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ORIGINAL ARTICLE

Opioids are responsible for neurochemical feminization of the brain in prenatally stressed male rats

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Abstract

OBJECTIVES: To study the role of opioids in early postnatal changes of the hypothalamic testosterone metabolism and catecholamine content underlying feminizing effect of prenatal stress on male sexual behavior in rats.

MATERIAL AND METHODS: 10 day-old male and female offspring from mothers given naltrexone prior to daily 1-hour restraint during the last gestational week were used in the study. Aromatase and 5α-reductase activities, noradrenaline and dopamine contents in the brain preoptic area and medial basal hypothalamus were studied by thin layer chromatography, radiometric and spectrofluorimetric techniques.

RESULTS: Sexual dimorphism of testosterone metabolism enzymes activity and catecholamine content in discrete brain regions of 10 day-old rat pups was found. Prenatal stress attenuated these gender-related differences. Naltrexone pre-treatment of stressed dams prevented modifying effect of prenatal stress on aromatase activity and noradrenaline content in the male preoptic area.

CONCLUSIONS: The results of the study demonstrate preventive effect of naltrexone on stress-induced alterations of testosterone aromatization and noradrenaline concentration in the developing brain preoptic area associated with neuroendocrine control of the male sexual behavior in adult rats. These findings indicate that endogenic opioids mediate detrimental effect of prenatal stress on neurochemical determinants of the brain sexual differentiation that may underlie feminization of the male sexual behavior.

Introduction

Since 1972, it has been known that prenatal stress feminizes and demasculinizes the sexual behavior of adult male rats [1]. Human males exposed to severe stress *in utero* seem to have an increased rate of homo- and bisexual behavior in adulthood [2]. It was hypothesized that alteration of androgen-dependent sex brain differentiation in the fetuses occurs due to testicular testosterone deprivation caused by exposure of pregnant dams to environmental stressful stimuli. This hypothesis has been supported with data on a decrease of blood testosterone titers in the rat male fetuses resulted from maternal stress during last week of gestation [3] and preventive effect of testosterone

replacement upon sexual behavior [4] and its early programming in the male offspring [5].

It is generally acknowledged that stress is associated with an activation of catecholamine system and the hypothalamic-pituitary-adrenal axis followed by elevation of catecholamines, ACTH, opioids, corticosteroids and other hormone titers in blood. Maternal corticosteroids can pass through placenta and affect the fetal brain additively to corticosteroids originated from the fetal adrenal glands. However, exposure of normal pregnant rats to glucocorticoid excess during the last gestational week does not change the tissue noradrenaline concentration in the preoptic area of male rat pups, although it attenuates sexual differences in aromatase activity [6]. These observations turned our attention toward opioids as possible mediators of prenatal stress effect on sexual differentiation of male brain.

Alongside with maternal and fetal corticosteroids and other hormones, neurotransmitters and neuromodulators, a large amount of opioids are being released during gestational stress. Opioids are known to exert suppressive effect on the hypothalamic-pituitary-gonadal axis. Therefore, they could be responsible for a decline of LH and testosterone secretion in the male fetuses. In accordance with this suggestion, β-endorphin levels changed in the hypothalamus of male and female rat fetuses resulted from maternal stress [7]. Mature male offsprings of the female rats treated with β-endorphin during the last gestational week developed behavioral abnormalities like prenatally stressed ones [8, 9]. On the other hand, naltrexone, opioid receptor blocker, while administered to pregnant dams shortly before each daily 45 min restraining combined with a bright light between days 14th and 21st of gestation preserved normal levels of male copulatory behavior in adult offspring [10, 11].

The mechanisms behind these observations have not been defined. Earlier the crucial role of the hypothalamic steroid aromatase [12, 13] and catecholamines [14–16] in perinatal androgen-dependent brain sex differentiation has been postulated in rodents. Prenatal stress feminizes neurochemical phenotype of the brain preoptic area of male offspring in early postnatal life [6, 14, 17]. This effect is obviously associated with activation of the hypothalamic-pituitary-adrenal axis because it can be prevented with dexamethasone blockade of its stress response [5]. Those alterations should contribute greatly to behavioral abnormalities because the brain preoptic area of rodents is identified as a neuroendocrine center of male sexual behavior.

The aim of this study was to elucidate whether pharmacological blockade of the opioid receptors is able to prevent neurochemical feminization of the male brain induced by prenatal stress. Here we present the results of studying testosterone metabolism and catecholamine contents in the brain preoptic area and medial basal hypothalamus in normal and prenatally stressed rat pups as well as in those from mothers treated with naltrexone prior to stress.

Material and Methods

Experiments were performed according to protocols approved by the Animal Care Commission at our Institute, in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, 1986).

Time-mated Wistar rats were subjected to 1 h strict immobilization during days 15 to 21 of gestation [4]. Some of them were injected subcutaneously with naltrexone hydrochloride (Sigma) in a dose of 10 mg/kg b.w. 30 min prior to each stress session. During restraining the animals were excited, they screamed and demonstrated piloerection, urination and defecation. Control mothers were housed in the vivarium with no handling. On the 10th postnatal day the male and female pups were quickly decapitated and tissue samples of the preoptic area and medial basal hypothalamus were collected and frozen at –20°C until assays. Tissue samples taken from 2–3 pups were combined to be used in each assay.

Aromatase and 5α-reductase activities were measured in the 1000 g supernatant of the 10% tissue homogenate in Tris-HCl pH 7.4 buffer solution after 1 h incubation in the presence of [1,2,6,7 – ^{3}H] testosterone (s.a. 3.74 TBq/mmol, Amersham) and NADPH generating system (2mM NADP, 10mM glucose-6-phosphate and 2 IU/ml glucose-6-phosphate dehydrogenase) [18]. The end products of testosterone conversion, estradiol and 5α-reduced metabolites, were separated by silicagel thin layer two-dimensional chromatography. ^{14}C-Estradiol has been used as an internal standard. Radioactivity of labeled estradiol, 5α-dihydrotestosterone and 5α-androstan-3α,17β-diol (3α-diol) was counted. Aromatase activity was measured as an amount of estradiol formed during 1 h incubation. 5α-reductase activity was calculated as a sum of 5α-dihydrotestosterone and 3α-diol generated from ^{3}H-testosterone after 1 h incubation. The enzyme activities were expressed per 1 g total protein.

Noradrenaline and dopamine levels in the brain tissues were determined by spectrofluorimetric assay [19]. Catecholamine contents were calculated per 1 g wet tissue.

All data are expressed as mean ± SEM. Student's *t*-criterion has been used for evaluation of the differences between experimental groups. $P < 0.05$ was considered as the borderline of statistic significance.

Results

Testosterone metabolism. The results of aromatase activity measuring are presented in Fig. 1. In 10 day-old normal pups, a marked sexual dimorphism in aromatase activity in the preoptic area with significantly higher enzyme activity in males was observed. This difference disappeared in prenatally stressed pups due to a decrease in enzyme activity in males down to normal female levels. In contrast, naltrexone treatment followed by stressing pregnant dams completely prevented a decrease in aromatase activity in the preoptic area of

males. Medial basal hypothalamus did not demonstrate gender-related difference in aromatase activity. Prenatal stress alone or combined with naltrexone pre-treatment did not influence aromatase activity in this area in either male and female pups.

Unlike aromatase, 5α-reductase activity in the preoptic area of normal male pups was 55.1% less as compared to that of females (270.9 ± 25.1 pmol 5α-reduced metabolites/h/g protein vs. 603.1 ± 140.3 pmol 5α-reduced metabolites/h/g protein, $p < 0.05$). In prenatally stressed male pups, the enzyme activity had no significant difference from that of females. A significant decrease of the enzyme activity in prenatally stressed females resulted in disappearance of sexual differences in 5α-reduced testosterone metabolites formation (305.6 ± 97.3 pmol 5α-reduced metabolites/h/g protein in females vs. 725.8 ± 211.9 pmol 5α-reduced metabolites/h/g protein in males, $p > 0.05$). In the medial basal hypothalamus prenatal stress led to 5α-reductase activity lowering in females and to its elevation in the preoptic area of males (females: 573.3 ± 188.3 pmol 5α-reduced metabolites/h/g protein in controls vs. 290.7 ± 58.6 pmol 5α-reduced metabolites/h/g protein in prenatally stressed; males: 365.6 ± 100.1 pmol 5α-reduced metabolites/h/g protein in controls vs. 970.5 ± 234.7 pmol 5α-reduced metabolites/h/g protein in prenatally stressed). Moreover, the sexual dimorphism in the enzyme activity emerged in this brain region, in contrast to its absence in normal rats. Naltrexone pre-medication did not affect the prenatal stress-induced pattern of 5α-reductase activity in both discrete brain structures studied.

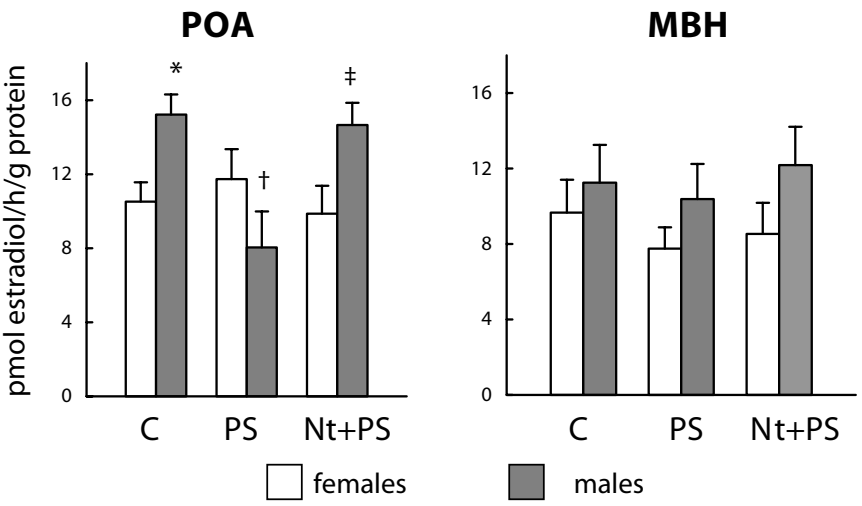

Figure 1. Prenatal naltrexone (Nt) effect on aromatase activity in the preoptic area (POA) and medial basal hypothalamus (MBH) of 10 day-old prenatally stressed (PS) rats. Data represent mean ± S.E.M. (pmol estradiol/h/g protein) for 4-8 determinations. * $p < 0.05$ versus control females, † $p < 0.05$ versus control males, ‡ $p < 0.05$ versus females Nt+PS.

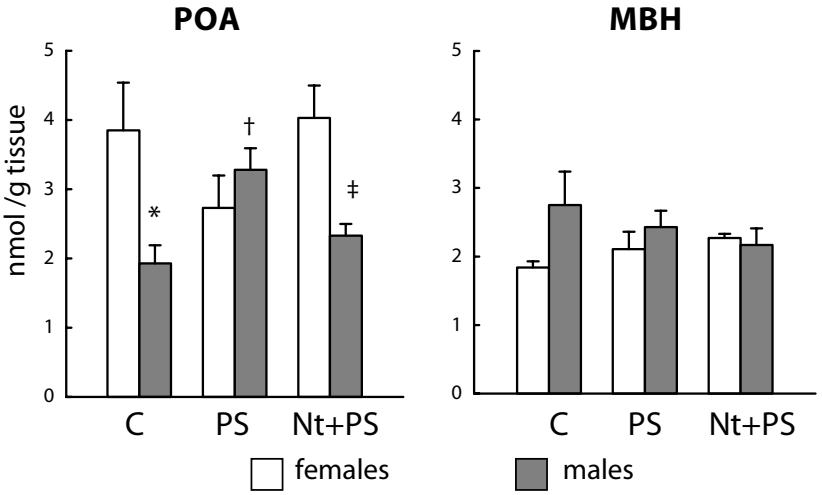

Figure 2. Prenatal naltrexone (Nt) effect on noradrenaline content in the preoptic area (POA) and medial basal hypothalamus (MBH) of 10 day-old prenatally stressed (PS) rats. Data represent mean ± S.E.M. (nmol/g tissue) for 4-8 determinations. * $p < 0.05$ versus control females, † $p < 0.05$ versus control males, ‡ $p < 0.05$ versus females Nt+PS.

Catecholamines. On the 10th postnatal day, normal males displayed a lower level of noradrenaline, but not dopamine, in the preoptic area in comparison with normal females (Fig. 2). Prenatal stress attenuated these gender-related differences by increasing noradrenaline concentrations up to normal or prenatally stressed female levels. This modifying effect of prenatal stress on males has been prevented by naltrexone. There were no changes in the preoptic dopamine levels in any experimental groups of male or female pups.

Dopamine concentrations in the medial basal hypothalamus of normal male pups were higher in comparison with those of females (3.82 ± 0.54 nmol/g tissue in males vs. 2.33 ± 0.11 nmol/g tissue in females, $p < 0.05$). Meantime, sex-related peculiarities in noradrenaline concentrations were not found in that brain region. Dopamine content in the medial basal hypothalamus in females increased being affected by prenatal stress resulting in disappearance of the sex differences (2.33 ± 0.11 nmol/g tissue in controls vs. 3.07 ± 0.27 nmol/g tissue in prenatally stressed, $p < 0.05$). Naltrexone had no impact on this effect of prenatal stress.

Discussion

From these observations, we postulate that prenatal stress selectively impairs the male rat brain preoptic area, which is related to neuroendocrine control of male sexual behavior.

Stress-induced changes depend upon the brain aromatase and noradrenaline, which are known as important neurochemical determinants of normal androgen-dependent brain masculinization. The female brain was much more resistant to the damaging effect of prenatal stress. Perhaps, a decrease in 5α-reduction of testosterone in the preoptic area of prenatally stressed female pups contributes to alteration of estrous cycles and fecundity in adulthood [20]. However these changes, as well as elevation of the enzyme activity and dopamine content in the male medial basal hypothalamus, have not been prevented by naltrexone, therefore, they are not attributed to endogenic opioids.

The results obtained in this study clearly demonstrate the protective effect of naltrexone on the pattern of testosterone aromatization and noradrenaline concentration in the brain of prenatally stressed males. There is a clear association between these results and the effects of naltrexone pretreatment of stressed pregnant dams [10] or prenatal β-endorphin administration to non-stressed ones [8] on the sexual behavior of adult offspring.

Opioids seem to play an important role in prenatally stress-induced feminization of developing male brain preoptic area due to their ability to inhibit LHRH-dependent release of the fetal pituitary LH followed by a decrease of testicular testosterone secretion.

In the rat hypothalamus, steroid aromatase is an androgen-inducible enzyme [21]. Its activity correlates with androgen levels during perinatal life in rats being at their highest by the end of embryonic development and during first postnatal days [18, 22]. A decrease of blood testosterone level induced by maternal stress in the preterm rat fetuses was accompanied by hypothalamic aromatase activity decline [23].

Taking into account the data on androgen-dependent changes in the hypothalamic catecholamine content during the critical period of sex brain differentiation in rats [14, 16, 24] we suppose that prenatal stress induced feminization of the brain catecholamines is caused by testosterone deprivation. Prenatal stress alters the steroid-catecholamine interrelationship within the developing neuroendocrine system and interferes with hormone-neurotransmitter imprinting of the sex-related brain structures.

Our experimental data establish the link between neurochemical determinants of sex brain differentiation and opioid-induced behavioral feminization in prenatally stressed males. We postulate that the observed neurochemical changes are mediated by opioid receptors.

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