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Adult partner preference and sexual behavior of male rats exposed prenatally to betamethasone

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ABSTRACT

The aim of this study was to investigate long-term effects of prenatal betamethasone exposure on sexual partner preference, testosterone level, and sexual behavior. Pregnant rats received 0.1 mg/kg of betamethasone or saline on the 12th, 13th, 18th, and 19th days of pregnancy. Parameters in male offspring were evaluated at 90 days of age. Male rats from the betamethasone group did not show any difference in sexual partner preference as expressed by the total number of visits to the female or male zone. However, these males spent significantly less total time and shorter duration per visit in the female zone than their controls. Therefore, prenatal exposure to betamethasone led to a significantly lower sexual female partner preference score compared to the control group. These animals also presented diminished testosterone levels in adulthood. Prenatal exposure to betamethasone induced a delay in the latency to first ejaculation, as well as a decrease in the numbers of postejaculatory intromissions, total intromissions and total ejaculations. Although 80% of the betamethasone-treated animals exhibited male sexual behavior, when they were castrated and pretreated with estrogen, 50% of them showed lordosis and accepted mounts of another sexually experienced male. These results suggest that the prenatal treatment with betamethasone, by increasing maternal corticosteroid level, may have diminished testosterone peak in male pups, a peak crucial to brain sexual differentiation. As a consequence, the prenatal betamethasone treatment reduced the testosterone level in adulthood and altered partner preference and sexual behavior.

1. Introduction

Corticosteroids are used widely in obstetric clinical practice in pregnant women at risk for preterm delivery, between 24 and 34 weeks' gestation. This therapy promotes fetal lung maturation, thus reducing the incidence of respiratory distress syndrome, which in turn decreases neonate mortality and morbidity [1–3]. Recent studies have shown that betamethasone is the drug of choice for antenatal corticosteroid treatments in humans [3,4]. The beneficial effects of this therapy are evident for the neonate. In spite of this, much less is known about the interference of high corticosteroid levels on the testosterone peak during the critical period of brain sexual differentiation. This process, by which permanent sex differences in the brain arise, is regulated by testosterone secreted from fetal and neonatal testes [5]. In male rats, testosterone surges markedly on days 18–19 of gestation [6–9] and again during the first few hours following parturition [8,10]. However, high corticosteroid levels could interfere with the testosterone peak, a peak crucial to brain sexual differentiation [8]. The process of brain masculinization requires the conversion of androgen to estrogen. Thus, the neural aromatization of androgens is known to be the critical step in the development and adult expression of male sexual behavior [11]. During the period of brain sexual differentiation, testosterone or its metabolites are fundamental for masculinization and defeminization of sexual behavior, for 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 [12]. Thus, in rats, behavioral masculinization results in the display of male-typical sexual behavior in adulthood (mounts, intromissions, and ejaculations). Behavioral defeminization results in loss of a cyclic release of gonadotropins necessary for ovulation and female-typical sexual behavior (proceptive and receptive behavior) [13]. All these data reinforce the notion that masculinization and defeminization are important neural processes whose absense may provoke damage to reproductive function.

On basis of these considerations, the aim of present study was to investigate long-term effects of prenatal betamethasone exposure on sexual partner preference, testosterone level, and sexual behavior in adulthood of male rats.

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2. Materials and methods

2.1. Animals

Wistar rats (*Rattus norvegicus albinus*) strain SPF were obtained from the Multidisciplinary Center for Biological Investigation, State University of Campinas (CEMIB — UNICAMP), Brazil. These animals were taken from the Animal Research Laboratory of Sao Paulo State University (UNESP/Botucatu) and used as the parent generation. After acclimatization in standard conditions (temperature at 25 ± 1 °C, humidity 55 ± 5% and light from 06:00 to 18:00 h), virgin adult female rats (up to 90 days old, 230 ± 10 g) were mated with one fertile untreated male of the same age. Daily inspections of the vaginal smear were then carried out, with the first day of pregnancy defined as the morning on which spermatozoa were found. These pregnant females were randomly assigned to two groups, according to treatment, as described below. The animals used in this study were maintained in accordance with the Ethical Principles in Animal Research adopted by Brazilian College of Animal Experimentation and were approved by the Botucatu Medical School/UNESP Animal Research Ethics Committee (Protocol number 429).

The experimental protocol is diagrammed in Fig. 1.

2.2. Experimental groups

On the 12th, 13th, 18th, and 19th days of pregnancy 10 rats per group received once a day either the vehicle (sterile physiological solution — control group) or 0.1 mg/kg [14] of betamethasone (betamethasone 21-phosphate, Sigma Co., USA — betamethasone group), diluted in sterile physiological solution by intramuscular injections. This dose was calculated as corresponding to the dose utilized in pregnant women. The treatment periods chosen for this study were based on the corticosteroid therapy employed in women at risk for premature delivery, and were adapted from Souza et al. [15]. Thus, the treatment was initiated at the end of the first half of pregnancy whereas the second course was applied during the brain sexual differentiation period.

The pups were born naturally and left undisturbed together with their own mothers until weaning, always 8 newborns/dam (4 females and 4 males to ensure the presence of both sexes in the litters). On the twenty-second postnatal day (PND22), the male rats from the control and betamethasone-treated groups were identified and housed in collective polypropylene cages (32 × 40 × 18 cm^3) each with a bedding of wood shavings, 4 animals/cage. For each set of experiments in adult life, one male sibling was chosen from each litter in order to decrease "litter effects".

On PND 90, for each parameter, ten males per group from different mothers (1 male/litter) were used in the sexual partner preference assessment, plasma testosterone quantification, and sexual behavior evaluation, since any of these procedures could interfere with the other ones.

2.3. Body weight and anogenital distance of male pups

Immediately after birth and at 22 days of age (weaning), male descendants from different mothers were weighed and their anogenital distance (length from anal opening to genital) obtained with a vernier-caliper.

2.4. Sexual partner preference evaluation in adulthood

The sexual partner preference evaluation apparatus utilizes a semicircular arena (100 × 50 cm) with 2 cages (25 × 15 cm) positioned on opposite sides, outside of the arena, in which the stimulus animals, a sexually active male and a receptive female in estrus, were placed. The partition between the stimulus animals and the experimental sexually inexperienced adult male rats (gonadally intact) consisted of a wire mesh allowing both animals to see, smell, and hear each other. The floor in front of the stimulus animals was demarcated in zones (30 × 20 cm) and the test lasted 20 min under red-light illumination during the first half of the dark phase of their cycle. The following measures were recorded: number of visits to each of the stimulus animal zones; total time spent within each of the stimulus animal zones; and the duration of each visit to each stimulus animal's zone (adapted from Vega-Matuszczyk and Larsson [16]). Following each test, a partner preference score was calculated by subtracting the time spent in the zone containing the sexually active male from the time spent in the zone containing the estrus female. Thus, a positive score indicates a preference for the estrous female, a negative score a preference for the sexually active male.

2.5. Plasma testosterone quantification

The adult male rats were anesthetized with sodium pentobarbital (40 mg/kg, ip). Blood from the abdominal aorta was collected (between 8:00 and 10:00 h), centrifuged (2500 rpm for 20 min at 2 °C), and the plasma stored at −20 °C until assayed. The hormonal level was measured by radioimmunoassay using Coat-A-Count Total Testosterone Kit (Diagnostic Products Co., Los Angeles, CA, USA) according to the manufacturer's instructions. The assay detection limit was 4.0 ng/dl, the intra-assay coefficient of variation was 4.8% and all samples were run in a single assay.

2.6. Sexual behavior evaluation in adult life

Male rats were housed in a large cage with 2 regular cycling females until 15 days of age. The male rats now sexually experienced were anesthetized with sodium pentobarbital (40 mg/kg, ip) and bilaterally castrated. Then, all of these males received testosterone propionate (Sigma Co., USA) at 1 mg/day, sc, 3 times a week, for 2 weeks [17]. The testosterone replacement schedule was set up so that the first injection was given on the day after orchidectomy, and the last one was always

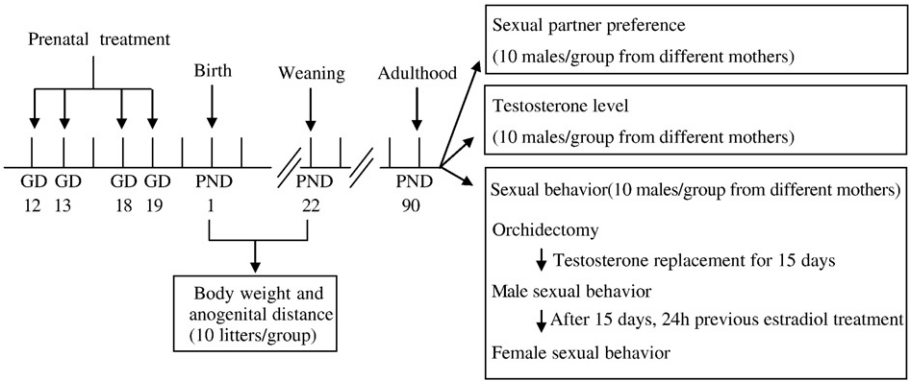

Fig. 1. Diagram of the experimental design: GD, gestational day; PND, postnatal day.