Pharmacology Biochemistry and Behavior, Vol. 63, No. 3, pp. 441–448, 1999

0091-3057/99/$–see front matter

PII S0091-3057(99)00041-6

Reduced Progesterone Metabolites Are Not Critical for Plus-Maze Performance of Lactating Female Rats

CAROL K. KELLOGG AND KATHY A. BARRETT

Department of Brain and Cognitive Sciences, University of Rochester, Rochester, NY 14627

Received 27 February 1998; Revised 7 December 1998; Accepted 22 December 1998

KELLOGG, C. K. AND K. A. BARRETT. *Reduced progesterone metabolites are not critical for plus-maze performance of lactating female rats*. PHARMACOL BIOCHEM BEHAV **63**(3) 441–448, 1999.—Lactation has been associated with anxiolysis in several tests of anxiety. These observations, considered together with observations that progesterone and its 5α-reduced metabolites are anxiolytic in cycling, nonlactating females, raised the question of whether the changes in anxiety-related behaviors that accompany lactation are driven by reduced progesterone metabolites. Lactating female rats were tested on the plus-maze on postpartum days 2 or 7, and demonstrated enhanced open-arm performance relative to cycling, nonlactating females. Hormonal analysis indicated that while serum levels of both progesterone and its 3α,5α-reduced metabolite were increased in lactating females, the turnover of progesterone to the metabolite was markedly reduced during lactation. Furthermore, treatment with a 5α-reductase inhibitor for 3 days prior to testing potentiated the open-arm performance in lactating females, implying that enhanced open-arm performance was not mediated by the reduction of progesterone or other steroids. Additionally, analysis of $GABA_A$ receptor function indicated that parturition and lactation did not alter the sensitivity of the receptor to GABA or to modulation by reduced steroids. The mechanisms driving enhanced plus-maze behavior in lactating females appear to differ from mechanisms identified in nonlactating females. © 1999 Elsevier Science Inc.

5α-Reductase Anxiety Vigilance $GABA_A$ Receptors Adaptive behavior

PROGESTERONE has been shown to be anxiolytic in females in a number of different animal models of anxiety (4,9,22), whereas withdrawal from daily progesterone treatment reportedly induces behavior indicative of experimental anxiety in females (14). The anxiolytic action of progesterone appears to be mediated by the conversion of progesterone to reduced metabolites: treatment with a 5α-reductase inhibitor but not a progesterone receptor antagonist prevented the anxiolytic action of progesterone (5). Consistent with the suggestion that reduced metabolites of progesterone mediate the anxiolytic effects of the parent hormone, administration of reduced metabolites themselves induces anxiolysis in both males and females (2,6,8). In females, reduced responses to anxiogenic stimuli have been correlated to stage of the estrous cycle, with female rats in proestrus (when progesterone levels are elevated) showing decreased defensive burying behavior relative to females in metestrus, diestrus, or estrus (9). Considered together, the results of these studies give rise to the hypothesis that the production of reduced progesterone metabolites represents an endogenous mechanism that plays a role in regulating responses to anxiogenic stimuli, particularly in female rats.

Pregnancy and lactation have also been shown to influence behavior in experimental tests of anxiety. We observed that exploration of the open arm in the elevated plus-maze test was increased in lactating females at postnatal days 2 and 7 relative to the performance of cycling females in either proestrus or diestrus (3). Other studies have reported a higher number of punished responses in a conflict paradigm in postpartuant than in virgin female rats (11,18), and behavior in the defensive burying paradigm varied as a function of pregnancy and lactation (21). These observations, considered together with the reports that progesterone and its reduced metabolites exert anxiolytic effects, raise the question of whether the changes in anxiety-related behavior that accompany pregnancy and lactation are driven by changing hormone levels during these periods.

Plasma progesterone levels in rats are high during the last

Requests for reprints should be addressed to Dr. Carol K. Kellogg, Room 186 Meliora Hall, Dept. of Brain and Cognitive Sciences, University of Rochester, Rochester, NY 14627.

week of gestation, fall precipitously within 24 h of parturition, remain at low levels until around day 2 postpartum, and then increase steadily throughout lactation eventually reaching pregnancy levels just before weaning (17,25). Because circulating progesterone is one source for the production of reduced progesterone metabolites in the brain, the brain levels of these metabolites might fluctuate with the changing level of the parent hormone during pregnancy and lactation. However, progesterone can also be produced in the brain by synthesis from cholesterol, a process that takes place in glial cells in the adult brain (15), and the levels of the reduced metabolites in the brain might not correlate with circulating levels of parent hormone.

The anxiolytic effects of the reduced progesterone metabolites have been shown to be mediated via modulation of the γ-aminobutyric acid$_A$ ($GABA_A$) receptor. For example, picrotoxin, an antagonist at the $GABA_A$ receptor, and the benzodiazepine antagonist, flumazenil, have both been shown to prevent the anxiolytic actions of reduced progesterone metabolites (3,10). Furthermore, the reduced fear associated with lactation resembles behavior elicited in virgin females given benzodiazepines, suggesting that $GABA_A$ receptors in the brain may be influenced by lactation (19). Binding at several sites around the $GABA_A$ complex is not altered in lactating females (11). However, it is possible that the sharp decrease in progesterone just prior to parturition may elicit a withdrawal-induced change in the sensitivity of $GABA_A$ receptors to reduced steroid metabolites. Such an effect might explain the enhanced anxiolysis observed on the day of parturition when plasma progesterone levels are low (21).

The goals of this study, therefore, were to 1) determine whether the responsiveness of $GABA_A$ receptors in the cerebral cortex to GABA and/or 3α-reduced pregnane steroids was altered in lactating females relative to nonlactating, intact females; and 2) evaluate the importance of the production of reduced progesterone metabolites to the anxiolysis associated with lactation.

METHOD

Animals

Female rats (250–300 g; Long–Evans, Harlan–Sprague–Dawley, Altamont, NY) were mated overnight with male rats of the same strain. The presence of a sperm-positive vaginal smear designated gestation day 0. Animals were maintained on a 12-h light/dark cycle (lights on at 0600 h) and provided with ad lib food and water. The day of birth was designated postnatal day 0. Testing was conducted on postnatal days 2 and 7, 7–8 h after the onset of light. Nonlactating, cycling females were used as controls. The stage of the estrous cycle was determined in the cycling females by vaginal lavage, and the tested females were found to be in stages of diestrus.

Behavioral Testing

To replicate our previous observations that lactating rats demonstrate enhanced anxiolytic activity (3), lactating females were tested either at postnatal days 2 or 7 for performance on the elevated plus-maze, and their performance was compared to that of the cycling, nonlactating females. The plus maze was made of wood and consisted of two runways that intersected at the center at right angles. Each arm of the maze measured 100 cm (length) by 10 cm (width). Two of the arms that were opposed to each other had walls that measured 40 cm in height (closed arms), whereas the other two arms had no walls (open arms). The maze was elevated 50 cm above the floor and positioned such that the open arms were centered under bright, incandescent lights. Females were placed in the center of the maze (10 × 10 cm), and the number of entries into each type of arm was counted (all four paws in the arm defining an entry) as was the time spent on each type of arm. The test was terminated 5 min after the female was placed in the center. The following measures were calculated: total number of arm entries, ratio of entries onto the open arms to the total entries, total time on open and closed arms, and ratio of time spent on open arms to total time spent on both arms. Changes in the total number of arm entries reflect a general index of activity, whereas changes in the ratio measures constitute indices of anxiety: percent increased open-arm performance reflects an anxiolytic state, while decreased percent open-arm performance reflects an anxiogenic state. A measure of general locomotor activity was also obtained by measuring activity in a novel, open field for 5 min prior to testing on the plus-maze. Animals were placed individually in a Plexiglas arena (41 × 25 × 41 cm) that was housed in a sound-attenuating chamber with an illumination intensity of 13–15 scotopic lx. Gross locomotor activity was detected by infrared photocells located along the walls of the arena at 5-cm intervals. The photocells were connected to a cumulative recorder, thereby providing an automated measure of gross locomotor activity.

Determination of $GABA_A$ Receptor Function

To assess responsiveness of the $GABA_A$ receptor as a function of lactation, GABA-stimulated $^{36}Cl^-$ uptake was evaluated in synaptoneurosomes prepared from the cerebral cortex of lactating dams on postpartum days 3 and 8, as well as from nonlactating, cycling females. All tissue was prepared 1 day following behavioral testing. Synaptoneurosomes were prepared in 4-(2-hydroxyethyl)-l-piperazineethanesulfonic acid (HEPES)-Tris buffer (20 mM, pH 7.4) as described by Schwartz et al. (23). Chloride uptake was initiated by addition of freshly prepared synaptoneurosomes (4 mg protein/ml, final incubation concentration) to reaction tubes (preincubated at 30°C for 10 min) containing 0.25 μCi of $^{36}Cl^-$ (specific activity 13.38–13.47 mCi/g; New England Nuclear, Boston, MA) and varying (nine total) concentrations of GABA (1–1000 μM). The final incubation volume was 250 μl. The reaction was terminated after a 10-s incubation period by addition of 5 ml ice-cold buffer containing picrotoxin (100 μM) to the assay tubes and filtration through Whatman GF/C glass fiber filters. Rinsed filters were placed in Ecoscint A (National Diagnostics) and the activity of $^{36}Cl^-$ determined by liquid scintillation spectrometry. The data were expressed as net $^{36}Cl^-$ uptake in nmol/mg protein (uptake in the presence of GABA minus uptake in the absence of any added GABA). The maximal stimulation of chloride uptake by GABA (nmol/mg protein) and the EC_{50} for GABA stimulation (μM) were determined for each individual experiment using Prism by GraphPad™. To evaluate the responsiveness of the $GABA_A$ receptor to neuroactive steroids, aliquots of each sample were incubated in tubes containing the reduced progesterone metabolite 3α-hydroxy-5β-pregnan-20-one (3α,5β-THP) at 500 nM in addition to GABA. Previous work from this laboratory has shown that this metabolite has anxiolytic properties when administered to animals tested in the plus maze (2).

Inhibition of 5α-Reductase

To examine the importance of the conversion of progesterone to reduced metabolites for the enhanced anxiolytic ef-

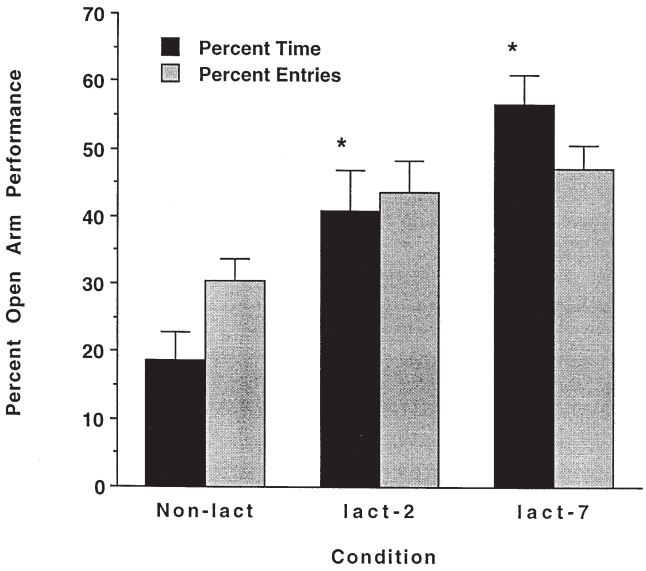

FIG. 1. Percent time on and percent entries onto the open arm of the plus-maze in nonlactating and lactating females at 2 and 7 days postpartum (lact-2, lact-7, respectively). Data are presented as mean ± SEM. *Indicates significant difference from same measure in nonlactating females.

fect of lactation, the effect of exposure to a 5α-reductase inhibitor, MK-434 (an azosteroid, Merck Research Laboratories), over postnatal days 5–7 on plus-maze performance on day 7 was examined. The drug was dissolved in 100% dimethylsulfoxide (DMSO) and administered at 50 mg/kg. Controls were given an equivalent volume of DMSO. All animals were tested on day 7, 4 h after the third daily injection. The weight of the litter was monitored over each of the 3 days to determine whether MK-434 treatment interfered with lactation. A 25–30% increase in litter weight took place over that period in both MK-434 and DMSO-exposed litters.

Hormonal Analysis

The serum concentrations of progesterone and the reduced progesterone metabolite, 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP), were determined in all females tested. In the one study, trunk blood was collected following decapitation prior to removal of the brain for analysis of chloride uptake. Trunk blood was collected from animals administered MK-434 or DMSO on the day following behavioral testing (24 h after the third injection). Progesterone was measured using Coat-A-Count kit for progesterone (Diagnostic Products Corp., Los Angeles, CA). 3α,5α-THP was measured using an RIA performed on ether extracts of serum: the assay was performed at the Southwestern Foundation, San Antonio, TX, by Dr. Perry Moore. All results were expressed as ng/ml.

Statistical Analysis

All data were analyzed by analysis of variance (ANOVA) using a commercially available package (StatView 512+™). Because aliquots of the same tissue were incubated under two different conditions (GABA alone or GABA plus 3α,5β-THP) in the chloride uptake assay, analysis for repeated measures was applied to that data. Statistical significance was noted when the probability of a Type I error was <0.05.

RESULTS

Experiment 1: Responsiveness of the $GABA_A$ Receptor During the First Week of Lactation

Before assessing function of the $GABA_A$ receptor during lactation, lactating females at postnatal day 2 or 7 as well as cycling, nonlactating females were tested on the plus-maze to verify our previous observations that lactation alters plus-maze performance (3). ANOVA of percent time on the open arms (Fig. 1) indicated that the percent time spent on the open arms varied as a function of lactation state ($p < 0.005$). Post hoc testing (Fisher PLSD) indicated that percent time on the open arms was greater at both postpartum day 2 and 7 than in cycling, nonlactating females, but that time on the open arms did not differ between the two lactating groups. ANOVA of the percent entries onto the open arms indicated only a marginal influence of lactation state ($p < 0.08$). Analysis of the data presented in Table 1 indicated that neither locomotor activity nor the total number of entries was altered significantly as a function of lactating state, indicating that general activity was not altered by lactation. The number of open arm entries was only marginally affected by lactation ($p < 0.06$), and the number of closed arm entries was unaffected. Neither total time nor time on the closed arms varied as a function of lactation. However, the time spent on the open arms varied with lactation state, with post hoc testing revealing that lactating females spent more overall time on the open arms than nonlactating females. These results, therefore, replicated previous observations that performance on the open arms of the plus-maze was enhanced in lactating females (3).

TABLE 1

LOCOMOTOR ACTIVITY AND PLUS MAZE PERFORMANCE IN NON-LACTATING AND LACTATING FEMALE RATS AT POSTPARTUM DAYS 2 AND 7

		Number			Time		
Condition	Activity	Total	Open	Closed	Total	Open	Closed
Non-lactating (4)	994.75 ± 42.86	12.0 ± 1.0	3.75 ± 0.75	8.25 ± 0.25	193.0 ± 29.2	34.75 ± 7.46	158.25 ± 28.70
Lactating-2 (6)	988.86 ± 32.88	15.3 ± 1.9	6.67 ± 0.99	8.67 ± 1.38	190.0 ± 14.7	75.67 ± 10.6*	114.33 ± 19.20
Lactating-7 (4)	1059.67 ± 56.30	15.7 ± 0.7	7.33 ± 0.33	8.33 ± 0.88	182.7 ± 15.1	102.33 ± 3.53*	80.33 ± 14.62

Data are expressed as mean ± SEM; activity scores are the number of photobeam interruptions per 5-min test session; time is reported in seconds; (number of animals tested).

*$p < 0.05$ relative to non lactating females.

TABLE 2

EFFECT OF LACTATION ON THE RESPONSIVENESS OF CORTICAL $GABA_A$ RECEPTORS TO PROGESTERONE METABOLITES: CHARACTERISTICS OF GABA-STIMULATED CHLORIDE UPTAKE

	EC_{50}[1]			Maximal Stimulation[2]		
Condition	GABA Only	GABA + THP*	THP:GABA	GABA Only	GABA + THP*	THP:GABA
Non-lactating	9.34 ± 0.73	6.51 ± 1.41	0.70 ± 0.16	27.88 ± 4.55	37.49 ± 3.15	1.34 ± 0.12
Lactating-3	11.81 ± 0.39	6.97 ± 0.64	0.59 ± 0.06	28.57 ± 4.58	37.19 ± 5.31	1.31 ± 0.12
Lactating-8	10.12 ± 0.08	7.32 ± 1.86	0.72 ± 0.19	29.04 ± 10.71	36.50 ± 6.19	1.36 ± 0.29

Data presented as mean ± SEM; [1] μM; [2]nmoles/mg protein.
*Significant main effect of incubation condition; THP = 3α,5β-THP.

On the morning following testing on the plus maze, GABA-stimulated chloride uptake was evaluated in cortical synaptoneurosomes. The mean for the EC_{50} and maximal stimulation calculated for each individual experiment is indicated in Table 2. A two-factor [incubation condition (GABA alone or GABA plus steroid) × state of lactation] ANOVA for repeated measures indicated that the EC_{50} for GABA stimulation varied significantly with incubation condition ($p < 0.005$). Post hoc evaluation indicated that the EC_{50} was significantly decreased in the presence of 3α,5β-THP, indicating that the sensitivity of the receptor for GABA was enhanced by the presence of the steroid. However, the EC_{50} was not altered significantly as a function of lactation state, and there was no significant interaction between lactation state and incubation condition. Additionally, two-factor ANOVA indicated a significant effect of incubation condition on the maximal stimulation by GABA ($p < 0.004$). Post hoc testing showed that the maximal stimulation was significantly increased in the presence of the neurosteroid. However, there was no overall effect of lactation state on maximal stimulation. Because aliquots of each sample were incubated in both conditions, the ratio of the response in the presence of GABA plus steroid to the response in the presence of GABA only was calculated for each experiment and the mean results for each group are indicated in Table 2. The ratio did not vary significantly across lactation condition. These data indicate that neither the sensitivity of the $GABA_A$ receptor to GABA nor to the modulatory effects of the progesterone metabolite, 3α,5β-THP, were altered in cortical synaptoneurosomes of lactating females when compared to nonlactating females.

The levels of progesterone and its reduced metabolite, 3α,5α-THP, were measured in serum collected at the same time brains were removed for analysis of $GABA_A$ receptor function, and the results are shown in Fig. 2. One-factor ANOVA indicated a significant effect of lactation state on the serum level of both progesterone and its reduced metabolite ($p < 0.008$ and $p < 0.003$, respectively). Post hoc analysis indicated that progesterone levels at postpartum day 8 were significantly higher than in nonlactating females, whereas the progesterone levels at postpartum day 2 did not differ significantly from either the nonlactating or lactation day 8 females. Levels of 3α,5α-THP were higher at day 8 than in both of the other groups. ANOVA of the ratio of 3α,5α-THP:progesterone, which provides an index of the turnover of progesterone to the metabolite, revealed a significant effect of lactation state ($p < 0.003$). Post hoc testing indicated that the ratio was significantly reduced in both groups of lactating females relative to nonlactating females. This observation implies that while plasma progesterone levels increased during lactation (at least by postpartum day 8), the conversion of the parent hormone to the reduced metabolite was decreased.

Experiment 2: Effect of Inhibition of 5α-Reductase on Plus-Maze Performance in Lactating Females

To evaluate the importance of production of 5α-reduced metabolites to responses on the plus-maze, lactating females were treated with a 5α-reductase inhibitor or vehicle for 3 days prior to testing at postpartum day 7. Percent time on the open arms and the percent entries onto the open arms are illustrated in Fig. 3 for the two groups. ANOVA of these data and that from untreated lactating females tested at postnatal day 7 (data shown in Fig. 1) indicated a significant effect of treatment on both percent time ($p < 0.02$) and on percent entries ($p < 0.04$). Post hoc testing revealed that neither of the treated groups differed in either percent time or percent en-

TABLE 3

LOCOMOTOR ACTIVITY AND PLUS MAZE PERFORMANCE IN LACTATING FEMALES AT POSTPARTUM DAY 7: EFFECT OF INHIBITION OF 5 ALPHA-REDUCTASE

		Number			Time		
Condition	Activity	Total	Open	Closed	Total	Open	Closed
MK-434 (4)	1080.25 ± 72.26	20.0 ± 1.2	11.80 ± 2.46	8.25 ± 1.50	215.8 ± 5.1	154.2 ± 18.5*	61.5 ± 18.89
DMSO (4)	936.25 ± 25.86	16.5 ± 1.0	6.25 ± 0.48	10.25 ± 0.95	182.8 ± 14.7	69.8 ± 6.52	113.0 ± 17.32
Lactating-7 (4)	1059.67 ± 56.30	15.7 ± 0.7	7.33 ± 0.33	8.33 ± 0.88	182.7 ± 15.1	102.3 ± 3.53	80.3 ± 14.62

Data expressed as mean ± SEM; activity scores are the number of photobeam interruptions per 5-min test session; time is reported in seconds; (number of animals tested). Data from uninjected lactating-7 females from Table 1 included for comparison.
*$p < 0.05$ compared to both other groups.

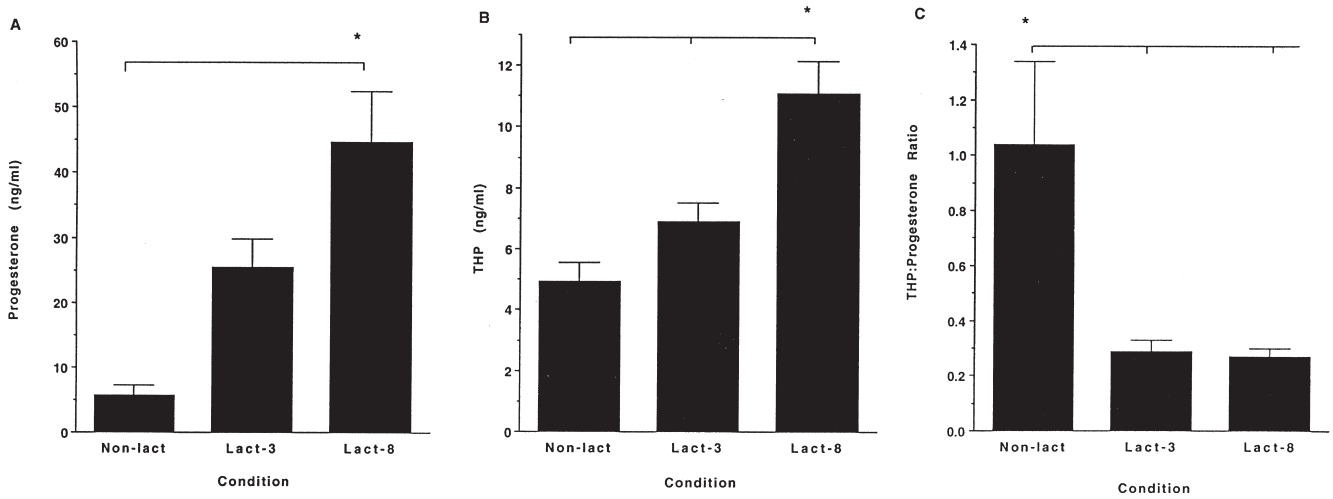

FIG. 2. The levels of progesterone and 3α,5α-THP (THP) and the ratio of THP:progesterone measured in nonlactating and lactating females at postpartum day 3 and 8 (1 day following testing on the plus maze). Data are presented as mean ± SEM. *Indicates a significant difference between marked group and groups indicated by a vertical dash mark.

tries from untreated lactating females, but both measures were greater in animals treated with MK-434 than with the vehicle (DMSO). However, analysis of amount of time on the open arms (Table 3) indicated a significant effect of treatment ($p < 0.004$), with lactating females exposed to MK-434 spending significantly more time on the open arms than either vehicle-treated or untreated lactating females. Neither locomotor activity nor total time nor total number of arm entries differed between the two treatment groups or from nontreated lactating females. Clearly, inhibition of 5α-reductase did not reduce performance on the open arm in lactating females but actually increased the total time spent on the open arms.

Steroid levels in the serum were compared between the two treated groups using an unpaired Student's *t*-test. As shown in Fig. 4, the serum progesterone level did not differ between the two groups, but the serum level of 3α,5α-THP was significantly lower in the MK-434–treated females ($p < 0.007$). Consequently, the ratio of 3α,5α-THP:progesterone was significantly reduced by MK-434 treatment, indicating the decreased conversion of progesterone to the metabolite following inhibition of 5α-reductase.

DISCUSSION

The results of these experiments do not support a role for reduced progesterone metabolites in mediating the apparent anxiolytic effects that have been observed in lactating females. While lactating females did exhibit enhanced open-arm performance on the plus-maze relative to nonlactating females, as was previously reported (3), analysis of serum hormone levels indicated that the turnover of progesterone to the reduced metabolite, 3α,5α-THP, was decreased in lactating females. Furthermore, while the behavior was similar at both postpartum days tested, the serum level of 3α,5α-THP was significantly higher at postpartum day 8 than at postpartum day 2, indicating a lack of correlation between the behavior and serum levels of the reduced progesterone metabolite. Additionally, inhibition of 5α-reductase, which decreased serum levels of the metabolite, did not reduce open-arm performance in lactating females.

It could be argued that the apparent lack of effect of inhibition of the reductase on plus maze performance in lactating female rats was due to incomplete inhibition of the enzyme. Even though treatment with MK-434 significantly reduced the serum level of 3α,5α-THP and decreased the turnover of progesterone to the reduced metabolite, the absolute level of 3α,5α-THP in MK-treated rats was still slightly higher than that measured in cycling, nonlactating females. It seems un-

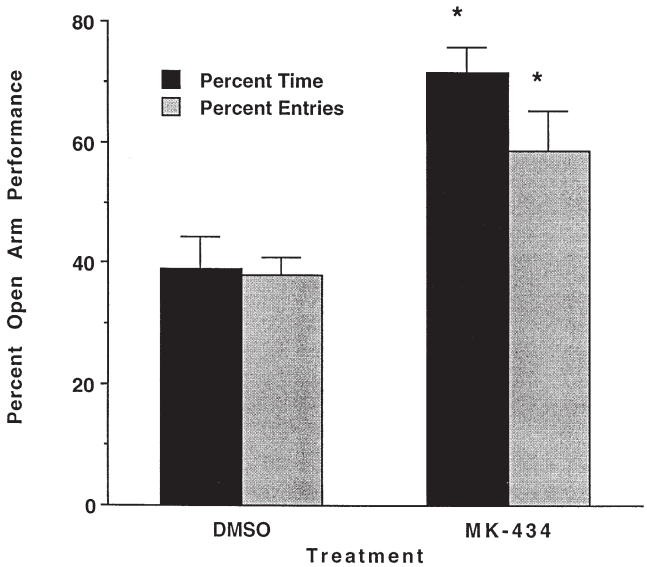

FIG. 3. Percent time on and percent entries onto the open arm of the plus-maze in lactating females tested on postpartum day 7. One group was treated from postpartum days 5 through 7 with the 5α-reductase inhibitor, MK-434 (50 mg/kg), while the control group received the diluent, DMSO, over the same period. Data are presented as mean ± SEM. *Indicates a signifcant difference from the control group.

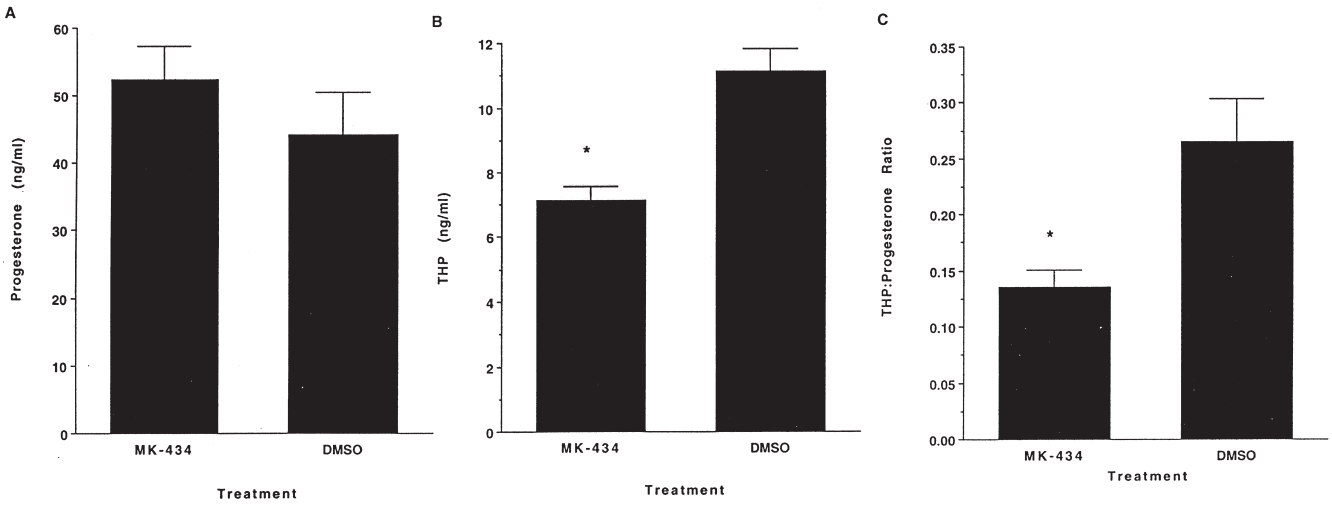

FIG. 4. The levels of progesterone and 3α,5α-THP (THP) and the ratio of THP:progesterone measured at postnatal day 8 (1 day following testing on the plus-maze) in lactating females administered MK-434 or DMSO over postpartum days 5–7. Data are presented as mean ± SEM. *Indicates a significant difference from the control group.

likely, however, that there is an absolute threshold level of reduced metabolite in the serum above which anxiolytic behavior will be observed. The results of a recent study by Smith et al. (24) also illustrate the poor correlation between serum 3α,5α-THP levels and plus-maze performance. In that study, the serum levels of the metabolite in 40-day-old ovariectomized rats were twice as high as the levels measured in the cycling, adult females in the present study, but the time spent on the open arms of the plus-maze was similar. Furthermore, while the induction of pseudopregnancy increased serum 3α,5α-THP levels, it did not alter plus maze performance. But treatment of pseudopregnant rats with an inhibitor of 5α-reductase decreased serum metabolite levels to that measured in the ovariectomized controls and also decreased open-arm performance. Absolute serum levels of reduced progestins, therefore, do not seem predictive of behavior. Brain levels of the metabolite were not measured in the current study, and one cannot generalize from serum to brain; however, the 5α-reductase inhibitor used is highly lipophilic in nature, so the drug should readily have penetrated into cells in the brain. Indeed, 3-day exposure to the same drug used in this study (MK-434) significantly reduced 5α-reductase activity in the telencephalon of birds (16), and treatment of adult rats with a compound similar to MK-434 (4-MA), prevented the effects of exogenous progesterone administration on plus-maze performance and cortical $GABA_A$ receptor function (5). Therefore, it seems highly likely that 5α-reductase was inhibited in the brains of lactating females in the current study.

The estimation of progesterone turnover to reduced metabolites, as was done in the current study by measuring the ratio of the metabolite to progesterone, may provide a better index of the activity of the enzyme than do absolute serum levels. Turnover of progesterone to the metabolite in the periphery was clearly reduced by 3-day exposure to MK-434 compared to either vehicle-injected or uninjected lactating females. However, the turnover of progesterone to the metabolite was also reduced by lactation itself. And even though treatment with MK-434 further reduced turnover in lactating females, the turnover may already have been so reduced that any further decrease was inconsequential. Because brain levels of the steroids were not obtained in this study, the effect of lactation on progesterone turnover in brain cells is not known. But a recent report indicates that the effect of lactation on progesterone turnover in various brain regions does not necessarily follow the direction measured in plasma (13). Although lactation reduced progesterone turnover to reduced metabolites in plasma relative to cycling females at diestrus 1 (the stage of the estrous cycle in that study where the plasma hormone levels best compare to those measured in cycling females in the current study), progesterone turnover increased only slightly in the hypothalamus, decreased in the amygdala, and increased in the hippocampus and cerebral cortex of lactating rats. The changes in behavior occurring with lactation may be related to far more complex mechanisms than the conversion of progesterone to its reduced metabolites.

The apparent lack of an influence of reduced progesterone metabolites on plus-maze performance in lactating females may have occurred because the plus-maze can detect behavior other than anxiety-related behavior exclusively. Although the plus-maze has been validated as a measure of anxiety in rats (20), the test may also detect other states. Consistent with this possibility, untreated lactating females spent 50% time on the open arms of the maze, indicating that the open arms were no more aversive than the closed arms. Compared to either vehicle-injected or uninjected lactating females at postnatal day 7, the females exposed to MK-434 spent significantly more time on the open arms of the plus-maze. Increased open-arm performance in lactating females may reflect the vigilance required of lactating females for the successful survival of their offspring, and thus be an adaptive behavioral response. Treatment with MK-434 may have potentiated this vigilance. Clearly, the impact of reduced progestins during lactation must be evaluated using several different tests.

Actions of progesterone other than via reduced metabolites also must be considered as possibly mediating the effects of lactation. The role of the progesterone receptor in mediating specific neural and behavioral changes associated with lactation has been investigated in several studies. Normal maternal behavior in lactating mice was disrupted by exposure of the dams to a progesterone receptor antagonist during preg-

nancy (26). interestingly, administration of the antagonist after parturition had a negligible effect, suggesting that progesterone action at the progesterone receptor during pregnancy serves as a priming mechanism for the onset of normal maternal behavior after parturition. Therefore, if progesterone plays a role in establishing anxiolysis or vigilance in lactating females, it may exert its impact prior to parturition. However, progesterone may not mediate all maternal behaviors. Lactating females show enhanced aggression towards conspecifics (19), but progesterone has been shown to be antiaggressive (7,12). On the other hand, progesterone may act in synergy with other aspects of lactation. Reduced cortical activation by *N*-methyl-D,L-aspartic acid has been reported in lactating females, and progesterone action at the progesterone receptor in synergy with suckling appears to be responsible (1). Reduced cortical activation in lactating females suggests that these females may process incoming environmental information differently from nonlactating females. The altered plus-maze behavior observed in lactating females may reflect altered cortical function.

Parturition and the onset of lactation, however, did not appear to induce a change in the sensitivity of cortical $GABA_A$ receptors to GABA or to modulation by progesterone metabolites. This observation is consistent with a previous report showing that binding to several recognition sites on $GABA_A$ receptors is not altered in lactating females (11). The observations that various drugs that disrupt function of $GABA_A$ receptors can reverse the increased aggression (19) and anticonflict behavior (18) measured in lactating rats may indicate only that the receptor is part of the neural circuitry involved in these responses, but does not necessarily indicate that changes in $GABA_A$ receptor function mediate the effect of lactation on these behaviors. The lack of effect of lactation state on cortical $GABA_A$ receptors contrasts with the effect of ovarian hormones in ovariectomized females on $GABA_A$ receptor function (3,5). The lack of effect of lactation state on $GABA_A$ receptor function considered together with the lack of effect of treatment with a 5α-reductase inhibitor on lactation-increased open-arm performance suggests that mechanisms underlying plus-maze performance in lactating females differ from those underlying progesterone-enhanced plus-maze performance in ovariectomized females.

In summary, although these results do not explicitly prove that reduced progesterone metabolites do not mediate the enhanced open-arm performance observed in lactating females, they do raise serious doubts as to the role of such metabolites. Although the reduction of progesterone to its metabolites may be ethologically advantageous as an endogenous mechanism to reduce anxiety during sexually receptive periods in cycling females, other mechanisms may mediate the adaptive behaviors observed in lactating females.

ACKNOWLEDGEMENTS

This work was supported in part by grant number DA07080 from the National Institute for Drug Abuse. The authors thank Dr. Linda Rhodes, Merck Research laboratories, Rahway, NJ, for the generous supply of MK-434.

REFERENCES

1. Abbud, R.; Hoffman, G. E.; Smith, M. S.: Cortical refractoriness to *N*-methyl-D,L-aspartic acid (NMA) stimulation in the lactating rat; Recovery after pup removal and blockade of progesterone receptors. Brain Res. 604:16–23; 1993.
2. Bitran, D.; Hilvers, R. J.; Kellogg, C. K.: Anxiolytic effects of 3α-hydroxy-5α(β)-pregnan-20-one: Endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. Brain Res. 561:157–161; 1991.
3. Bitran, D.; Hilvers, R. J.; Kellogg, C. K.: Ovarian endocrine status modulates the anxiolytic potency of diazepam and the efficacy of γ-aminobutyric acid-benzodiazepine receptor-mediated chloride ion transport. Behav. Neurosci. 105:653–662; 1991.
4. Bitran, D.; Purdy, R. H.; Kellogg, C. K.: Anxiolytic effect of progesterone is associated with increases in cortical allopregnanolone and $GABA_A$ receptor function. Pharmacol. Biochem. Behav. 45:423–428; 1993.
5. Bitran, D.; Shiekh, M.; McLeod, M.: Anxiolytic effect of progesterone is mediated by the neurosteroid allopregnanolone at brain $GABA_A$ receptors. J. Neuroendocrinol. 7:171–177; 1995.
6. Brot, M. D.; Akwa, Y.; Purdy, R. H.; Keeo, G. F.; Britton, K. T.: The anxiolytic-like effects of the neurosteroid allopregnanolone: Interactions with $GABA_A$ receptors. Eur. J. Pharmacol. 325:1–7; 1997.
7. Canonaco, M.; Vallenti, A.; Maggi, A.: Effects of progesterone on [^{35}S]t-butylbicyclophosphorothionate binding in some forebrain areas of the female rat and its correlation to aggressive behavior. Pharmacol. Biochem. Behav. 37:433–438; 1990.
8. Carboni, E.; Wieland, S.; Lan, N. C.; Gee, K. W.: Anxiolytic properties of endogenously occurring pregnanediols in two rodent models of anxiety. Psychopharmacology (Berlin) 126:173–178; 1996.
9. Fernandez-Guasti, A.; Picazo, O.: Changes in burying behaviour during the oestrous cycle: Effect of oestrogen and progesterone. Psychoneuroendocrinology 17:681–689; 1992.
10. Fernandez-Guasti, A.; Picazo, O.: Flumazenil blocks the anxiolytic action of allopregnanolone. Eur. J. Pharmacol. 281:113–115; 1995.
11. Ferreira, A.; Hansen, S.; Nielsen, M.; Archer, B.; Minor, B. G.: Behavior of mother rats in conflict tests sensitive to antianxiety agents. Behav. Neurosci. 103:193–201; 1989.
12. Fraile, I.; McEwen, B. S.; Pfaff, D. W.: Progesterone inhibition of aggressive behavior in hamsters. Physiol. Behav. 39:225–229; 1987.
13. Frye, C. A.; Banyon, L. E.: Mating stimuli influence endogenous variations in the neurosteroids 3α,5α-THP and 3α-diol. J. Neuroendocrinol. (in press).
14. Gallo, M. A.; Smith, S. S.: Progesterone withdrawal decreases latency to and increases duration of electrified prod burial: A possible rat model of PMS anxiety. Pharmacol. Biochem. Behav. 46:897–904; 1993.
15. Garcia-Segura, L. M.; Chowen, J. A.; Naftolin, F.: Endocrine glia: Roles of glial cells in the brain actions of steroid and thyroid hormones and in the regulation of hormone secretion. Front. Neuroendocrinol. 17:180–211; 1996.
16. Grisham, W.; Tam, A.; Greco, C. M.; Schlinger, B. A.; Arnold, A. P.: A putative 5 alpha-reductase inhibitor demasculinizes portions of the zebra finch song system. Brain Res. 750:122–128; 1997.
17. Grota, L. J.; Eik-Nes, K. B.: Plasma progesterone concentrations during pregnancy and lactation in the rat. J. Reprod. Fertil. 13:83–91; 1967.
18. Hansen, S.: Mechanisms involved in the control of punished responding in mother rats. Horm. Behav. 24:186–197; 1990.
19. Hansen, S.; Ferreira, A.; Selart, M. E.: Behavioural similarities between mother rats and benzodiazepine-treated non-maternal animals. Psychopharmacology (Berlin) 86:344–347; 1985.
20. Pellow, S.; Chopin, P.; File, S. E.; Briley, M.: Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14:149–167; 1985.
21. Picazo, O.; Fernandez-Guasti, A.: Changes in experimental anxiety during pregnancy and lactation. Physiol. Behav. 54:295–299; 1993.

22. Rodriguez-Sierra, J. F.; Howard, J. L.; Pollard, G. T.; Hendricks, S. E.: Effects of ovarian hormones on conflict behavior. Psychoneuroendcrinology 8:293–300; 1984.
23. Schwartz, R. D.; Suzdak, P. D.; Paul, S. M.: Gamma-aminobutyric (GABA)- and barbiturate-mediated Cl^- uptake in rat brain synaptoneurosomes: Evidence for rapid desensitization of the GABA receptor-coupled chloride ion channel. Mol. Pharmacol. 30:3369–3375; 1986.
24. Smith, S. S.; Gong, Q. H.; Li, X.; Moran, M. H.; Bitran, D.; Frye, C. A.; Hsu, F.-C.: Withdrawal from 3α-OH-5α-pregnan-20-one using a pseudopregnancy model alters the kinetics of hippocampal $GABA_A$-gated current and increases the $GABA_A$ receptor α4 subunit in association with increased anxiety. J. Neurosci. 18:5275–5284; 1998.
25. Sutter-Dub, M. T.; Leclercq, R.; Sutter, B. C.; Jacquot, R.: Plasma glucose, progesterone and immunoreactive insulin levels in the lactating rat. Horm. Metab. Res. 6:297–300; 1974.
26. Wang, M.-W.; Crombie, D. L.; Hayes, J. S.; Heap, R. B.: Aberrant maternal behaviour in mice treated with a progesterone receptor antagonist during pregnancy. J. Endocrinol. 145:371–377; 1995.