Participation of Endogenous Opioids in Pathogenesis of Early Neuroendocrine Manifestations of Prenatal Stress Syndrome

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We studied sex dimorphism in the content of norepinephrine and activity of enzymes involved in testosterone metabolism in the preoptic hypothalamic area of 10-day-old rats. Prenatal stress eliminated sex-related differences in these indices. These disturbances were absent in rats subjected to prenatal stress under conditions of opioid receptor blockade with naltrexone. These data attests to the important role of opioids in the pathogenesis of prenatal stress syndrome.

Key Words: *prenatal stress; catecholamines; testosterone metabolism; hypothalamus; opioids; sex-related brain differentiation*

Stress during the last week of pregnancy produces prenatal stress syndrome in the offspring, which is characterized by neuroendocrine disorders of stress-reactivity and sex-related differentiation of the brain. Delayed manifestations of this exposure to stress are alterations of sexual behavior: demasculinization and/or feminization in males and reduced fertility in females. These disturbances are related to stress-induced changes in the levels of catecholamines and sex steroids in females and their offspring during the critical period of sex-related brain differentiation [5].

Recent studies demonstrated the involvement of opioids in the pathogenesis of prenatal stress syndrome. There are also data on increased β-endorphin content in the adenohypophysis and hypothalamus of rat fetuses and newborns, whose mothers were exposed to stress during the last week of pregnancy [6]. Opiates administered during pregnancy can simulate some effects of prenatal stress on sexual behavior [8]. Pharmacological blockade of opioid receptors during stress exposure of pregnant rats prevents feminization in mature male offspring [7]. However, the neuroendocrine basis of these phenomena remains unclear.

Taking into consideration the important role of cooperative interaction of catecholamines and steroid metabolites during the first 10 days of postnatal development for normal process of sexual differentiation of the brain in rats [2] and the data on possible involvement of opioids into behavioral disturbances caused by prenatal stress, we studied the content of norepinephrine, dopamine, and activity of enzymes involved into testosterone metabolism in the preoptic hypothalamic area (PHA) of 10-day-old males and females of rats, whose mothers were exposed to stress during pregnancy under conditions of pharmacological blockade of opioid receptors. The choice of this cerebral structure was determined by the fact that the neuroendocrine center regulating sexual behavior in males and cyclic secretion of gonadotropins in females is located in PHA.

MATERIALS AND METHODS

The experiments were carried out on Wistar rats weighing 180-200 g. Estrous females were caged (1:2)

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with mature males. Pregnancy was counted from the day when spermatozoa were detected in vaginal smears. The pregnant rats were subdivided into 3 groups: 1) intact females; 2) females exposed to 1-h immobilization stress daily during gestational days 15–21; and 3) females exposed to immobilization stress after pharmacological blockade of opioid receptors with naltrexone. Naltrexone (Sigma) was injected subcutaneously in a dose of 10 mg/kg 30 min before immobilization. According to previous data [4], injection of naltrexone in a 5-fold higher dose (50 mg/kg) to pregnant rats produced no negative effects on the course of pregnancy and on the embryonic and postnatal development of the offspring. This fact made it possible to carry out the experiments without the control group of pregnant rats receiving naltrexone alone.

The experiments were carried out on 10-day-old pups (n=120) from intact and stressed females. Activity of testosterone metabolism enzymes and catecholamine content were examined in pooled PHA samples from 2-3 rats. Activity of aromatase complex enzymes estrogen synthetase (EC 1.14.14.1) and 5α-reductase (3-oxo-5α-steroid: $NADP^+$-en-oxidoreductase, EC 1.3.1.22) was determined in the supernatant after centrifugation of 10% PHA tissue homogenate at 1000g. The products of reaction formed after one-hour incubation of tissue samples in the presence of NADPCN-generated system (NADP, 2 mM; glucose-6-phosphate, 10 mM, and glucose-6-phosphate dehydrogenase, 2 U/ml) with [1,2,6,7-^{3}H]-testosterone (Amersham) were divided by two-way chromatography in thin silica gel, after which the spots corresponding to estradiol-17β, 5α-dihydrotestosterone, and 5α-androstane-3α,17β-diol, were radiometried. Aromatase activity was assessed by concentration of estradiol-17β (pmol), and 5α-reductase activity was measured by the concentration of 5α-reduced testosterone metabolites (5α-dihydrotestosterone and 5α-androstane-3α,17β-diol) formed over 1 h per 1 g wet tissue [1]. Catecholamine content in PHA was determined by spectrophotometry. The results were analyzed statistically using Student's t test.

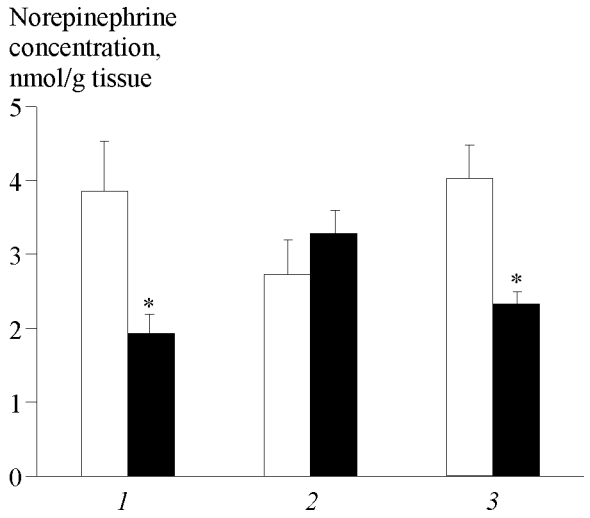

Fig. 1. Concentration of norepinephrine (nmol/g tissue) in preoptic hypothalamic area of rat female (light bars) and male (solid bars) in 10-day-old pups. Here and in Fig. 2: *1*) control; *2*) prenatal stress; *3*) prenatal stress+naltrexone. *p<0.05 compared to females.

RESULTS

The results corroborated previous data on peculiarities of sex-related dimorphism in norepinephrine content and testosterone metabolism in PHA of 10-day-old intact rats [5]. In particular, females demonstrated higher level of norepinephrine and 5α-reductase activity, while males had higher aromatase activity (Figs. 1 and 2). The experiments revealed no sex-related differences in the content of dopamine in PHA (males: 3.47±0.73 nmol/g tissue; females: 3.30±0.76 nmol/g tissue, p>0.05).

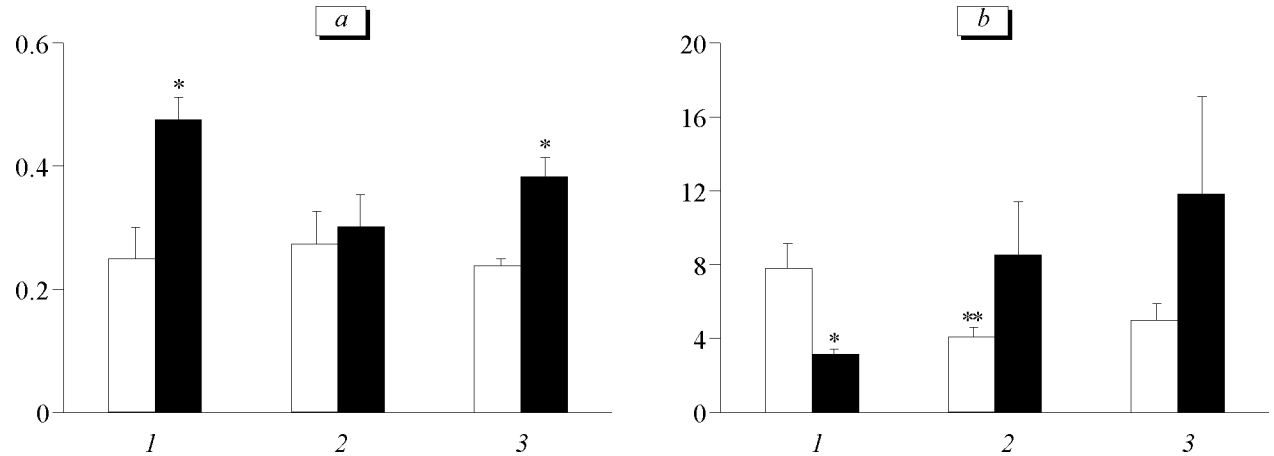

Fig. 2. Activity of testosterone metabolic enzymes in hypothalamic preoptic area in 10-day-old female (light bars) and male (solid bars) rat pups. *a*) aromatase (pmol estradiol/h/g tissue; *b*) 5α-reductase (pmol 5α-oxidated metabolites/h/g tissue). **p<0.05 compared to intact females.

Prenatal stress eliminated sex-related differences in the content of norepinephrine and activity of both enzymes of testosterone metabolism. The concentration of norepinephrine in PHA of prenatally stressed males increased to a level characteristic of intact females. At the same time, prenatal stress had no effect on dopamine concentration (3.14±0.18 and 2.99± 0.24 nmol/g tissue in stressed males and females, respectively, $p>0.05$).

In males, prenatal stress decreased aromatase activity in PHA to a level observed in intact or prenatally stressed females. In females, stress reduced 5α-reductase activity to a level characteristic of intact males.

Stress stimulation of pregnant rats treated with naltrexone produced no adverse effects on the content of norepinephrine and aromatase activity in PHA: typical sex-related differences in the concentrations of these enzymes were preserved in 10-day-old pups (Figs. 1 and 2). However, naltrexone did not prevent the stressed-induced changes of 5α-reductase activity. The content of dopamine in PHA of experimental pups did not differ from the corresponding control values (3.09±0.38 and 3.76±0.47 nmol/g tissue in males and females, respectively).

Thus, our findings suggest the involvement of endogenous opioids in the mediation of the effects of prenatal stress on norepinephrine content and intensity of aromatization of androgens in male PHA during the critical period of sex-related cerebral differentiation. They also demonstrated possible mechanisms of disturbances in imprinting determining differentiation of the neuroendocrine regulation of sexual behavior, which depend on androgens produced by fetal testes.

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