Pharmacology Biochemistry and Behavior, Vol. 58, No. 3, pp. 637–642, 1997

0091-3057/97 $17.00 + .00

ELSEVIER

PII S0091-3057(97)00004-X

Ketanserin and Anxiety Levels: Influence of Gender, Estrous Cycle, Ovariectomy and Ovarian Hormones in Female Rats

GABRIELA DÍAZ-VÉLIZ,[1] TATIANA ALARCÓN, CLAUDIA ESPINOZA, NELSON DUSSAUBAT AND SERGIO MORA

Unidad de Farmacología, Campus Oriente, Facultad de Medicina, P.O. Box 16038, Universidad de Chile, Santiago, Chile

Received 12 February 1995; Revised 2 December 1996; Accepted 31 December 1996

DÍAZ-VÉLIZ, G., N. T. ALARCÓN, C. ESPINOZA, N. DUSSAUBAT AND S. MORA. *Ketanserin and anxiety levels: Influence of gender, estrous cycle, ovariectomy and ovarian hormones in female rats.* PHARMACOL BIOCHEM BEHAV **58**(3) 637–642, 1997.—The influence of gender, estrous cycle, ovariectomy and ovarian hormones on the behavioral effects of the 5-HT_2 receptor antagonist, ketanserin (KET), was studied. Intact males, female rats in the four stages of the estrous cycle and ovariectomized (OVX) female rats 14 days after surgery were used. The OVX rats received progesterone [PROG, 25 mg/kg, subcutaneously (SC)] and/or estradiol benzoate (EB, 10 μg/kg, SC). KET (3 mg/kg, SC) was injected 30 min before testing. All the animals were subjected to the following behavioral tests: exploration of an elevated plus-maze and retention of a passive-avoidance response. KET enhanced the exploration of the open arms in diestrous female rats but inhibited this behavior during the other stages of the cycle and in OVX rats injected either with oil or EB. This dose of KET was ineffective in males and in OVX rats injected with PROG. Furthermore, KET inhibited the retention of the passive avoidance response in males, in diestrous and metestrous female rats and in OVX rats injected with oil. In estrous females and in OVX rats injected with EB, KET enhanced the passive-avoidance response. These results demonstrate that the sensitivity to KET differs with the gender, estrous cycle and hormonal treatment and suggest that central serotonergic activity is influenced by the hormonal status of the animal. © 1997 Elsevier Science Inc.

Ketanserin Elevated plus-maze Passive avoidance Estrous cycle Estradiol Progesterone

MANY aspects of behavior and neural functions in the rat are influenced by the hormonal changes that occur during the estrous cycle or following ovariectomy and ovarian hormone administration. Previous behavioral studies have shown that a two-way active avoidance performance is improved at diestrus but deteriorated at estrus (13). Furthermore, ovariectomy enhances avoidance conditioning, whereas systemic administration of a single dose of estradiol benzoate impairs this behavior (14). Progesterone prevents the impairment of the two-way avoidance acquisition at estrus and antagonizes the depressant effects of estradiol (15). The avoidance acquisition has been considered a valid animal model of anxiety. Acute administration of benzodiazepines improves avoidance performance, mainly during early acquisition, and this effect can be explained as the consequence of an anxiolytic action (5). These results led us to suggest that the changes in the avoidance acquisition observed across the estrous cycle or induced by exogenous administration of ovarian hormones could reflect variations of basal anxiety levels. Moreover, experimental evidence has shown that estradiol and progesterone exert anxiolytic effects in paradigms that are typically used to assess the anxiolytic potency of drugs (19,25,26,31). The elevated plus-maze test used in the present study has been behaviorally, physiologically and pharmacologically validated as an animal model of anxiety in rats (28–30). Recently, we showed that exploration of the plus-maze differed according to the stages of the estrous cycle and the environmental light intensity (25). In fact, open-arm exploration by rats in proestrus and estrus was higher than exploration by rats in other stages of the cycle when the test was conducted under low-light con-

[1] To whom requests for reprints should be addressed.

ditions. Nevertheless, we found that under low-light intensity there was no significant effect of ovarian hormones on the plus-maze exploration by ovariectomized (OVX) rats (25).

The effects of gonadal steroids on the serotonergic (5-HT) nervous system of mammals are well established (24). Higher levels of 5-HT and 5-HIAA were found in the brains of female rats than in the brains of male rats (9). In addition, the 5-HT synthetic capacity is higher in the female rat brain (9,16). Neurochemical and electrophysiological studies have shown modifications related to the estrous cycle in the activity of 5-HT brain systems (32). During the estrous cycle of the rat, there are changes in serotonin binding (3); cortical [^{3}H]5-HT binding is low on the morning of proestrus and then increases during estrus (32,34). Furthermore, treatment of OVX rats with estradiol produces a rapid decrease in 5-HT_1 receptors, with a concomitant increase in 5-HT_2 receptors (4,34).

Pharmacological and behavioral evidence suggests that serotonergic neurons may be involved in the control of anxiety (11,12,20,21). A reduction in 5-HT neurotransmission reduces anxiety, whereas stimulation of the 5-HT system results in an anxiogenic effect (10,11). Moreover, there is evidence for an anxiolytic effect of 5-HT_2 antagonists as assessed by the elevated plus-maze model (10,20). However, there is strong evidence in support of a role for central 5-HT in passive avoidance behavior. Specifically, stimulation of the central 5-HT system has been shown to interfere with passive-avoidance retention (18,33). Systemic administration of 5-HT receptors antagonists both nonselective (metergoline, methysergide) and 5-HT_2 selective (pirenperona, ketanserin and mianserin) have been shown to facilitate the memory of a previously learned avoidance response (1,2).

The present study was designed to explore whether variations of gonadal hormones might influence nonsexual behavioral effects of acute serotonergic antagonist administration. We studied the influence of ketanserin (KET), a selective 5-HT_2 antagonist, on two different paradigms: exploration of an elevated plus-maze and retention of a passive-avoidance response. In both experiments, intact males, cycling female rats at four stages of the estrous cycle, OVX rats and OVX rats with administration of ovarian hormones were used.

METHODS

Subjects

Twenty male and 160 female Sprague–Dawley rats, weighing 180–200 g, were used throughout the experiments. They were housed in groups of six, with free access to food and tap water. The rats were maintained on a 12-h light period beginning at 0800 h.

Intact female rats ($n = 80$) were subjected daily to vaginal smears to determine the different stages of the estrous cycle. Only rats exhibiting at least three consecutive regular 4-day cycles were included in this study. The female rats were assigned to four groups according to the stage of the estrous cycle on the experimental day: proestrus, diestrus, estrus and metestrus. Eighty female rats were bilaterally ovariectomized under light ether anesthesia. Vaginal smears were taken for at least 4 days before commencement of hormone administration; rats were invariably found in diestrus, confirming the completeness of ovariectomy. Fourteen days after surgical removal of the ovaries, rats were injected with progesterone (PROG, 25 mg/kg) or corn oil (1 ml/kg). This dose of PROG has demonstrated to influence behavioral changes in intact and ovariectomized rats, which are not seen with lower doses without inducing ataxia or sedative effects (8,15). Three hours after PROG, rats were also injected with estradiol benzoate (EB, 10 μg/kg) or corn oil (1 ml/kg). Both hormones were dissolved in corn oil and injected subcutaneously (SC) in the dorsal region of the neck. Behavioral experiments started 6 h after PROG injection.

Male and OVX rats were handled on several consecutive days before the experiments in the same manner as the intact females to exclude handling-induced differences between groups.

Drug

Ketanserin tartrate (KET; Research Biochemicals Inc., Natick, MA) was dissolved in 0.9% saline and administered SC (3 mg/kg) in an injection volume of 1 ml/kg 30 min before testing. Control rats received the same volume of saline. Rats were injected with KET or saline only once and were tested between 1000 and 1400 h.

Elevated Plus-Maze

The apparatus consisted of two open (50 × 10 cm each) and two enclosed (50 × 10 × 20 cm each) arms connected by an open central area (10 × 10 cm) arranged such that the two arms of each type were opposite each other. The maze was elevated to a height of 100 cm. Twenty-five minutes after KET or saline injection, each rat was placed in a glass chamber (35 × 35 × 35 cm) for 5 min. According to Pellow et al. (28,29), this procedure induces an elevation of the total arm entries on the maze. Each rat was then placed on the center of the maze, facing toward one enclosed arm. During a 5-min test period, the following responses were recorded: (a) number of open-arm entries, (b) number of enclosed-arm entries, (c) time spent in open arms and (d) time spent in enclosed arms. A rat was considered to have entered an arm if all four limbs had left the central area of the maze. Because illumination seems to play a crucial role in the plus-maze behavior of rats (30), the test was conducted under low-light intensity (approximately 10 lux). Both the glass chamber and the maze were wiped thoroughly after each trial. Because in this test anxiety is reflected in the unconditioned aversion to heights and open spaces, the percentages of entries and time spent in the open arms provide measures of fear-induced inhibition of exploratory activity. This ratio is increased by anxiolytics and reduced by anxiogenic compounds (28–30).

Passive-Avoidance Behavior

This test was carried out in a two-way shuttle box (Lafayette Instrument Co., Lafayette, IN) composed of two stainless steel modular testing units of equal dimensions (30 × 20 × 20 cm), with a manual guillotine door placed between units. One compartment remained illuminated and the other was darkened. The dark compartment was equipped with a 18-bar grid floor connected to a Master Shock Supply (Lafayette Instrument Co.). On day 1, each rat was habituated to the apparatus by placing it into the lighted compartment, away from the guillotine door, and the rat allowed to enter the dark compartment. Time (latency) to enter the dark compartment was recorded, and 10 s later the rat was returned to its home cage. Because rats prefer dark to light, they enter within 5–15 s. On day 2 (training), each rat was placed into the lighted compartment, and when it entered the dark compartment the guillotine door was lowered and an unavoidable foot shock (0.35

mA × 2 s) was applied through the grid floor. The rat was removed from the apparatus 10 s later. On day 3 (retention), each animal was placed into the lighted compartment, and its latency to reenter the dark compartment (cutoff time = 300 s) was recorded. Rats were injected with KET or saline 30 min before retention (on day 3). This procedure is widely used to measure memory alterations following drug administration. Injecting the drug shortly before the retention test may affect retrieval processes, but other noncognitive factors, such as fear or anxiety, are almost certainly involved. Some anxiolytics given before the retention test inhibit retention of passive avoidance by lowering the latency to enter the dark compartment (11).

Early on day 3, rats were injected with PROG, EB, KET or the corresponding solvents 6 h, 3 h and 30 min before the plus-maze test, respectively. Immediately after the injection, each rat was placed into the shuttle box, and the retention test was performed. The order of tests was always the same in all rats.

Data Analysis

The results of the elevated plus-maze test were expressed as the means and standard errors of the time spent on open arms as a percentage of total time on all arms, and the percentage of open-arm to total-arm entries. Results were analyzed by using a two-way analysis of variance (ANOVA) followed by a post hoc Newman-Keuls multiple comparison test. Passive-avoidance latencies were expressed as medians and interquartile ranges. Because of the arbitrary cutoff latency used, the results were evaluated by using nonparametric Kruskal–Wallis ANOVA, and the differences between groups were estimated by individual Mann–Whitney *U*-tests. A minimum acceptable level of significance was set at $p < 0.05$.

RESULTS

Effect of KET on the Elevated Plus-Maze in Intact Rats

The ANOVA revealed significant effects of KET treatment [$F(1, 90) = 4.27$; $p < 0.05$] and hormonal status (gender and stage of the estrous cycle) [$F(4, 90) = 2.45$; $p < 0.05$], on the percentage of entries onto the open arms of the elevated plus-maze. The interaction between both factors was also significant [$F(4, 90) = 5.81$; $p < 0.005$]. Post hoc analysis between saline-treated groups showed a significant increase of the percentage of entries onto the open arms during the stages of proestrus and estrus vs. diestrus ($p < 0.05$ in both cases). Administration of KET failed to induce changes in the exploration of the plus-maze in male rats but significantly increased this behavior in diestrous rats ($p < 0.05$) and induced a significant decrease ($p < 0.05$) in the other three stages of the cycle as compared with saline control rats (Fig. 1, top panel).

Figure 1 (bottom panel) shows a significant effect of KET treatment [$F(1, 90) = 3.40$; $p < 0.05$] and hormonal status [$F(4, 90) = 8.32$; $p < 0.005$] in the percentage of time spent on the open arms of the elevated plus-maze. The interaction between KET and hormonal status was also significant [$F(4, 90) = 4.07$; $p < 0.005$]. Post hoc comparisons revealed a significant increase in the percentage of time spent on the open arms in proestrous and estrous rats vs. male rats and female rats at diestrus ($p < 0.05$ in all cases). KET produced a significant increase in the percentage of time spent in the open arms of diestrous rats ($p < 0.05$) and a decrease in rats at proestrus, estrus and metestrus ($p < 0.05$ in all cases) as compared with saline control rats. KET failed to produce significant changes in male rats.

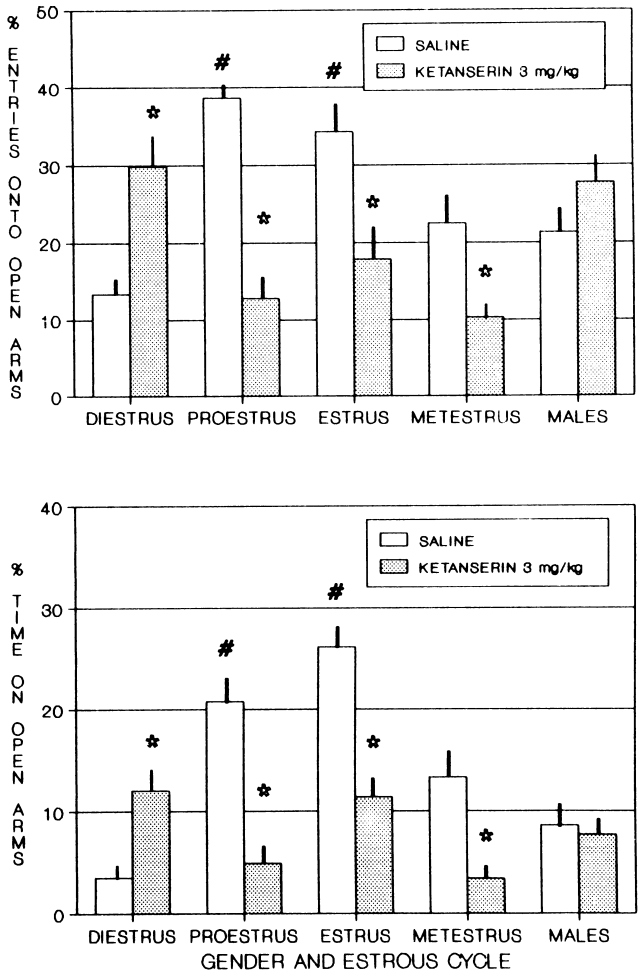

FIG. 1. Effects of KET (3 mg/kg, SC) on plus-maze activity in intact male and female rats. Top: Entries onto open arms as percentage of total arm entries. Bottom: Time on open arms as percentage of total time in both arms. Data represent the means ± SEM of 10 rats per group. Comparisons were made by using a two-way ANOVA and the post hoc Newman-Keuls test. *$p < 0.05$ vs. respective control group; differences from other saline groups: #$p < 0.05$.

Effect of KET on the Elevated Plus-Maze in OVX Rats

Two-way ANOVA revealed significant effects of KET treatment [$F(1, 72) = 5.36$; $p < 0.05$], on the percentage of entries onto the open arms of the elevated plus-maze. The interaction between KET and hormonal treatment was also significant [$F(3, 72) = 2.85$; $p < 0.05$]. KET significantly decreased the percentage of entries onto the open arms in OVX rats and after EB treatment ($p < 0.05$ in both cases); no other effects were significant (Fig. 2, top panel).

Inspection of the data in Fig. 2 (bottom panel) indicates a significant effect of KET treatment [$F(1, 72) = 5.76$; $p < 0.05$] on the percentage of time spent on the open arms of the elevated plus-maze. KET significantly decreased the percentage of time on the open arms in OVX rats and after EB treatment ($p < 0.05$ in both cases).

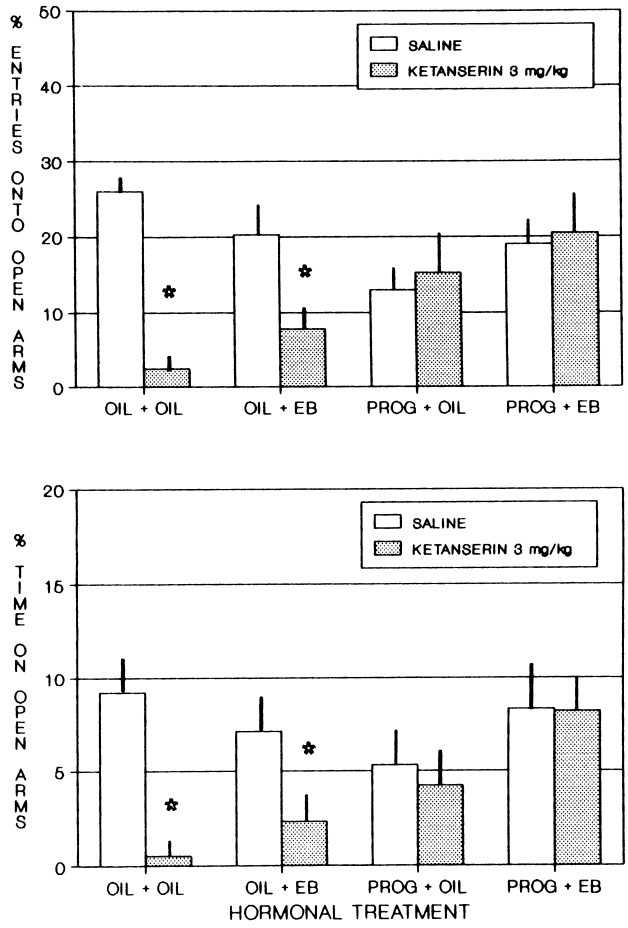

FIG. 2. Effect of KET (3 mg/kg, SC) on plus-maze activity in OVX rats treated with EB (10 μg/kg), PROG (25 mg/kg) or PROG + EB. Top: Entries onto open arms as percentage of total arm entries. Bottom: Time on open arms as percentage of total time in both arms. Data represent the means ± SEM of 10 rats per group. Comparisons were made by using two-way ANOVA and the post hoc Newman-Keuls test. *$p < 0.05$ vs. respective control group.

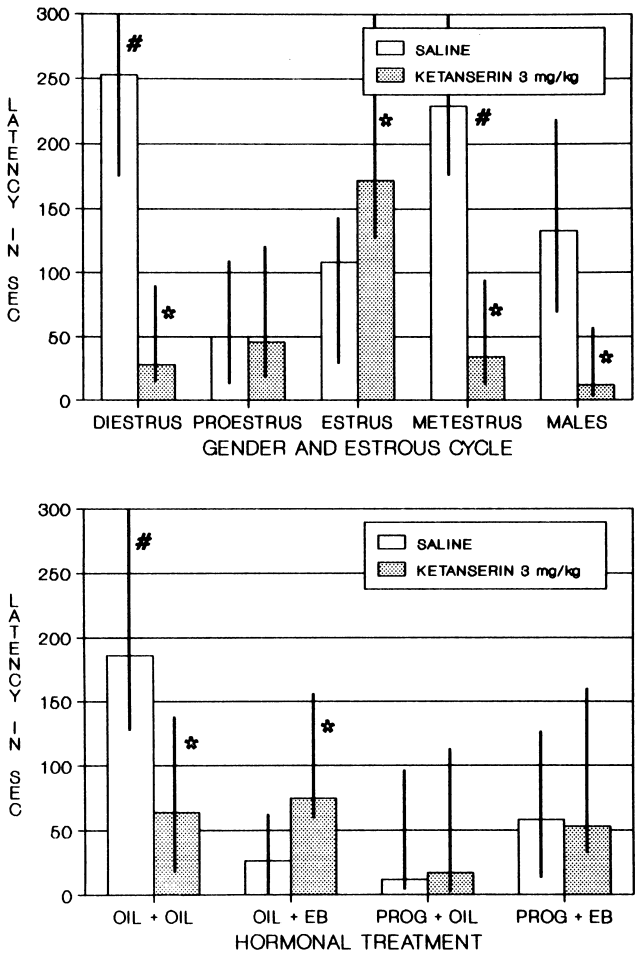

FIG. 3. Effects of KET (3 mg/kg, SC) on the retention of a passive-avoidance response. Top: Influence of the hormonal status (male and intact cycling female rats). Bottom: Influence of treatment with EB (10 μg/kg), PROG (25 mg/kg) or PROG + EB. Data are expressed as medians and interquartile range of passive avoidance latencies (n = 10 rats per group). Comparisons were made by using nonparametric Kruskal–Wallis ANOVA and the post hoc Mann–Whitney U-test. *$p < 0.05$ vs. respective control group; differences from other saline groups: #$p < 0.05$.

Effect of KET on Passive-Avoidance Behavior in Intact Rats

Kruskal–Wallis ANOVA showed significant changes in retention of a passive-avoidance response induced by KET, gender and the estrous cycle [$H(9) = 20.215$; $p < 0.005$]. Avoidance latencies were significantly enhanced in diestrous and metestrous rats ($p < 0.05$ in both cases) compared with the other saline groups (Fig. 3, top panel). KET treatment significantly facilitated passive-avoidance behavior in estrous rats ($p < 0.05$), but the avoidance latencies were significantly lowered in male rats ($p < 0.05$) and in female rats at diestrus ($p < 0.005$) and metestrus ($p < 0.05$).

Effect of KET on Passive-Avoidance Behavior in OVX Rats

Kruskal–Wallis ANOVA revealed significant effects of ovarian hormones on passive-avoidance retention [$H(7) = 16.612$, $p < 0.01$]. Both PROG and EB significantly inhibited passive-avoidance behavior in OVX saline-treated rats ($p < 0.01$ in both cases) (Fig. 3, bottom panel). KET administration exerted a significant decrease in avoidance latencies in OVX oil-treated rats ($p < 0.05$); however, in OVX rats primed with EB, KET facilitated passive-avoidance behavior ($p < 0.05$). Administration of KET to OVX rats that also received PROG was without significant effect (Fig. 3, bottom panel).

DISCUSSION

The present data, in agreement with previous findings (25), showed that the gender, estrous cycle and ovarian hormones modify exploration of the elevated plus-maze and the retention of a passive-avoidance conditioned response.

In the present experiment, we observed changes in anxiety levels along the different phases of the estrous cycle. The results from the elevated plus-maze experiments revealed that cycling female rats at diestrus showed the highest levels of anxiogenic-like behavior, whereas lower anxiety levels during proestrus and estrus were observed. KET induced an anxiogenic-like behavior, except in diestrous rats, in which KET showed an anxiolytic effect. This anxiogenic-like effect of KET

also was evident in oil-treated and EB-replaced OVX females, but not in those animals treated with PROG alone or with EB. Although we failed to demonstrate a significant effect of both EB and PROG in the present study, previously we demonstrated that PROG has an anxiolytic-like effect under strong-light conditions (25). Finally, KET did not influence anxiety levels in male rats. These observations are consistent with the findings of other investigators (10–12,19,20), but they contrast with results that failed to demonstrate significant changes in plus-maze exploration during the estrous cycle or after ovariectomy (26). Discrepancies can be attributed to differences in the experimental conditions adopted. Low environmental illumination, multiple handling, pretest exposure to an unfamiliar environment and the fact that all the animals received a shock from passive-avoidance training the day before also may have influenced basal anxiety level and the subsequent response to KET.

Some studies have reported that modifications of serotonergic activity, depending on the dose used, can induce either anxiolytic-like or anxiogenic-like effects in some reward–punishment conflict paradigms (23). The present results suggest that both anxiogenic-like and anxiolytic-like effects of KET also can be observed, depending on the hormonal status of the rat. In the normal cycling rat, KET shows anxiogenic-like effects under high estrogen (proestrus) or low PROG levels (estrus and metestrus) (6,7). In contrast, KET induces an anxiolytic-like behavior in the plus-maze that is evident under low estrogen and moderate PROG levels (diestrus) (6,7). This finding agrees with a study that used the burying behavior test and that demonstrated low anxiety levels during proestrus, presumably related to the peak of steroid hormones (19). The crucial role of PROG in these effects is further supported by the experiments performed in OVX rats. KET increases anxiogenic-like behavior under low estrogen and low PROG (OVX + oil) and under high estrogen (OVX + EB), but this effect is prevented when the OVX animals are treated with PROG. Ovarian hormone fluctuations that occur across the estrous cycle (6,7) could account for the changes in the anxiety levels in the female rats (25,26). In addition, our results suggest that the activity of 5-HT system involved in the regulation of anxiety could change according to the hormonal status, as it has also been postulated by other investigators (3,4,19,31,32).

Retention of the passive-avoidance behavior also differed according to hormonal status of the rat. Cycling female rats in diestrus and metestrus showed a higher retention of the passive-avoidance response, and KET significantly reduced this retention level. In the OVX rats, an originally high retention latency was also reduced by KET. In contrast, KET increased the retention level in female rats during estrus and in EB-replaced OVX rats. The drug showed no effect in both cycling female rats in proestrus and PROG-replaced OVX females, suggesting an interaction between KET and PROG in this behavior, similar to that observed in the plus-maze.

There is literature on the sex differences in passive-avoidance behavior, with male rats typically performing better than female rats (17,22). In the present study, males performed comparably to females at their hormonal peak in the cycle, but the performance of males was poorer than that of females at diestrus and metestrus, stages with lower estrogen levels. Although administration of the 5-HT_2 selective antagonists significantly facilitated a passive-avoidance response (1,2, 11,33), other investigators failed to show a similar elevation in latencies (27). The facilitatory or inhibitory effects of 5-HT antagonists have been found to be dependent on time of drug administration with respect to training (1,2). In the present study, KET was administrated 30 min before the retention test, which is 24 h after the training. Thus, the drug may be affecting performance by its action on processes other than those underlying memory storage. Interpretation of the effects produced by administration of 5-HT receptor antagonists is complicated because the passive-avoidance performance can be influenced by many factors. For instance, inhibition of the retention induced by KET during diestrus could be due to a disruption of the retrieval processes or be a consequence of the anxiolytic properties of the drug. However, facilitation of the retention induced by KET during estrus and in OVX + EB rats could be confounded with the anxiogenic effects of the drug, as assessed by the elevated plus-maze test. Efforts to develop anxiolytic drugs without effects on memory have failed, leading to the question of an unavoidable link among anxiety, memory and learning. According to Hamon (21), because the same limbic structures (amygdala, hippocampus and septum) are implicated in both anxiety and memory, it is probably illusory to expect anxiolytics (or anxiogenics) to have no effects on cognition. Different animal models may be tapping different aspects of anxiety. A possible conclusion is that the behavior observed in the two models used in the present study does not reflect a unitary concept of anxiety. The elevated plus-maze test possesses a clear advantage over the inhibition of passive-avoidance behavior because it is based on the spontaneous response to an unfamiliar situation and does not use noxious stimuli (electric foot shock) as passive avoidance does. Because animals are expossed to the plus-maze only once, there is no influence of cognitive factors.

In conclusion, the present study supports the suggestion that ovarian hormones modulate anxiety levels, cognitive alterations probably related to anxiety and subsequent responses to KET in male and female rats.

ACKNOWLEDGEMENTS

This work was supported by grants B-3416-9433 from DTI U. de Chile and 193-1071 from FONDECYT, Chile.

REFERENCES

1. Altman, H. J.; Normile, H. J.: Enhancement of the memory of a previously learned aversive habit following pre-test administration of a varity of serotonergic antagonists in mice. Psychopharmacology 90:24–27; 1986.
2. Altman, H. J.; Normile, H. J.: Different temporal effect of serotonergic antagonists on passive avoidance retention. Pharmacol. Biochem. Behav. 28:353–359; 1987.
3. Biegon, A.; Bercovitz, H.; Samuel, D.: Serotonin receptor concentration during the estrous cycle of the rat. Brain Res. 187:221–225; 1980.
4. Biegon, A.; McEwen, B. S.: Modulation by estradiol of serotonin receptors in brain. J. Neurosci. 2:199–205; 1982.
5. Boix, F.; Fernández Teruel, A.; Tobeña, A.: The anxiolytic action of benzodiazepines is not present in handling-habituated rats. Pharmacol. Biochem. Behav. 31:541–546; 1988.
6. Butcher, R. L.; Collins, W. E.; Fugo, N. W.: Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. Endocrinology 94:1704–1708; 1974.
7. Cabrera, R.; Díaz, A.; Pinter, A.; Belmar, J.: In vitro progester-

one effects on ^{3}H-dopamine release from rat corpus estriatum slices obtained under different endocrine conditions. Life Sci. 53:1767–1777; 1993.
8. Canonaco, M.; Valenti, A.; Maggi, A.: Effects of progesterone on [^{3}5S] t-butylbicyclophospho-rothionate binding in some forebrain areas of the female rat and its correlation to aggressive behavior. Pharmacol. Biochem. Behav. 37:433–438; 1990.
9. Carlsson, M.; Svensson, K.; Eriksson, E.; Carlsson, A.: Rat brain serotonin: Biochemical and functional evidence for a sex difference. J. Neural Transm. 63:297–313; 1985.
10. Ceulemans, D. L. S.; Hoppenbrouwers, M. L. J. A.; Gelders, Y. G.; Reyntjens, A. J. M.: The influence of ritanserin, a serotonin antagonist, in anxiety disorders: A double-blind placebo-controlled study versus lorazepam. Pharmacopsychiatry 18:303–305; 1985.
11. Chopin, P.; Briley, M.: Animal models of anxiety: The effects of compounds that modify 5-HT neurotransmission. Trends Pharmacol. Sci. 8:383–388; 1987
12. Critchley, M. A. E.; Handley, S. L.: Effects in the X-maze anxiety model of agents acting at 5HT1 and 5HT2 receptors. Psychopharmacology 93:502–506; 1987.
13. Díaz-Véliz, G.; Soto, V.; Dussaubat, N.; Mora, S.: Influence of the estrous cycle, ovariectomy and estradiol replacement upon the acquisition of conditioned avoidance responses in rats. Physiol. Behav. 46:397–401; 1989.
14. Díaz-Véliz, G.; Urresta, F.; Dussaubat, N.; Mora, S.: Effects of estradiol replacement in ovariectomized rats on conditioned avoidance responses and other behaviors. Physiol. Behav. 50:61–65; 1991.
15. Díaz-Véliz, G.; Urresta, F.; Dussaubat, N.; Mora, S.: Progesterone effects on the acquisition of conditioned avoidance responses and other motoric behaviors in intact and ovariectomized rats. Psychoneuroendocrinology 19:387–394; 1994.
16. Dickinson, S. L.; Curzon, G.: 5-Hydroxytryptamine-mediated behavior in male and female rats. Neuropharmacology 25:771–776; 1986.
17. Drago, F., Bohus, B.; Scapagnini, U.; De Wied, D.: Sexual dimorphism in passive avoidance behavior of rats: Relation to body weight, age, shock intensity and retention interval. Physiol. Behav. 24:1161–1164; 1980.
18. Essman, W. B.: Age dependent effects of 5-hydroxytryptamine upon memory consolidation and cerebral protein synthesis. Pharmacol. Biochem. Behav. 1:7–14; 1973.
19. Fernández-Guasti, A.; Picazo, O.: The actions of diazepam and serotonergic anxiolytics vary according to the gender and the estrous cycle phase. Pharmacol. Biochem. Behav. 37:77–81; 1990.
20. Gibson, E. L.; Barnfield, A. M. C.; Curzon, G.: Evidence that mCPP-induced anxiety in the plus-maze is mediated by post-synaptic 5-HT_2c receptors but not by sympathomimetic effects. Neuropharmacology 33:457–465;1994.
21. Hamon, M.: Neuropharmacology of anxiety: Perspectives and prospects. Trends Pharmacol. Sci. 15:36–39;1994.
22. Heinsbroek, R. P. W.; Feenstra, M. G. P.; Boon, P.; Van Haaren, F.; Van de Poll, N. E.: Sex differences in passive avoidance depend on the integrity of the central serotonergic system. Pharmacol. Biochem. Behav. 31:499–503; 1988.
23. Hjorth, S.; Söderpalm, B.; Engel, J. A.: Biphasic effect of L-5-HTP in the Vogel conflict model. Psychopharmacology 92:371–375; 1987.
24. Maggi, A.; Perez, J.: Role of female gonadal hormones in the CNS: Clinical and experimental aspects. Life Sci. 37:893–906; 1985.
25. Mora, S.: Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology; 21:609–620; 1996.
26. Nomikos, G. G.; Spiraki, C.: Influence of oestrogen on spontaneous and diazepam-induced exploration of rats in an elevated plus maze. Neuropharmacology 27:691–696; 1988.
27. Ögren, S. O.; Johansson, C.: Separation of the associative and non-associative effects of brain serotonin released by p-chloroamphetamine: Dissociable serotonergic involvement in avoidance learning, pain and motor function. Psychopharmacology 86:12–26; 1985.
28. 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.
29. Pellow, S.; File, S.: Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: A novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24:525–529;1986.
30. Reibaud, M.; Bohme, G. A.: Evaluation of putative anxiolytics in the elevated plus-maze test. Methods Neurosci. 14:230–239; 1993.
31. Rodríguez-Sierra, J. F.; Hagley, M. T.; Hendricks, S.: Anxiolytic effects of progesterone are sexually dimorfic. Life Sci. 38:1841–1845; 1986.
32. Uphouse, L.; Williams, J.; Eckols, K.; Sierra, V.: Cortical changes in serotonin receptors during the female rat estrous cycle. Brain Res. 381:376–381; 1986.
33. Wetzel, W.; Getsova, V. M.; York, R.; Matthies, H.: Effect of serotonin on Y-maze retention and hippocampal protein synthesis in rats. Pharmacol. Biochem. Behav. 12:319–322; 1980.
34. Williams, J.; Uphouse, L.: Serotonin binding sites during proestrus and following estradiol treatment. Pharmacol. Biochem. Behav. 33:615–620; 1989.