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Progesterone impairs social recognition in male rats

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ABSTRACT

The influence of progesterone in the brain and on the behavior of females is fairly well understood. However, less is known about the effect of progesterone in the male system. In male rats, receptors for progesterone are present in virtually all vasopressin (AVP) immunoreactive cells in the bed nucleus of the stria terminalis (BST) and the medial amygdala (MeA). This colocalization functions to regulate AVP expression, as progesterone and/or progestin receptors (PR)s suppress AVP expression in these same extrahypothalamic regions in the brain. These data suggest that progesterone may influence AVP-dependent behavior. While AVP is implicated in numerous behavioral and physiological functions in rodents, AVP appears essential for social recognition of conspecifics. Therefore, we examined the effects of progesterone on social recognition. We report that progesterone plays an important role in modulating social recognition in the male brain, as progesterone treatment leads to a significant impairment of social recognition in male rats. Moreover, progesterone appears to act on PRs to impair social recognition, as progesterone impairment of social recognition is blocked by a PR antagonist, RU-486. Social recognition is also impaired by a specific progestin agonist, R5020. Interestingly, we show that progesterone does not interfere with either general memory or olfactory processes, suggesting that progesterone seems critically important to social recognition memory. These data provide strong evidence that physiological levels of progesterone can have an important impact on social behavior in male rats.

Introduction

There is a notable lack of knowledge about the role of the hormone progesterone in males. The vast body of our knowledge on progesterone and progestin receptor (PR) function comes from studies in females (Blaustein, 2008; Priest and Pfaff, 1995). Although progesterone has always been considered a "female hormone", adult male rats have circulating levels of progesterone around 1.5–2 ng/mL (Andersen et al., 2004; Auger and Vanzo, 2006), compared to a range of 3–35 ng/mL in females that is seen throughout the rat estrous cycle (Weisz and Ward, 1980). Also, depending on the type of stressful event encountered, progesterone levels in males can approach 6 ng/mL (Andersen et al., 2004), suggesting a potential functional significance of this hormone in males. Recent studies demonstrate that progesterone and its receptor play an important, yet understudied, role in male behavior and physiology (Wagner, 2006). It is also important to note, that as males have higher levels of steroid receptor coactivators, which enhance steroid hormone action in many brain regions (Bian et al., 2011), it is likely that lower levels of progesterone are sufficient to elicit a physiological response within the male brain.

It has been shown that PRs are found in virtually every AVP-immunoreactive (AVP-ir) cell within the bed nucleus of the stria terminalis (BST) and medial amygdala (MeA) (Auger and De Vries, 2002). Indeed, this co-localization has functional implications for AVP regulation in the BST and MeA cells, as progesterone treatment results in a suppression of AVP-ir labeling within these cells, and two of the projection sites of these cells, the lateral septum (LS) and lateral habenula (LH) (Auger and Vanzo, 2006). Taken together, these data suggest an important role for progesterone regulation of AVP expression; however, it is unclear from these data if progesterone can regulate AVP-dependent behaviors.

One behavior that is linked to AVP in the LS, a site that receives AVP projections from the BST and MeA, is social recognition behavior. Social recognition paradigms capitalize on an animal's innate motivation to investigate unfamiliar conspecifics (Ferguson et al., 2002). An animal's ability to recognize a conspecific after an initial exposure typically lasts only 30 minutes; however, subcutaneous injections of AVP in rats and mice can facilitate social recognition by lengthening this social memory to 2 hours after initial exposure to a conspecific (Bielsky and Young, 2004; Dantzer et al., 1987). Social recognition is also enhanced by site specific infusions of an AVP agonist or impaired by infusions of AVP antagonists directly into the lateral septum (Dantzer et al., 1988). Furthermore, castration, which depletes AVP in the BST and MeA, and in projection sites of these cells, also impairs social recognition (Bluthe et al., 1990). These studies demonstrate the importance of AVP in social recognition. As social recognition

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behavior is clearly AVP-dependent, and progesterone treatment functions to suppress AVP-ir expression within the BST and MeA (Auger and Vanzo, 2006), we hypothesized that progesterone treatment would impair social recognition within the male brain.

Methods

Animals

Adult male Sprague–Dawley rats were bred in our animal facility from breeding stock obtained from Charles River (Charles River Laboratories, Inc., Wilmington, MA). Juvenile male stimulus animals (between 20 and 30 days old) were purchased directly from Charles River. All animals were group housed in our animal facility, unless otherwise noted, on a 12 h light/12 h dark cycle with lights off at 11:00 am, and had free access to food and water. This research was approved by the University of Wisconsin Animal Care and Use Committee. Different cohorts of animals were used in each experiment described below unless otherwise noted.

Drug treatments

All of the experiments described in this paper involved drug treatment administration via subcutaneous injection. Injections occurred during the "lights on" portion of the light cycle for three consecutive days. RU-486 (Steraloids, Newport R.I.; 5 mg) was dissolved in 0.4 mL of vehicle (5% benzyl alcohol, 15% benzyl benzoate, sesame oil, all from Sigma-Aldrich Co., St Louis, MO, USA). Progesterone (Steraloids, Newport R.I.; 1 mg) was dissolved in 0.1 mL of sesame oil. R5020 (Perkin Elmer, Boston MA; 20 μg) was dissolved in 0.1 mL of sesame oil. 0.4 mL of vehicle was used as a control for RU-486 and 0.1 mL of sesame oil was used as a control for progesterone and R5020.

Behavioral testing and statistical analyses

Testing took place during the "lights off" portion of the light cycle under dim red light in our behavior room. All behavior was digitally recorded, unless otherwise noted, and then analyzed by a trained researcher blind to all treatments using The Observer (Noldus Information Technologies, Leesburg, VA) behavioral observation software. Sigma Stat 3.5 was used to conduct all statistical analyses. For the habituation–dishabituation study, a two-way repeated measures ANOVA was used to compare treatment by trial. A one-way ANOVA was run on habituation scores, which were calculated by subtracting the amount of investigation in trial 4 from the amount of investigation in trial 5. In the food-finding test, groups were compared using a Student's *t* test. Paired *t* tests were used to statistically analyze all other experiments.

Experiment 1: habituation–dishabituation, RU-486

Adapted from Winslow and Camacho (1995). Four-month-old male rats ($n=23$) were pretreated with RU-486 or vehicle, and then 2 hours later treated with progesterone or oil, for 3 days. During the 3 days of injections, the animals were separated from their cage mates and singly housed. On the third day of pretreatment and treatment, animals underwent behavioral testing 4 hours after the last round of injections. Testing occurred in the home cages of the adult male subjects. The test for each subject involved five, 1 minute trials. During the first four trials, the same juvenile male rat was placed in the subject's cage. On the fifth trial, a novel juvenile male rat was placed in the subject's cage. A 10 minute intertrial interval occurred between each of the five trials. Adult investigation of the juvenile was scored to include direct contact between the nose of the adult and the body of the juvenile and close (within 1 cm) following behavior. Cage directed behavior was also scored as a measure of basic locomotor and investigatory activity.

Experiments 2 and 3: social discrimination, RU-486, R5020

The social discrimination paradigm was adapted from Engelmann et al. (1995). In experiment 2, 3 month old male rats ($n=40$) received the same pretreatment with RU-486 or vehicle, and treatment with progesterone or oil as described above in the methods for experiment 1. In experiment 3, a different set of 3 month old male rats ($n=40$) received injections of progesterone, R5020 or oil for 3 days. Again, during the 3 days of injections, the animals were separated from their cage mates and singly housed. On the third day, injections of progesterone or oil were administered 4 hours before behavioral testing, while injections of R5020 were given 2 hours before behavioral testing; this time course for R5020 was empirically found to be the most behaviorally effective. Testing occurred in the home cages of the adult male rats. In trial 1, a male juvenile rat was placed in the home cage of the adult rat and the adult was allowed to freely investigate for 5 minutes. After 5 minutes, the juvenile was removed and the adult was alone in its cage for 30 minutes. After the 30 minute intertrial interval, the juvenile from trial 1 plus a novel juvenile were placed in the adult's cage, and the adult was again free to investigate for 5 minutes. The juvenile rats were distinguishable to the researcher scoring the video by unique tail marks drawn with permanent marker. Adult investigation of the juvenile(s) was scored to include direct contact between the nose of the adult and the body of the juvenile and close following behavior. Cage directed behavior was also scored as a measure of basic locomotor and investigatory activity.

Experiment 4: olfactory tests, preputial preference test

Four-month-old male rats ($n=40$) received the same pretreatment with RU-486 or vehicle, and treatment with progesterone or oil as described above in the habituation–dishabituation paradigm. During the 3 days of injections, the animals were separated from their cage mates and singly housed. This test utilized preputial glands that were surgically removed from sacrificed male rats about 20–30 days old. The preputial glands were homogenized in ice cold Tris buffered saline (TBS) and then centrifuged (Thompson et al., 2007). The supernatant (preputial extract) was then removed and stored at −80 °C. On the day of behavior testing, the preputial extract was thawed and used in a preference test. While in its home cage, each adult male rat was exposed to two Nestlets (Ancare, Bellmore, N.Y.): one with 40 μL of preputial extract on it, the other with 40 μL of TBS. The subject rat was freely allowed to investigate both Nestlets for 5 minutes. Behavior was digitally recorded and scored for direct contact between the subject and the Nestlet. Animals used in this experiment were the same cohort as that used in experiment 2.

Experiment 4: olfactory tests, food-finding test

This paradigm was adapted from Mencio-Wszalek et al. (1992). 6.5 month old rats ($n=20$) were treated with either progesterone or oil for 3 days. On the third day, injections occurred 4 hours before behavioral testing. A piece of chocolate chip cookie (average weight of 7.6 g) was buried at the center of a clean cage under a 1 cm layer of fresh bedding. Subjects were placed in the corner of the test cage and given a maximum of 10 minutes to uncover the cookie. The latency until the subject uncovered the buried cookie was measured in seconds with a stop watch.