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An Antiprogestin, CDB4124, Blocks Progesterone's Attenuation of the Negative Effects of a Mild Stress on Sexual Behavior

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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 Fisher rats were injected with 10 µg estradiol benzoate. Two days later, rats were injected subcutaneously (sc) with the progesterone receptor antagonist, CDB4124 (17 α-acetoxy-21-methoxy-11β-[4-N,N-dimethylaminophenyl]-19-norpregna-4,9-dione-3,20-dione) (60 mg/kg), or vehicle (20% DMSO + propylene glycol). One hr 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.

Keywords

sexual receptivity; lordosis; ovariectomized rats; proceptivity; stress; progesterone receptors

1.0 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

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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 anxiolytic action, often attributed to positive effects of progesterone metabolites at GABA_A receptors [26–31]. Progesterone is metabolized by 5 α -reductase into 5 α -dihydroprogesterone (5 α -DHP) and then into allopregnanolone (3 α -hydroxy-5 β -pregnan-20-one; 3 α ,5 β -THP) by 3 α -hydroxysteroid dehydrogenase (3 α -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 5 α -reductase inhibitor, finasteride [41]. These findings appeared to rule out the necessity for progesterone metabolites. Moreover, progesterone's effect was blocked by the progesterone receptor antagonist, RU486 (11 β -(4-dimethylamino)phenyl-17 β -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 [42, 43]. Stress activates the hypothalamic-pituitary-adrenal axis leading to an increased secretion of corticosterone which can alter female rat sexual behavior [44, 45], and progesterone can bind to glucocorticoid, as well as progesterone, receptors [43]. 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 adrenocorticotrophic hormone and corticosterone [46, 47]. 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 [48–50] but has limited antiglucocorticoid activity [49, 50]. Therefore, if progesterone acts through progesterone receptors to attenuate the negative effects of the mild stressor, CDB4124 should prevent this protection.

2.0 Materials and Methods

2.1 Materials

Estradiol benzoate, progesterone and sesame seed oil were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). CDB4124 (17 α -acetoxy-21-methoxy-11 β -[4-N,N-dimethylaminophenyl]-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 two week acclimation to the animal facility, females (70 to 90 days of age) were anesthetized with AErrane[®] and ovariectomized as previously described [39]. Two weeks later, rats were injected with 10 μ g 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 hr later, rats were injected sc with 500 μ g 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, 41]. The dose of CDB4124 was based on work by Beckley et al. [51]. Testing occurred 4 to 5 hr 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 hr 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 min of behavioral testing, as described in 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 posthoc comparisons were performed manually. An alpha of 0.05 was required for rejection of the null hypothesis and the statistical reference was Zar [52].

3.0 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 (Figure 1; ANOVA for treatment, $F_{2,26} = 5.40$, $p = 0.011$). However, with posthoc comparisons, only ECV and EVP rats were significantly different from each other (Tukey's $q_{26,3} = 5.15$, $p = 0.05$). There were also significant treatment effects for lordosis quality ($F_{2,26} = 4.66$, $p = 0.019$) before restraint but all quality scores were relatively high (The means $\pm$ S.E. 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; Chi Square = 6.02, $df = 2$, $p = 0.05$). There was no significant difference between progesterone-treated rats with or without CDB4124 ($p > 0.05$). Resistance was present in all conditions and this was most prevalent in the ECV rats and least prevalent in EVP, but there were no significant differences among groups (Chi Square = 2.23, $df = 2$, $p > 0.05$).

3.2 Effects of restraint

One rat from the ECV group failed to receive mounts after restraint and was omitted from the following analyses. Consistent with earlier studies [37, 41], 5 min of restraint reduced L/M ratios relative to the pretest (ANOVA for time, $F_{3,75} = 8.06$, $p = 0.01$) (See Figure 1). After restraint, both ECV and ECP rats had L/M ratios lower than their pretest while L/M ratios of EVP rats were not significantly changed. This led to a significant main effect of treatment ($F_{2,25} = 5.11$, $p = 0.014$). ECV rats differed from their pretest at both the 5 and 10 min intervals while ECP rats were significantly lower at the first 5 min interval ($q_{3,75} = 4.65$, $p = 0.05$). EVP rats were never different from their pretest ($p > 0.05$). Rats without progesterone priming (ECV rats) were significantly different from EVP rats throughout the testing period (Tukey's, $F_{2,25} = 3.83$, $p = 0.05$). L/M ratios of progesterone -treated rats given CDB4124 were significantly lower than those of rats given progesterone without CDB4124 at 5 and 10 min after restraint (Tukey's, $q_{2,25} = 3.83$, $p = 0.05$).

Three rats (1 ECV and 2 ECP) had L/M ratios of zero after restraint so that the quality could not be assessed. These 3 rats were omitted from the ANOVA for lordosis quality. For the remaining rats, there was a significant effect of time ($F_{3,66} = 7.92$, $p = 0.001$) but the effect of treatment narrowly escaped statistical significance ($F_{2,22} = 3.42$, $p = 0.051$). The means $\pm$ S.E lordosis quality after restraint were: ECV = 2.27 ± 0.26 , ECP = 2.60 ± 0.09 , and EVP = 2.91 ± 0.04 , $p = 0.04$). Treatment effects after restraint were not the result of differential

mounting by the males ($p > 0.05$). The means $\pm$ S.E. for number of mounts during the 15 min after restraint were: 18.5 ± 1.7 , 19.5 ± 2.5 , and 17.5 ± 1.7 .

Proceptivity after restraint is shown in Table 1. EVP rats were significantly different from the two other groups (for ECV and ECP, respectively, Chi Square = 5.22 and 9.98, $p = 0.022$ and 0.001). Similarly, with the exception of the EVP group, a majority of the rats showed resistance after restraint so that EVP rats were significantly different from the other two groups (Chi Square = 9.98, $df = 1$, $p = 0.001$).

4.0 Discussion

Consistent with prior studies [37, 41], the current findings support the suggestion of progesterone receptor involvement in progesterone's attenuation of the negative sexual behavioral effects of mild restraint. In prior studies, RU486, which has both antiglucocorticoid and antiprogesterin activity [42, 43], was used. The current studies with the antiprogesterin, CDB412, which does not have significant antiglucocorticoid activity [49, 50], implicates progesterone receptors, and not glucocorticoid receptors, in progesterone's attenuation of the effects of restraint. In ovariectomized Fischer rats, 10 μ g estradiol benzoate (a commonly used dose of estradiol benzoate for hormonal priming) is adequate for induction of high levels of lordosis responding [37] and this was not altered by the treatment with CDB4124. The lack of a progesterone receptor antagonist to prevent estradiol benzoate-facilitated lordosis behavior is consistent with prior experiments demonstrating that RU486 does not interfere with lordosis behavior induced only by estradiol [37, 53]. It is, therefore, not surprising that CDB4124 had no effect on lordosis behavior prior to restraint. It is surprising that the progesterone receptor antagonist failed to reduce proceptivity before restraint since proceptivity is minimal in the absence of progesterone [1, 53, 54] and is thought to be progesterone receptor dependent [6, 54, 55]. This could reflect the relatively low levels of proceptive behavior in Fischer females compared to some other rat strains such as Sprague-Dawley rats [56] so that further reductions were undetectable. However, this is unlikely since there were robust differences between rats that did and did not receive progesterone. An additional possibility is that, in spite of progesterone receptor antagonism, progesterone metabolites may have been sufficient to facilitate proceptivity. Although progesterone receptors are clearly important for progesterone's induction of proceptivity [6], progesterone metabolites have previously been shown to increase proceptivity in estrogen-primed, ovariectomized rats [41, 57, 58].

In contrast to the lack of effect of CDB4124 on sexual behaviors before restraint, robust effects of the antiprogesterin were evident on sexual behaviors after restraint. L/M ratios were significantly reduced in rats primed only with estradiol benzoate and in rats primed with estradiol benzoate plus CDB4124 plus progesterone but not in rats primed with estradiol benzoate and progesterone without the progesterone receptor antagonist. These findings are similar to a prior report that RU486 reduced progesterone's protection against effects of mild restraint [37] and implicate the progesterone receptor as a mediator of progesterone's attenuation of the lordosis-inhibiting effects of restraint. CDB4124 also blocked progesterone's ability to reduce the effects of restraint on proceptive behavior. CDB4124's reversal of progesterone's attenuation of restraint-induced effects is consistent with a suggestion that progesterone receptors are also important for the continuation of appetitive and/or precopulatory behaviors after restraint.

Although 10 μ g estradiol benzoate leads to high lordosis responding in ovariectomized Fischer rats, such females are often resistant to the male's attempts and such resistance is amplified after restraint. This behavioral pattern was present in the current study where resistance both before and after restraint was highest in rats given estradiol benzoate priming

and lowest in rats with estradiol benzoate and progesterone priming. CDB4124, alone, did not increase resistance after restraint but did reverse the effect of progesterone. CDB414's reversal of progesterone's effect on the female's resistance to the male's attempts to mount after restraint, as shown in Table 1, allows the suggestion that progesterone receptors are also important in the female's willingness to mate after restraint.

Since CDB4124 did not inhibit progesterone's facilitation of sexual behaviors prior to restraint but did prevent progesterone's positive effects after restraint, these findings indicate that the mechanisms responsible for progesterone's protection from effects of mild stress may be dissociable from those responsible for induction of the behaviors. Many actions of progesterone mediated through progesterone receptors and progesterone metabolites are site-specific [6, 59]. Therefore, the current results could also indicate that different brain areas are involved in progesterone's protection against the deleterious effects of stress and those brain areas required for emergence of the behaviors.

Progesterone's recognized anxiolytic action could also explain the steroid's protection against the effects of restraint. However, in many studies, progesterone's anxiolytic effects have been attributed to progesterone metabolites such as allopregnanolone [25–27, 36]. Allopregnanolone can substitute for progesterone in reducing effects of mild restraint [41], but this effect of allopregnanolone was reduced by prior treatment with RU486 [60]. This led to speculation about the role of progesterone receptors in the effect of allopregnanolone and is consistent with suggestions by other investigators that progesterone receptors are important for the antianxiety effects of progesterone [21]. The current findings lend support to this possibility.

In prior studies, we have suggested that progesterone's effect on the serotonergic system contributes to its attenuation of the behavioral responses to restraint. Serotonin (5-HT) within the mediobasal hypothalamus inhibits female rat sexual behavior by activation of 5-HT_{1A} receptors (see for review [61]) and the 5 min restraint experience amplifies the effect of a 5-HT_{1A} receptor agonist on lordosis behavior [62]. We have suggested that a stress-induced elevation of extracellular 5-HT leads to activation of these 5-HT_{1A} receptors and consequent inhibition of lordosis behavior [62]. Progesterone reduces extracellular 5-HT in a variety of brain areas, including the medial basal hypothalamus [63, 64], so that progesterone's attenuation of the effects of restraint may result from a reduction in 5-HT's activation of 5-HT_{1A} receptors. Consistent with this possibility is evidence that progesterone reduces the lordosis-inhibiting potency of a 5-HT_{1A} receptor agonist [40].

In summary, results of the current experiment reinforce prior suggestions that progesterone acts via progesterone receptors to reduce the negative effects of a mild stress on female rat sexual behavior. Such findings do not rule out a role for progesterone metabolites but do seem to indicate that such metabolites are not sufficient. While much emphasis has been placed on the mechanisms responsible for progesterone's role in the emergence of female sexual behavior, the current studies allow the suggestion that mechanisms responsible for progesterone's induction of and those responsible for preventing sexual behavioral effects of stress may not be identical. Understanding the mechanisms responsible for these different roles of progesterone may further knowledge of how progesterone can exert such diverse effects on both reproductive and nonreproductive behaviors.

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Research Highlights

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

Figure 1. CDB4124 and lordosis behavior after restraint

Ovariectomized Fisher inbred rats were primed with 10 μ g estradiol benzoate. Two days later, rats received one of three treatments: (1) (ECV; n = 8) estradiol benzoate, CDB4124, sesame seed oil vehicle, (2) (ECP; n = 12) estradiol benzoate, CDB4124, progesterone, and (3) (EVP; n = 8) estradiol benzoate, DMSO/propylene glycol vehicle, progesterone. Four hr after the third injection, rats were pretested for sexual behavior and immediately restrained for 5 min. They were then returned to the male's cage for 15 consecutive min of sexual behavioral testing. Data are the mean $\pm$ S.E. lordosis to mount ratios before and for 3 consecutive 5-min intervals after restraint. * indicates a significant difference from the pretest, within treatment condition. ** indicates a significant difference from EVP group, within time interval.

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Table 1

Proceptivity and Resistance Before and After Restraint

TREATMENT	N	% Proceptive Before Restraint	% Proceptive After Restraint	% Resistive Before Restraint	% Resistive After Restraint
ECV	8	0/9 = 0%	0/9 = 0% ^a	4/9 = 44.4%	8/8 = 100% ^a
ECP	12	5/7 =41.6% ^a	1/12 = 8.3% ^a	3/12 = 25.0%	9/12 = 75.0% ^a
EVP	8	4/8 = 50% ^a	3/8 = 37.5%	1/8 = 12.5%	2/8 = 25.0%

^a indicates significant difference from EVP rats;