Published in final edited form as:

Behav Brain Res. 2015 April 1; 282: 95–102. doi:10.1016/j.bbr.2015.01.004.

DOSE-DEPENDENT EFFECTS OF THE ANTIPROGESTIN, RU486, ON SEXUAL BEHAVIOR OF NATURALLY-CYCLING FISCHER RATS

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Abstract

Regularly cycling Fischer female rats were treated with either a low (5 mg/kg) or high (5 mg/RAT; approximately 30 mg/kg) dose of the antiprogesterin, RU486, before the morning of proestrus or on the morning of proestrus. The emergence of sexual behavior after treatment with RU486 was examined in a mating test with a sexually active male rat. Lordosis behavior was remarkably resistant to the effects of RU486. Only the high dose of RU486 given the evening before proestrus, approximately 22 hours before mating, reduced lordosis behavior. Independent of dose or time of treatment, proceptivity was reduced and resistance to the male's attempts to mount was increased by RU486 treatment. In addition, the effect of a 5 min restraint stress on sexual behavior was examined. In contrast to the relative resistance of lordosis behavior of unrestrained rats to RU486 treatment, RU486 treated rats showed a significant decline in lordosis behavior after restraint. These findings allow the suggestion that the emergence of lordosis behavior is relatively resistant to the antiprogesterin while the maintenance of lordosis behavior after restraint may require participation of intracellular progesterone receptors.

Keywords

progesterone; lordosis behavior; proceptivity; restraint; stress; intracellular progesterone receptors

1.0 Introduction

During the female reproductive cycle, estradiol and progesterone cooperate to regulate female sexual behavior and ovulation [1–3]. In female rats, sexual activity encompasses a set of behaviors that indicate the female's willingness to mate, solicit interest from the male partner and lead to successful copulation [4–7]. In ovariectomized female rats, copulation (measured by the lordosis response) can be elicited with only estradiol priming, but progesterone is required for emergence of proceptivity/sexual motivation [1, 4, 7, 8].

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Nevertheless, with a large dose of estradiol and/or repeated estradiol priming, even proceptivity (a measure of the female's solicitation/motivation) can occur in the absence of progesterone priming [9–12].

Progesterone regulates reproductive function by binding to its intracellular cognate receptors [13–15], by binding to membrane progesterone receptors [16–18], and through progesterone metabolites such as allopregnanolone [19, 20]. Several lines of evidence allow the suggestion that intracellular progesterone receptors play an important role in progesterone's ability to facilitate lordosis behavior [21–24]. For example, when ovariectomized rats are hormonally primed with progesterone following a dose of estradiol benzoate that is insufficient for induction of lordosis behavior: (a) compounds, such as RU486 (11 β -(4-dimethylamino)phenyl-17 β -hydroxy-17-(1-propynyl)estra-4,9-dien-3-one), which can antagonize intracellular progesterone receptor-mediated events, block progesterone's facilitation of lordosis behavior [20, 25, 26]; (b) hypothalamus infusion of antisense oligonucleotides to intracellular progesterone receptors blocks the facilitation [24, 27]; and (c) progesterone's facilitation is reduced or absent in progesterone receptor knock out (PRKO) rodents [22, 28, 29]. However, when ovariectomized rats are treated with doses of estradiol benzoate that are sufficient to elicit lordosis behavior without progesterone priming, RU486 does not reduce lordosis behavior and has led to suggestions that intracellular progesterone receptors may not be required for estrogen-induced lordosis behavior [20, 26, 30–32]. In support of such a suggestion, ovariectomized PRKO mice still exhibit lordosis behavior in response to estradiol benzoate, alone [28]. Therefore, estrogen-dependent initiation of lordosis behavior may involve mechanisms that are distinct from those that mediate the estradiol and progesterone-facilitated behavior.

The physiological significance of estrogen's ability to induce lordosis behavior in the absence of intracellular progesterone receptors is not clear. Since the naturally cycling rat produces both estradiol and progesterone in preparation for ovulation and sexual receptivity, and since estrogen induces intracellular progesterone receptors [33, 34], it is not unreasonable to assume that progesterone receptors are critical for the facilitation of sexual receptivity in the intact rat. However, there have been surprisingly few tests of this assumption. When effects of antiprogestins, such as RU486, have been reported in intact rats, most have been focused on gonadotropic and ovarian events rather than on behavioral effects [35–37]. In addition, in studies where behavioral effects have been examined, RU486 has often been administered on multiple days [38]. Such a repeated treatment regimen makes it difficult to compare findings in the intact rat with those reported in hormonally-primed ovariectomized rat when RU486 has been administered 1–2 hours before the progesterone priming. Moreover, under these conditions, behavioral effects may be confounded with effects of the drug at ovarian sites.

To our knowledge, in only one report [39] has sexual behavior been examined after a single RU486 treatment of naturally cycling, virgin proestrous rats. In that study, 1 mg/kg RU486 was administered to Wistar rats on proestrus before the LH surge and behavior was monitored 1–2 hours after lights off. There was a significant reduction of the lordosis quotient (lordosis to mount ratio $\times$ 100), and although no data were presented, the authors reported that proceptivity was also reduced. In a related experiment, when lactating Sprague

Dawley females were treated with 5 mg/kg RU486 over 3 days postpartum, the development of postpartum estrous behavior was reduced [40].

While these few studies may be marshaled as evidence that intracellular progesterone receptors are required for development of lordosis behavior in naturally cycling proestrous rats, the amount of estrogen that is required to elicit lordosis behavior in the absence of progesterone varies greatly among rat/mouse strains [12, 41–43]. For example, ovariectomized Fischer female rats may show high sensitivity because a single injection of 10 µg estradiol benzoate to naive ovariectomized females elicits near maximal lordosis behavior (but not proceptivity) in a majority of females [44, 45] while comparable treatment is not reported to elicit high lordosis responding in Sprague Dawley or Wistar females [20, 46].

Although these strains have not been directly compared for hormonal induction of sexual behavior, Fischer females are especially vulnerable to estrogen-induced pituitary tumors and have heightened sensitivity to a variety of estrogen-like compounds [47–51]. Therefore, Fischer rats appear to have a higher sensitivity to estrogens than either the Sprague Dawley or Wistar strains in which behavioral effects of RU486 have been examined in intact females. Thus, it might be important to evaluate the effect of RU486 in intact females from a high estrogen-responsive strain.

In the following study, naturally-cycling Fischer female rats were injected with a high (5 mg/RAT; approximately 30 mg/kg) or low (5 mg/kg) dose of RU486 under treatment paradigms designed to mimic conditions where RU486 has been reported to disrupt lordosis behavior in intact rats or in ovariectomized rats, hormonally primed with estradiol benzoate and progesterone. Sexual behavior was evaluated 1–3 hr after lights off on proestrus. It was hypothesized that RU486's effect on the emergence of lordosis behavior would be less robust than has been reported for Sprague Dawley or Wistar rats.

In addition to progesterone's contribution to estrogen's facilitation of lordosis behavior, progesterone also appears to reduce the female's response to stress [52–54]. When ovariectomized Fischer rats are hormonally primed with 10 µg estradiol benzoate to elicit high lordosis responding, a brief 5 min restraint stress significantly reduces lordosis behavior [55]. The addition of progesterone priming completely eliminates this effect of restraint and intracellular progesterone receptors may be required [44, 55]. Importantly, restraint does not reduce lordosis behavior in naturally-cycling proestrous rats [55]. Since progesterone reduces the response to the 5 min restraint, we have proposed that this anti-stress effect of progesterone can account, in part, for progesterone's ability to facilitate low-level lordosis behavior of estrogen-primed ovariectomized rats and may be required for this anti-stress effect [44, 45, 56].

Therefore, an additional objective of the study was to determine if RU486 would enhance the effects of the 5 min restraint on female sexual behavior of naturally cycling proestrous Fischer rats. To this aim, when RU486-treated rats showed high lordosis responding (L/M 0.7), they were restrained for 5 min and then retested for sexual receptivity. It was

hypothesized that, in contrast to the emergence of lordosis behavior, the progesterone-dependent protection from the 5 min restraint would be reduced by RU486.

2.0 Materials and Methods

All procedures were approved by the TWU IACUC committee in accordance with the PHS Guide.

2.1 Materials

RU486 (11 β -(4-dimethylamino)phenyl-17 β -hydroxy-17-(1-propynyl)estra-4,9-dien-3-one) and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Propylene glycol was obtained from Eastman Kodak Company (Rochester, NY). Decapicone® restrainers were from Braintree Scientific, Inc. (Braintree, MA). Other supplies came from Fisher Scientific (Houston, TX).

2.2 Animals, housing and treatment procedures

Adult, Fischer (F-344) female rats were purchased from Charles River Laboratories (Wilmington, MA) and were housed in polycarbonate shoebox cages in a colony room with lights on from 12:00 midnight to 12:00 pm with food and water available *ad lib*. Females were allowed at least 2 weeks for adjustment to the animal facility. Thereafter, when females were 125–150 days of age, their vaginal smears were taken daily as previously described [57]. When females had shown at least 3 regular estrous cycles, they were injected subcutaneously (s.c.) with 5 mg/kg RU486 (low dose) or 5 mg/RAT (high dose; approximately 30 mg/kg) (in 15% DMSO, propylene glycol) or the DMSO/propylene glycol vehicle. For the lower 5 mg/kg dose, injections were given under three different conditions: (1) between 4:30 and 6:30 on the evening before the predicted day of proestrus (approximately 22 hours before testing) (loRUPM); (2) on the evening (4:30–6:30) before the predicted day of proestrus (approximately 22 hours before testing) and between 8:30 and 9:30 on the morning of predicted proestrus (approximately 7 hours before testing) (loRUPMAM); or (3) between 4:30 and 6:30 on the two consecutive evenings before predicted proestrus (loRU2days). For the higher 5 mg/RAT dose, injections were (1) the evening before proestrus (e.g. 22 hours before testing) (hiRUPM) or (2) the morning of proestrus (7 hours before testing) (hiRUAM). Proestrus was defined by a vaginal smear consisting primarily of nucleated and cornified cells and few or no leukocytes.

Twenty adult, sexually experienced Sprague Dawley males were used in the study. Males were singly housed in the same experimental room as the females.

2.3 Behavioral testing

Between 2:00 and 4:00 pm on the day of proestrus, females were placed into the home cage of a sexually experienced male rat and sexual behavior was monitored until 10 mounts had occurred or for a maximum of 15 min. Sexual receptivity was measured as the lordosis to mount ratio (L/M ratio; number of lordosis responses divided by number of male mounts). Lordosis quality (the magnitude of the lordosis response) was scored on a scale of 1 to 4 as previously described [55]. 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. When females showed at least one hop/dart sequence or one fighting/boxing sequence, that behavior was categorized as present.

2.4 Restraint procedures

Immediately after completion of the pretest, females that had shown high lordosis responding (defined as an L/M ratio ≥ 0.70) were restrained for 5 min as previously described [55]. The female was placed head first into a Decapicone® so that her nose was flush with the small opening at the tip of the cone. The base of the cone was gathered around the female's tail and secured with tape. Immediately after the restraint experience, females were placed back into a male's cage for 10 consecutive min of behavioral testing, as described in 2.3.

2.5 Uterine ballooning and persistent vaginal cornification

Persistent vaginal cornification and the prolonged presence of uterine ballooning (edematous swelling) occurs in rats treated with estrogens in the absence of progesterone [58–61] and are characteristic of RU486 treatment of naturally cycling rats [39, 58]. Persistent vaginal cornification was monitored by vaginal smears as described in 2.2. Uterine balloon was assessed by visual inspection for the presence of uterine swelling and a translucent appearance.

2.6 Statistical procedures

Data for the loRU486 and hiRU486 doses were analyzed separately.

2.6.1 Emergence of sexual behavior—For evaluation of the effect of RU486 on the emergence of sexual behavior, data for L/M ratios and lordosis quality during the pretest before any restraint had occurred were compared with ANOVA with type of treatment as the independent factor. Since L/M data for this pretest were not normally distributed, the data were subjected to square root transformation prior to ANOVA. The proportions of rats showing receptivity (defined as L/M ≥ 0.70), proceptivity and resistance were compared by Chi-Square procedures.

2.6.2 Response to restraint—When effects of restraint were examined, only rats that showed L/M ratios ≥ 0.7 in the pretest before restraint were included. L/M ratios and lordosis quality before and after restraint were compared by two-way repeated measures ANOVA with time relative to restraint as the repeated factor and type of treatment as the independent factor. Post-hoc comparisons were made with Tukey's test. Proceptivity and resistance were compared by Chi-Square procedures. First, data before and after restraint were independently evaluated with Chi-Square procedures for treatment effects. Any potential interaction between treatment and restraint was evaluated with heterogeneity Chi-Square procedures with treatment and time relative to restraint as factors. Data were analyzed with SPSS and the statistical reference was Zar [62].

3.0 Results

3.1. Effect of RU486 on the emergence of sexual behavior

All rats had proestrous-type (nucleated and cornified cells with minimal or no leukocytes) on the day of testing and, with the exception of rats given 5 mg/RAT RU486 the night before proestrus (22 hours before testing), the emergence of lordosis behavior was remarkably resistant to effects of RU486.

3.1.1. Receptivity

3.1.1.1 Lower dose of 5 mg/kg (loRU486): For the lower 5 mg/kg dose of RU486, most females (regardless of treatment) showed high levels of lordosis behavior during the pretest (Table 1). When receptivity was defined as an L/M ratio ≥ 0.7 , 100% of vehicle-treated rats were sexually receptive (L/M ≥ 0.7) and treatment with RU486 did not significantly reduce this percentage (Chi Square, $df = 3$, $p = 0.062$). Group differences in L/M ratios ($F_{3,36} = 2.4$, $p = 0.076$) and lordosis quality scores were small and were not statistically significant ($F_{3,33} = 2.43$, $p = 0.08$) (Table 1).

3.1.1.2 Higher 5 mg/RAT dose (hiRU486): A larger effect of RU486 was seen when the dose of RU486 was increased to 5 mg/RAT (approximately 30 mg/kg). For this experiment, only three groups were investigated: control group ($n = 8$), 5 mg RU486 given the evening before sexual receptivity (22 hours before testing) (hiRUPM) ($n = 10$), and rats given 5 mg RU486 the morning before sexual receptivity (7 hours before testing) (hiRUAM) ($n = 8$). Most (62.5%) of rats treated with RU486 (hiRUAM) the morning of proestrus showed sexual receptivity, but 70% of rats treated the evening before proestrus (hiRUPM) had no or low lordosis behavior or were so vicious that the male was unable to mount (Table 1).

Regardless of the injection time, lordosis behavior was lower in hiRU486-treated rats than in the control (ANOVA $F_{2,21} = 3.46$, $p = 0.05$), but only hiRUPM rats were significantly different from the control (Tukey's $q_{21,3} = 3.69$, $p = 0.05$). Lordosis quality for rats that did show lordosis behavior was not altered by treatment ($p > 0.05$).

3.1.2 Proceptivity and resistance—Motivational parameters (e.g. proceptivity and resistance) were considerably more sensitive to RU486 treatment than were L/M ratios and lordosis quality. For both doses of RU486, all treatments with RU486 reduced proceptivity and increased resistance relative to the vehicle control (All Chi Square, $p = 0.02$) (See Table 2).

3.2 Effects of RU486 on the response to restraint

3.2.1 Lordosis behavior—Rats with a pretest L/M ratio before restraint that was greater than 0.7 (e.g. receptive rats, Table 1) were restrained. As expected from prior studies with ovariectomized rats, hormonally primed only with estradiol benzoate, all treatments with RU486 significantly reduced L/M ratios after 5 min restraint. Lordosis behavior of control rats was unaffected by restraint (Figure 1).

3.2.1.1 Lower 5 mg/kg dose: With the lower dose of 5 mg/kg RU486, restraint reduced L/M ratios in all rats treated with RU486 (see Figure 1A). There were main effects of type of treatment ($F_{3,30} = 4.12$, $p = 0.015$) and time relative to restraint ($F_{1,30} = 22.33$, $p = 0.001$). All groups of rats treated with loRU486 were significantly different from the pretest and vehicle control (all $q_{40,4} = 4.27$, $p = 0.05$). There was not a significant interaction between time and treatment ($F_{3,30} = 1.65$, $p > 0.05$).

3.2.1.2 Higher 5 mg/RAT dose: For the higher (5 mg/RAT) RU486 dose, effects of restraint on L/M ratios were similar to those of the lower dose (See Figure 1B). It is important to note that only 3 rats from the hiRU486 group met the criterion for restraint. There were significant effects of time relative to restraint ($F_{1,14} = 14.65$, $p = 0.002$) and treatment ($F_{2,14} = 5.86$, $p = 0.014$). The interaction was also significant ($F_{2,14} = 4.61$, $p = 0.03$). Both hiRU486 treatments were significantly different from their pretest as well as from the control (Tukey's, all $p = 0.05$).

3.2.2 Lordosis quality

3.2.2.1 Lower 5 mg/kg dose: For the lower dose of RU486, there was a significant main effect of treatment on lordosis quality ($F_{3,28} = 4.03$, $p = 0.017$) and of time relative to restraint ($F_{1,28} = 6.93$, $p = 0.014$). However, these effects were relatively small and could be attributed to the lower lordosis quality scores of rats given loRU486 for two consecutive evenings before proestrus. This was the only group showing a significant decline after restraint ($F_{2,28} = 5.31$, $p = 0.05$) (See Figure 2A).

3.2.2.2 Higher 5 mg/RAT dose: Even the higher dose of RU486 had minor effects on lordosis quality after restraint. Although there were significant effects of time ($F_{1,14} = 20.46$, $p = 0.001$), treatment ($F_{2,14} = 3.77$, $p = 0.05$) and their interaction ($F_{2,14} = 4.69$, $p = 0.028$), only the hiRUPM group was significantly from the control (Tukey's, $q = 8.93$, $p = 0.05$). hiRUPM and hiRUAM did not differ (See Figure 2B).

3.2.3 Proceptivity and resistance after restraint—Effects of RU486 on proceptivity and resistance before and after restraint are shown in Table 3. For the low dose of RU486, there were significant treatment effects on proceptivity and resistance before and after restraint ($p = 0.05$) and no evidence for an interaction between treatment and restraint (heterogeneity Chi-Square, $p > 0.05$). For the high dose treatment, there was a significant treatment effect on proceptivity after restraint ($p = 0.05$) but not before restraint ($p > 0.05$). In contrast, there were significant treatment effects for resistance before restraint ($p = 0.05$), but not after restraint. In neither case was there a significant heterogeneity Chi-Square.

3.2.4 Vaginal cyclicity after testing—Following treatment with the lower dose of RU486, 6 loRU486-treated and 4 vehicle-treated rats were euthanized on estrus (the day after testing), and the presence or absence of uterine ballooning was examined. Uteri of loRU486-treated rats showed evidence of fluid retention typical of RU486-treated rats [58, 63, 64] while this was not the case for the control rats. In 13 loRU486-treated rats and 9 control rats, vaginal smears were monitored for at least 1 week after testing. Nine of these

rats (4/6 loRUPM; 2/4 loRU2days; 3/3 loRUPMAM) exhibited persistent vaginal estrus while the remaining 4 rats showed normal estrous cyclicity. Controls cycled normally.

Following the higher dose, vaginal smears of all rats were monitored after testing. In spite of their relatively low L/M ratios, 5/10 of the hiRUPM rats had sperm in the vaginal smear the day after testing. Thereafter, 3 of these ceased vaginal cycling and showed a continuous presence of leukocytes (but not cornified or nucleated cells) until euthanasia (15–19 days after testing). However, upon euthanasia, none of the rats exhibited a productive pregnancy. The two other rats with obvious sperm after testing showed normal vaginal cyclicity. Of the 5 rats that did not show sperm in the vaginal smear, 3 had persistent vaginal estrus and two showed normal vaginal cyclicity.

Of the 8 hiRUAM rats, 4 showed persistent vaginal estrus after testing even though one of the rats with persistent vaginal estrus had shown sperm in the vaginal smear. The remaining 4 rats showed normal vaginal cyclicity after testing even though 2 had shown sperm in the vaginal smear. None of these rats evidenced productive pregnancy when they were euthanized and none exhibited pregnancy-type vaginal smears.

4.0 Discussion

The current studies were designed to examine two separate but related questions: (1) the effect of RU486 on the emergence of sexual behavior in naturally cycling Fischer rats and (2) the effect of RU486 on the sexual behavioral response of proestrous rats to a 5 min restraint stress. In contrast to expectations with ovariectomized rats hormonally primed with estradiol and progesterone, RU486 had surprisingly minor effects on the emergence of lordosis behavior of naturally cycling Fischer rats. Only when the high dose of RU486 was given 22 hours before testing was lordosis behavior significantly reduced. However, for this hiRUPM group, the decline in lordosis behavior was substantial so that only 3/10 rats showed pre-restraint L/M scores sufficient to be included in the restraint procedure. Unlike effects of RU486 on the emergence of lordosis behavior, all RU486 treatments reduced proceptivity and increased resistance to the male's attempts to mount. These findings are consistent with the idea that, even in naturally cycling unrestrained rats, estrogen, alone, can induce lordosis behavior in an intracellular progesterone receptor-independent manner while progesterone receptors are required for sexual motivation.

Unlike the resistance of lordosis behavior in unrestrained rats to RU486, the antiprogesterin significantly reduced lordosis behavior following a 5 min restraint stress. Although there were significant treatment effects on proceptivity and resistance after restraint, these differences essentially mirrored treatment effects before restraint and there was little evidence for an interaction between RU486 and stress.

In the one report of RU486 effects on lordosis behavior of virgin, naturally cycling intact Wistar female rats [39], a dose of RU486 as low as 1 mg/kg reduced lordosis behavior to an L/M ratio of approximately 0.3. The current findings are only partially consistent with this earlier study in that the higher 5 mg/RAT RU486 treatment, given the evening before proestrus, did reduce or eliminate sexual receptivity on proestrus. However, the failure of

any of the treatments with the lower (5 mg/kg) dose of RU486 to substantially reduce lordosis behavior is in marked contrast to the findings of Telleria et al. This seems to indicate that lordosis behavior of Fischer females is less sensitive to effects of RU486 than are the Wistar rats used by Telleria et al. However, proceptivity (a behavior thought to be dependent on progesterone receptor action [1, 4, 30, 65]) of Fischer females was significantly reduced even at the lower dose of RU486. The high proportion of females showing persistent vaginal estrus and the occurrence of uterine ballooning following RU486 treatment evidence that intracellular progesterone receptor action was reduced. Moreover, the ability of RU486 to enhance effects of restraint on lordosis behavior is consistent with the assumption that intracellular progesterone receptor action was reduced by RU486 even when lordosis behavior before restraint was comparatively unaffected.

The different outcomes between the current and prior experiment could reflect differences in the testing procedures since Telleria et al. only counted mounts that were accompanied by pelvic thrusting while all mounts were counted in the current study. Yet given the resistance of females to the male's attempts to mount following RU486 treatment, it would have been expected that inclusion of all mounts would have led to lower, rather than higher, L/M ratios. Regardless of the explanation for different findings between the current and prior experiment, the current studies allow the suggestion that lordosis behavior, but not proceptivity, of unrestrained Fischer females may be relatively resistant to blockage of intracellular progesterone receptors by RU486. Such a possibility is consistent with the report by Soma et al [30] where estrogen-induced sexual behavior of Long-Evans ovariectomized/ adrenalectomized females occurred coincident with increased neuroprogesterone synthesis and where RU486 blocked the emergence of proceptivity but not lordosis behavior [30].

In contrast, RU486 had robust effects on the response to restraint. In previous experiments [44, 45, 55], we have reported that a mild 5 min restraint reduced lordosis behavior in ovariectomized rats that were hormonally primed with 10 µg estradiol benzoate but not in naturally cycling rats or in ovariectomized rats primed with estradiol benzoate and progesterone. A role for intracellular progesterone receptors in this resistance to the lordosis-inhibiting effects of the restraint was suggested by the ability of both RU486 and of the more selective progesterone receptor antagonist, CDB4124, to block effects of progesterone [44, 56]. In the current experiment, in all treatments with RU486, lordosis behavior was reduced by restraint.

It is important to note that no additional, independent measure of progesterone receptor action was examined so it is possible that Fischer females have such high levels of endogenous progesterone that only the higher dose of RU486 was able to effectively compete with the endogenous progesterone. If so, the progesterone receptors that were occupied by progesterone rather than RU486 must have been adequate for facilitation of lordosis behavior but insufficient to produce proceptivity or to reduce the response to stress. If this is true, it implies that higher intracellular progesterone receptor occupation is required for proceptivity and for protection against the restraint than is required for facilitation of lordosis behavior.

Alternatively, proceptivity and resistance to stress may be especially dependent on intracellular progesterone receptors while facilitation of lordosis behavior may allow a greater participation of membrane progesterone receptors that are not antagonized by RU486 [18, 66, 67]. Although classical intracellular progesterone receptors within the ventromedial nucleus have been clearly implicated in lordosis behavior [25, 67–69]; in other brain areas such as the medial preoptic area and ventral tegmental area, membrane progesterone receptors and/or neurotransmitter receptors such as GABA_A may be sufficient to facilitate the behavior following progesterone treatment [18, 70, 71].

Emergence of lordosis behavior without progesterone could also reflect ligand-independent events such as have been evidenced following treatments with neurotransmitters or progesterone metabolites [13]. For example, the progesterone metabolite, allopregnanolone, increases estradiol-induced lordosis behavior even in PRKO mice [72]. While some of these effects may include indirect activation of the intracellular progesterone receptor, others may not [72–74]. Clearly, various agents (e.g. progesterone metabolites, neurotransmitters) can substitute for progesterone in facilitating lordosis behavior of rats primed with estrogen doses that are insufficient for facilitation of the behavior [13, 75–77]. Other reproductive events, such as the preovulatory LH surge, may also be increased by estrogen independently of intracellular progesterone receptors. In a recent study with naturally cycling rats, estrogen's facilitation of the luteinizing hormone surge was found to be independent of treatment with RU486 [78].

Since rat and mouse strains show differences in levels and/or receptors of neurotransmitters which may be able to substitute for progesterone in reproductive behavior [75, 79–83], it is possible that strain differences in ligand-independent mechanisms might account for the apparent greater sensitivity of Fischer females for estradiol-induced lordosis behavior. Fischer rats have a high sensitivity to stress [81, 84–86] and differ from other strains on several measures of serotonergic and/or dopaminergic functioning [81, 87–91], two neurotransmitters known to influence progesterone action and/or to be influenced by progesterone [13, 92–94].

Both serotonin and dopamine can influence the production of cAMP that can influence progesterone receptor mediated action [21, 95, 96]. By phosphorylating SRC-1, 8-bromo-cAMP (a cAMP analogue) enhances ligand-independent actions of the intracellular progesterone receptor [97]. It is, therefore, important to note that RU486 has both agonist and antagonist actions [98, 99] and agonist action is accentuated by 8-bromo-cAMP [97]. Therefore, we cannot rule out the possibility that RU486's agonist action is higher in Fischer than in Sprague Dawley or Wistar females.

The current studies also provide evidence supporting the suggestion that progesterone's anxiolytic/anti-stress effects could be a significant contributor to progesterone's ability to enhance female lordosis behavior. For successful reproduction to occur, the female's response to stress must be reduced. It is, therefore, not unreasonable to expect progesterone to reduce the response to stress in order to enable the maintenance of lordosis behavior. In the paradigm for sexual behavioral testing, resistance to the male is evidenced by both escape and defensive behaviors and is a characteristic behavior of naturally cycling rats

during diestrus and of ovariectomized rats that are primed only with estradiol benzoate even when lordosis behavior is present. In the current experiment, even in unrestrained rats, behavior of rats treated with RU486 resembled such estradiol benzoate-primed, ovariectomized rats.

In summary, although the lordosis reflex is dependent only on estrogen, the probability of it occurring is increased by progesterone so that lower doses of estrogen are required [100]. Since progesterone facilitation requires, at least in part, involvement of intracellular progesterone receptors [1], it has generally been assumed that lordosis behavior of naturally cycling rats is dependent on intracellular progesterone receptor action. The current studies with RU486 suggest that, even in naturally cycling unrestrained females, the different sexual behaviors are differentially responsive to an intracellular progesterone receptor antagonist. In the absence of restraint, proceptivity was reduced and resistance to the male's attempts to mount was increased by RU486 while lordosis behavior was relatively unaffected. However, in the presence of restraint, lordosis behavior was also reduced by RU486. Therefore, the current studies allow the suggestion that lordosis behavior of naturally cycling rats may be less dependent on intracellular progesterone receptors than previously anticipated. Whether these findings will extend to rat strains other than the Fischer strain will require further investigation.

Acknowledgement

Appreciation is expressed to Ms. Karolina Blaha-Black for animal care and to Ms. Cindy Hiegel for technical assistance. Appreciation is also extended to Dr. Jutatip Guptarak and Dr. Chandra S. J. Miryala and to members of the Uphouse lab for reading prior versions of the manuscript. Research supported by NIH HD28419.

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Highlights

1. The effects of the antiprogestin, RU486, were examined in proestrous Fischer rats.
2. 5 mg/kg RU486 reduced proceptivity but not lordosis behavior.
3. 5 mg/RAT RU486 reduced lordosis behavior only when given the night before proestrus.
4. All treatments with RU486 enhanced the response to 5 min restraint.

Figure 1. Restraint effects on sexual receptivity following treatment with RU486

Data are the mean $\pm$ S.E. lordosis/mount ratios for naturally cycling Fischer rats treated with vehicle (CONTROL), 5 mg/kg RU486 (1A) or 5 mg/RAT RU486 (1B) before (closed bars) or after (open bars) a 5 min restraint experience. In 1A are data for rats injected with vehicle (CONTROL, N = 13) or 5 mg/kg RU486 at the following times: (1) between 4:30 and 6:30 on the evening before the predicted day of proestrus (loRUPM, N = 18); (2) on the evening (4:30–6:30) before the predicted day of proestrus and between 8:30 and 9:30 on the morning of predicted proestrus (loRUPMAM, N = 7); or (3) between 4:30 and 6:30 on the two

consecutive evenings before predicted proestrus (loRU2days, N = 6). In 1B, data are for rats injected with vehicle (CONTROL, N = 8) or 5 mg/RAT RU486 at the following times: (1) evening before proestrus (hiRUPM, N = 6) or (2) the morning of proestrus (hiRUAM, N = 3). Asterisks indicate a significant difference from the L/M ratio before restraint (within group) and difference from the control behavior after restraint.

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Figure 2. Restraint effects on lordosis quality following treatment with RU486

Data are the mean $\pm$ S.E. lordosis quality scores for naturally cycling Fischer rats treated with vehicle (CONTROL), 5 mg/kg RU486 (1A) or 5 mg/RAT RU486 (1B) before (closed bars) or after (open bars) a 5 min restraint experience. In 2A are data for rats injected with vehicle (CONTROL, N = 13) or 5 mg/kg RU486 at the following times: (1) between 4:30 and 6:30 on the evening before the predicted day of proestrus (loRUPM, N = 8); (2) on the evening (4:30–6:30) before the predicted day of proestrus and between 8:30 and 9:30 on the morning of predicted proestrus (loRUPMAM, N = 7); or (3) between 4:30 and 6:30 on the

two consecutive evenings before predicted proestrus (loRU2days, N = 6). In 2B, data are for rats injected with vehicle (CONTROL, N = 8) or 5 mg/RAT RU486 at the following times: (1) evening before proestrus (hiRUPM, N = 6) or (2) the morning of proestrus (hiRUAM, N = 3). Asterisks indicate a significant difference from the lordosis quality score before restraint (within group) and difference from the control behavior after restraint.

TABLE 1

Effects of RU486 on the emergence of sexual behaviors

Treatment	N	% receptive ^a	L/M ratio Mean $\pm$ S.E.	Lordosis quality Mean $\pm$ S.E
5 mg/kg (loRU486)				
CONTROL	13	100	1.0 $\pm$ 0	3.0 $\pm$ 0.01
loRUPM	12	75	0.71 $\pm$ 0.14	2.85 $\pm$ 0.09
loRUPMAM	7	100	0.87 $\pm$ 0.04	2.78 $\pm$ 0.01
loRU2days	8	75	0.78 $\pm$ 0.08	2.73 $\pm$ 0.13
5 mg/RAT (hiRU486)				
CONTROL	8	100	1.0 $\pm$ 0	3.03 $\pm$ 0.02
hiRUPM	10	30 ^b	0.45 $\pm$ 0.15 ^b	2.92 $\pm$ 0.10
hiRUAM	8	62.5	0.67 $\pm$ 0.15	2.87 $\pm$ 0.05

^a defined as L/M ratio $\geq$ 0.70^b significantly different from control

TABLE 2

Effects of RU486 on proceptivity and resistance

Treatment	N	% proceptive	% resistive
5 mg/kg (loRU486)			
CONTROL	13	92.3	0
loRUPM	12	41.7 ^a	75 ^a
loRUPMAM	7	14.3 ^a	85.7 ^a
loRU2days	8	25.0 ^a	75 ^a
5 mg/RAT (hiRU486)			
CONTROL	8	100	0%
RUPM	10	30 ^a	90% ^a
RUAM	8	50 ^a	66.7% ^a

^a significantly different from control

TABLE 3

Effects of RU486 on proceptivity and resistance after restraint

Treatment	Number Restrained	% proceptive before restraint	% proceptive after restraint	% resistive before restraint	% resistive after restraint
5 mg/kg (loRU486)					
CONTROL	13	92.3	100 ^a	0	23
loRUPM	8	62.5	50 ^a	50 ^a	37.5
loRUPMAM	7	14.3 ^a	28.5 ^a	85.7 ^a	100 ^a
loRU2	6	33.3 ^a	16.6 ^a	50 ^a	83.3 ^a
5 mg/RAT (hiRU486)					
CONTROL	8	100	87.5	0	50
hiRUPM	3	100	33.3	66.6 ^a	100
hiRUAM	6	66.6	16.6 ^a	100 ^a	83.3

^a significantly different from control