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Life Sciences 78 (2006) 2767 – 2771
Life Sciences
www.elsevier.com/locate/lifescie

Could neonatal testosterone replacement prevent alterations induced by prenatal stress in male rats?

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Received 16 August 2005; accepted 31 October 2005

Abstract

The present study was designed to examine whether testosterone replacement is able to prevent some effects of maternal restraint stress — during the period of brain sexual differentiation — on endocrine system and sexual behavior in male rat descendants. Pregnant rats were exposed to restraint stress for 1 h/day from gestational days 18 to 22. At birth, some male pups from these stressed rats received testosterone propionate. The neonatal testosterone replacement was able to prevent the reduction in anogenital distance at 22 days of age observed in pups from stressed pregnant rats as well as prevents the decrease in testosterone levels during the adulthood of these animals. Testosterone replacement in these males also presented an improvement in sexual performance. In this way, testosterone replacement probably through increasing neonatal level of this hormone was able to prevent the later alterations caused by the prenatal stress during the period of brain sexual differentiation.

Keywords: Prenatal stress; Brain sexual differentiation; Testosterone; Sexual behavior; Male rat

Introduction

Sexual differentiation of the hypothalamus of male and female rats involves complex phenomena and an important participation of estrogen, as well as androgens (Dohler, 1991). In male rats, testosterone surges markedly on days 18–19 of gestation (Weisz and Ward, 1980) and again during the first few hours following parturition (Baum et al., 1988; Corbier et al., 1978; Lalau et al., 1990; Slob et al., 1980). During this period of brain sexual differentiation, testosterone or its metabolites are fundamental for masculinization and defeminization of sexual behavior, for the establishment of gonadotropin secretion patterns, and also for various morphological indices. In the absence of testosterone or its metabolites, sexually dimorphic structures and functions are feminized (Rhees et al., 1997).

The stress response induced by physical or emotional challenges has been recognized as a profoundly disruptive factor in reproductive function in both males and females (Velazquez-Moctezuma et al., 1993; Wang et al., 1995; Retana-Marques et al., 1998; Rhees et al., 1999; Ward et al., 2002). The prenatal stress during the critical stage of hypothalamic differentiation is related to reduced fertility and fecundity (Anderson et al., 1986) and leads to altered sexual behavior (Ward, 1984) in male offspring through an increased corticosterone level. During stressful situations the activation of the hypothalamus–pituitary–adrenal axis was greater in prenatally stressed animals compared to non-stressed controls. Thus, prenatal stress partly affects adult behavior by altering the regulation of the hypothalamus–pituitary–adrenal axis (Szuran et al., 2000). Prenatal stress can alter masculine function even more directly by reducing plasma testosterone levels in adult males (Anderson et al., 1985; Pollard and Dyer, 1985). It has been hypothesized that prenatal stress disrupts the normal maternal hormonal milieu and suppresses the fetal testosterone peak on gestational days (GD) 18 and 19, a necessary peak for the later expression and maintenance of male sexual behavior

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0024-3205/$ - see front matter © 2005 Published by Elsevier Inc.
doi:10.1016/j.lfs.2005.10.035

(Ward and Weisz, 1984). High plasma levels on corticosterone are also associated with depression of the circulating androgen level (Kime et al., 1980; Moore and Miller, 1984; Nowel, 1980; Tokarz, 1987).

In humans, it is well established that adverse prenatal or early childhood events have a profound influence on neurodevelopment, altering central nervous system function later in life (Fride and Weinstock, 1988) and potentially contributing disorder and cognitive and psychiatric disorders (Fumagalli et al., 2005). At gestation (22–23 days) the gross appearance and histological detail in rat brain is like that of a human fetus of about 14–17 weeks (Bayer et al., 1993). Thus, some neuronal systems that are present at birth in the human continue to develop in the rat for several days or weeks after parturition (Weinstock, 2001).

The data from the present study confirm also the results obtained in previous study realized in male rats offspring exposed to prenatal stress (Gerardin et al., 2005) by showing a reduction in anogenital distance at 22 days old as well as a reduction in plasmatic testosterone level and a delay in the latencies of first mount and intromission and a decrease in number of ejaculations in adulthood. How to prevent this alteration becomes fundamental importance. So, we propose to evaluate whether neonatal testosterone replacement could restore some alterations caused by prenatal stress during the hypothalamus sexual differentiation. Thus, the aim of present study was to replicate this previous study in order to verify whether neonatal testosterone replacement may be able to prevent endocrine and sexual alterations caused by prenatal stress.

Materials and methods

Animals and experimental groups

Wistar rats were used as the parent generation. They were kept in a controlled environment with temperature at 25 ± 1 °C; humidity of 55 ± 5%; 12 h light/dark cycle (lights on at 6:00 a.m.) and had free access to regular lab chow and tap water. Virgin female rats (200 ± 10 g) were mated overnight. The onset of pregnancy was confirmed by the presence of spermatozoa in vaginal smears on the following morning and was considered day 1 of gestation. On GD22, all dams were weighed, anaesthetized with sodium pentobarbital (40 mg/kg, ip), and laparotomized to obtain male pups, which were divided according to treatment, as described below.

- Control group: some male pups ($n = 18$) were obtained from different dams that were not manipulated during gestation. At birth these male received only vehicle (s.c.).
- Stressed group: some male pups ($n = 18$) were obtained from different dams that were restrained in a Plexiglas cylinder (with variable diameter and 16 cm length) for 1 h from gestational day (GD) 18 to 22. The removable restraining shield was readjusted to the tightest setting the expanding body size of the pregnant animals would allow. At birth these male received only vehicle (s.c.).
- Stressed group + TP: some male pups ($n = 17$) were obtained from different dams that were restrained according to the same procedure in stressed group. At birth these male received testosterone propionate dissolved in corn oil, 10 μg/animal (s.c.).

The pups from the different groups were immediately fostered to recipient dams (8 pups/recipient dams) that had not been manipulated during the gestation and delivered on the same day. The pups were culled to six males and two females to ensure the presence of both sexes in the litters. They were left with each dam until weaning (23 days of age). For each set of experiments, a maximum of two male siblings was taken from each litter in order to avoid "litter effects".

The animals used in this study were maintained in accordance with Ethical Principles in Animal Research adopted by the Brazilian College of Animal Experimentation and approved by the Bioscience Institute/UNESP Ethical Committee for Animal Research (Protocol number: 065/03).

Body weight and anogenital distance during the pre-weaning period of male pups

At birth and on postnatal day 22 (PND 22), the average offspring's body weight of five litters was done. At birth and on postnatal day 22 (PND 22), ten male pups per group were utilized to obtain the anogenital distance through a vernier-caliper.

Plasmatic testosterone quantification on postnatal day 75 (PND 75)

Blood samples were collected through the abdominal aorta into heparin-coated syringes (always at 09:00 a.m.) from seven male rats per group. Blood was centrifuged (2500 rpm for 20 min at 2 °C) and the plasma testosterone concentration determined by competitive immunoassay using the IMMULITE® Total Testosterone Test (Diagnostic Products Corporation, USA). The antibody used was highly specific for testosterone and the test had an analytical sensitivity of 0.10 ng/ml. The intra-assay coefficient of variation was 4.8%.

Sexual behavior evaluation on PND 75

At least ten sexually inexperienced male pups per group were observed under red-light illumination during the dark phase of their cycle. For the test, female rats in their estrus phase (induced by estradiol benzoate −20 μg/kg, i.p., 24 h before test) were used (Arteche et al., 1997). Each male was placed into a Plexiglass cage, and after 5 min, the female was introduced. For 30 min, the following parameters were recorded: mount (the male normally mounts from the rear, sometimes posing his forelegs over the female's back, and makes rapid anteroposterior pelvic thrusts), intromission (vaginal penetration, this behavior starts with a mount, but suddenly the male makes a deep thrust forward and stops pelvic thrusting, then vigorously withdraws and always licks