Hormonal protection of gender-related peculiarities of testosterone metabolism in the brain of prenatally stressed rats

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Abstract

OBJECTIVES: Prenatal stress results in demasculinization and feminization of sexual behavior in adult male rats. Earlier the preventive effect of testosterone replacement of an androgen deficiency in male fetuses during exposure to stress has been demonstrated. Nevertheless the neuroendocrine mechanisms underlying hormonal protection of the brain sexual differentiation are not clear.

MATERIAL AND METHODS: Time-mated rats were undergone to 1 h strict immobilization during days 15 to 21 of gestation. Another group of stressed dams was injected with testosterone propionate on the 17th, 19th and 21st gestational days subcutaneously (5 mg/kg b.w.) 30 min prior to restraining. Aromatase and 5α-reductase activities were determined in the preoptic area and medial basal hypothalamus of 10-day old offspring.

RESULTS: Aromatase activity in the preoptic area declined in affected with prenatal stress males and reached the normal female level. It completely restored in prenatally stressed males under influence of prenatal testosterone replacement. 5α-reductase activity decreased in the preoptic area of prenatally stressed females. In those pretreated with testosterone propionate, 5α-reductase activity was significantly elevated above normal level.

CONCLUSIONS: The data obtained demonstrate a preservation of aromatase activity in the preoptic area of males resulted from testosterone replacement. Presumably testosterone exerts its protective effect on the male sexual behavior by prevention of neurochemical feminization of the brain preoptic area and, perhaps, due to some other mechanisms.

ORIGINAL ARTICLE

INTRODUCTION

During perinatal development gonadal steroids and neurotransmitters exert programming effect on sexual differentiation of the brain [1–3]. Various environmental stimuli are capable of disturbing this process. In particular, maternal stress caused by restraining rats during the last week of gestation changes the hormone and neurotransmitter levels in fetal blood and the hypothalamus [4]. Prenatally stressed males display low rates of male copulatory behavior and enhancement of female lordotic responses in adulthood [5–7]. Increased incidence of homosexual behavior has been found in human males whose mothers were exposed to severe stress during pregnancy [8, 9]. In rats, stressful stimuli in late gestation are capable of altering sex-dependent differences in adaptive behavior like anxiety, learning and depression [10,11]. Various neuroendocrine mechanisms including changes in biogenic monoamines [12], stress- and noradrenergic reactivity of the hypothalamic-pituitary-adrenal axis [13,14] and others could contribute to these behavioral abnormalities.

In the hypothalamus and some other brain tissues, steroid aromatase catalyzes testosterone conversion into estradiol-17β [15] while 5α-reductase is responsible for producing 5α-dihydrotestosterone and other 5α-reduced C_{19}-steroids [16]. These products of local testosterone metabolism contribute to maturation of neuroendocrine reproductive system. The difference between aromatase activities in the hypothalamus of male and female pups is an essential neurochemical feature of normal androgen-dependent sexual differentiation of developing brain in the rodents [17,18]. From our previous studies it was postulated that prenatal stress attenuates these differences by decreasing aromatase activity in the brain preoptic area in males [14,19]. No changes of these indices were found in the medial basal hypothalamus [14,19]. These observations established a link between pathogenic stressful conditions and behavioral demasculinization and feminization in males, because preoptic area is known as the neuroendocrine center regulating male sexual behavior in the rodents.

Behavioral abnormalities in prenatally stressed rats can be prevented by bilateral adrenalectomy in mothers [20] or pharmacological inhibition of the maternal and fetal hypothalamic-pituitary-adrenal responses to stressful stimuli with dexamethasone [7]. Similar preventive effect of testosterone replacement therapy of an androgen deficiency in male fetuses during exposure to stress has been demonstrated [6]. Interestingly, neonatal testosterone treatment of prenatally stressed male rats can improve altered sexual performance in adulthood [21]. Neuroendocrine mechanisms underlying hormonal protection of the brain sexual differentiation are not clear. We supposed that it should refer to maintaining normal aromatase activity in the brain tissues. If this is a case, crucial role of neural aromatase activity disorders in the pathogenesis of sexual differentiation abnormalities in prenatally stressed males could be strongly supported.

In a study designed to test this assumption steroid aromatase and 5α-reductase activities were measured in discrete neuroendocrine regions of the brain in 10 day-old normal or prenatally stressed male and female rat offspring or those whose mothers were given testosterone propionate followed by restraint stress during the last gestational week.

MATERIAL AND METHODS

Experiments were performed according to protocols approved by the Animal Care Commission at the 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 undergone to 1 h strict immobilization during days 15 to 21 of gestation. Another group of stressed dams was injected with 1% testosterone propionate oil solution on the 17th, 19th and 21st gestational days subcutaneously (5 mg/kg b.w.) 30 min prior to restraining. Control mothers were housed in the vivarium and injected with the vehicle acording to above protocol. The pups aged 10 days from all animal groups were quickly decapitated. The preoptic area and medial basal hypothalamus were isolated by brain dissection, then combined by 2–3 tissue samples and frozen at –20 °C until be assayed. For aromatase and 5α-reductase activities determination, the aliquots of 1 000 g supernatant of the 10% tissue homogenates were incubated during 1 h in Tris-HCl buffer (pH 7.4) containing [1,2,6,7-^{3}H]testosterone (s.a. 3.74 TBq/mmol, Amersham, UK) as the enzyme substrate in the presence of NADP·H generating system (2 mM NADP, 10 mM glucose-6-phosphate and 2 IU/ml glucose-6-phosphate dehydrogenase) [22]. [4-^{14}C]Estradiol was used as an internal standard. Tritiated estradiol and 5α-reduced androgens which have been derived from testosterone were isolated by two-dimensional thin layer chromatography on silica gel plates Silufol UV_{254} (Kavalier, Czech Republic) as described previously [19]. Radioactivity of isolated steroids was recorded in a two-channel β-spectrometer (Beckman LS 500TA, USA). Protein contents were measured in the supernatant aliquots by Lowry method. Aromatase activity was calculated as an amount of estradiol and 5α-reductase activity as a sum of 5α-dihydrotestosterone and 3α-androstandiol produced for 1 h per or 1 g protein.

All data are presented 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

As it is shown in the Figure, control rat pups demonstrated sexual dimorphism of steroid aromatase activity in the brain preoptic area with its higher levels in males as compared to those of females. The enzyme activity declined on average by 37.9% in males as affected with

prenatal stress and reached the normal female level. It completely restored and even rose by 20.3% above normal level in prenatally stressed males under influence of prenatal testosterone treatment. No changes of aromatase activity have been obtained in the brain preoptic area in prenatally stressed female rats as well as in those treated with testosterone propionate.

Prenatal stress did not affect aromatase activities in the medial basal hypothalamic tissue samples both in males and females, and so did not in those exposed prenatally to testosterone propionate. There were no significant sexual differences in the aromatase activities in the medial basal hypothalamus neither in normal, nor in experimental animal groups.

The data of measuring 5α-reductase activities are presented in the Table. No sexual differences in these measures have been found in both brain regions in the controls. They became evident in the preoptic area in prenatally stressed offspring due to decrease of the enzyme activity in females by 68.2%. Prenatal testosterone propionate induced abnormally high levels of 5α-reductase in this brain region in female pups with no changes in males.

As far as the medial basal hypothalamus is concerned, the only change that has been revealed in experimental animal groups was a decrease of the 5α-reductase activity by 46.3% in prenatally stressed female offspring in comparison to the controls.

DISCUSSION

There are increasing bodies of evidence that the brain preoptic area is *locus minoris resistentiae* in prenatally stressed male rats. Under influence of prenatal stress or glucocorticoids [23,24], male rats demonstrate early microstructural, physiological and neurochemical feminization of this neuroendocrine region [4] which is in a good association with sexual behavior disorders in adulthood [5,25]. Our data on the effect of prenatal stress on steroid aromatase activity in the brain preoptic area strongly support a primary role of this enzyme in the disorders of sexual brain differentiation in male rodents. This suggestion is supported by recent publication on the impact of prenatal administration of the steroid aromatase inhibitor on formation of sexual dimorphism in anxiety levels and other behavioral responses in rats [26].

In the rat brain, steroid aromatase is up-regulated with testosterone [27]. Androgens increase aromatase activity in the fetal and newborn rat brain [28]. Its expression decreases under conditions of testosterone deprivation and are being normalized as stimulated with an androgen. Tranzient testosterone deficiency in the fetal blood circulation induced by maternal stress is believed to be mediated with opioids and responsible for behavioral demasculinization in male offspring, which could be perinatally determined, at least in part, by a decrease in the brain steroid aromatase activity. This decrease can be prevented with naltrexone, the opioid receptor blocker [29], presumably, due to maintenance of testicular androgen secretion. A positive effect of testosterone replacement therapy of transient fetal androgen deficiency on the brain physiology and chemistry confirms a great importance of testosterone production decline in male rat fetuses caused by prenatal stress for disturbing androgen-dependent sex brain differentiation.

The present experimental data demonstrate a preservation of aromatase activity in the preoptic area of males and thus of its sexual difference resulted from combination of prenatal stress with testosterone replacement

Table. Prenatal testosterone propionate effect on 5α-reductase activities in the preoptic area and medial basal hypothalamus of 10-day old prenatally stressed rats.

Animal group	Preoptic area	Medial basal hypothalamus
Control		
Females	1 185.2±139.5 (5)	966.9±146.3 (5)
Males	971.7±191.9 (5)	869.2±225.3 (5)
Prenatal stress		
Females	377.1±22.1* (4)	519.1±128.3* (4)
Males	941.3±160.0# (4)	686.7±96.5 (4)
Testosterone + prenatal stress		
Females	2 460.6±343.4*#† (5)	886.4±129.1 (5)
Males	1 222.2±218.1 (6)	945.6±296.3 (6)

Data represent mean ± S.E.M. (pmol 5α-reduced metabolites/h/g protein). Number of measurements is indicated in parentheses. *p<0.05 versus control females; #p<0.05 versus prenatally stressed females; †p<0.05 versus 'testosterone + prenatal stress' males.

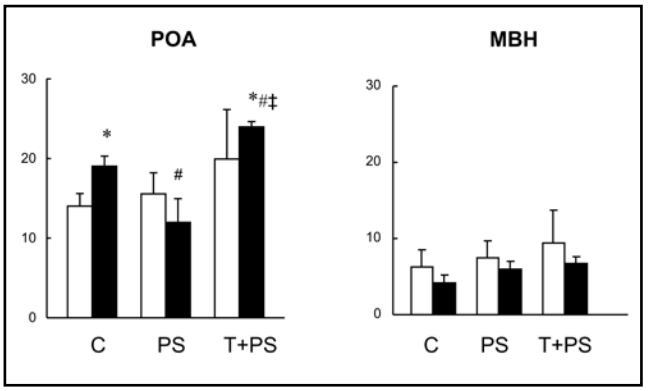

Figure. Prenatal testosterone (T) effect on aromatase activity in the preoptic area (POA) and medial basal hypothalamus (MBH) of 10 day-old prenatally stressed (PS) female (light bars) and male (dark bars) rats. Data represent mean ± S.E.M. (pmol estradiol/h/g protein) for 4–6 determinations. *p<0.05 versus control females, #p<0.05 versus control males, ‡p<0.05 versus PS males .

therapy. The testosterone propionate treatment schedule that has been used in our experiments was successfully used by Dörner *et al*. [9] for prevention of the sexual behavior demasculinization and feminization in prenatally stressed male rats. Therefore, testosterone exerts its protective effect on the male sexual behavior by prevention of neurochemical feminization of the brain preoptic area and, perhaps, due to some other mechanisms.

In this study, formation of 5α-reduced testosterone metabolites slowed down in the brain preoptic area of prenatally stressed female offspring, and mechanisms underlying these changes have not been defined. 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 [30]. Testosterone propionate treatment induced a great rise of 5α-reductase activity which is in line with well-known data on androgen induction of this enzyme. However the possible contribution of this phenomenon to neuroendocrine function and/or behavior regulation in adult females is not specified.

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