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RU486 facilitates or disrupts the sensitization of sexual behaviors by estradiol in the ovariectomized Long–Evans rat: Effect of timecourse

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ABSTRACT

An acute injection of estradiol benzoate (EB) to the ovariectomized (OVX) rat activates low levels of lordosis, and subsequent progesterone (P) administration augments lordosis and recruits a complete pattern of sexual behavior including appetitive behaviors (e.g., hops/darts and solicitations). However, repeated injections of 5 μg or 10 μg EB (but not 2 μg EB), administered every 4 days to sexually-experienced OVX rats results in a behavioral sensitization, such that lordosis quotients (LQs) and appetitive behaviors progressively increase. We have shown that adrenal P does not play a critical role because behavioral sensitization to EB is not prevented by adrenalectomy. Here we tested whether P receptors play a role by examining the effect of chronic administration of the P receptor antagonist RU486 at a dose that reliably inhibits sexual behavior in fully primed OVX rats. Females were treated with EB (5 or 10 μg), and 5 mg RU486 dissolved in 0.4 mL vehicle (VEH; 80% sesame oil, 15% benzyl benzoate, 5% benzyl alcohol) 48 h and 5 h prior to each of 7 tests, respectively, occurring at 4-day intervals in unilevel 4-hole pacing chambers. Control animals were treated with 2, 5, or 10 μg EB + VEH. As expected, sensitization did not occur in females treated with 2 μg EB + VEH, and those females received fewer intromissions and ejaculations than all other groups. RU486 did not prevent the sensitization of LQ, moderate and high lordosis magnitudes (LM2 and LM3) or appetitive sexual behaviors on early tests, and in fact potentiated appetitive behaviors, LQ, LM2 and LM3, consistent with its facilitative actions in females treated with EB-alone, as we and others have reported previously. However, despite the initial facilitation, blocking P receptors by chronic administration of RU486 inhibited the maintenance of behavioral sensitization to EB.

Introduction

Sexual behavior in ovariectomized (OVX) rats can be fully reinstated by administration of estradiol benzoate (EB) followed by progesterone (P), 48 h and 4 h prior to testing, respectively (Beach et al., 1942; Boling and Blandau, 1939). Whereas acute doses of EB dose-dependently increase lordosis, appetitive sexual behaviors such as hops, darts, solicitations, and ear wiggles are increased dose dependently by P following EB (Beach et al., 1942; Whalen, 1974). Although the acute administration of EB alone partially reinstates sexual behavior in OVX rats, it is recognized that they become more sensitive behaviorally to EB (above 3 μg) with repeated administration (Babcock et al., 1988; Beach and Orndoff, 1974; Blaustein et al., 1987; Clark and Roy, 1983; Davidson et al., 1968; Gerall and Dunlap, 1973; Jones et al., 2013; Jones and Pfaus, 2014; Kow and Pfaff, 1975; Parsons et al., 1979; Sinchak and Micevych, 2001; Whalen and Nakayama, 1965). We recently characterized the development of EB sensitization in sexually-experienced OVX Long–Evans rats receiving 2, 5, or 10 μg EB at 4- or 8-day intervals, and found that 2 μg EB does not induce sensitization, and that the effect is most robust in those treated with 10 μg EB every four days (Jones et al., 2013). Although the underlying mechanisms are not well understood, Parsons et al. (1979) determined that increased behavioral sensitivity to EB is not due to the accumulation of EB in plasma. They administered crystalline 17β-estradiol (E2) or cholesterol to OVX rats via subcutaneous capsules for one week followed by their removal. In those females pre-exposed to E2, plasma levels of E2 fell to control levels within 12 h but they were more behaviorally receptive in response to E2 administered five days after removal compared to controls, indicating that E2 induced long-term changes in neuronal responsivity to subsequent E2 treatment.

Estradiol binding to receptors (ERs) in the ventromedial hypothalamus (VMH) promotes the display of lordosis in response to mounting by the male, and induces P receptor (PR) synthesis in a number of hypothalamic regions, including the VMH and the medial preoptic area (mPOA) (MacLusky and McEwen, 1978; Pfaff, 1980). Subsequent PR activation within the VMH potentiates lordosis, whereas the activation of PR within the mPOA stimulates appetitive sexual behaviors (Beyer et al., 1997; Glaser et al., 1983; Hoshina et al., 1994; Mani et al., 1994a; Rubin and Barfield, 1983; Sakuma, 1994, 2008). Given the importance of PR in the display of sexual behavior, we examined whether adrenal P contributes to the sensitization of sexual behaviors by EB and found that

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sexually-experienced OVX rats that had also been adrenalectomized (ADX) and were treated with 5 or 10 μg EB every four days displayed behavioral sensitization (Jones et al., 2013). This suggests that adrenal P does not play a role, as previously shown with repeated daily injections of EB (Davidson et al., 1968). This is also in agreement with studies showing that the PR antagonist RU486 (administered 5 h prior to testing) fails to disrupt the potentiated lordosis response following repeated administration of EB, in estradiol-induced (continuous exposure to estradiol-alone by subcutaneous capsules), and estradiol-facilitated (EB followed 44 h later by a bolus dose of estradiol administered in place of P, 4 h prior to testing) paradigms (Blaustein et al., 1987; Micevych et al., 2008). Indeed, mice lacking P receptors (PR knockouts) display similar (albeit slightly lower) levels of lordosis in response to repeated administration of EB (Mani et al., 1996). However, those studies that failed to find an effect of RU486 on lordosis administered it acutely just prior to testing and at least 44 h after E2 was initially administered.

Although peripheral P is not involved in estradiol's sensitization of sexual behaviors, a role for PR cannot be ruled out. First, PR can be activated by agents other than P, for example by second-messenger effects of neurotransmitters such as dopamine (Mani et al., 1994a), or neuropeptides such as gonadotropin releasing hormone (Mani et al., 1994b; Moss and McCann, 1973; Waring and Turgeon, 1992). PRs also have a low binding affinity for estradiol (MacLusky and McEwen, 1980; Parsons et al., 1984). Moreover, PR activation is involved in the short-term enhancement of lordosis by mating or vaginocervical stimulation (VCS), as this effect is blocked by RU486 (Auger et al., 1997). PRs may also be activated by neuroprogesterone, particularly with respect to appetitive sexual behaviors. Estradiol induces the production of neuroprogesterone in hypothalamic astrocytes (Micevych et al., 2003; Sinchak et al., 2003), and administration of RU486 disrupts the potentiation of hops, darts, and ear wiggles using an estradiol-facilitated paradigm in OVX–ADX animals (Micevych et al., 2008). This suggests that PRs play a role in the potentiation of appetitive sexual behaviors by repeated administration of EB. It is important to note that in studies that failed to find an effect of RU486 on the facilitation of lordosis, RU486 was administered once, days after the initial exposure to estradiol and after the animals had sensitized. As such, it is unclear whether repeated blockade of PR beginning at the onset of the sensitization paradigm would interfere with the development of the sensitization of sexual behaviors by 5 μg or 10 μg EB repeatedly administered SC at 4-day intervals.

The goal of this study was to examine whether chronic administration of RU486, administered at a dosing regimen that is known to block P-activated sexual behaviors, would interfere with the sensitization of sexual behaviors in the OVX Long–Evans rat treated with 5 or 10 μg EB at 4-day intervals. We first confirmed that RU486 inhibits the P facilitation of sexual behavior (Experiment 1) by treating sexually experienced EB-primed OVX Long–Evans rats with either RU486 or vehicle 1 h prior to P administration. In Experiment 2, we investigated whether chronic blockade of PR in sexually experienced OVX Long–Evans rats treated with 5 μg or 10 μg EB every 4 days would disrupt the development of the sensitization of sexual behaviors by chronic administration of EB.

Material and methods

Animals

Long–Evans rats were purchased from Charles River Canada (St-Constant, QC). Females were pair-housed in shoebox cages lined with a mixture of betachip and corncob bedding, and males were housed in groups of four lined with betachip. All animals had standard laboratory chow (Charles River #5075) and tap water freely available, and were kept in the same colony room which was maintained at 21 °C, on a 12-hour reverse day–night cycle (lights off at 8:00 AM). Animals were given one week to acclimate to the animal care facility. All the rats were treated in accordance with the guidelines of the Canadian Council on Animal Care and approval for all experimental procedures was granted by the Concordia University Animal Ethics Committee.

Ovariectomy

Bilateral ovariectomies (OVX) were performed under a 4:3 mixture of ketamine hydrochloride (50 mg/mL; Ketaset©, Wyeth Canada) and xylazine hydrochloride (4 mg/mL; Rompum ©, Bayer Healthcare) injected intraperitoneal (1 mL/kg body weight), via a single lumbar incision, and identified by ear punch. Polysporin© was applied to the incision site, and post-operative care included 2.5 mg/kg of *Flunixin meglumine* (Banamine©), 0.1 mL/rat of benzylpenicillin (Penicillin G), and 2 mL/rat of 0.9% saline, followed by a one-week post-operative recovery prior to sex training.

Steroid hormones and RU486 preparation and administration

EB (10 μg) and P (500 μg) were dissolved in 0.1 mL reagent grade sesame oil. EB doses (2 μg and 5 μg) were always diluted down from 10 μg EB solution, and administered 48 h prior to testing. Progesterone was administered 4 h prior to testing. RU486 (mifepristone; 17β-hydroxy-11β-[4-dimethylaminophenyl]-17α-[ι-propynyl]-estra-4,9-dien-3-one; 5 mg/0.4 mL; Sigma-Aldrich) was dissolved in 80% reagent grade sesame oil, 15% benzyl benzoate and 5% benzyl alcohol (Blaustein et al., 1987; as in, Pleim et al., 1990), 15 h prior to use, to ensure adequate dissolution, and injected 5 h prior to behavioral testing. Preparation of this dose of RU486 has been demonstrated to attenuate female sexual behavior when given before a P injection (Blaustein et al., 1987; Brown and Blaustein, 1984; Vathy et al., 1989). All treatments were administered SC.

Throughout the course of the experiment, animals developed subdermal lumps or scabs at the injection sites (regardless of treatment condition), therefore, injection sites were alternated in quadrants to reduce irritation. Using a set of scrub females from our laboratory, it was suspected that a component in the vehicle solution (not sesame oil nor RU486) induced those lesions, as lesions were also observed in scrubs treated with the VEH but not sesame oil. All animals were closely monitored and scabs were treated with iodine and polysporin as needed. Following any signs of distress or impaired general health, animals were immediately removed from the experiment. Additional details regarding chronic administration of this vehicle solution are reported elsewhere (Jones, S.L., Gardner Gregory, J., Pfaus, J.G., in preparation).

Sexual behavior training and testing

Behavioral training and testing were carried out during the middle-third of the dark cycle, in unilevel pacing chambers (38 × 60 × 38 cm) lined with betachip and bisected by clear Plexiglas® divider with 4 square holes cut into the bottom. The holes were adjusted to allow passage of only the smaller female, restricting the male to one compartment. Males were first placed on one side of the chamber, and allowed a 5-minute habituation period prior to introduction of the female to the empty compartment. All training and test sessions were 30 min in duration, were video-recorded using a Sony Handycam Digital camera and subsequently scored using a personal computer and the Behavioral Observation Program customized for sexual behavior in the pacing chamber (Cabilio, 1996) with the experimenter blind to treatment condition.

Experiment 1. RU486 inhibits sexual behavior in females primed fully with EB + P

Sexually experienced females (N = 19) used as stimulus animals in unrelated studies in our lab, were primed with 10 μg EB 48 h, and 500 μg P 4 h prior to testing. To verify that in our hands RU486 inhibits sexual behavior, half the animals were given RU486 (n = 9) or the vehicle