Pharmacology Biochemistry and Behavior, Vol. 46, pp. 897-904, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Progesterone Withdrawal Decreases Latency to and Increases Duration of Electrified Prod Burial: A Possible Rat Model of PMS Anxiety

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Received 26 January 1993

GALLO, M. A. AND S. S. SMITH. *Progesterone withdrawal decreases latency to and increases duration of electrified prod burial: A possible rat model of PMS anxiety.* PHARMACOL BIOCHEM BEHAV 46(4) 897-904, 1993.—The purpose of this study was to determine whether withdrawal from chronic exposure to the female sex steroid progesterone (P) alters response of female rats to an electrified prod using the defensive burying paradigm, considered a rat model of anxiety. Withdrawal from chronic exposure to 500 μg P (daily, SC, for four days) resulted in a significant decrease in the latency (77%, $P < 0.05$) to prod burial and an increase in duration (75%, $P < 0.05$) of this reflexive response, compared with the behavior of oil-injected controls. These results are consistent with the idea that withdrawal from chronic exposure to P increases behaviors that accompany anxiety. At a lower dose (50 μg), withdrawal from chronically administered P produced significant changes in response to this paradigm only when the steroid was given concomitantly with estradiol (2 μg, SC, for two days). Prior exposure to indomethacin, which blocks the conversion of P to its metabolite 3α,5α-tetrahydroprogesterone (3-α-hydroxy-5-α-pregnan-20-one), prevented P withdrawal from altering response in the defensive burying paradigm. This finding suggests that it may be withdrawal from this metabolite, rather than P, which increases behaviors associated with increased anxiety.

Progesterone Pregnanolone 3α,5α-tetrahydroprogesterone $GABA_A$ Defensive burying paradigm Defensive withdrawal paradigm Anxiety Premenstrual syndrome Hormone Withdrawal Rat

WITHDRAWAL from sustained exposure to the female sex steroid P may play a role in triggering anxiety (11) related to the premenstrual syndrome (PMS). Dennerstein et al. (7) have described a correlation between decreasing and lower than normal levels of circulating P and increases in anxiety, premenses. Conversely, their studies demonstrated that administration of P reversed this symptomatology (7). Other studies have demonstrated that P may have tranquilizing effects in humans (5,16) and in rats (2,32).

The anxiolytic actions of P are most likely due to local formation of the 3-α-hydroxy-5-α-reduced metabolite 3α,5α-tetrahydroprogesterone (3α,5α-THP). Local intraventricular administration of this metabolite has been shown to decrease anxiety in rats, using performance in the elevated plus maze as a test of anxiety (1). In addition, the anxiolytic actions of systemically injected P have been shown to be associated with increased levels of 3α,5α-THP in the cortex (2). This metabolite binds to a novel site (19,23,24,30,41) on the the $GABA_A$ receptor (25). It can then amplify GABA-mediated Cl^- conductance in cultured hippocampal neurons (12,21), much in the same manner as the anxiolytic barbiturates (20,26). These actions may be physiologically relevant, as the parent compound, P, markedly potentiates GABA-mediated inhibition of cerebellar Purkinje cells at circulating levels similar to those seen endogenously (34,37,38). This neuromodulatory action of the steroid also appears to be mediated by the GABA-active metabolite 3α,5α-THP (35,36).

Withdrawal from chronic exposure to other psychoactive GABA-active agents, such as ethanol, benzodiazepines, and barbiturates, can result in anxiogenic and, in extreme cases, convulsant effects (3,14,22). The purpose of the present study was to determine whether withdrawal from chronic exposure to P is also anxiogenic. P was tested for withdrawal properties rather than 3α,5α-THP because P more readily crosses the blood-brain barrier, where it can then be rapidly converted to the GABA-active metabolite (17). In addition, levels of P are

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known to decrease prior to PMS-related anxiety state (7). Systemic levels of 3α,5α-THP do not change prior to this reported change in mood (31).

The hypothesis that P withdrawal is anxiogenic was tested using the defensive burying (6,39,40) and the defensive withdrawal (4) paradigms as rat models of anxiety. For the present study, the operational definition of "anxiety" is a significant change in response for either of these two paradigms. For the defensive burying paradigm, a decrease in the latency and an increase in the duration of response to an electrified prod has been established by a number of investigators to be consistent with an increase in "anxiety" (6,39,40). For the defensive withdrawal paradigm, an increase in the number of crossings from the open field to the dark compartment and an increase in the time spent in the dark compartment have been operationally defined as objective behaviors that accompany anxiety (4). Changes in response to both paradigms were used in the present study to evaluate the hypothesis that P withdrawal increases "anxiety." The results from the present study may be useful for studying the etiology and potential treatment of PMS-related increases in anxiety.

METHODS

Subjects

Naive female rats (Long Evans), 200–225 g, served as subjects. The rats were housed in groups of three under a reverse light/dark cycle (14 h light, 10 h dark). Food and water were available continuously in an environmentally controlled animal facility (23°C). Rats were tested during the dark part of the circadian cycle.

Defensive Burying Paradigm

Apparatus. All testing was performed using a Plexiglas test chamber (46 × 26 × 25 cm) evenly filled with 5 cm Bed O'Cobs, a commercial bedding material made of ground corn cob. An electrified prod (20 × 3.5 cm) was placed on top of the bedding approximately 10 cm from the back wall of the chamber. Electric current (350 V) was administered through two wires that were attached to one end of the prod. The intensity of the current administered from the power source was measured by a voltage meter that was connected to a 10-KΩ resistor in series. Rats received electrical shocks ranging from 0.05 to 1.0 mA in intensity. The behavior of each rat was monitored for 20 min from a separate acoustically isolated room via a closed circuit television, and events were recorded on a videocassette recorder.

Protocol. On the day of testing, rats were habituated in the Plexiglas test chamber in groups of two or three for a 60-min period several hours before the experiment. General activity level of each rat was also assessed during this time in terms of locomotion, grooming, and wall rears. Prior to testing of each animal, the chamber was cleaned with 70% ethanol and the bedding replaced. Rats were tested individually. Contact with the electrified prod typically elicited a flinch away from the prod and withdrawal to the back of the chamber, followed by a burying response after a variable latency. Immediately after shock administration the power supply was turned off. Times for both the latency to and the duration of the burying response were assessed.

Analysis and Interpretation of Data

Increases in anxiety in this model are thought to be reflected by a decrease in the latency and an increase in the duration of prod burial compared with oil vehicle-injected controls (6,29). The statistical significance of the data was evaluated using the Mann–Whitney *U* test for nonparametric data, since these data did not follow a normal distribution.

In a few cases, rats failed to bury the prod. Failure to bury the prod could result from 1) a decrease in anxiety state or 2) an increase in the fear response, exhibited as immobilization. Thus, due to the difficulty in interpreting this response, these data were not figured into the statistical analysis.

Drug Administration

Animals received four or five consecutive days of drug or vehicle (sesame oil) injections SC (Fig. 1). Animals tested for steroid withdrawal were tested 24 h following the last injection of the steroid or vehicle. In some cases (nonwithdrawal or replacement conditions), rats were tested 20–30 min following the last dose of steroid or vehicle. One group of animals served as controls for Experiments 1–4. All injections were administered while the animals were anesthetized with halothane fluothane (2-bromo-2-chloro-1,1,1-trifluoroethane, 2.5% in oxygen), which aided in reducing stress and pain prior to testing.

Prior to each experiment, vaginal smears were performed on each rat using the lavage technique (34) to determine the

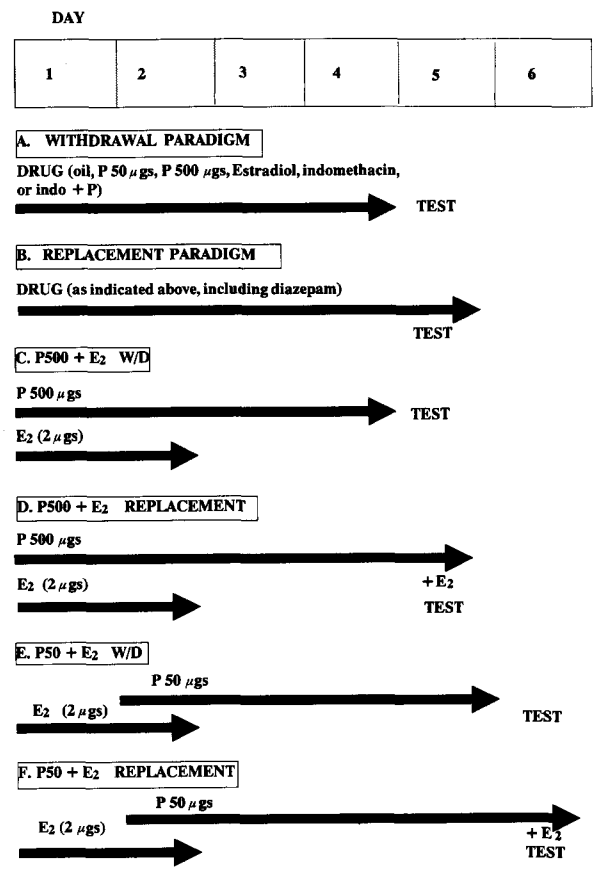

FIG. 1. Drug injection schedule for Experiments 1–5. Animals were injected for four to six days with steroid and/or indomethacin or vehicle (oil), as indicated. Specific comments regarding these injection paradigms are described in the Methods section.

stage of the estrous cycle. Rats were tested during all phases of the cycle and no difference was observed (data not shown), probably due to the exogenous hormone treatment which reset their normal cycles. Thus the data presented are representative of all estrous stages.

The study was divided into five groups of experiments:

1. *Diazepam validation study.* Diazepam (5 mg/kg, SC) or oil vehicle was administered for five consecutive days (Fig. 1, schedule B). Testing was performed on the fifth day. Diazepam is a known anxiolytic agent (8), and this study was performed to validate the defensive burying protocol as a model of anxiety.
2. *P withdrawal—500 μg.* Rats received either P (500 μg), estradiol (2 μg), or both steroids over a four-day period as illustrated in Fig. 1 (schedule A or C). In some cases, rats were injected with vehicle alone. Testing was then performed on day 5, 24 h after the last steroid injection (withdrawal conditions). A separate group of rats received a fifth steroid injection (estradiol and P) on day 5, 20–30 min prior to testing (steroid replacement group—Fig. 1, schedule D). The addition of estradiol has been shown to facilitate the anxiolytic properties of P (32).
3. *P withdrawal—50 μg.* One group of rats received P (50 μg) for four days. Performance in the defensive burying paradigm was then assessed on day 5, 24 h after the last steroid injection (Fig. 1, schedule A). In a second group of rats, estradiol (2 μg) was injected for two days beginning on the day before the first P injection (estrogen-primed—Fig. 1, schedule E). This was followed by the combination of P and estradiol on day 2. Rats were reexposed to P (50 μg) for days 3, 4, and 5; anxiety state was then tested on day 6. In a third group of estradiol-primed animals, P plus estradiol was injected on day 6 (replacement group), 20–30 min prior to testing (Fig. 1, schedule F). Finally, oil-injected animals served as controls. Both estrogen priming and the use of the lower dose of P more closely approximate physiological conditions compared to those described in Experiment 2.
4. *Indomethacin effects on P withdrawal.* Indomethacin (0.1 mg/kg, SC, in castor oil) was administered in combination with P (500 μg) for four consecutive days to two groups of rats. Withdrawal or replacement of both indomethacin and P occurred on day 5 of the injection schedule (Fig. 1, schedule A or B). Indomethacin alone was injected as a control. Indomethacin blocks formation of 3α,5α-THP from P (27). Thus this study was conducted to determine the role of this metabolite in mediating the ability of P withdrawal to alter response in the defensive burying paradigm.
5. *P withdrawal—defensive withdrawal paradigm.* The defensive withdrawal paradigm has also been shown to be a sensitive test of anxiety in the rat (4). This paradigm was implemented in our study to confirm results obtained using the defensive burying paradigm (Experiments 1–4). Testing was conducted in a clear Plexiglas open field (38 × 20 × 41 cm) illuminated by an incandescent lamp and cleaned with ethanol after each individual test. A narrow opening (4 × 4 cm) .connected the open field to a dark Plexiglas chamber (24 × 21.5 × 21 cm). The day before testing, rats were habituated to the open field containing the dark chamber for 10 min. During the test, the time spent in the dark chamber and number of crossings into the dark chamber were monitored for 20 min. Results from steroid-treated animals were then compared to similar values from oil-injected controls, and the statistical significance determined using Student's *t* test. Increases in anxiety are thought to be accompanied by an increase in the time in the dark chamber versus the time for exploration of the open field. To determine the general activity level, the number of wall rears and grooming sessions were monitored during the experimental protocol. Rats tested using the defensive withdrawal paradigm were injected for four days with either P (500 μg) or vehicle (sesame oil) and tested on day 5, 24 h after the last injection of the steroid (Fig. 1, schedule A).

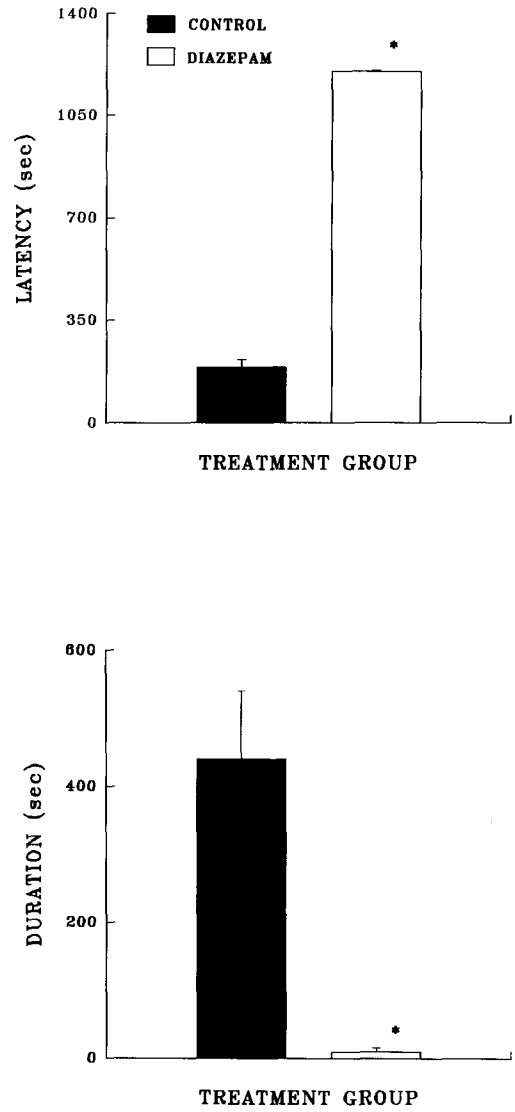

FIG. 2. Effects of diazepam on response to the defensive burying paradigm. In this and the following figures, changes in the latency (upper panel) and duration (lower panel) of prod burial are presented. These values were determined for various treatment, hormone or drug withdrawal, and hormone replacement groups, as indicated. Significant decreases in the latency and increases in the duration of prod burial compared with control values are thought to reflect behaviors which accompany anxiety. In all cases, the injection schedule is indicated in the Methods section. Diazepam (5 mg/kg) administration produced a 528% ($P < 0.001$) increase in latency and a 97% ($P < 0.001$) decrease in duration as compared to control values. $n = 7$ animals per group; $n = 10$ for control group. *$P < 0.001$ vs. control.

RESULTS

Experiment 1

Chronic administration of diazepam (5.0 mg/kg) for five days increases behaviors associated with a decrease in "anxiety" as previously demonstrated by other investigators (6,8, 39). This was reflected in the present study by a significant increase in latency (528%, $P < 0.001$; see Fig. 2) and decrease in the duration of prod burial (by 97%, $P < 0.001$) exhibited by diazepam-treated rats, compared to controls. These results confirm previous reports and thus validate the use of the defensive burying paradigm as a test of "anxiety" in our study.

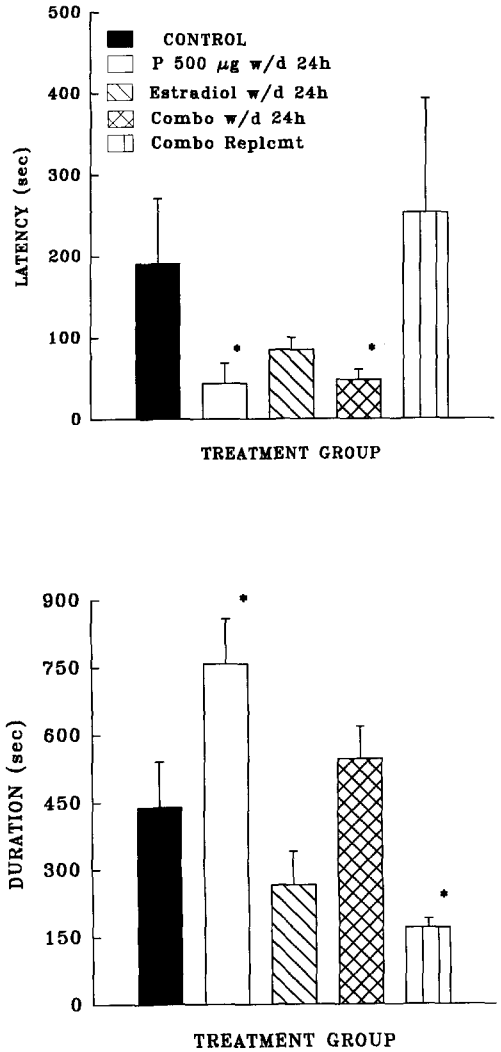

FIG. 3. Withdrawal from chronic administration of progesterone at a dose of 500 µg significantly alters response to the defensive burying paradigm. Withdrawal from the higher dose of P (500 µg) administered without estradiol (2 µg) decreased the latency and increased the duration of prod burial, as compared to control values ($P < 0.05$). The addition of estradiol did not enhance the withdrawal potential of P at this dose, in terms of the parameters assessed. Combination replacement of P (500 µg) plus estradiol (2 µg) prevented this response to the paradigm. In contrast, withdrawal from chronic exposure to estradiol (2 µg) alone did not significantly alter either behavioral parameter. n = 5–10 animals per group. *$P < 0.05$ vs. control (n = 10).

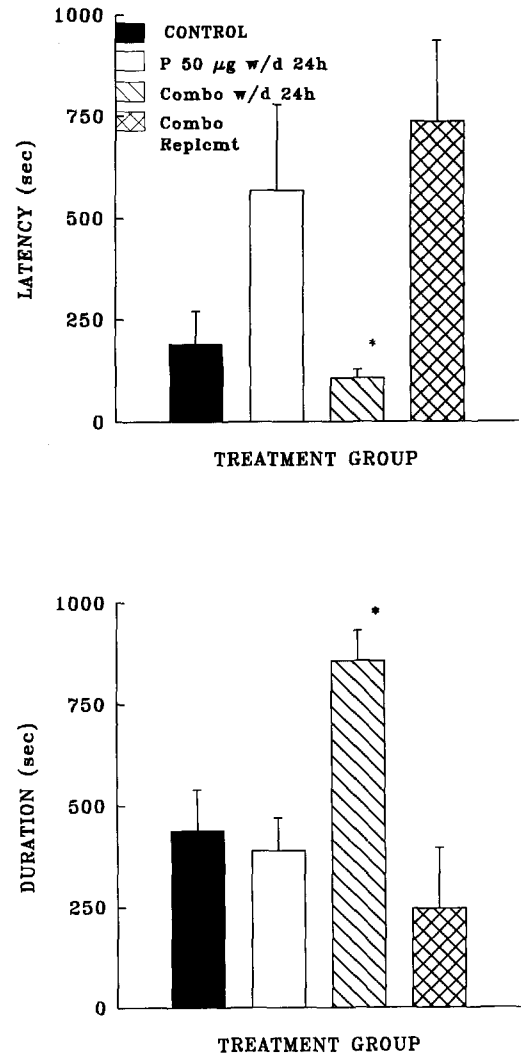

FIG. 4. Withdrawal from 50 µg progesterone alters response to the defensive burying paradigm only in the presence of estradiol. Withdrawal from combined administration of P (50 µg) plus estradiol (2 µg, Combo w/d) produced significant decreases in the latency ($P < 0.05$) and increases in the duration ($P < 0.001$) of prod burial, as compared to control values. Estradiol was administered for two days, beginning one day prior to the onset of the P injection schedule. Replacement with P (50 µg) plus estradiol (2 µg) on the fifth day (Combo Replcmnt) completely prevented the response to this paradigm observed after withdrawal from P. n = 6 animals per group. *$P < 0.05$ vs. control.

Experiment 2

Withdrawal from chronic administration of the higher dose of P (500 µg) produced a 77% ($P < 0.05$) decrease in latency and a 75% increase ($P < 0.05$) in duration of prod burial, compared to control values (see Fig. 3). Replacement therapy with P (500 µg) and estradiol (2 µg) administered to rats on the day of testing (Fig. 3, Combo Replcmt) prevented this effect. This was reflected by a 33% increase in latency and a 61% decrease ($P < 0.05$) in the duration of prod burial as

compared to control values. Administration of 500 μg P alone on day 5 also prevented the change in response to this paradigm observed after P withdrawal (data not shown). Withdrawal from combined administration of both P (500 μg) and estradiol (2 μg, Combo w/d 24h) resulted in a 75% ($P < 0.01$) decrease in latency, but an insignificant increase in duration as compared to control values. Withdrawal from administration of estradiol (2 μg) alone produced insignificant changes in both the latency (55% decrease) and duration (40% decrease) of prod burial.

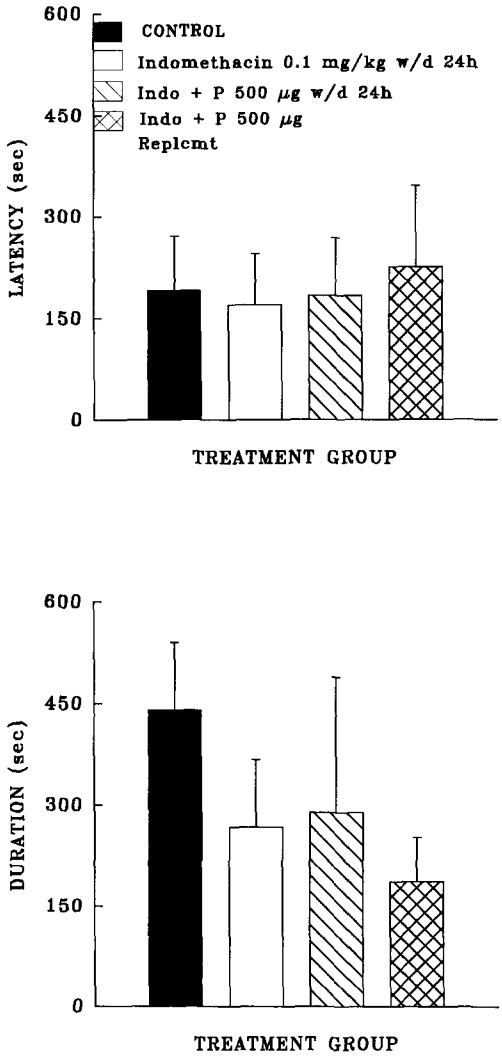

FIG. 5. Indomethacin administration prevents the observed effect of P withdrawal on response to the electrified prod. Withdrawal from combined administration of P (500 μg) with indomethacin (0.1 mg/kg) produced insignificant changes in the latency and duration of prod burial compared to control values. As indomethacin blocks formation of the 3α,5α-THP metabolite from P, these data suggest that it is actually withdrawal from the metabolite and not P which produces a change in burial response to this paradigm. Withdrawal from indomethacin alone produced a 39% decrease in duration of prod burial, an effect not significantly different from control values. n = 5 animals per group.

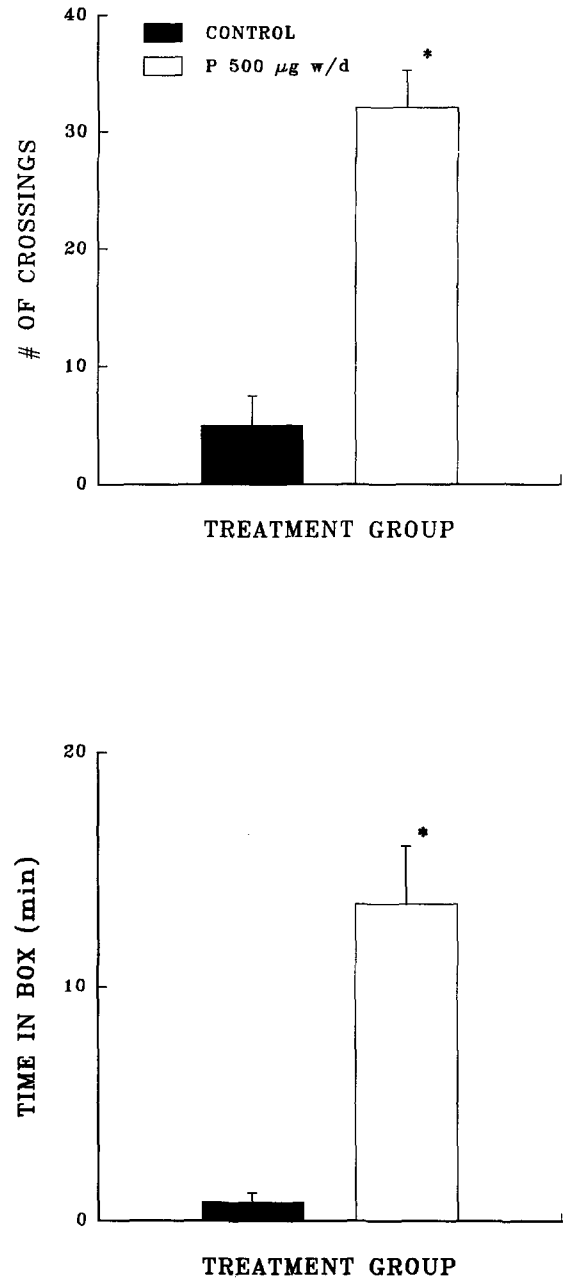

FIG. 6. Effects of P withdrawal on response to the defensive withdrawal paradigm. Changes in the 1) frequency of crossings from light to dark compartments (upper panel) and 2) time spent in the dark compartment (lower panel) are depicted for control and P-treated rats. Twenty-four hours after withdrawal from chronic administration of P (500 μg), injected SC for four days, significant increases in both parameters were observed, compared with control values. These data confirm results obtained using the defensive burying paradigm. n = 10 animals per group. *$P < 0.001$ vs. control.

Experiment 3

Withdrawal from P (50 μg) chronically administered to an estrogen-primed animal (Combo w/d, Fig. 4) produced a 44% ($P < 0.05$) decrease in latency and a 95% ($P < 0.001$) increase in duration of prod burial, compared with controls. In

this case, rats were tested 24 h after the last injection of the steroid. Replacement with P (50 μ) in combination with estradiol (2 μg) on day 5 (Fig. 4, Combo Replcmt) prevented this increase in anxiety, suggesting that withdrawal from these steroids, and not chronic exposure to these agents, alters response to this paradigm. In contrast, withdrawal from chronic administration of P (50 μg) alone did not alter response to the electrified prod: These rats exhibited increases in latency and decreases in duration of prod burial which were not significantly different from control values.

Experiment 4

Indomethacin blocks formation of the 3α,5α-THP metabolite from P by inhibiting the enzyme 3-α-hydroxysteroid oxidoreductase (27). No significant changes in latency or duration of prod burial were seen after withdrawal from the combined administration of both indomethacin and P (500 μg) as compared to control values (see Fig. 5). Indomethacin and P (500 μg) replacement on day 5 of the injection schedule also failed to significantly alter latency and duration of prod burial as compared to indomethacin control and untreated control values.

Experiment 5

As a second animal model of anxiety, the defensive withdrawal paradigm was used to confirm the results obtained using the defensive burying paradigm. As illustrated in Fig. 6, withdrawal from P, administered at a dose of 500 μg for four days, resulted in significant increases in (upper panel) the number of crossings from the light into the dark chamber (a 10-fold increase versus control values, $P < 0.001$) as well as (lower panel) the amount of time spent in the dark chamber compared with oil-injected controls (12.5 vs. 0.5 min, respectively, $P < 0.005$). These results confirm findings from the previous paradigm, suggesting that withdrawal from P alters behaviors using a second established rat model of anxiety. Furthermore, these changes occurred in the absence of corresponding changes in general activity level, as the number of wall rears, grooming sessions, and locomotor activity were not significantly altered by hormone treatment (data not shown).

DISCUSSION

Results from the present study indicate that withdrawal from chronic administration of P, at a dose of 500 μg, significantly decreases the latency to and increases the duration of prod burial using the defensive burying paradigm. Using the operational definition employed by this study and previously established by a number of other investigators (6,29,39,40), these changes are consistent with behaviors which would accompany an *increase* in anxiety. The defensive burying paradigm has been shown to be a sensitive and specific test for evaluating the anxiolytic actions of drugs such as the benzodiazepines (6,29), drugs which increase the latency and decrease the duration of the burying response. In contrast, drugs which suppress $GABA_A$ receptor function and are considered to be "anxiogenic," such as the β-carbolines, exert the opposite effect on the response to this paradigm (40)—that is, decrease the latency and increase the duration of the burying response. However, treatment with analgesic doses of morphine does not alter either parameter (39), suggesting that the defensive burying paradigm is a selective test for anxiety and not a measure of pain threshold. Indeed, alterations in pain threshold should not be a complicating factor in the present study as decreases in sensory threshold have been reported transiently after injections of estradiol and P (15), but not during withdrawal from these hormones. In addition, activity level was not altered by P withdrawal. Therefore, the present results suggest that withdrawal from chronic administration of P results in behaviors which accompany increases in anxiety.

This anxiogenic effect of P withdrawal was further confirmed using a second, specific rat model of anxiety, the defensive withdrawal paradigm. For this paradigm, an increase in the number of crossings from the open field into the dark chamber as well as an increase in the time spent in the dark chamber have also been operationally defined as behaviors which accompany anxiety (4). Similar pharmacological tests using GABA-active agents such as the benzodiazepines and β-carbolines have been used to justify the use of this paradigm and interpret the response (4). The pharmacological specificity of the drug-induced response in this paradigm has also confirmed its use as a test specific for behaviors consistent with changes in anxiety. Again, changes in activity level did not accompany changes in response using this paradigm following withdrawal from P. Therefore, results from two different and specific animal models of anxiety strongly suggest that P withdrawal is anxiogenic.

The psychotropic actions of P or its metabolite have been suggested by a number of earlier reports, both in humans and rat models of anxiety (1,2,5,32,42). However, the finding that withdrawal from chronic exposure to P is anxiogenic is novel and has implications for mood disorders related to hormonal fluctuations: PMS anxiety, postpartum dysphoria, and postmenopausal dysphoria. In all cases, changes in mood follow withdrawal from P alone or in combination with estradiol. The present findings may be especially relevant for PMS-related anxiety in the human, since P levels during the luteal phase remain elevated for 12–14 days and then decline rapidly prior to the premenstrual period. Thus the rapid withdrawal from this anxiolytic steroid following chronic exposure may result in a transient increase in anxiety. It may be, however, that it is actually withdrawal from the 3α,5α- THP metabolite, and not P per se, which is anxiogenic, as P is quickly metabolized to 3α,5α-THP within the central nervous system (CNS) (17,18). The finding that anxiolytic progestins exhibit withdrawal properties following chronic exposure further suggests similarities between these 3-α-hydroxy C21 steroids and other depressant, anxiolytic agents such as ethanol, benzodiazepines, and barbiturates (3,14,22).

Recent clinical studies which suggest that P does not ameliorate PMS symptoms (9) are not necessarily contradictory to the present findings. First, these studies did not identify women with PMS anxiety as the predominant symptom; P therapy may not alleviate other dysphoric symptoms. Second, subjective questionnaires were employed rather than a physiological measure of anxiety (i.e., heart rate). Third, forms of P which were administered may have been incompletely absorbed or were nonmetabolizable.

At the lower dose of P tested (50 μg), estrogen priming was required for P withdrawal to be anxiogenic. Conversely, combined replacement therapy with P and 17-β-estradiol prevented this anxiogenic action. These results are especially worthy as this is a physiological means of administration of the steroids: 1) The dose of P used (50 μg) would produce circulating levels of the steroid close to those observed endogenously, and 2) during the estrous (and menstrual) cycles, levels of estradiol peak prior to endogenous elevations of P. Thus estradiol priming prior to P administration more closely approximates a physiological steroid milieu.

The presence of estradiol may be essential in facilitating conversion of P to the active 3α,5α-THP metabolite, as has been demonstrated by Karavolas et al. (17,18) in various CNS areas. Consistent with this idea, Rodriguez-Sierra et al. (32) demonstrated anxiolytic actions of systemic P only in estrogen-primed animals. That the 3-α-hydroxy-5-α-reduced metabolite—and not the parent compound, P—may be active in altering anxiety state is suggested by the finding that indomethacin, a drug known to block 3-α-hydroxysteroid oxidoreductase (27), and thus formation of 3α,5α-THP, completely prevented the anxiogenic effect of P withdrawal in the present study.

It is 3α,5α-THP, and not P, which allosterically enhances $GABA_A$ receptor function (10,12,21,36), an event that can produce significant anxiolytic actions (1,2,42). It seems very likely, therefore, that the anxiogenic actions of P withdrawal are, in fact, due to withdrawal from the metabolite. This possibility is strengthened by the finding that anxiolytic actions of systemically administered P have been found to be correlated with elevated cortical levels of 3α,5α-THP (2). The actual brain levels of metabolite need to be assessed in our paradigm before this can be determined conclusively. Further support for this idea is provided by the finding that circulating levels of P and CNS levels of 3α,5α-THP are well correlated (31), suggesting rapid conversion of the parent compound to its neuroactive metabolite. As 3α,5α-THP does not cross the blood-brain barrier as readily as P due to the presence of a hydroxy moiety at the C3 position, the withdrawal properties of this agent following chronic administration would be more difficult to test directly. In addition, during the luteal phase of the menstrual cycle, it is circulating P from the corpus luteum which most likely enters the brain, where it is then rapidly metabolized to 3α,5α-THP. Thus studies examining withdrawal properties of systemic increases in P may more closely replicate physiological conditions preceding PMS anxiety.

Alternatively, other actions of estradiol may contribute to the observed effects of P withdrawal in this paradigm. Estrogen priming is known to increase the number of cytosolic/nuclear receptors for P (28). However, it is not clear that these receptors mediate changes in behavior which may be relevant for the behaviors tested in this study. In addition, estradiol can alter the function of a number of monoamine systems (28), an action which could alter anxiety state and thus affect the response to the defensive burying paradigm. This latter action of the steroid cannot be ruled out until further studies are conducted. However, as administration of estradiol alone did not alter the response to the electrified prod, it seems unlikely that estradiol exerts direct effects on CNS systems to alter mood.

Indomethacin also acts to inhibit cyclooxygenase, the enzyme required for prostaglandin synthesis. Although indomethacin treatment may ameliorate some of the physical symptoms of PMS, this has not been demonstrated conclusively (33). In addition, neither chronic administration of nor withdrawal from this agent should be expected to alter anxiety state. The data from the present study demonstrate that indomethacin administration does not in fact alter either the duration or latency parameters assessed using the defensive burying paradigm. Thus its interaction with steroid metabolic pathways is a more likely mechanism for prevention of the anxiogenic effect of P withdrawal observed in the present study.

The results from the present study suggest that P, administered with estradiol, may be an effective treatment for women suffering from the anxiety subtype of PMS. That a GABA-active agent would ameliorate symptoms of women suffering from PMS anxiety is further suggested by studies demonstrating the clinical effectiveness of GABA-active drugs such as alprazolam in treating such women (13). Alternatively, administration of indomethacin during the luteal phase would be expected to prevent conversion of P to the metabolite, and thus prevent the anxiogenic actions of P withdrawal during the premenstrual period.

ACKNOWLEDGEMENTS

The authors are grateful to D. Bitran, R. Valentino, and S. De Boer for their helpful comments during the progress of this study. The authors also express their thanks to J. Li for helpful technical assistance and to J. Hudson for clerical assistance.

REFERENCES

1. Bitran, D.; Hilvers, R. J.; Kellogg, C. K. Anxiolytic effects of 3α-hydroxy-5α[β] - pregnane-20-one: Endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. Brain Res. 561:157–161; 1991.
2. Bitran, D.; Purdy, R. H.; Kellogg, C. K. The anxiolytic effect of progesterone is associated with increases in cortical allopregnanolone and $GABA_A$ receptor function. Pharmacol. Biochem. Behav. 45:423–428; 1993.
3. Buck, K. J.; McQuilkin, S. J.; Harris, R. A. Modulation of γ-aminobutyric $acid_A$ receptor-operated chloride channels by benzodiazepine inverse agonists is related to genetic differences in ethanol withdrawal seizure severity. J. Neurochem. 57:2100–2105; 1991.
4. Butler, P. D.; Weiss, J. M.; Stout, J. C.; Nemeroff, C. B. Corticotropin-releasing factor produces fear-enhancing and behavioral activating effects following infusion into locus coeruleus. J. Neurosci. 10:176–183; 1990.
5. Carter Little, B.; Matta, R. J.; Zahn, T. P. Physiological and psychological effects of progesterone in men. J. Nerv. Ment. Dis. 159:256–267; 1983.
6. DeBoer, S. F.; van der Gugten, J.; Slanger, J. L. Behavioral and hormonal indices of anxiolytic and anxiogenic drug action in the shock prod defensive burying/avoidance paradigm. In: Animal models in psychopharmacology. Adv. Pharmacol. Sci., Boston: Birkhauser/Verlag; 1991, 81–96.
7. Dennerstein, L.; Spencer-Gardner, C.; Gotts, G.; Brown, J. B.; Smith, M. A.; Burrows, G. D. Progesterone and premenstrual syndrome: A double blind crossover trial. Br. Med. J. 290:1617–1621, 1985.
8. File, S. E. The benzodiazepine receptor and its role in anxiety. Br. J. Psychiatry 152:599–600; 1988.
9. Freeman, E.; Rickels, L.; Sondheimer, S. J.; Polansky, M. Ineffectiveness of progesterone suppository treatment for premenstrual syndrome. JAMA 264:349–354; 1990.
10. Gee, K. W.; Chang, W. C.; Brinton, R. E.; McEwen, B. S. GABA-dependent modulation of the Cl^- ionophore by steroids in rat brain. Eur. J. Pharmacol. 136:419–423; 1987.
11. Halbreich, U.; Endicott, J. Methodological issues in studies of premenstrual changes. Psychoneuroendocrinology 10:15–23; 1985.
12. Harrison, N. L.; Majewska, M. D.; Harrington, J. W.; Barker, J. L. Structure-activity relationships for steroid interaction with a γ-aminobutyric $acid_A$ receptor complex. J. Pharmacol. Exp. Ther. 241:346–353; 1987.
13. Harrison, W. M.; Endicott, J.; Nee, J. Treatment of premenstrual dysphoria with alprazolam. Arch. Gen. Psych. 47:270–275; 1990.

14. Hauser, P.; Devinsky, O.; DeBellis, M.; Theodore, W. H.; Post, R. M. Benzodiazepine withdrawal delirium with catatonic features. Occurrence in patients with partial seizure disorders. Arch. Neurol. 46:696–699; 1989.
15. Henkin, R. I. Sensory changes during the menstrual cycle. In: Pories, W.; Strain, W., eds. Biorhythms and human reproduction. New York: Wiley; 1974:277–285.
16. Hermann, W. M.; Beach, R. C. Experimental and clinical data indicating the psychotropic properties of progestins. Postgrad. Med. J. 54:82–87; 1978.
17. Karavolas, H. J.; Hodges, D. R. Metabolism of progesterone and related steroids by neural and neuroendocrine structures. In: Costa, E.; Paul, S. M., eds. Neurosteroids and brain function. New York: Thieme Medical Publishers; 1991:135–145.
18. Karavolas, H. J.; Nuti, K. M. Progesterone metabolism by neuroendocrine tissues. In: Naftolin, F.; Ryan, K. J.; Davies, T. J., eds. Subcellular mechanisms in reproductive neuroendocrinology. Amsterdam: Elsevier; 1976:305–326.
19. Lan, N. C.; Gee, K. W.; Bolger, M. B.; Sheng Chen, J. Differential responses of expressed recombinant human γ-aminobutyric acid$_A$ receptors to neurosteroids. J. Neurochem. 57:1818–1821; 1991.
20. MacDonald, R. L.; Weddle, M. G.; Gross, R. A. Benzodiazepine, β-carboline, and barbiturate actions on GABA responses. In: GABAergic transmission and anxiety, Adv. Biochem. Psychopharmacol. Raven Press, 1986, 67–78.
21. Majewska, M. D.; Harrison, N. L.; Schwartz, R. D.; Barker, J. L.; Paul, S. M. Steroid hormone metabolites are barbiturate-like modulators of the GABA receptor. Science 232:1004–1007; 1986.
22. McCaslin, P. P.; Morgan, W. M. Anticonvulsive activity of several excitatory amino acid antagonists against barbital withdrawal-induced spontaneous convulsions. Eur. J. Pharmacol. 147: 381–386; 1988.
23. Morrow, A. L.; Pace, J. R.; Purdy, R. H.; Paul, S. M. Characterization of steroid interactions with γ-aminobutyric acid receptor-gated chloride ion channels: Evidence for multiple steroid recognition sites. Mol. Pharmacol. 37:263–270; 1990.
24. Olsen, R. W. Discussion. In: Chadwick, D.; Widdows, K., eds. Steroids and neuronal activity; Chichester, England: John Wiley and Sons; 1990:104.
25. Olsen, R. W.; Tobin, A. J. Molecular biology of GABA$_A$ receptors. FASEB J. 4:1469–1480; 1990.
26. Olsen, R. W.; Venter, J. C., eds. Benzodiazepine/GABA receptors and chloride channels, structural and functional properties. New York: Alan R. Liss; 1986.
27. Penning, T. M.; Sharp, R. B.; Krieger, N. R. Purification of 3α-hydroxysteroid dehydrogenase from rat brain cytosol: Inhibition by nonsteroidal anti-inflammatory drugs and progestins. J. Biol. Chem. 260:15266–15272; 1985.
28. Pfaff, D. W.; McEwen, B. S. Actions of estrogens and progestins on nerve cells. Science 219: 808–814; 1983.
29. Pinel, J. P. J.; Treit, D. Burying as a defensive response in rats. J. Comp. Physiol. Psychol. 92:708–712; 1978.
30. Puia, G.; Santi, M.; Vicini, S.; Pritchett, D. B.; Purdy, R. H.; Paul, S. M.; Seeburg, P. H.; Costa, E. Neurosteroids act on recombinant human GABA$_A$ receptors. Neuron 4:759–765; 1990.
31. Purdy, R. H.; Moore, P. H., Jr.; Rao, P. N.; Hagino, M.; Yamaguchi, T.; Schmidt, P.; Rubinow, D. R.; Morrow, A. L.; Paul, S. M. Radioimmunoassay of 3α-hydroxy-5α- pregnane-20-one in rat and human plasma. Steroids 55:290–296; 1990.
32. Rodriguez-Sierra, J. F.; Howard, J. L.; Pollard, G. T.; Hendrickes, S. E. Effect of ovarian hormones on conflict behavior. Psychopharmacology 9:293–300; 1984.
33. Shapiro, S. S. Treatment of dysmenorrhoea and premenstrual syndrome with nonsteroidal anti-inflammatory drugs. Drugs 36: 475–490, 1988.
34. Smith, S.S. Progesterone enhances inhibitory responses of cerebellar Purkinje cells mediated by the GABA$_A$ receptor subtype. Brain Res. Bull. 23:317–322; 1989.
35. Smith, S. S. Discussion. In: Chadwick, D.; Widdows, K., eds. Steroids and neuronal activity. Chichester, England: John Wiley and Sons; 1990:74–82.
36. Smith, S. S. The effects of estrogen and progesterone on GABA and glutamate responses at extrahypothalamic sites. In: Costa, E.; Paul, S. M., eds. Neurosteroids and brain function. New York: Thieme Medical Publishers; 1991:87–94.
37. Smith, S. S.; Waterhouse, B. D.; Chapin, J. K.; Woodward, D. J. Progesterone alters GABA and glutamate responsiveness: A possible mechanism for its anxiolytic action. Brain Res. 400:353–359; 1987.
38. Smith, S. S.; Waterhouse, B. D.; Woodward, D. J. Locally applied progesterone metabolites alter neuronal responsiveness in the cerebellum. Brain Res. Bull. 18:739–747; 1987.
39. Treit, D.; Pinel, J. P. J.; Fibiger, H. C. Conditioned defensive burying: A new paradigm for the study of anxiolytic agents. Pharmacol. Biochem. Behav. 15:619–626; 1981.
40. Tsuda, A.; Ida, Y.; Nishimura, H.; Tanaka, M. Anxiogenic effects of β-CCE as measured in two different conditioning paradigms. Psychobiology 17:202–206; 1989.
41. Turner, D. M.; Ransom, R. W.; Yang, J. S.; Olsen, R. W. Steroid anesthetics and naturally occurring analogs modulate the γ-aminobutyric acid receptor complex at a site distinct from barbiturates. J. Pharmacol. Exp. Ther. 248:960–966, 1989.
42. Wieland, S.; Lan, N. C.; Mirasedeghi, S.; Gee, K. W. Anxiolytic activity of the progesterone metabolite 5α-pregnane-3α-ol-20-one. Brain Res. 565:263–268; 1991.