Published in final edited form as:

Psychoneuroendocrinology. 2011 July ; 36(6): 824–833. doi:10.1016/j.psytneu.2010.11.004.

Progesterone receptor antagonist CDB-4124 increases depression-like behavior in mice without affecting locomotor ability

Ethan H. Beckley¹, Angela C. Scibelli¹, and Deborah A. Finn^{1,2}

¹ Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR 97239

² Portland Veterans Affairs Medical Center, Portland, OR 97239

Abstract

Progesterone withdrawal has been proposed as an underlying factor in premenstrual syndrome and postpartum depression. Progesterone withdrawal induces forced swim test (FST) immobility in mice, a depression-like behavior, but the contribution of specific receptors to this effect is unclear. The role of progesterone's GABA_A receptor-modulating metabolite allopregnanolone in depression- and anxiety-related behaviors has been extensively documented, but little attention has been paid to the role of progesterone receptors. We administered the classic progesterone receptor antagonist mifepristone (RU-38486) and the specific progesterone receptor antagonist CDB-4124 to mice that had been primed with progesterone for five days, and found that both compounds induced FST immobility reliably, robustly, and in a dose-dependent fashion. Although CDB-4124 increased FST immobility, it did not suppress initial activity in a locomotor test. These findings suggest that decreased progesterone receptor activity contributes to depression-like behavior in mice, consistent with the hypothesis that progesterone withdrawal may contribute to the symptoms of premenstrual syndrome or postpartum depression.

Keywords

premenstrual syndrome; postpartum depression; CDB-4124; mifepristone; forced swim test; progesterone receptors; allopregnanolone

Introduction

Depressive disorders are more common in females than males, indicating that sex steroids may contribute to sex differences in depression (Hyde et al., 2008; Kessler, 2003; Noble, 2005; Steiner et al., 2003), and to reproductive-related depressive syndromes such as postpartum depression or perimenstrual affective disorders (premenstrual syndrome or premenstrual dysphoric disorder) (Payne et al., 2009). The timing of these syndromes coincides with particular hormone fluctuations, which has led some to hypothesize that reproductive-related depression may be a sort of hormone withdrawal syndrome (Kammerer

Corresponding author: Ethan H. Beckley, Oregon Health & Science University, 3181 SW Sam Jackson Park Road, Mail Code L-470, Portland, OR 97239 USA, beckleye@ohsu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

et al., 2006; Meaden et al., 2005; Pearlstein et al., 2005; see also, Gehlert et al., 1999; Gonda et al., 2008). The delay between peak luteal progesterone concentrations and peak symptom severity suggests that progesterone withdrawal may be a contributing factor in perimenstrual affective disorders (e.g., Halbreich et al., 1986; Redei & Freeman, 1995) or postpartum depression (MacDonald et al., 1991).

Several methods have been developed to model hormone withdrawal in laboratory animals. There is no standardized nomenclature for the different methods, but we propose general terms to distinguish some of the methods used (see Table 1). Passive, metabolic, surgical, and estrous-cycle dependent methods are the most common approaches for hormone withdrawal, and have consistently been associated with increased depression- or anxiety-like behaviors in laboratory animals (Bekku et al., 2006; Bitran & Smith, 2005; de Chaves et al., 2009; Devall et al., 2009; Gallo & Smith, 1993; Löfgren et al., 2009; Navarre et al., 2010; Schneider & Popik, 2007; Stoffel & Craft, 2004). However, the temporal correlation between steroid withdrawal and depression symptoms in humans or rodents does not in itself identify which receptor systems are involved, since many steroids have multiple receptor targets. The current paper utilizes steroid-receptor antagonists to selectively attenuate progesterone receptor activity to address the specific role of progesterone receptors in the effects of progesterone withdrawal on depression-like and locomotor behavior. We describe this approach as “precipitated withdrawal” to be consistent with other instances where a withdrawal syndrome is induced by blocking a signal at the receptor level rather than removing the signaling molecule from systemic circulation (e.g., precipitated cannabis withdrawal, Budney & Hughes, 2006; precipitated opioid withdrawal, Sadée et al., 2005).

As discussed above, hormone withdrawal treatments in laboratory animals are commonly reported to result in increases in depression-like or anxiety-like behaviors. One standard measure is the forced swim test (FST), in which immobility behavior is thought to indicate a depression-like state. We recently reported that passive progesterone withdrawal increases FST immobility (Beckley & Finn, 2007). However, changes in progesterone concentrations also affect the concentrations of metabolite steroids such as allopregnanolone (ALLO), a positive allosteric modulator of γ -aminobutyric acid type-A receptors (GABA_A receptors). ALLO binds with high affinity to GABA_A receptors where it increases the open-time of the chloride channel (Belelli & Lambert, 2005). Our passive progesterone withdrawal procedure results in decreased plasma progesterone (Beckley & Finn, 2007), but since progesterone is a precursor for ALLO, passive progesterone withdrawal also dramatically reduces brain concentrations of ALLO (Beckley & Finn, unpublished data).

The metabolic relationship between concentrations of ALLO and progesterone led some to hypothesize that ALLO withdrawal might underlie FST immobility during progesterone withdrawal. Administering the 5 α -reductase inhibitor finasteride to block the conversion of progesterone to ALLO (a method of metabolic ALLO withdrawal) resulted in increased FST immobility to a level consistent with the immobility observed during passive progesterone withdrawal (Beckley & Finn, 2007). Related evidence has led many in the field to suggest a role for ALLO in depressive-like behavior in laboratory rodents (e.g., Dong et al., 2001; Molina-Hernández et al., 2005). Furthermore, two studies have shown that progesterone receptors are not required for progesterone to have anxiolytic effects on rodent behavior (Frye et al., 2006; D. S. Reddy et al., 2005). Thus, much of the existing research that has examined relationships between progesterone and depression has focused on progesterone as a precursor for ALLO. While previous studies have convincingly demonstrated that intracellular progesterone receptors are not necessary for certain behavioral effects of progesterone, those studies have not rigorously ruled out a possible role for progesterone receptors in affective behaviors.

The present set of experiments tested whether the nonselective progesterone receptor antagonist mifepristone (RU-38486) and the selective progesterone receptor antagonist CDB-4124 would increase FST immobility when administered in a precipitated withdrawal procedure. Mifepristone is a high-potency, high-affinity ligand for the progesterone receptor, but it is neither purely antagonistic at progesterone receptors, nor is it selective for progesterone receptors. In some cases mifepristone has progesterone-enhancing or progesterone-agonist effects (Chien, 2009; Taylor et al., 1998), and it is a potent anti-glucocorticoid (Attardi et al., 2004). CDB-4124 has decreased binding affinity and decreased anti-progesterone potency compared to mifepristone, based on its ability to inhibit the effects of the synthetic progestin R5020 (promegestone) (Attardi et al., 2004). Nonetheless, CDB-4124 is still a very potent progesterone receptor antagonist (Benagiano et al., 2008a), and compared to mifepristone has greatly reduced binding affinity for glucocorticoid receptors as well as decreased *in vivo* anti-glucocorticoid effects (Attardi et al., 2004). We also tested the effects of CDB-4124 or finasteride on locomotor activity to determine whether FST immobility was related to a general suppression of activity. Together, the results of these findings provide new information about the potential involvement of progesterone receptors in depression-like behavior of mice.

Materials and Methods

Animals

DBA/2J female mice were purchased from Jackson Laboratory—West (Sacramento, CA) and experiments took place at the Veterans Affairs Medical Center (VAMC) in Portland, OR. Tests were conducted when the mice were aged approximately 11–12 weeks old. Typical body mass for mice in these experiments ranged from 20 g to 25 g and was within the normal range for DBA/2J mice. Procedures were approved by the Portland VAMC Institutional Animal Care and Use Committee, and conformed to the guidelines of “Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral research” (National Research Council, 2003).

All mice were housed in Maxi-Miser #1 cages (Thoren Caging Systems, Hazelton, PA) with EcoFresh bedding (Absorption Corp, Bellingham, WA). Mice were maintained on 12 hr/12 hr light/dark cycles with lights on at 0600 h except mice in Experiment 4, during which the light cycle was phased forward a total of 1 hr over the course of two weeks following the change from Pacific Standard Time to Pacific Daylight Time. Animal rooms were temperature controlled at approximately 21 ± 1 °C. Mice had unrestricted access to LabDiet 5001 Rodent Diet food (PMI International) and tap water. All mice received ear punches for identification prior to commencement of injections and had over a week to acclimate to the facilities prior to onset of injections. Mice were otherwise experimentally and surgically naive, and had intact ovaries.

Drugs

All experiments employed an 8-day procedure in two treatment phases. The first treatment phase lasted 5 days and consisted of administering progesterone or vehicle injections. The second phase (days 6–8) consisted of continued administration of progesterone or vehicle injections but introduced additional injections to cause precipitated withdrawal of progesterone receptor activity or metabolic withdrawal of GABA_A receptor activity. Progesterone, mifepristone, and finasteride were purchased from Steraloids (Newport, RI). CDB-4124 was a gift from Dr. Ronald Wiehle and Repros Therapeutics Inc. (The Woodlands, TX). All injections of progesterone were 5 mg/kg.

Except where noted, the volume of each injection was 10 mL/kg. All injections were administered intraperitoneally and were administered from 1000–1230 h. The vehicle for each experiment was 20% w/v 2-hydroxypropyl- β -cyclodextrin, supplied by Onbio (Richmond Hill, Ontario, Canada), prepared in 0.9% saline solution (Baxter Healthcare, Deerfield, IN). On any given day within an experiment mice were given an equal number of injections regardless of treatment group, and vehicle injections were used to equalize the number of injections. The vehicle (VEH) group in Experiment 4 received two injections of the vehicle on days 6–8. On days when mice received more than one injection both injections were administered less than a minute apart.

Forced Swim Test (FST)

We performed the FST as previously described (Beckley & Finn, 2007). Mice were transferred to holding cages after their last injections on day 8. Testing occurred from 1300–1700 hr, during which each mouse was tested in a cylindrical water tank measuring 21.5 cm $\times$ 24.5 cm (inner diameter $\times$ height), which was refilled with 25 ± 2 °C tap water for each mouse to a height of 15 ± 1 cm. Subjects were dropped into the tank from a height of approximately 20 cm above the upper rim of the tank, which was sufficient to submerge the mice underwater. A video camera placed above the tank recorded each mouse's behavior for 6 min until she was removed and transferred to a holding cage. All FST behavior reported here was scored from videotape by a single observer in a way that the rater never knew the treatment condition of a given mouse. For each test, only the last 4 minutes of the 6 min test session were scored to minimize effects of the novel environment (as suggested by some sources, e.g., Kurtuncu et al., 2005), and to use a scoring system consistent with other researchers (e.g., Lucki et al., 2001). Since mice tested in the FST spend most of their time swimming and exhibit bouts of immobility of variable length, total immobility was measured with a stopwatch that enabled the scorer to record cumulative time spent immobile. Immobility was recorded when the experimenter judged the mouse to be exhibiting no overt behaviors other than breathing or postural movements necessary to maintain floating.

Locomotor Activity

Locomotor activity was measured in Experiment 4 using Accuscan activity monitors (Accuscan Activity Instruments Inc., Columbus, OH) in 30-min sessions. Mice were transferred to holding cages after their last injections on day 8, and testing took place from 1300–1500 hr, during which mice were placed into one of 16 clear acrylic boxes measuring 40 cm $\times$ 40 cm $\times$ 30 cm (length $\times$ width $\times$ height), which were in turn housed in shelters that blocked light and attenuated sound. Incandescent 3.3 W light bulbs provided light inside the testing chamber, and a fan masked outside noise and provided ventilation. Activity monitors had an 8 $\times$ 8 array of photocell beams and detectors raised 2 cm above the floor. Beam breaks from mouse movement were automatically recorded and converted by Accuscan software into total distance traveled (cm).

Statistical Analyses

Experiments were analyzed using one-way analysis of variance (ANOVA) except where noted. Post hoc tests were performed only if justified by an omnibus significant difference from one-way ANOVA or a significant interaction from ANOVA with repeated measures. The α -level was set at .05 for all analyses. A small percentage of mice (< 2% of the tested mice) were removed based on automated outlier analyses performed in SYSTAT (version 11). One mouse was removed from one experiment (from the 60 mg/kg group of Experiment 2) based on unusual swimming posture and breathing, but removal or inclusion of this mouse did not alter which comparisons were significant. Reported sample sizes reflect these exclusions. For Experiment 4, ANOVA with repeated measures was used to analyze

differences in locomotor activity during 6-min time bins. Sphericity was assessed using Mauchly's test of sphericity and by inspecting the Greenhouse-Geisser and Huynh-Feldt estimates of ϵ . Significant ANOVA with repeated measures were followed with one-way ANOVA of the individual time bins.

Experiment 1—CDB-4124 dose response

The purpose of this experiment was to determine if progesterone withdrawal could increase FST immobility without concurrent withdrawal of its metabolic derivatives. We used the specific progesterone receptor antagonist CDB-4124 to precipitate progesterone receptor withdrawal, thus avoiding concomitant withdrawal of other steroids. Mice were injected daily with progesterone on days 1–8 of the experiment. One group received additional injections of vehicle on days 6–8 of the experiment (0 mg/kg group), while other groups received additional injections of CDB-4124 on these days in doses of 20 mg/kg, 40 mg/kg, or 60 mg/kg.

Experiment 2—CDB-4124 dose response replication

The purpose of this experiment was to confirm an immobility-inducing effect of 60 mg/kg CDB-4124 and to test for a similar effect at a higher dose. Mice were administered daily injections of progesterone on days 1–8, and on days 6–8 received additional daily injections of vehicle (0 mg/kg group), 60 mg/kg CDB-4124 (60 mg/kg group), or 80 mg/kg CDB-4124 (80 mg/kg group). Vehicle and CDB-4124 injections were administered in volumes of 20 mL/kg in this experiment.

Experiment 3—Mifepristone dose response

The purpose of this study was to use a second progesterone receptor antagonist to confirm an immobility-inducing effect of precipitated progesterone withdrawal. All mice received daily injections of progesterone on days 1–8. One group received additional injections of vehicle on days 6–8 of the experiment (0 mg/kg group), while additional groups received daily injections of 60 mg/kg mifepristone or 80 mg/kg mifepristone (60 mg/kg and 80 mg/kg groups, respectively). Vehicle and mifepristone injections were administered in volumes of 20 mL/kg in this experiment.

Experiment 4—Effect of CDB-4124 or finasteride on locomotion

CDB-4124 (current paper) and finasteride (Beckley & Finn, 2007) were shown to increase FST immobility, but it is not clear from FST studies whether immobility results from gross motor changes or from higher-order changes, such as changes in motivation. The purpose of this experiment was to determine whether CDB-4124 or finasteride would suppress spontaneous locomotor activity, which could explain increases in FST immobility. Mice received daily injections of vehicle on days 1–8 (VEH group), or daily injections of progesterone on days 1–8 of the experiment. Of the mice that received progesterone, on days 6–8 of the experiment one group also received daily vehicle injections (PRO group), one received daily 100 mg/kg finasteride injections (FIN group), and one group received daily 60 mg/kg CDB-4124 injections (CDB group).

Results

Experiment 1—CDB-4124 dose response

Experiment 1 revealed that CDB-4124 increased FST immobility (see Figure 1). A significant omnibus difference in FST immobility was detected by ANOVA ($F_{3,44} = 3.73$, $p < .05$). Post hoc comparisons revealed that FST immobility was significantly increased ($p < .05$) in the 60 mg/kg group compared to the 0 mg/kg (vehicle) group.

Experiment 2—CDB-4124 dose response replication

FST immobility differed among groups in Experiment 2 ($F_{2,28} = 15.6, p < .05$). CDB-4124 significantly increased FST immobility in both the 60 mg/kg and 80 mg/kg groups compared to the 0 mg/kg (vehicle) group (both $p < .05$), although immobility did not differ between the two drug groups (see Figure 2).

Experiment 3—Mifepristone dose response

In Experiment 3, FST immobility was different among the experimental groups ($F_{2,33} = 4.44, p < .05$). Post hoc Tukey tests revealed that immobility was significantly increased among mice receiving 80 mg/kg mifepristone compared to the 0 mg/kg (vehicle) group ($p < .05$; see Figure 3).

Experiment 4—Effect of CDB-4124 or finasteride on locomotion

Locomotor activity in Experiment 4 was divided into six-minute bins and analyzed with ANOVA with repeated measures. Based on Mauchly's test and estimates of sphericity violation (ϵ statistic) we concluded that the sphericity assumption was met. There was a significant time (bin) by drug interaction, ($F_{12,156} = 2.34, p < .05$). In an analysis of total distance travelled (activity across time bins), Tukey post hoc tests did not reveal a difference in locomotor activity between mice in VEH and PRO groups (*ns*). Tukey post hoc tests revealed that total locomotor activity was significantly decreased in FIN group mice compared to all other groups (all $p < .05$). CDB group mice exhibited significantly decreased overall locomotor activity compared to PRO and VEH group mice ($p < .05$), but increased locomotor activity compared to FIN group mice (see Figure 4a and 4b).

Given the significant interaction, these findings led us to examine locomotor activity in smaller time bins using ANOVA. Group differences in locomotor activity were evident in each of five 6-min time bins ($F_{3,39}$ range: 17.9–38.7, all $p < .05$). Tukey post hoc tests revealed the following pairwise differences ($p < .05$ if stated to be significant): PRO and VEH were not significantly different from one-another in any time bin. CDB group mice did not differ significantly from PRO or VEH in time bin 1 (Figure 4b and 4c). However, CDB mice had significantly lower locomotor activity compared to PRO group mice in bins 2 and 3, and compared to VEH group mice in bins 2–5. Locomotor activity was significantly lower in the FIN group compared to all other groups in all time bins.

Discussion

The present series of experiments made use of the FST to determine potential depression-inducing effects of precipitated withdrawal of progesterone receptor activity in female mice with high-physiological levels of progesterone. The FST is perhaps the best-validated rodent model of depression, and can discriminate between pharmacological compounds that do or do not act as antidepressants in humans with a high degree of accuracy (Cryan et al., 2002). FST immobility is also decreased by stimuli that are non-pharmacological antidepressant therapies in humans, including sleep deprivation (Lopez-Rodriguez et al., 2004), electroconvulsive shock (Li et al., 2007), and light exposure (Schulz et al., 2008). Lastly, FST immobility is increased by a wide variety of treatments that have depression-inducing effects in humans (e.g., Rygula et al., 2008; Mazarati et al., 2008; Stevenson et al., 2009; Wu & Lin, 2008). Based on these findings, it is common and justified to interpret changes in FST immobility in two complementary ways: (1) that increases and decreases in FST immobility index relative levels of depression-like symptoms in the laboratory subject, and (2) that these changes in FST immobility predict how a human's affective state might change under comparable circumstances.

CDB-4124 is a potent progesterone receptor antagonist with reduced antiglucocorticoid activity (Attardi et al., 2002). In our studies, we detected a significant increase in FST immobility when CDB-4124 was administered in doses of 60 or 80 mg/kg. Increased sample sizes also revealed a significant increase in FST immobility in mice that received 40 mg/kg CDB-4124 (Beckley & Finn, unpublished data). These findings suggest that CDB-4124 induced a depression-like state in mice, proportional to the dose administered.

We had previously reported that finasteride (metabolic ALLO/GABA_A receptor withdrawal) increased FST immobility in a way that appeared to mimic progesterone withdrawal (Beckley & Finn, 2007), suggesting that GABA_A receptors contribute to the effect of progesterone withdrawal on FST immobility. Our current findings present an alternate explanation for how passive progesterone withdrawal increases FST immobility. Locomotor activity was used to help clarify whether finasteride or CDB-4124 might influence the ability of mice to perform in the FST. We reasoned that if either (or both) CDB-4124 and finasteride increased FST immobility by interfering with locomotor ability, this finding would confound the interpretation of FST results. Although finasteride increases FST immobility, similar to CDB-4124, we noted previously that finasteride suppressed locomotor activity immediately after injection (Gabriel et al., 2004). The current results show that finasteride had a robust locomotor depressant effect using the same treatment regimen that increased FST immobility, which complicates the interpretation of the finding that it increased FST immobility.

CDB-4124 was associated with overall decreased locomotor during the 30-min testing session, but analyzing these data in smaller time intervals revealed that activity was unaffected in the initial 6 min of testing. We chose to analyze locomotor activity in 6-min bins specifically so that we could compare activity in the activity chambers to behavior in the 6-min FST session, and the initial 6-min bin is the most relevant time point for comparisons between the locomotor study and the forced swim test studies. Since activity was unchanged by CDB-4124 in the first 6 min of locomotor activity testing, these data suggest that CDB-4124 did not alter FST immobility in Experiments 1 and 2 by causing outright motor deficits. Swimming (in the FST) and walking (in the locomotor activity test) may require different neural processes, or even different muscle groups, so the locomotor activity test does not provide conclusive evidence that CDB-4124 is entirely devoid of locomotor effects. Nonetheless, the behaviors are similar, and we believe that the present findings provide preliminary evidence that CDB-4124 did not grossly affect locomotor ability. The results of Experiment 4 therefore add credence to the conclusion that CDB-4124 increases FST immobility because of emotional or motivational changes consistent with a depression-like state.

Only mice treated with finasteride exhibited locomotor activity that was decreased starting in bin 1. It is not clear whether finasteride affected muscle performance, perception of novelty, or some other factor that would mediate a mouse's motivation or ability to explore a novel environment, but for some reason the locomotor activity of these mice was atypical even in the earliest phase of the test. Mice treated with progesterone or vehicle served as a control, and compared to these control groups the mice treated with CDB-4124 had a typical reaction to the novel environment as demonstrated by the indistinguishable locomotor activity among these three groups. All four groups showed a decline in distance travelled over time, presumably due to habituation to the environment. Although CDB-4124 showed decreased total locomotion during the session compared to the PRO group, this overall effect was due to locomotor differences in time bins 2 and 3, and CDB-4124 did not cause a significant decrease in locomotion compared to the PRO group during time bins 4 and 5. Thus, it could be said that mice treated with CDB-4124 not only had an initial reaction to the novel environment that was comparable to mice treated with progesterone, but that they also

experienced the same overall magnitude of habituation. The key difference between these groups seems to be neither their reaction to novelty, nor the extent of habituation to the new environment, but the rate of change. It could be hypothesized that quicker habituation to an environment could be related to a sort of “giving up” akin to behavioral despair.

The prototypic progesterone receptor antagonist mifepristone was also tested for immobility-inducing effects in the FST. The finding that mifepristone increased FST immobility offers additional support for the hypothesis that progesterone receptors underlie the effect of progesterone withdrawal on depression-like behavior. However, there is considerable interest in using mifepristone to treat some forms of depression (see Benagiano et al., 2008b; Flores et al., 2006), stemming from the fact that it is also a glucocorticoid receptor antagonist (Attardi et al., 2004; Benagiano et al., 2008a). Recent studies have shown antidepressant-like effects of mifepristone in mice (Galeeva et al., 2007), rats (Korte et al., 1996; Wulsin et al., 2010), and chicks (Sufka et al., 2009).

While mifepristone may have an antidepressant effect under certain conditions, the physiological context of the subjects should be considered when comparing such studies to the current work. Thus, to reconcile the discrepant findings regarding antidepressant effects of mifepristone compared to the current data indicating increased depression-like behavior, we point to a unique characteristic of the current experimental preparations. In the current work mice were injected repeatedly with progesterone prior to mifepristone administration, and thus had a very different physiological state when they received mifepristone compared to studies showing an antidepressant effect of mifepristone. For example, in the study by Wulsin and colleagues (2010), the subjects were male rats with no other experimental manipulation. These male rats can be presumed to have relatively low concentrations of circulating progesterone, so the activity of progesterone receptors would be minimal and difficult to diminish further. In this circumstance, the antidepressant-like effect of mifepristone was likely due to its antiglucocorticoid effects (consistent with the authors' interpretation). Additionally, it is important to recognize that not all drugs have linear dose-response curves, so the antidepressant-like effect of 10 mg/kg mifepristone in rats (Wulsin and coworkers) does not preclude a depressogenic-like effect of a higher dose of mifepristone (e.g., 80 mg/kg in the current study). Collectively, the physiological context of the animal and the specific dose tested may each contribute to divergent biological and behavioral outcomes when animals are administered mifepristone.

The current results support the hypothesis that progesterone priming permits mifepristone to elicit a depression-like response, and the similar findings with CDB-4124 suggests that both elicit this response by a progesterone receptor antagonist mechanism. Based on our current and previous studies (Beckley & Finn, 2007), it seems that plasma progesterone concentrations in the high physiological range are sufficient to permit increased FST immobility during a passive or precipitated withdrawal technique. Thus, we hypothesize that the presence of progesterone in the high physiological range is necessary for mifepristone to induce depression via anti-progesterone effects that are not observed when progesterone concentrations are lower. This hypothesis suggests that progesterone concentrations must be relatively high, such as the high physiological range, so that there is some endocrine signal that may be withdrawn, consistent with the progesterone withdrawal hypothesis of reproductive depression.

The specific mechanism of progesterone priming is not known at this point. There are conflicting data regarding the effects of progesterone on the regulation of progesterone receptors in the brain. Some studies suggest that progesterone causes down regulation of progesterone receptors in the brain (Mann & Babb, 2005; Parsons et al., 1981). Other studies suggest that progesterone may cause no change (Bieganski et al., 1983) or even increase

(Francis et al., 2002; Numan et al., 1999) expression of its own receptors, depending on the methods employed. These contradictory findings may indicate that there are additional factors that alter the effect of progesterone on progesterone receptor expression. We hypothesize that progesterone receptors were at least not completely down regulated in the mice we tested, despite having received progesterone injections for several days, because the selective progesterone receptor antagonist CDB-4124 was shown to induce dose-dependent changes in behavior.

CDB-4124 or mifepristone may not have depressogenic-like effects when progesterone levels are not relatively high (as in the discussion above). While this does decrease the generalizability of our current findings, we believe that the specific case where progesterone levels are relatively-high is an important case because of the high physiological levels of progesterone that are observed in the luteal phase of the menstrual cycle or during pregnancy. Thus, while these findings may have limited relevance to depressive disorders in general, our precipitated withdrawal approach provides new evidence regarding the role of progesterone receptors in the mouse progesterone withdrawal syndrome that may shed light on reproductive depression in women such as premenstrual syndrome and postpartum depression.

Schmidt and colleagues (1991) administered mifepristone to women with premenstrual syndrome and assessed its effects on premenstrual symptoms. They found that mifepristone increased the severity ratings of all premenstrual symptoms. However, because mifepristone is a progesterone receptor antagonist they interpreted this finding as an indication that endocrine functions during the menstrual cycle were *not* involved in premenstrual syndrome (i.e., since blocking the receptor did not block the symptoms, progesterone must not be involved). In light of the present data, we suggest that the study by Schmidt and colleagues could instead be used to support the opposite conclusion: that removal of progesterone may contribute to premenstrual syndrome, and that administration of a progesterone blocker increased these symptoms because administering the antagonist mimicked removal of progesterone. This is also the interpretation of the study by Schmidt and coworkers that was made by MacDonald and coworkers (1991).

The current set of experiments provides new information about the role of progesterone receptors in depression-like behavior in mice. There is also a considerable literature that addresses the effects of estrogens on depression (for review see Solomon & Herman, 2009). Specifically, a great number of studies have reported antidepressant effects of estradiol on FST immobility in mice and rats (e.g., Dhir & Kulkarni, 2008; Molina- Hernández et al., 2009; Tasset et al., 2008). The current experiments did not directly address the role of estrogens in depression-like behavior because the focus was on progesterone receptors, but future research will need to incorporate estrogen and progesterone manipulations to gain a better understanding of the role of steroid interactions in depression-like behavior. Although the current research did not directly address the role of estrogens in depression-like behavior, the data may help to explain the antidepressant-like effects of estradiol in the FST. One of the primary physiological effects of estrogen is the induction of progesterone receptor gene expression (Hewitt & Korach, 2000; Pinter et al., 1996). One might hypothesize that estrogen's antidepressant characteristics are mediated by its induction of progesterone receptors. If so, reducing progesterone receptor activity would have a depressogenic effect, which is the effect observed in the current study. Therefore, future efforts should assess whether progesterone receptor antagonists such as CDB-4124 or mifepristone can block the antidepressant-like effect of estrogens in mice and rats.

These data implicate progesterone receptors in the depression-like behavior of mice made to withdraw from progesterone. Few studies have attempted to identify the specific genes that

are regulated by progesterone receptors, but it is clear that progesterone regulates a vast number of genes in the brain, including genes for proteins involved in neurotransmission, cell survival, and signal transduction (Auger et al., 2006; A. P. Reddy & Bethea, 2005). For example, a recent study with monkeys reported that progesterone plus estrogen treatment increased mRNA for superoxide dismutase (SOD1), a protein that increases cell survival by scavenging free radicals, in neurons from the dorsal raphe nucleus, compared to monkeys treated with only estrogen (Bethea & A. P. Reddy, 2008). Since the dorsal raphe nucleus is a key region for serotonin signaling, the finding that progesterone treatment significantly increases mRNA for a cell survival protein such as SOD1 could indicate that progesterone helps maintain normal dorsal raphe function and, therefore, helps to maintain normal emotional states. Given that the current data indicate that progesterone withdrawal increases depression-like symptoms in mice, one potential mechanism could be through the loss of factors like SOD1 and corresponding dysfunction of emotion-regulation centers.

In conclusion, progesterone receptor antagonism was found to robustly and reliably increase FST immobility in a manner that mimicked the effect of passive progesterone withdrawal. The finding that CDB-4124 increased FST immobility at a dose that did not alter initial locomotor behavior in a novel environment suggests that this increase in FST immobility was not due to an alteration of the animals' general locomotor ability. Future experiments should incorporate additional measures of emotion-related behavior in mice during progesterone withdrawal to more fully characterize the progesterone withdrawal syndrome in mice. Tests that are relatively independent of locomotion such as the sucrose preference test could further clarify the relationships between CDB-4124, finasteride, and depression-like states in mice. The current experiments provide preliminary evidence that progesterone receptor withdrawal can induce depression-like behavior, and we hope that these studies will stimulate increased attention to the potential involvement of progesterone receptors in perimenstrual or postpartum depression.

Acknowledgments

The authors thank Dr. Ronald Wiehle and Repros Therapeutics for their generous gift of CDB-4124. We also thank Dr. Tamara J. Phillips for granting us the use of her locomotor activity monitors.

References

- Attardi BJ, Burgenson J, Hild SA, Reel JR. In vitro antiprogesterone/antiglucocorticoid activity and progestin and glucocorticoid receptor binding of the putative metabolites and synthetic derivatives of CDB-2914, CDB-4124, and mifepristone. *J Steroid Biochem Mol Biol*. 2004; 88:277–288. [PubMed: 15120421]
- Attardi BJ, Burgenson J, Hild SA, Reel JR, Blye RP. CDB-4124 and its putative monodemethylated metabolite, CDB-4453, are potent antiprogesterone with reduced antiglucocorticoid activity: In vitro comparison to mifepristone and CDB-2914. *Mol Cell Endocrinol*. 2002; 188:111–123. [PubMed: 11911951]
- Auger CJ, Jessen HM, Auger AP. Microarray profiling of gene expression patterns in adult male rat brain following acute progesterone treatment. *Brain Res*. 2006; 1067:58–66. [PubMed: 16376865]
- Beckley EH, Finn DA. Inhibition of progesterone metabolism mimics the effect of progesterone withdrawal on forced swim test immobility. *Pharmacol Biochem Behav*. 2007; 87:412–419. [PubMed: 17597197]
- Bekku N, Yoshimura H, Araki H. Factors producing a menopausal depressive-like state in mice following ovariectomy. *Psychopharmacology*. 2006; 187:170–180. [PubMed: 16788811]
- Belelli D, Lambert JJ. Neurosteroids: Endogenous regulators of the GABA_A receptor. *Nat Rev Neurosci*. 2005; 6:565–575. [PubMed: 15959466]
- Benagiano G, Bastianelli C, Farris M. Selective progesterone receptor modulators 1: Use during pregnancy. *Expert Opin Pharmacother*. 2008a; 9:2459–2472. [PubMed: 18778184]

- Benagiano G, Bastianelli C, Farris M. Selective progesterone receptor modulators 3: Use in oncology, endocrinology and psychiatry. *Expert Opin Pharmacother*. 2008b; 9:2487–2496. [PubMed: 18778186]
- Bethea CL, Reddy AP. Effect of ovarian hormones on survival genes in laser captured serotonin neurons from macaques. *J Neurochem*. 2008; 105:1129–1143. [PubMed: 18182058]
- Bitran D, Smith SS. Termination of pseudopregnancy in the rat produces an anxiogenic-like response that is associated with an increase in benzodiazepine receptor binding density and a decrease in GABA-stimulated chloride influx in the hippocampus. *Brain Res Bull*. 2005; 64:511–518. [PubMed: 15639547]
- Budney AJ, Hughes JR. The cannabis withdrawal syndrome. *Curr Opin Psychiatry*. 2006; 19:233–238. [PubMed: 16612207]
- Chien CH, Lai JN, Liao CF, Wang OY, Lu LM, Huang MI, et al. Mifepristone acts as progesterone antagonist of non-genomic responses but inhibits phytohemagglutinin-induced proliferation in human T cells. *Hum Reprod*. 2009; 24:1968–1975. [PubMed: 19401324]
- Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: Recent developments and future needs. *Trends Pharmacol Sci*. 2002; 23:238–245. [PubMed: 12008002]
- de Chaves G, Moretti M, Castro AA, Dagostin W, da Silva GG, Boeck CR, et al. Effects of long-term ovariectomy on anxiety and behavioral despair in rats. *Physiol Behav*. 2009; 97:420–425. [PubMed: 19321148]
- Devall AJ, Liu ZW, Lovick TA. Hyperalgesia in the setting of anxiety: Sex differences and effects of the oestrous cycle in Wistar rats. *Psychoneuroendocrinology*. 2009; 34:587–596. [PubMed: 19059728]
- Dhir A, Kulkarni SK. Antidepressant-like effect of 17 β -estradiol: Involvement of dopaminergic, serotonergic, and (or) sigma-1 receptor systems. *Can J Physiol Pharmacol*. 2008; 86:726–735. [PubMed: 18841177]
- Dong E, Matsumoto K, Uzunova V, Sugaya I, Takahata H, Nomura H, et al. Brain 5 α -dihydroprogesterone and allopregnanolone synthesis in a mouse model of protracted social isolation. *Proc Natl Acad Sci USA*. 2001; 98:2849–2854. [PubMed: 11226329]
- Flores BH, Kenna H, Keller J, Solvason HB, Schatzberg AF. Clinical and biological effects of mifepristone treatment for psychotic depression. *Neuropsychopharmacology*. 2006; 31:628–636. [PubMed: 16160710]
- Francis K, Meddle SL, Bishop VR, Russell JA. Progesterone receptor expression in the pregnant and parturient rat hypothalamus and brainstem. *Brain Res*. 2002; 927:18–26. [PubMed: 11814428]
- Frye CA, Sumida K, Dudek BC, Harney JP, Lydon JP, O'Malley BW, et al. Progesterone's effects to reduce anxiety behavior of aged mice do not require actions of intracellular progesterone receptors. *Psychopharmacology*. 2006; 186:312–322. [PubMed: 16538472]
- Gabriel KI, Cunningham CL, Finn DA. Allopregnanolone does not influence ethanol-induced conditioned place preference in DBA/2J mice. *Psychopharmacology*. 2004; 176:50–56. [PubMed: 15083256]
- Galeeva AY, Pivina SG, Tuohimaa P, Ordyan NÉ. Involvement of nuclear progesterone receptors in the formation of anxiety in mice. *Neurosci Behav Physiol*. 2007; 37:843–848. [PubMed: 17922250]
- Gallo MA, Smith SS. Progesterone withdrawal decreases latency to and increases duration of electrified prod burial: A possible rat model of PMS anxiety. *Pharmacol Biochem Behav*. 1993; 46:897–904. [PubMed: 7906038]
- Gehlert S, Chang CH, Hartlage S. Symptom patterns of premenstrual dysphoric disorder as defined in the Diagnostic and Statistical Manual of Mental Disorders-IV. *J Womens Health*. 1999; 8:75–85. [PubMed: 10094084]
- Gonda X, Telek T, Juhász G, Lazary J, Vargha A, Bagdy G. Patterns of mood changes throughout the reproductive cycle in healthy women without premenstrual dysphoric disorders. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008; 32:1782–1788. [PubMed: 18721843]
- Halbreich U, Endicott J, Goldstein S, Nee J. Premenstrual changes and changes in gonadal hormones. *Acta Psychiatr Scand*. 1986; 74:576–586. [PubMed: 3825581]

- Hewitt SC, Korach KS. Progesterone action and responses in the α ERKO mouse. *Steroids*. 2000; 65:551–557. [PubMed: 11108859]
- Hyde JS, Mezulis AH, Abramson LY. The ABCs of depression: Integrating affective, biological, and cognitive models to explain the emergence of the gender difference in depression. *Psychol Rev*. 2008; 115:291–313. [PubMed: 18426291]
- Kammerer M, Taylor A, Glover V. The HPA axis and perinatal depression: A hypothesis. *Arch Womens Ment Health*. 2006; 9:187–196. [PubMed: 16708167]
- Kessler RC. Epidemiology of women and depression. *J Affect Disord*. 2003; 74:5–13. [PubMed: 12646294]
- Korte SM, De Kloet ER, Buwalda B, Bouman SD, Bohus B. Antisense to the glucocorticoid receptor in hippocampal dentate gyrus reduces immobility in the forced swim test. *Eur J Pharmacol*. 1996; 301:19–25. [PubMed: 8773442]
- Kurtuncu M, Luka LJ, Dimitrijevic N, Uz T, Manev H. Reliability assessment of an automated forced swim test device using two mouse strains. *J Neurosci Methods*. 2005; 149:26–30. [PubMed: 15967510]
- Li B, Suemaru K, Cui R, Araki H. Repeated electroconvulsive stimuli have long-lasting effects on hippocampal BDNF and decrease immobility time in the rat forced swim test. *Life Sci*. 2007; 80:1539–1543. [PubMed: 17306836]
- Löfgren M, Johansson IM, Meyerson B, Turkmen S, Bäckström T. Withdrawal effects from progesterone and estradiol relate to individual risk-taking and explorative behavior in female rats. *Physiol Behav*. 2009; 96:91–97. [PubMed: 18793660]
- Lopez-Rodriguez F, Kim J, Poland RE. Total sleep deprivation decreases immobility in the forced-swim test. *Neuropsychopharmacology*. 2004; 29:1105–1111. [PubMed: 14970835]
- Lucki I, Dalvi A, Mayorga AJ. Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. *Psychopharmacology*. 2001; 155:315–322. [PubMed: 11432695]
- MacDonald PC, Dombroski RA, Casey ML. Recurrent secretion of progesterone in large amounts: An endocrine/metabolic disorder unique to young women? *Endocr Rev*. 1991; 12:372–401. [PubMed: 1760994]
- Mann PE, Babb JA. Neural steroid hormone receptor gene expression in pregnant rats. *Brain Res Mol Brain Res*. 2005; 142:39–46. [PubMed: 16253385]
- Mazarati A, Siddarth P, Baldwin RA, Shin D, Caplan R, Sankar R. Depression after status epilepticus: Behavioural and biochemical deficits and effects of fluoxetine. *Brain*. 2008; 131:2071–2083. [PubMed: 18559371]
- Meaden PM, Hartlage SA, Cook-Karr J. Timing and severity of symptoms associated with the menstrual cycle in a community-based sample in the Midwestern United States. *Psychiatry Res*. 2005; 134:27–36. [PubMed: 15808287]
- Molina-Hernández M, Tellez-Alcántara NP, Garcia JP, Olivera Lopez JI, Jaramillo MT. Anti-depressant like actions of intra-accumbens infusions of allopregnanolone in ovariectomized Wistar rats. *Pharmacol Biochem Behav*. 2005; 80:401–409. [PubMed: 15740782]
- Molina-Hernández M, Tellez-Alcántara NP, Olivera-Lopez JI, Jaramillo MT. Olanzapine plus 17 β -estradiol produce antidepressant-like actions in rats forced to swim. *Pharmacol Biochem Behav*. 2009; 93:491–497. [PubMed: 19583976]
- National Research Council of the National Academies. Guidelines for the care and use of mammals in neuroscience and behavioral research. National Academics Press; Washington, DC: 2003.
- Navarre BM, Laggart JD, Craft RM. Anhedonia in postpartum rats. *Physiol Behav*. 2010; 99:59–66. [PubMed: 19850056]
- Noble RE. Depression in women. *Metab Clin Exp*. 2005; 54(Suppl 1):49–52. [PubMed: 15877314]
- Numan M, Roach JK, del Cerro MC, Guillamón A, Segovia S, Sheehan TP, Numan MJ. Expression of intracellular progesterone receptors in rat brain during different reproductive states, and involvement in maternal behavior. *Brain Res*. 1999; 830:358–371. [PubMed: 10366694]
- Parsons B, McGinnis MY, McEwen BS. Sequential inhibition of progesterone: Effects on sexual receptivity and associated changes in brain cytosol progestin binding in the female rat. *Brain Res*. 1981; 221:149–160. [PubMed: 7272758]

- Payne JL, Palmer JT, Joffe H. A reproductive subtype of depression: Conceptualizing models and moving toward etiology. *Harv Rev Psychiatry*. 2009; 17:72–86. [PubMed: 19373617]
- Pearlstein T, Yonkers KA, Fayyad R, Gillespie JA. Pretreatment pattern of symptom expression in premenstrual dysphoric disorder. *J Affect Disord*. 2005; 85:275–282. [PubMed: 15780697]
- Pinter JH, Deep C, Park-Sarge OK. Progesterone receptors: Expression and regulation in the mammalian ovary. *Clin Obstet Gynecol*. 1996; 39:242–235.
- Reddy AP, Bethea CL. Preliminary array analysis reveals novel genes regulated by ovarian steroids in the monkey raphe region. *Psychopharmacology*. 2005; 180:125–140. [PubMed: 15731897]
- Reddy DS, O'Malley BW, Rogawski MA. Anxiolytic activity of progesterone in progesterone receptor knockout mice. *Neuropharmacology*. 2005; 48:14–24. [PubMed: 15617723]
- Redei E, Freeman EW. Daily plasma estradiol and progesterone levels over the menstrual cycle and their relation to premenstrual symptoms. *Psychoneuroendocrinology*. 1995; 20:269–267. [PubMed: 7777655]
- Rygula R, Abumaria N, Havemann-Reinecke U, Rüther E, Hiemke C, Zernig G, et al. Pharmacological validation of a chronic social stress model of depression in rats: Effects of reboxetine, haloperidol and diazepam. *Behav Pharmacol*. 2008; 19:183–196. [PubMed: 18469536]
- Sadée W, Wang D, Bilsky EJ. Basal opioid receptor activity, neutral antagonists, and therapeutic opportunities. *Life Sci*. 2005; 76:1427–1437. [PubMed: 15680308]
- Schmidt PJ, Nieman LK, Grover GN, Muller KL, Merriam GR, Rubinow DR. Lack of effect of induced menses on symptoms in women with premenstrual syndrome. *N Engl J Med*. 1991; 324:1174–1179. [PubMed: 2011161]
- Schneider T, Popik P. Attenuation of estrous cycle-dependent marble burying in female rats by acute treatment with progesterone and antidepressants. *Psychoneuroendocrinology*. 2007; 32:651–659. [PubMed: 17561352]
- Schulz D, Aksoy A, Canbeyli R. Behavioral despair is differentially affected by the length and timing of photic stimulation in the dark phase on an L/D cycle. *Prog Neuropsychopharmacol Biol Psychiatry*. 2008; 32:1257–1262. [PubMed: 18485555]
- Solomon MB, Herman JP. Sex differences in psychopathology: Of gonads, adrenals, and mental illness. *Physiol Behav*. 2009; 97:250–258. [PubMed: 19275906]
- Steiner M, Dunn E, Born L. Hormones and mood: From menarche to menopause and beyond. *J Affect Disord*. 2003; 74:67–83. [PubMed: 12646300]
- Stevenson JR, Schroeder JP, Nixon K, Besheer J, Crews FT, Hodge CW. Abstinence following alcohol drinking produces depression-like behavior and reduced hippocampal neurogenesis in mice. *Neuropsychopharmacology*. 2009; 34:1209–1222. [PubMed: 18563059]
- Stoffel EC, Craft RM. Ovarian hormone withdrawal-induced “depression” in female rats. *Physiol Behav*. 2004; 83:505–513. [PubMed: 15581673]
- Sufka KJ, Warnick JE, Pulaski CN, Slauson SR, Kim YB, Rimoldi JM. Antidepressant efficacy screening of novel target in the chick anxiety-depression model. *Behav Pharmacol*. 2009; 20:146–154. [PubMed: 19300238]
- Tasset I, Peña J, Jimena I, Feijóo M, del Carmen Muñoz M, Montilla P, et al. Effect of 17 β -estradiol on olfactory bulbectomy-induced oxidative stress and behavioral changes in rats. *Neuropsychiatr Dis Treat*. 2008; 4:441–449. [PubMed: 18728794]
- Taylor RN, Savouret JF, Vaisse C, Vigne JL, Ryan I, Hornung D, et al. Promegestone (R5020) and mifepristone (RU486) both function as progestational agonists of human glycodeclin gene expression in isolated human epithelial cells. *J Clin Endocrinol Metab*. 1998; 83:4006–4012. [PubMed: 9814484]
- Wu TH, Lin CH. IL-6 mediated alterations on immobile behavior of rats in the forced swim test via ERK1/2 activation in specific brain regions. *Behav Brain Res*. 2008; 193:183–191. [PubMed: 18573547]
- Wulsin AC, Herman JP, Solomon MB. Mifepristone decreases depression-like behavior and modulates neuroendocrine and central hypothalamic-pituitary-adrenocortical axis responsiveness to stress. *Psychoneuroendocrinology*. 2010; 35:1100–1112. [PubMed: 20149549]

Figure 1. CDB-4124 increased FST immobility

Mice received daily injections of progesterone on days 1–8. CDB-4124 was also administered on days 6–8. CDB-4124 doses are indicated in the figure. The 0 mg/kg group received vehicle injections instead of drug. CDB-4124 (60 mg/kg) increased FST immobility when co-administered with progesterone, $*p < .05$ difference between to 0 mg/kg and 60 mg/kg groups ($n = 12$ /group).

Figure 2. CDB-4124 increased FST immobility

Mice received injections of progesterone on days 1–8 of the experiment. Mice received additional injections of CDB-4124 on days 6–8 in doses indicated by the group names. The 0 mg/kg group received vehicle injections instead of drug. CDB-4124 (60 mg/kg and 80 mg/kg) increased FST immobility compared to 0 mg/kg ($*p < .05$) ($n = 9–11$ /group).

Figure 3. Mifepristone increased FST immobility

Mice received injections of progesterone on days 1–8 of the experiment. Mifepristone was co-administered on days 6–8 of the experiments as indicated by the group names. The 0 mg/kg group received vehicle injections instead of drug. Mifepristone (80 mg/kg) increased FST immobility compared to 0 mg/kg, $*p < .05$ ($n = 12$ /group).

Figure 4. CDB-4124 decreased overall locomotor activity but not initial locomotor ability
 (A) Total locomotor activity across the 30-min session. * $p < .05$ FIN lower than CDB group, and CDB lower than PRO or VEH groups. (B) Activity across the 30-min session displayed in 6-min time bins (bin 1 = minutes 1–6, bin 2 = minutes 7–12, etc.). Significant differences are not shown due to graphical complexity; see Results for narrative description of differences. (VEH = open square, black line; PRO = gray square, gray line; FIN = open circle, gray line; CDB = gray circle, black line). (C) Locomotor activity was decreased by FIN (* $p < .05$) compared to all other groups, but activity in the CDB was unchanged compared to PRO and VEH groups in the first 6 min of activity testing ($n = 10$ –12/group).

Table 1

Methods of inducing hormone withdrawal in laboratory animals.

Withdrawal type	Method
Passive	Administer hormones for a period of time, then discontinue administration and allow hormones to withdraw as a result of endogenous clearance mechanisms
Metabolic	Administer precursor hormones for a period of time, then co-administer drugs to inhibit metabolism of the precursor, thus withdrawing hormone of interest by blocking its production
Surgical	Allow the animal to produce its own hormones (often after inducing pseudopregnancy), then surgically remove the steroidogenic glands
Estrous cycle dependent	Allow the animal to produce and withdraw from its own hormones according to its estrous cycle
Precipitated	Administer hormones for a period of time, then co- administer drugs that antagonize the target receptors of interest