Hormones and Behavior 71 (2015) 91–95

Contents lists available at ScienceDirect

Hormones and Behavior

journal homepage: www.elsevier.com/locate/yhbeh

Paternal behavior in the Mongolian gerbil (*Meriones unguiculatus*): Estrogenic and androgenic regulation

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ARTICLE INFO

Article history:
Received 22 August 2014
Revised 13 April 2015
Accepted 15 April 2015
Available online 29 April 2015

Keywords:
Mongolian gerbil
Paternal behavior
Testosterone
Estradiol
Dihydrotestosterone
Meriones unguiculatus

ABSTRACT

Here, we analyzed the effects of testosterone (T) and its metabolites, estradiol (E_2) and dihydrotestosterone (DHT), on the onset of paternal behavior in virgin male Mongolian gerbils (*Meriones unguiculatus*). We hypothesized that T and E_2, but not DHT, would facilitate the onset of paternal behavior. Seventy males displaying aggression toward pups were selected through a paternal behavior screening test. Forty males were bilaterally castrated. Of them, 10 were implanted with T, 10 with E_2, and 10 with DHT, and 10 received no treatment. Another 30 males underwent a sham procedure. In these gerbils, T, E_2 and DHT were measured to obtain the basal levels of these hormones. After treatment, the paternal behavior test was conducted again. Blood samples were obtained immediately after the administration of the test for the quantification of T, E_2 and DHT by radioimmunoassay. Surprisingly, 100% of the males that received T, E_2 and DHT implants stopped being aggressive and became paternal. Castrated and sham-operated males displayed no changes in their aggressive behaviors. This is the first report that T and its metabolites are involved in neuroendocrine mechanisms that inhibit aggression toward pups and facilitate paternal behavior in virgin male Mongolian gerbils. In addition, this is the first report of regulation of paternal behavior in a rodent by estrogenic and androgenic pathways.

Introduction

In mammals, females alone generally care for the young, whereas males increase their reproductive success by pairing with several females, foregoing paternal care (Trivers, 1972). However, in 5% of vertebrate species, the males do provide care for their offspring. Paternal care is most prevalent in primates, carnivores and rodents (Kleiman and Malcolm, 1981).

Most rodent virgin males, including those species with paternal care, are infanticidal (Hrdy, 1979; Hausfater and Hrdy, 1984; Gubernick et al., 1994). Such behavior must be inhibited before the birth of their young to allow for offspring survival (Hrdy, 1979; Vom Saal and Howard, 1982). The mechanisms implicated in the inhibition of infanticide and facilitation of paternal behavior have been suggested to involve neuroendocrine changes that are activated by stimuli from females and pups (Brown, 1985; Reburn and Wynne-Edwards, 1999).

In some rodents, hormones, such as prolactin and testosterone (T), have been associated with paternal care behavior (Wynne-Edwards, 2010). In the dwarf hamster (*Phodopus campbelli*), male plasma T levels increase before the female gives birth and decrease after offspring are born (Wynne-Edwards and Reburn, 2000). A decrease in T levels has also been observed in human males when they become fathers (Berg and Wynne-Edwards, 2001; Fleming et al., 2002; Burnham et al., 2003; Gray et al., 2006; Alvergne et al., 2009; Van Anders et al., 2012), although this decrease coincides with the less frequent sexual intercourse of these new fathers (Gettler et al., 2013). Subsequent studies in the dwarf hamster have indicated that T levels do not decrease when males are engaged in paternal care (Schum and Wynne-Edwards, 2005). In other mammals, such as the cotton-top tamarin (*Saguinus oedipus*), urinary T levels remain elevated after parturition, when the males display paternal behavior (Ziegler and Snowdon, 2000). In the California mouse (*Peromyscus californicus*), castration reduces the degree of paternal care, whereas castrated males that receive exogenous T replacement display an increase in paternal behavior (Trainor and Marler, 2001). In this rodent species, castrated males with sexual experience treated with T or estradiol (E_2) display an increase in paternal behavior compared with males that receive dihydrotestosterone (DHT) or empty implants. Further, the inhibition of aromatase, an

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http://dx.doi.org/10.1016/j.yhbeh.2015.04.009
0018-506X/

enzyme that converts T to E_2, blocks the positive effect of T on paternal behavior (Trainor and Marler, 2002). In the California mouse, increased aromatase activity in the medial preoptic area, a brain area known to regulate parental behavior in rodents, has been associated with the onset of this behavior. These results suggest that T is taken up by neurons of the brain regions that are involved in the regulation of paternal behavior and is converted locally to E_2, regulating this behavior through the estrogenic pathway (Trainor and Marler, 2002; Trainor et al., 2003). However, in the Mongolian gerbil, virgin castrated males have been shown to spend more time performing paternal activities than sham-castrated males or castrated males with T implants when cohabiting with a female and pups (Clark and Galef, 1999).

One model establishing the hormonal basis of maternal behavior is termed "ovariectomy and hormonal replacement in virgin females" (Siegel and Rosenblatt, 1975; Bridges and Russel, 1981; Bridges, 1984; Numan et al., 2006). Using the same experimental model and aiming to expand upon the previous findings in the California mouse, we hypothesized that T and E_2, but not DHT, facilitate the onset of paternal behavior. To test this hypothesis, we analyzed the effects of these hormones on the onset of paternal behavior in virgin male Mongolian gerbils (*Meriones unguiculatus*). In this monogamous rodent species, males exhibit high levels of paternal care (Agren, 1984; Dewsbury, 1981; Elwood, 1975). Therefore, it is an excellent model for the study of the hormonal regulation of parental care.

Materials and methods

Animals

In this study, we used virgin male Mongolian gerbils, aged 80 to 210 days old. Gerbils were weaned between 25 and 28 days old. The animals were obtained from a breeding colony kept at the Facultad de Estudios Superiores Iztacala, UNAM. The colony was maintained under an inverted photoperiod of 12:12 h (light–dark cycle; onset of light at 1800 h), at an ambient temperature of between 17 and 21 °C. The gerbils were fed pellets for small rodents (Harlan Laboratories) and tap water ad libitum. At the beginning of the study, two or three gerbils of the same sex were housed in a polycarbonate cage (37 × 27 × 15 cm) with sawdust bedding. All cohabitating males were assigned to the same treatment group to avoid close contact with males in different hormonal conditions. The males used in this study were selected for aggressiveness toward pups. Virgin males of the Mongolian gerbil, similar to those of the California mouse, may be aggressive, indifferent or paternal toward foreign pups of the same species (De Jong et al., 2012). The criteria for aggressive male behavior included sniffing and attacking pups and moving them violently. In addition, pups may be bitten if they are not withdrawn. Paternal males sniff and touch pups with the nose and groom and crouch beside them. During the paternal behavior screens, each male was placed in a polycarbonate cage (48 × 32 × 15 cm) with clean sawdust bedding. After 10 min, three 2- to 4-day-old pups were introduced into the cage. The pups were withdrawn quickly when they were moved violently by the male gerbil. The paternal behavior selection tests were performed at 10 to 15 days before the beginning of the experimental manipulations. Eighty males were subjected to a paternal behavior screen, resulting in the identification of 70 (87.5%) aggressive males, of which 10 were bilaterally castrated, 10 underwent bilateral castration + T replacement, 10 received bilateral castration + E_2 replacement and 10 underwent bilateral castration + DHT replacement. Another 30 males were subjected to a sham surgical procedure. In these gerbils, T, E_2 and DHT were measured to determine the basal levels of these hormones in the plasma.

All experiments were carried out in accordance with the ethical guidelines of the Official Mexican Standard, which regulates technical specifications for the production, care and use of laboratory animals (Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación, 2001), and followed the ethical guidelines of the American Society of Mammalogists for animal care and use (Animal Care and Use Committee, 1998).

Surgeries and implants

Gerbils were anesthetized with 80 mg/kg pentobarbital before surgery and the placement of T, E_2 or DHT implants. Only sterile surgical instruments were used. Seventy percent alcohol was applied to disinfect the scrotum, and a small midline incision was made, through which the testes were exteriorized. The spermatic vessels were tied with 6.0 silk sutures, and the testes were removed. The incision was closed with 6.0 silk sutures. In the sham-operated gerbils, the skin of the scrotum was incised to bring the testes out and extend them back, and the incision was closed with sutures only. Acetylsalicylic acid (~100 mg/kg) was administered orally as an analgesic during the first 12 h following surgery. At 24 h after surgery, the gerbil was returned to the home cage. Silastic tube implants (Silastic Laboratory Tubing, i.d. 1.47 o.d. 1.96 mm) packed with 10 mm testosterone propionate, β-estradiol 3-benzoate or dihydrotestosterone (5α-androstan-17-β-ol-3-one) were produced (Sigma Aldrich, St. Louis, MO, USA), and the ends were sealed with silicone. In the California mouse, 10 mm implants of these steroid hormones have been shown to successfully exert positive effects on paternal behavior (Trainor and Marler, 2001).

Hormone assay

Blood samples (250 μl) were collected from the retro-orbital sinus using heparinized capillary tubes under light ether anesthesia immediately after the parental behavior test (second exposure to pups). Blood collection was performed over a minute at between 11:00 and 14:30 h. Plasma was separated by centrifugation and stored at −70 °C. Hormonal analysis was conducted by radioimmunoassay (RIA). The T level was measured with a Siemens kit to determine the level total testosterone as ^{125}I testosterone (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) at a sensitivity of 4 pg/ml. The intra-assay and inter-assay coefficients of variation were 2.7% and 5.6%, respectively. E_2 was quantified using a Siemens kit (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA) at a sensitivity of 8 pg/ml. The intra-assay and inter-assay coefficients of variation were 4.9% and 5.4%, respectively. DHT was measured (Beckman Coulter) with a sensitivity of 6.0 pg/ml. Prior to RIA, DHT was extracted with a mixture of N-hexane/ethanol (98:2 vol/vol). The intra-assay and inter-assay coefficients of variation were 5.5% and 7.5%, respectively.

Radioactivity was measured using a model 1282 Compugamma gamma counter (LKB-Wallac, Turku, Finland).

Paternal behavioral test

Behavioral tests were performed at 10 to 12 days after surgery and T, E_2 or DHT replacement, for which each male was placed in a polycarbonate cage according to the above-described paternal behavior screening method. Approximately 12 pups were evaluated during each test session. The pups were tested once and then returned to both parents. Paternal behavior was observed for 30 min. A single observer recorded the latency of paternal behavior onset (the length of time until the male contacted the pups) and the time spent crouching and grooming the pups. Additionally, sniffing of or contact with the pups was recorded (when a male approached a pup, it usually sniffed and touched it). Behavioral observations were carried out for between 11 and 14 h during the dark period. Observations were made under red light illumination.