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Research report

An antiprogestin, CDB4124, blocks progesterone's attenuation of the negative effects of a mild stress on sexual behavior

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HIGHLIGHTS

- Ovariectomized rats show lordosis behavior following 10 μg estradiol benzoate.
- Sexual behavior is reduced after 5 min of restraint.
- Progesterone attenuates the negative effects of the restraint.
- The progesterone receptor antagonist, CDB4124, blocks progesterone's effect.

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ABSTRACT

These experiments were designed to test the hypothesis that a progesterone receptor antagonist would block progesterone's ability to reduce the negative effects of a 5 min restraint on female rat sexual behavior. Ovariectomized Fischer rats were injected with 10 μg estradiol benzoate. Two days later, rats were injected subcutaneously (sc) with the progesterone receptor antagonist, CDB4124 (17α-acetoxy-21-methoxy-11β-[4-N,N-dimethyaminopheny]-19-norpregna-4,9-dione-3,20-dione) (60 mg/kg), or vehicle (20% DMSO + propylene glycol). One hour later, rats were injected sc with 500 μg progesterone or vehicle (sesame seed oil). Rats were assigned to one of three different treatment conditions: (1) (ECV) estradiol benzoate, CDB4124, sesame seed oil vehicle, (2) (ECP) estradiol benzoate, CDB4124, progesterone, and (3) (EVP) estradiol benzoate, DMSO/propylene glycol vehicle, progesterone. That afternoon sexual behavior was examined before and after a 5 min restraint experience. Before restraint, lordosis behavior was comparable across treatment conditions but only progesterone-treated rats exhibited proceptive behavior. CDB4124 did not block progesterone's induction of proceptivity. However, after restraint, CDB4124 attenuated the positive effects of progesterone on all sexual behaviors examined. The restraint experience inhibited sexual behavior in rats treated with estradiol benzoate and CDB4124 and in rats treated with estradiol benzoate, CDB4124, and progesterone but not in rats given estradiol benzoate and progesterone without CDB4124. These findings are consistent with the hypothesis that progesterone receptors mediate progesterone's ability to reduce the negative sexual behavioral effects of a mild stressor.

1. Introduction

In naturally cycling female rats, estradiol and progesterone cooperate to regulate the complex behavioral and physiological sequence of events required for reproductive success [1–3]. Sexual behavior is temporally linked to ovulation so that the probability of pregnancy is increased [3]. Female rat sexual activities include appetitive, precopulatory and consummatory behaviors that are differentially regulated by gonadal hormones [1,4,5]. Only estradiol is required for lordosis behavior (the consummatory response) while progesterone can facilitate lordosis behavior; and progesterone may be required for certain appetitive and precopulatory activities [1,5,6]. Gonadal hormones alter behavior and physiology through both classical and nonclassical pathways [7–12]. The classical pathway includes the hormones's interaction with an intracellular receptor that functions as a nuclear transcription factor [13,14]. Nonclassical pathways include a variety of intracellular signaling cascades that are precipitated by membrane events consequent to hormonal exposure [9,15–17] but may also be initiated through ligand-independent mechanisms [8,18].

In addition to their role in reproduction, estradiol and progesterone modulate a variety of nonreproductive behaviors [19–25]. Progesterone, in particular, is recognized to have potent

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anxiolytic action, often attributed to positive effects of progesterone metabolites at $GABA_A$ receptors [26–31]. Progesterone is metabolized by 5α-reductase into 5α-dihydroprogesterone (5α-DHP) and then into allopregnanolone (3α-hydroxy-5β-pregnan-20-one; 3α,5β-THP) by 3α-hydroxysteroid dehydrogenase (3α-HSD) [32,33]. Substantial evidence has implicated allopregnanolone in progesterone's antianxiety effects [28,31,34–36]. However, progesterone's protection against the lordosis-inhibiting effects of a mild restraint experience may not require progesterone metabolites [37,38].

Sexual behavior of ovariectomized Fischer female rats that are hormonally primed only with estradiol benzoate shows a transient (5–10 min) decline in sexual receptivity after a 5 min restraint experience while no such decline occurs in rats treated with both estradiol benzoate and progesterone [39,40]. Progesterone's positive effects were mimicked by the nonmetabolizable progestin, medroxyprogesterone [37], and were not blocked by the 5α-reductase inhibitor, finasteride [38]. These findings appeared to rule out the necessity for progesterone metabolites. Moreover, progesterone's effect was blocked by the progesterone receptor antagonist, RU486 (11β-(4-dimethylamino)phenyl-17β-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one) [37]. From these findings, we tentatively concluded that progesterone receptors were required for progesterone's ability to reduce the effect of restraint. However, RU486 is not selective for progesterone receptors but also antagonizes glucocorticoid receptors [41,42]. Stress activates the hypothalamic–pituitary–adrenal axis leading to an increased secretion of corticosterone which can alter female rat sexual behavior [43,44], and progesterone can bind to glucocorticoid, as well as progesterone, receptors [42]. It is, therefore, possible that RU486 may have reduced the effect of progesterone through antagonism of glucocorticoid receptors. Additional information is needed with a more selective progesterone receptor antagonist before definitively concluding that progesterone receptors are required for progesterone's protection against the negative effects of restraint stress on sexual behavior. Identification of the mechanisms responsible for progesterone's protection is important because this protection enables the female to continue mating in spite of acute stress. Social interactions, including sexual activity, activate the hypothalamic–pituitary–adrenal axis and increase plasma levels of adrenocorticotropic hormone and corticosterone [45,46]. This is not surprising since the mating experience includes the introduction to a novel environment and consequent elevation of arousal. Therefore, resistance to a mild stressor should be beneficial for the female's reproductive fitness.

In the following experiment, the ability of CDB4124 to block progesterone's effect in a mild restraint paradigm was examined. CDB4124 has antiprogestin activity in a variety of functional assays [47–49] but has limited antiglucocorticoid activity [48,49]. Therefore, if progesterone acts through progesterone receptors to attenuate the negative effects of the mild stressor, CDB4124 should prevent this protection.

2. Materials and methods

2.1. Materials

Estradiol benzoate, progesterone and sesame seed oil were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). CDB4124 (17α-acetoxy-21-methoxy-11β-[4-N,N-dimethyaminopheny]-19-norpregna-4,9-dione-3,20-dione) was a generous gift from Dr. Ronald Wiehle and Repros Therapeutics Inc. (The Woodlands, TX). Propylene glycol was obtained from Eastman Kodak Company (Rochester, NY). Isoflurane (AErrane®) was purchased from Butler Schein Animal Health (Dublin, OH). Decapicone® restrainers were from Braintree Scientific, Inc. (Braintree, MA). Other supplies came from Fisher Scientific (Houston, TX).

2.2. Animals, housing and surgical procedures

Virgin, adult Fischer (F-344) female rats (Charles River Laboratories, Wilmington, MA) were housed in polycarbonate shoebox cages in a colony room with lights off from 12 noon to 12 midnight. Food and water were available *ad lib*. After a 2 week acclimation to the animal facility, females (70–90 days of age) were anesthetized with AErrane® and ovariectomized as previously described [39]. Two weeks later, rats were injected with 10 μg estradiol benzoate. Two days later, rats were injected subcutaneously (sc) with the progesterone receptor antagonist, CDB4124 (60 mg/kg), or vehicle (20% DMSO + propylene glycol). One hour later, rats were injected sc with 500 μg progesterone or vehicle (sesame seed oil). Rats were assigned to one of three treatment conditions: (1) (ECV) estradiol benzoate, CDB4124, sesame seed oil vehicle, (2) (ECP) estradiol benzoate, CDB4124, progesterone, and (3) (EVP) estradiol benzoate, DMSO/propylene glycol vehicle, progesterone. Estradiol benzoate and progesterone were dissolved in sesame seed oil and injected in a volume of 0.1 ml/rat. CDB4124 was dissolved in 20% DMSO/propylene glycol (vol/vol) at a concentration of 30 mg/ml and was injected in a volume of 2 ml/kg. Hormonal priming conditions were based on prior studies [37,38]. The dose of CDB4124 was based on work by Beckley et al. [50]. Testing occurred 4–5 h after the progesterone or sesame seed oil injection. All procedures were approved by the TWU IACUC committee in accordance with the PHS guide.

2.3. Testing for sexual behavior

Four hours after sesame seed oil or progesterone treatment, females were pretested for sexual behavior in the home cage of a sexually active Sprague–Dawley male. Behavior was monitored until the male had achieved 10 mounts or for a maximum of 10 min. Sexual receptivity (L/M ratio; number of lordosis responses divided by number of male mounts) and lordosis quality (sum of lordosis quality scores divided by number of lordosis responses) were scored as previously described [39]. Proceptivity (defined as the presence of hopping and darting) and resistance (defined as fighting, boxing, rolling over, trying to escape the cage) were measured as present or absent.

2.4. Restraint procedures

Immediately after the pretest, females were restrained for 5 min in a Decapicone® as previously described [39]. The female was placed head first into the cone, the base of which was gathered around the female's tail and secured tightly with tape. Immediately after the restraint experience, females were placed back into a male's cage for 15 consecutive minutes of behavioral testing, as described in Section 2.3.

2.5. Statistical procedures

Data for L/M ratios, lordosis quality and number of mounts were grouped into the pretest and 5 min intervals after restraint and were evaluated by two-way repeated measures ANOVA. Time relative to restraint was the repeated factor and treatment was the independent factor. Post hoc comparisons were made with Tukey's test. Proceptivity and resistance were compared by Chi-square and Fisher's exact test procedures. Data were analyzed with SPSS v.17 for Macintosh and post hoc comparisons were performed manually. An alpha of 0.05 was required for rejection of the null hypothesis and the statistical reference was Zar [51].

3. Results

3.1. Characteristics of rats before restraint

A total of 31 rats were used in the study. One rat given estradiol benzoate and CDB4124 (ECV rats) and one given estradiol benzoate, CDB4124, and progesterone (ECP rats) were not receptive during the pretest and were excluded from the remaining descriptions. Rats in all treatment conditions showed high L/M ratios before restraint but L/M ratios were slightly, but significantly, higher in rats given progesterone (Fig. 1; ANOVA for treatment, $F_{2,26} = 5.40$, $p \leq 0.011$). However, with post hoc comparisons, only ECV and EVP rats were significantly different from each other (Tukey's $q_{26,3} = 5.15$, $p \leq 0.05$). There were also significant treatment effects for lordosis quality ($F_{2,26} = 4.66$, $p \leq 0.019$) before restraint but all quality scores were relatively high (The means ± SE lordosis quality scores before restraint were: ECV = 2.4 ± 0.03, ECP = 2.83 ± 0.05, and EVP = 2.98 ± 0.01).

None of the ECV rats showed proceptivity in the pretest while approximately 45% of rats treated with progesterone were proceptive leading to a significant treatment effect (Table 1;