposite ones. By successful solution of the problem to accelerate brain growth, the physiologists would obtain a valuable model of rapidly developing brain, which can be used to study, specifically, the potencies to greater development of the brain. Probably, the reported findings can be used to create the prevention methods for clinically observed cases of delayed brain development in embryogeny (first of all for male fetus), and for correction of the long-term effects of this pathology.

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