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Sex and Estrous Cycle-Dependent Changes in Neurosteroid and Benzodiazepine Effects on Food Consumption and Plus-Maze Learning Behaviors in Rats

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REDDY, D. S. AND S. K. KULKARNI. *Sex and estrous cycle-dependent changes in neurosteroid and benzodiazepine effects on food consumption and plus-maze learning behaviors in rats.* PHARMACOL BIOCHEM BEHAV **62**(1) 53–60, 1999.—Experiments were designed to investigate the influence of estrous cycle and gender of the rat on the effects of a γ-aminobutyric acid type A ($GABA_A$) receptor active neurosteroid, 3α-hydroxy-5α-pregnan-20-one (allopregnanolone), the benzodiazepine, triazolam, and a $GABA_A$ receptor antagonistic neurosteroid, Δ5-androsten-3β-ol-17-one sulfate (dehydroepiandrosterone sulfate), on food intake and elevated plus-maze learning behaviors. Allopregnanolone (0.25 mg/kg, SC) and triazolam (0.25 mg/kg, IP) produced a hyperphagic effect, while dehydroepiandrosterone sulfate (5 mg/kg, SC) elicited an anorectic effect. However, allopregnanolone was more potent in diestrous females, whereas triazolam exhibited significantly higher hyperphagic potency in estrus females. The extent of anorexia following dehydroepiandrosterone sulfate was alike in male and female rats. The triazolam- and allopregnanolone-induced hyperphagic effect was blocked by bicuculline (1 mg/kg, IP), a selective $GABA_A$ receptor antagonist. In contrast to triazolam, the hyperphagic effect of allopregnanolone was insensitive to flumazenil (5 mg/kg, IP), a benzodiazepine antagonist. Vehicle-treated diestrous rats displayed moderately higher latencies in the elevated plus-maze learning task than estrus or proestrus females. Although allopregnanolone and triazolam elicited equipotent learning deficits in plus-maze learning in male and female rats, the magnitude of impairment-induced by triazolam was significantly higher in diestrous females than proestrus females. Dehydroepiandrosterone sulfate enhanced memory performance only in male rats. Although the use of the elevated plus-maze as a learning paradigm with benzodiazepines and neurosteroids may be sensitive to changes in anxiety, the differential data suggest that neurosteroid-induced effects are at least partly specific to learning behavior. These results confirm the role of estrous cycle and sex of rats in modifying the potency of neurosteroids and benzodiazepines on food consumption and learning and memory processes. © 1998 Elsevier Science Inc.

Neurosteroids Estrous cycle Allopregnanolone Dehydroepiandrosterone sulfate Triazolam Food intake Memory Plus-maze Rat

THE widespread use of agents acting through the γ-aminobutyric acid type A ($GABA_A$) receptor complex has severe limitations because of their potential to produce adverse side effects ranging from mild memory impairment to delusions. The $GABA_A$ receptors have allosteric modulatory sites for therapeutically useful drugs such as benzodiazepines, barbiturates, and neurosteroids (19). Neurosteroids are those steroids that are both synthesized in the central nervous system (CNS), either de novo from cholesterol or from steroid hormone precursors, and that accumulate in the nervous system to levels that are at least in part independent of steroidogenic gland secretion rates. The term "neuroactive steroid" applies to both endogenous and synthetic steroids that rapidly alter CNS excitability. A number of highly potent neuroactive steroids exist, including the A-ring–

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reduced metabolites of progesterone and deoxycorticosterone, 3α-hydroxy-5α-pregnan-20-one (allopregnanolone) and 3α, 21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone) (37,38). Pregnenolone sulfate (5-pregnen-3β-ol-20-one sulfate), and dehydroepiandrosterone sulfate (Δ5-androsten-3β-ol-17-one sulfate) are considered as neurosteroids even though their synthesis has not been demonstrated in the brain, because their concentration in brain persists long after removal of gonads and adrenals (37,38). Recent evidence suggests that allopregnanolone and allotetrahydrodeoxycorticosterone are positive allosteric modulators of the $GABA_A$ receptor acting via a unique site termed the "neurosteroid binding site" on the receptor complex (14,19,20). These neuroactive steroids produce potent anxiolytic (32,33,41), antistress (28,35), anticonvulsant (17), antiaddictive (30,31), and neuroprotective properties (12,34). The neurosteroids pregnenolone sulfate and dehydroepiandrosterone sulfate antagonize $GABA_A$ receptor function (19,20). In the animal paradigms of learning and memory processes, allopregnanolone impaired (21), while dehydroepiandrosterone sulfate enhanced the acquisition and retention performance (11), respectively. Studies from our laboratory and others suggest that low doses of allopregnanolone produce hyperphagia, while dehydroepiandrosterone sulfate elicits anorectic effects in food deprived rodents (6,36). The hyperphagic and memory impairment effects of neurosteroids and benzodiazepines are proposed to occur through the potentiation of $GABA_A$ receptors (6,11,21,36). However, little is known as yet whether these behavioral measures might be affected by natural variations such as estrous cycle or sex of the animals.

Multiple lines of evidence suggest that ovarian steroids profoundly influence the behavioral and neurochemical responses of the $GABA_A$ receptor complex (8–10,23). Because brain and plasma levels of allopregnanolone vary with progesterone levels (5), it is hypothesized that events dependent on GABAergic function will be enhanced during stage of the estrous cycle characterized by high levels of circulating progesterone. However, recent in vitro study has indicated that the potency of allopregnanolone as a modulator of the $GABA_A$ receptor complex is inversely related to the endogenous levels achieved during the estrous cycle (9). In addition, ovarian steroids modify the behavioral and neuroendocrine responses of the central benzodiazepine receptor (3). Therefore, the studies reported here were conducted to determine whether sex and estrous cycle modify the responsiveness and magnitude of neurosteroid and benzodiazepine effects on cognition and feeding behavior in rats. In this study, we examined the effect on food intake and memory processes of a benzodiazepine, triazolam, and two neurosteroids that act in different ways on the $GABA_A$ receptor complex. Allopregnanolone is positive allosteric modulator of $GABA_A$ receptor complex, whereas dehydroepiandrosterone sulfate behaves as an allosteric antagonist. Memory processes were evaluated in the elevated plus-maze learning task (16) that has been validated in our laboratory (29,40).

METHOD

Animals

Adult male and female Wistar rats (180–200 g; Central Animal House, Panjab University, Chandigarh, India) were housed in groups of five in laboratory conditions. They were maintained under a 12 L:12 D cycle, with food and water available ad lib. Food was removed from the home cages 24 h before testing for all the food intake experiments. However, water was available during the 24-h food deprivation. Animals were handled daily to reduce stress. Maintenance of animals and experimental procedures were in compliance with the guidelines established by the NIH Guide for the Use of Laboratory Animals. All the experiments were performed between 0900 and 1200 h at the ambient temperature. The stage of the estrous cycle was determined daily from vaginal smears that were taken at 0930 h, approximately 1 h before the behavioral test. Only those females showing two regular, 4–5 day cycles were selected. The groups were established according to the different vaginal–cytology estrous cycle phases as follows: proestrous (round, nucleated cells), estrus (cornified cells), metestrous (round, nucleated cells, cornified cells, and leukocytes), and diestrous (predominance of leukocytes).

Food Consumption Behavior

On the day of the feeding experiment, cytological results from vaginal smears were used to assign the female rats into one of the four groups. They were weighed before testing, and drugs were administered only if the body weight deviated less than 5% from other animals. Age and body weight matched male counterparts were used to assess the sex-related effects. The test food for the feeding experiment was standard rat chow modified for palatability by adding 10% sucrose (6). Animals were transferred to individual test cages in the testing room and allowed to acclimatize for 30 min before the administration of the test drug. Thirty minutes after administration of the allopregnanolone (0.25 mg/kg, SC), dehydroepiandrosterone sulfate (5 mg/kg, SC) or triazolam (0.25 mg/kg, IP), 40 g of test food was presented in a glass Petri dish and placed inside the test cages. The amount of food consumed by each animal was measured for 60 min, and during this interval only the test food was available. Water was freely available through out the acclimatization but not testing period. The animals were returned to their home cage and the test food consumed was determined to the nearest 0.1 g, with correction for spillage.

Additional experiments were performed using bicuculline (1 mg/kg, IP) and flumazenil (5 mg/kg, IP) to determine whether the effects on food intake of allopregnanolone and triazolam involve the $GABA_A$/benzodiazepine receptor complex.

Transfer Latency on Elevated Plus-Maze Test

Cognitive behavior was evaluated by using the elevated plus-maze learning task (16,29,40), which measures spatial long-term memory. Transfer latency (the time in which the rat takes to move from the open arm to the enclosed arm) was utilized as an index of learning and memory processes. The method used was as described previously (29). Briefly, the apparatus consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm) elevated to a height of 50 cm from the floor. The animals were placed individually at the end of either of the open arms and the transfer latency was noted on the first day. The animals were allowed to explore the apparatus for 20 s to become acquainted with the maze. On the second day, 24 h after the first exposure, transfer latency was again noted. Each animal was used only once. The neurosteroids allopregnanolone (0.25 mg/kg, SC), dehydroepiandrosterone sulfate (5 mg/kg, SC) or triazolam (0.25 mg/kg, IP) were administered 30 min prior to the assessment of the first day trial. An increase in acquisition/learning processes was defined as a decreased transfer latency on the second day trial relative to the first day trial. Failure to decrease the transfer latency on the second day trial was interpreted as an impairment of learning process.

Drugs

Allopregnanolone, dehydroepiandrosterone sulfate and bicuculline (Sigma, St. Louis, MO), triazolam (Upjohn, Kalamazoo, MI) and flumazenil (Hoffmann–La Roche, Basel, Switzerland) were used in the present study. All drug solutions were made in a 0.1% Tween 80 and diluted with saline. Control groups received vehicle treatment. The selection of doses was based on our previous studies (5,7,10,14). A dose–response curve was previously determined for each of the drugs tested in each paradigm. Triazolam (0.05–0.25 mg/kg, IP) and allopregnanolone (0.25–2 mg/kg, SC) produced a hyperphagic effect, while, dehydroepiandrosterone sulfate (1–10 mg/kg, SC) elicited a hypophagic effect, in a dose-related manner (34). Both triazolam (0.05–0.25 mg/kg, IP) and allopregnanolone (0.25–2 mg/kg, SC) impaired, whereas dehydroepiandrosterone sulfate (1–10 mg/kg, SC) significantly facilitated the elevated plus-maze learning task, respectively (Reddy and Kulkarni, unpublished observations). From the dose response of each drug tested, the single most effective dose of triazolam (0.25 mg/kg, IP), allopregnanolone (0.25 mg/kg, SC), and dehydroepiandrosterone sulfate (15 mg/kg, SC) was selected and studied for its possible differential effects under the influence of estrous cycle and sex of the rat.

Data Analysis

All results are expressed as mean ± SEM. All behavioral data were analyzed using two-way analysis of variance (ANOVA), with the stage of the estrous cycle of the rat as the within-groups repeated measures variable, and the drug treatment as the between groups categorical variable. Significant interactions were analyzed using post hoc multiple comparison test. Males were compared to the females averaged across their estrous cycle by using two-way ANOVA followed by post hoc multiple comparison test. The bicuculline, flumazenil, and neurosteroid interaction data was subjected to one-way ANOVA followed by Duncans multiple range test by using the STAT program on an IBM computer. Statistical significance was attributed at $p < 0.05$.

RESULTS

Food Intake

The influence of estrous cycle phases on the allopregnanolone, dehydroepiandrosterone sulfate, and triazolam induced changes in food intake is illustrated in Fig.1. A two-way ANOVA revealed that all three drugs induced significant effects on the food intake, $F(3, 64) = 44.65$, $p < 0.01$, and that these effects change with the estrous cycle phases, $F(3, 64) = 9.56$, $p < 0.05$. The interaction between these two factors was also significant, $F(9, 64) = 4.23$, $p < 0.05$. Post hoc analysis revealed that the stage of the estrous cycle had no effect on baseline food intake ($p > 0.1$) in vehicle-treated controls rats. A hyperphagic effect was observed after allopregnanolone (0.25 mg/kg, SC) or triazolam (0.25 mg/kg, IP), while dehydroepiandrosterone sulfate (5 mg/kg, SC) elicited an anorectic effect at each stage of the estrous cycle. In estrous, triazolam significantly increased food intake ($p < 0.05$), relative to metestrous, diestrous, and proestrous. In contrast, allopregnanolone significantly enhanced food intake during diestrous stage ($p < 0.05$), relative to estrous, metestrous, and proestrous. The dehydroepiandrosterone sulfate-induced hypophagic effect was not dependent on the stage of the estrous cycle ($p > 0.1$) (Fig. 1).

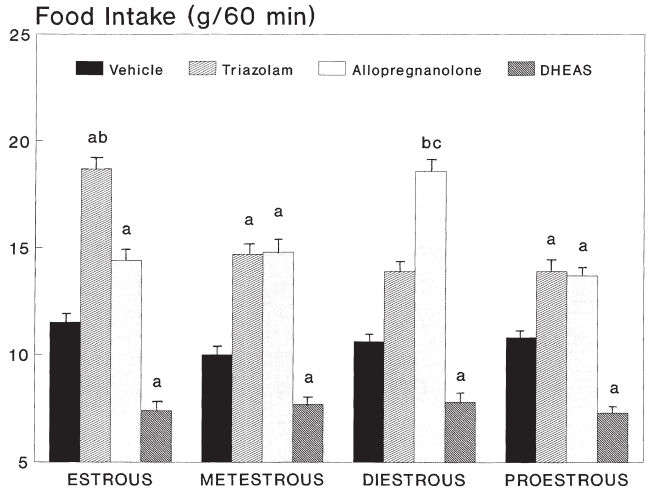

FIG. 1. Influence of estrous cycle on the hyperphagic effect of allopregnanolone and triazolam, and hyophagic effect of dehydroepiandrosterone sulfate in 24-h food-deprived rats. After cytological confirmation of the estrous stage, rats were injected with vehicle or allopregnanolone (0.25 mg/kg, SC) or triazolam (0.25 mg/kg, IP) or dehydroepiandrosterone sulfate (DHEAS) (5 mg/kg, SC), and palatable food intake was assessed after 30 min for the next 60 min. Each bar represents the mean ± SEM (n = 5–8 animals per group). Comparisons were made by using two-way ANOVA followed by post hoc multiple comparison test: $^{a}p < 0.05$ compared to respective estrous cycle stage vehicle control group; $^{b}p < 0.05$ when compared to female rats in metestrus, diestrus, and proestrus stage; $^{c}p < 0.05$ when compared female rats in estrus, metestrus, and proestrus stage.

Because sex and estrous cycle phase represent two separate variables, the effect of test drugs on food-intake in males were compared to females averaged across their cycles to address the sex difference arguments. Figure 2 shows the influence of sex on allopregnanolone, dehydroepiandrosterone sulfate, and triazolam induced changes in food intake. A two-way ANOVA revealed that all three drugs induced significant effects on the food intake, $F(3, 28) = 39.59$, $p < 0.01$, and that these effects depend on the sex of the rat, $F(1, 28) = 5.27$, $p < 0.05$. The interaction between these two factors was also significant, $F(7, 28) = 3.92$, $p < 0.05$. Post hoc tests revealed no sex differences on the baseline food intake in vehicle-treated animals ($p > 0.1$). The magnitude of hyperphagic effect of triazolam and allopregnanolone were significantly ($p < 0.05$) higher in male than the females averaged across their cycle. The extent of hypophagia following dehydroepiandrosterone sulfate was similar in male and female rats (Fig. 2).

To determine whether the hyperphagic effects of allopregnanolone and triazolam involve the $GABA_A$/benzodiazepine receptor complex, triazolam (0.25 mg/kg, IP) and allopregnanolone (0.25 mg/kg, SC) were tested again in the presence of bicuculline (1 mg/kg, IP), a selective $GABA_A$ receptor antagonist, and flumazenil (5 mg/kg, IP), a benzodiazepine antagonist. As shown in Table 1, one-way ANOVA revealed a significant effect of bicuculline and flumazenil in male, $F(8, 39) = 10.82$, $p < 0.01$ and estrus female rats, $F(8, 36) = 7.48$, $p < 0.01$. Post hoc analysis indicated that bicuculline (1 mg/kg, IP) did not affect the food intake when administered alone, but significantly ($p < 0.05$) prevented the allopregnanolone- and triazolam-induced hyperphagic effect in male and estrus female rats. Flumazenil did not modify the food intake behav-

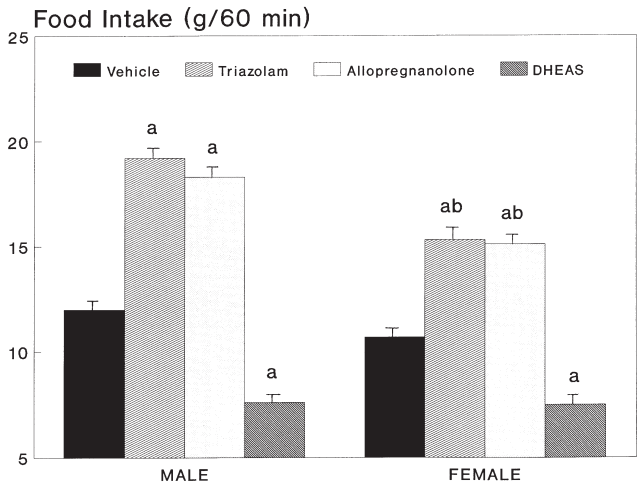

FIG. 2. Influence of sex on the hyperphagic effect of allopregnanolone and triazolam, and hyophagic effect of dehydroepiandrosterone sulfate in 24-h food-deprived rats. Rats were given vehicle, allopregnanolone (0.25 mg/kg, SC), triazolam (0.25 mg/kg, IP) or dehydroepiandrosterone sulfate (DHEAS) (5 mg/kg, SC) and food intake was assessed after 30 min for the next 60 min. Each bar represents the mean ± SEM (n = 10–12 animals per group). Males were compared to the females averaged across their estrous cycle by using two-way ANOVA followed by post hoc multiple comparison test: [a]$p < 0.05$ compared to respective sex vehicle control group; [b]$p < 0.05$ when compared to male rats.

TABLE 1

INFLUENCE OF BICUCULLINE, A SELECTIVE GABA-A RECEPTOR ANTAGONIST, AND FLUMAZENIL, A BENZODIAZEPINE ANTAGONIST, ON THE HYPERPHAGIC EFFECT OF NEUROSTEROID ALLOPREGNANOLONE AND BENZODIAZEPINE TRIAZOLAM IN MALE AND ESTRUS FEMALE RATS

	Food Intake (g/60 min)	
Treatment (mg/kg)	Male	Female
Vehicle	12.8 ± 0.78	11.5 ± 0.94
Triazolam (0.25)	19.2 ± 1.46*	18.7 ± 0.68*
Allopregnanolone (0.25)	17.9 ± 0.70*	14.6 ± 0.54*
Flumazenil (5)	13.0 ± 0.71	12.6 ± 1.05
Triazolam (0.25) + flumazenil (5)	13.2 ± 0.84†	14.0 ± 0.86†
Allopregnanolone (0.25) + flumazenil (5)	17.1 ± 0.92*	13.8 ± 0.76*
Bicuculline (1)	12.2 ± 0.75	11.3 ± 0.66
Triazolam (0.25) + bicuculline (1)	13.0 ± 1.01†	13.1 ± 0.97†
Allopregnanolone (0.25) + bicuculline (1)	13.6 ± 0.58†	13.1 ± 0.92†

Bicuculline (1mg/kg, IP) and flumazenil (5 mg/kg, IP) were administered 30 min prior to allopregnanolone (0.25 mg/kg, SC)) or triazolam (0.25 mg/kg, IP). Values are mean = SEM (n = 5–8 per group). Comparisons were made by using one-way ANOVA followed by post hoc Duncan multiple range test.

*$p < 0.05$ compared to respective vehicle-tested group; †$p < 0.05$ when compared to their respective control treatments alone (i.e., triazolam- or allopregnanolone-treated groups).

ior when administered alone, but completely blocked the hyperphagic effect of triazolam in male and female rats ($p < 0.05$). However, the hyperphagic effect of allopregnanolone was insensitive to flumazenil in either groups (Table 1).

Plus-Maze Learning

Figure 3 shows the effect of neurosteroids and triazolam on the plus-maze learning task in female rats in various estrous phases. A two-way ANOVA revealed a significant effect of drug treatment on the first day transfer latency, $F(3, 64) = 37$, $p < 0.01$. However, the estrous cycle stage, $F(3, 64) = 1.32$, and the interaction of drug treatment and estrous cycle phases, $F(9, 64) = 0.59$, were not significant in the plus-maze acquisition trial. The results of second day transfer latency trials (Fig. 3 lower panel), revealed that neurosteroids and triazolam produced significant effects on the plus-maze retention trial, $F(3, 64) = 49.51$, $p < 0.01$, and that these effects change with the stage of the estrous cycle, $F(3, 64) = 6.72$, $p < 0.05$. The interaction between these two factors was also significant, $F(9, 64) = 3.56$, $p < 0.05$. Post hoc analysis revealed that the stage of the estrous cycle had a significant effect on the vehicle response in the elevated plus-maze retention task ($p < 0.05$). Diestrous females showed a significantly greater transfer latencies in the plus-maze learning task, relative to estrous and proestrous rats ($p < 0.05$ in each case). Both allopregnanolone and triazolam produced significant transfer deficits at each stage of the estrous cycle compared to vehicle groups. In diestrous females, triazolam showed significantly higher transfer deficits ($p < 0.05$), relative to other estrous phases. Dehydroepiandrosterone sulfate did not significantly modify the performance of female rats across various phases of estrous cycle ($p > 0.1$), relative to vehicle groups (Fig. 3).

The influence of sex on the neurosteroids and triazolam-induced changes in the elevated plus-maze learning task was shown in Fig. 4. A two-way ANOVA revealed a significant effect of drug treatment on the second day trial, $F(3, 28) = 41.54$, $p < 0.01$. These effects on the retention trial vary with sex, $F(1, 28) = 5.43$, $p < 0.05$, and the interaction between these two factors was also significant, $F(7, 28) = 3.96$, $p < 0.05$. Post hoc analysis revealed that the mean latencies on the elevated plus-maze learning task with triazolam and allopregnanolone did not differ significantly between male and female rats ($p > 0.1$), but were significantly higher than those obtained in the respective vehicle-treated groups ($p < 0.05$). In contrast, a significant decrease in the transfer latency was observed with dehydroepiandrosterone sulfate in male ($p < 0.05$), but not in female rats ($p > 0.1$), relative to vehicle controls (Fig. 4).

DISCUSSION

The present study demonstrates that the effects of neurosteroids and benzodiazepines on food intake and cognitive processes in the elevated plus-maze are influenced by the gender and hormonal changes that occur in female rats during the estrous cycle. The hyperphagic effects of allopregnanolone and triazolam are highest in diestrous and estrus, respectively. In contrast, dehydroepiandrosterone sulfate has an hypophagic effect that is not dependent on the estrous cycle. In the elevated plus-maze learning task, impairment of memory by triazolam and allopregnanolone are not sex dependent, while dehydroepiandrosterone sulfate significantly facilitated learning in a sex-dependent manner. The neurosteroid allopreg-

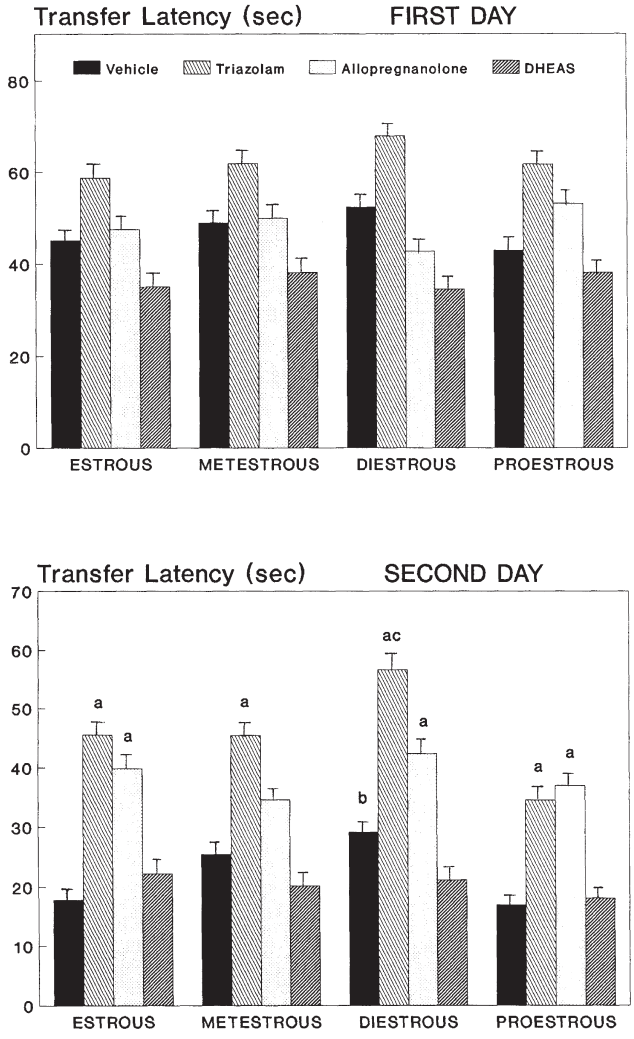

FIG. 3. Influence of estrous cycle on the effect of allopregnanolone, triazolam, and dehydroepiandrosterone sulfate in the elevated plus-maze learning task in rats. After cytological confirmation of the estrus stage, rats were injected with vehicle or allopregnanolone (0.25 mg/kg, SC) or triazolam (0.25 mg/kg, IP) or dehydroepiandrosterone sulfate (DHEAS) (5 mg/kg, SC), and the transfer latencies to the enclosed arms are assessed after 30 min on the first day (upper panel) and on the second day 24 h after the first trial (lower panel). Each bar represents the mean ± SEM (n = 5–8 animals per group). Comparisons were made by using two-way ANOVA followed by post hoc multiple comparison test: $^{a}p < 0.05$ compared to respective estrous cycle stage vehicle control group; $^{b}p < 0.05$ when compared to estrus and proestrus vehicle control; $^{c}p < 0.05$ when compared to the same treatment in proestrus females.

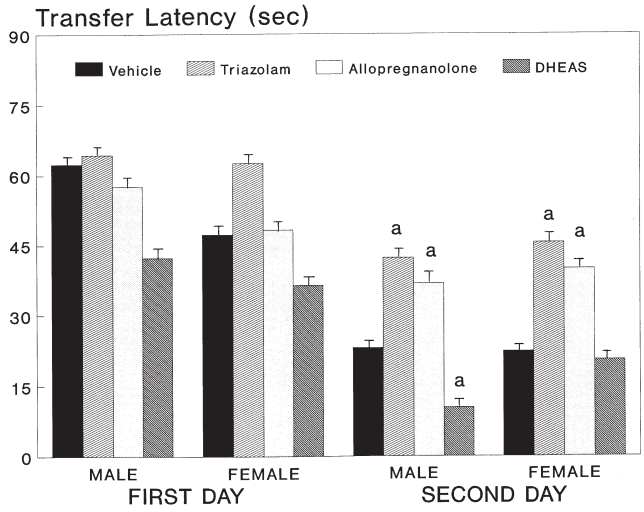

FIG. 4. Influence of sex on the effect of allopregnanolone, triazolam, and dehydroepiandrosterone sulfate in the elevated plus-maze learning task in rats. Rats were given vehicle, allopregnanolone (0.25 mg/kg, SC), triazolam (0.25 mg/kg, IP) or dehydroepiandrosterone sulfate (DHEAS) (5 mg/kg, SC), and the transfer latencies to the enclosed arms are assessed after 30 min on the first day (upper panel) and on the second day 24 h after the first trial (lower panel). Each bar represents the mean ± SEM (n = 5–8 animals per group). Males were compared to the females averaged across their estrous cycle by using two-way ANOVA followed by post hoc multiple comparison test: $^{a}p < 0.05$ compared to respective sex vehicle control group.

nanolone is a potent positive allosteric modulator of $GABA_A$ receptor complex (26) and produces anxiolytic and hyperphagic effects in food-deprived rodents (6,36). Recently, physiologically significant fluctuations in the endogenous levels of allopregnanolone during stress and estrous cycle have been reported (5, 8–10, 23). Although estrous cycle-dependent differences in anxiety levels, pain sensitivity, seizure susceptibility, and sensitivity to benzodiazepines and barbiturates is known (4,8,10,13), there is no information concerning the potency of neurosteroids and benzodiazepines on food intake and learning behaviors with regards to sex and estrous cycle.

The present results indicate that the estrous cycle- and gender-related differences in the hyperphagic effect of allopregnanolone and triazolam are related to their actions at the $GABA_A$ receptor complex. This contention can be supported by the observations that bicuculline, a selective $GABA_A$ receptor antagonist, significantly prevented the allopregnanolone- and triazolam-induced hyperphagic effect in both male and female rats. Further, the results indicate that the hyperphagic effects of allopregnanolone are most likely mediated via the stimulation of $GABA_A$ receptor by binding to a site other than benzodiazepine binding site, because flumazenil, a benzodiazepine antagonist, failed to modify the hyperphagic effects of allopregnanolone. However, the triazolam-induced hyperphagia was effectively reversed by preadministration of flumazenil, indicating the involvement of benzodiazepine receptors in the actions of triazolam. These results confirm the findings of previous studies indicating $GABA_A$ receptor-mediated effects of neurosteroids and benzodiazepines on food intake (6,36). Furthermore, they suggest that benzodiazepines and neurosteroids have unique binding sites on the $GABA_A$ receptor complex.

The hyperphagic effect of allopregnanolone was higher in diestrous relative to other estrous cycle stages consistent with recent in vitro data indicating that allopregnanolone is more potent in diestrous (9). Given the inverse relationship between the neurosteroid level and potency at the $GABA_A$ receptor complex (9,10), it is not surprising to observe a high hyperphagic potency for allopregnanolone in the diestrous stage, which is characterized by low levels of estrogens and progesterone. However, the ethanol-like discriminative stimulus ef-

fects of allopregnanolone are high during the luteal phase of the menstrual cycle in monkeys, characterized by higher levels of progesterone (15), suggesting that changes in endogenous levels of ovarian steroids differentially alter the behavioral sensitivity to allopregnanolone. In contrast, the hyperphagic potency of the benzodiazepine, triazolam, was higher in estrus, which is characterized by falling levels of both estrogen and progesterone, relative to other stages. Consistent with these data, Bitran and Dowd (3) have reported that high progesterone levels in estrous cycle can produce marked increase in benzodiazepine binding. Progesterone, by metabolizing to the neurosteroid allopregnanolone (5), may increase benzodiazepine binding in vitro (3) and potentiate the behavioral effects (3). In addition, in vitro studies show that neurosteroids, including allopregnanolone, may increase the affinity of benzodiazepines at the $GABA_A$ receptor, as indicated by their ability to enhance [^{3}H]flunitrazepam binding (22). Thus, the high potency of triazolam in estrus stage may be due to neurosteroid potentiation of triazolam binding at the $GABA_A$ receptor complex. Triazolam elicits potent effects on mood in women in the luteal phase of the menstrual cycle (42), further supporting the high hyperphagic potency of triazolam in estrus females. Both triazolam and allopregnanolone produced equipotent hyperphagia in proestrous rats. Although progesterone levels in late proestrous are high, the results of our study in early proestrous are consistent with a previous study that showed low anxiolytic-like effects in early proestrous (8).

Males had similar hyperphagia to both allopregnanolone and triazolam. The magnitude of hyperphagia was higher in male than female rats, indicating differential influence of gonadal hormonal status on food consummatous behavior. Consistent with these observations, previous work found a significant sex difference in pentylenetetrazol seizure threshold in rats (18). In view of the brain regional variations in the distribution of $GABA_A$ receptor subunit mRNAs (26), and the potency of allopregnanolone (23) and diazepam (3) as positive allosteric modulators of the $GABA_A$ receptor complex, there may be brain regional differences in the presence of $GABA_A$ receptors that preferentially recognize either neurosteroids or benzodiazepines. Thus, it is possible that these receptors may be differentially influenced by the gonadal hormones and, consequently, the hyperphagic potency of allopregnanolone and triazolam.

The estrous cycle- and gender-related differences in food intake behavior found in the present studies did not generalize across the two neurosteroids studied. Male and female rats exhibited equipotent anorectic effect to dehydroepiandrosterone sulfate. Dehydroepiandrosterone sulfate is both an allosteric antagonist of $GABA_A$ receptors (19), and agonist of *N*-methyl-D-apartate (NMDA) receptors acting via the sigma site (25). It also alters the release of neurotransmitters such as serotonin and norepinephrine in specific brain regions (2). Therefore, these differences between these neurosteroids in their behavioral patterns may be related to the different mechanisms involved, which are influenced differentially by hormonal status. Because the hyperphagic effects of allopregnanolone and triazolam are proposed to occur through $GABA_A$ receptor, changes in the sensitivity of the $GABA_A$ receptor complex during the estrous cycle may explain the differential effects of neurosteroids and benzodiazepines. However, although the NMDA receptor complex has been implicated (36), the precise mechanisms involved in the anorectic effect of dehydroepiandrosterone sulfate are largely unknown. Further, to date there is no evidence for gonadal hormone-induced changes in the functional sensitivity of NMDA receptor to its agonists.

In addition, the present study demonstrates significant estrous cycle- but not sex-related differences in the impairment of memory by triazolam and allopregnanolone in the elevated plus-maze learning task in rats. Although vehicle-treated females exhibited moderately higher latencies, they were no different from the vehicle-treated males. Triazolam produced a higher magnitude of memory impairment, evidenced by prolonged transfer latencies on second day trial, in diestrous females than estrous, metestrous or proestrus females. Unlike in estrous, diestrous and proestrous females, allopregnanolone failed to prolong the latencies in metestrous females compared to vehicle control. Further, triazolam was relatively more potent than allopregnanolone in impairing the plus-maze learning task across the estrous cycle in female rats. This indicates that the mechanisms involved in the triazolam and allopregnanolone-induced learning impairment may differ from that of hyperphagia, as the potency of triazolam in modulating the cognitive task is high in diestrous compared to its high hyperphagic potency in estrous. Acetylcholine and glutamate are important neurotransmitters involved in learning and memory processes (29). Allopregnanolone inhibits the release of acetylcholine in the hippocampus in conscious rats (7). In the present study, dehydroepiandrosterone sulfate has improved the elevated plus-maze learning performance in male, but not in female rats, indicating that the effect is sex dependent. These observations further highlight the significance of gonadal hormones in the learning task and implicate the higher endogenous levels of $GABA_A$ active neurosteroids in females (26) in the effect of dehydroepiandrosterone sulfate in the plus-maze learning task. This notion can be supported by the in vitro pharmacological antagonism between endogenous pregnane neurosteroids and dehydroepiandrosterone sulfate at the $GABA_A$ receptor complex (20,27). In addition, the higher androsterone levels of males may also contribute to the sex-related differences in the learning and memory processes.

Transfer latency in the elevated plus-maze task has been validated as an index of learning and memory for a variety of agents (16,40), including benzodiazepine ligands (24,29). Although the effects of neurosteroids and benzodiazepines on anxiety may influence learning performance in this task, the changes in transfer latencies observed cannot be solely attributed to their effects on anxiety behavior, because the transfer latency in the elevated plus-maze was not affected by anxiolytics and anxiogenics (24), and a reduction in anxiety is clearly not necessarily associated with an impairment in the plus-maze learning task. For example, 8-OH-DPAT, a 5-HT_{1A} receptor agonist and anxiolytic agent, did not affect the transfer latencies on either day (24). In the present study, DHEAS (5 mg/kg), at doses that do not elicit an anxiogenic effect in the elevated plus-maze test (32), significantly facilitated the learning task. Taken together, these data suggest that the neurosteroid-induced effects on learning are at least partly independent of their anxiogenic or anxiolytic effects on the elevated plus-maze. However, it remains possible that the effects of higher doses of neurosteroids may be associated with nonspecific behavioral effects that may interfere with the interpretation of drug effects in the learning task.

Further, the training and retention was assessed under different conditions or stages of the estrous cycle. However, test substances may affect acquisition when administered before training, while retention can be altered by treatments given after learning trials (memory storage) or before retention test (memory retrieval) (29). In our study, each drug was given 30 min prior to the test on the first day to assess the influence of

estrous cycle or gender on the effects of neurosteroids and benzodiazepine on learning performance. Thus, under our test conditions, the learning performance was assessed in a single estrous cycle phase. However, it is difficult to rule out whether the memory storage or retrieval phases might have been influenced by the changing hormones.

These differential behavioral effects of neurosteroids and benzodiazepines are consistent with [^{35}S]TBPS binding assays during the estrous cycle and functional sensitivity of the $GABA_A$ receptors to neuroactive steroids (10,23). The doses utilized of either neurosteroid should not have caused sedation or ataxia because similar doses did not affect locomotor activity and rota-rod performance (33,35). One of the other limitations in our study is that we have not delineated the role of each of the ovarian hormones by performing ovariectomy and administration of steroid regimens that produced steroid levels comparable to those found in the stages of the estrous cycle. However, it is speculated that variations in estrous cycle-related progesterone metabolite levels and their influence on $GABA_A$ receptor function may underlie certain cyclic behavioral differences (4,9,13). The changes in $GABA_A$ receptor binding after estradiol have been attributed to genomic mechanisms (39). Further, these estrous cycle and sex-related differences in the neurosteroid potency were not pharmacokinetic, because plasma levels do not differ between male and different estrous cycle females injected with allopregnanolone (9). However, it is possible that the metabolic clearance of triazolam may have been slowed in estrus (1), thus generating higher concentration of triazolam or its active metabolites that may produce the highest hyperphagia in estrous stage. This explanation is unlikely, because triazolam produced its highest acquisition deficits in diestrous, but not in estrus females.

In conclusion, these results confirm the significant role of sex and estrous cycle in modifying the effect of neurosteroids allopregnanolone, dehydroepiandrosterone sulfate, and benzodiazepine triazolam on food consumption and elevated plus-maze learning behaviors in rats. Although the effects of triazolam and neurosteroids on anxiety behavior may be an impediment to the interpretation of their effects on the elevated plus-maze learning task, the differential data suggest that the actions are at least partly specific to learning behavior.

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