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Effects of LHRH on Avoidance Conditioning in Normally Cycling and Ovariectomized Female Rats

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MORA, S., N. DUSSAUBAT AND G. DÍAZ-VÉLIZ. *Effects of LHRH on avoidance conditioning in normally cycling and ovariectomized female rats.* PHARMACOL BIOCHEM BEHAV **61**(3) 221–228, 1998.—Several studies have demonstrated that the peptide LHRH can modify behavior in the male rat. Peripheral and intracerebral infusions of LHRH impair the acquisition of conditioned avoidance responses (CARs) and increase some spontaneous motor behaviors, such as head shaking and grooming. The present study was undertaken to detect the effects of LHRH on the acquisition of CARs and spontaneous motility in normally cycling and ovariectomized (OVX) Sprague–Dawley female rats. Normally cycling females were separated in four groups, according to the stage of the estrous cycle. Ovariectomized female rats were pretreated, 48 h before the experiment, with estradiol benzoate (10 μg/kg) or corn oil. LHRH (6.25, 25, or 50 μg/kg) was subcutaneously injected and the behavioral tests began 1 h after. Low doses of LHRH stimulated the acquisition of CARs during proestrus, estrus, and metestrus, whereas higher doses impaired conditioning in all the four stages of the cycle. High doses of LHRH impaired acquisition in OVX rats treated with oil and potentiated the depressant effects of EB on this behavior. The effects of LHRH on spontaneous motor activity were either stimulatory or inhibitory, according to the hormonal status and the dose administered. High doses of LHRH decreased motor responses in the diestrous rat. All the doses of LHRH increased the number of headshakes during proestrus, estrus, and metestrus, while the other motor responses were scarcely or not affected by LHRH in these stages. In OVX rats LHRH increased rearing, head shaking, and grooming behavior. These results support a role of LHRH in the modulation of conditioned and spontaneous behavior. They could provide an explanation to the behavioral changes observed across the estrous cycle and those observed after EB priming in OVX rats. © 1998 Elsevier Science Inc.

LHRH Estrous cycle Estradiol Ovariectomy Avoidance conditioning Motor responses

THE hypothalamic decapeptide luteinizing hormone releasing hormone (LHRH) was originally thought to function only as a neurohormone that regulates the reproductive system by stimulating the release of gonadotropins from the anterior pituitary, but it is now clear that LHRH has diverse functions. Within the central nervous system (CNS), LHRH is currently thought to act as a neurotransmitter or neuromodulator. This notion is supported by findings showing that LHRH is distributed throughout the brain (23), and that LHRH receptors are present in several brain areas (15,37). Dense hippocampal concentrations of LHRH receptors have been described, and LHRH binding sites were also found in other limbic structures (20). In addition, it has been well established that both centrally and peripherally administered LHRH exerts direct actions in CNS of both male and female rats.

The first behavioral effect of LHRH to be demonstrated was the facilitation of sexual behavior in male (11) and female rats (33). This LHRH action was found independent of gonadotropins, ovarian hormones, and the pituitary–adrenal axis, because the peptide was active in hypophysectomized, ovariectomized, and adrenalectomized rats (34,36). A significant research about the behavioral effects of LHRH in rats has been carried out in our laboratory. We demonstrated that, in the two-way active avoidance paradigm, the peripheral LHRH

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administration before training impairs the acquisition in intact (24) and castrated (26) male rats. LHRH given after the acquisition trial significantly improves the retention of the task (27). The effect on memory recall was also observed in a passive avoidance paradigm, in which LHRH facilitated and impaired retention of the response according to the dose and the intensity of the foot shock applied during training (27).

The mechanism of action of LHRH on behavior is still unknown, but there is evidence suggesting that LHRH could interact with dopamine (DA) systems in the brain to exert a wide range of effects. LHRH antagonizes the improved performance induced by amphetamine in the acquisition of the active avoidance response (25). Pretreatment with L-DOPA antagonized the inhibitory effects of LHRH on this behavior, and attenuated the antagonism between LHRH and amphetamine (28). In addition, in vitro evidence demonstrates an inhibitory action of LHRH upon DA synthesis and release in the corpus striatum (29,41). The direct infusion of LHRH into several brain regions (lateral ventricle, hippocampus, nucleus accumbens, and corpus striatum) also impairs the acquisition of the active avoidance performance; nevertheless, only the intrastriatal LHRH injection induced an immediate dose-related disruption of the response (30). This supports the idea that the DA nigrostriatal system could be a locus of the LHRH actions in the rat brain.

Other investigators have also suggested a possible action of LHRH on learning and memory (5–7). In a conditioned place-preference procedure, LHRH can function as a dose-dependent appetitive stimulus in male but not in female rats, showing that LHRH can also cause behavioral effects by associative learning, thus influencing the organism during the process of behavioral adaptation (5). In addition, being an appetitive stimulus in rats, LHRH, injected either intraperitoneally (6) or intracerebrally (7), might become a discriminative stimulus in a two-lever, food-reinforced drug discrimination procedure. The administration of an analog of LHRH, D-Trp6-LHRH, enhanced the acquisition of the visual discrimination of the T-maze (appetitive learning) in young mature female rats (35). Thus, LHRH may also affect positively reinforced tasks.

Two-way active avoidance conditioning changes according to the hormonal fluctuations occurring across the estrous cycle of the female rat (8). Thus, conditioning is facilitated at diestrus, but it is decreased at proestrus and almost abolished at estrus and metestrus. The disruption in the acquisition of the conditioned task during certain stages of the estrous cycle could be mediated through increased levels of endogenous LHRH. The finding that the administration of a LHRH antagonist at noon on the day of proestrus prevents the impairment in acquisition observed in the subsequent days (31) supports this possibility.

The present work was designed to investigate the behavioral effects of LHRH in female rats and to study the influence of the estrous cycle, ovariectomy and estradiol replacement upon these effects. The neuropeptide was SC injected in normally cycling rats, ovariectomized rats (OVX), and ovariectomized primed with estradiol (OVX + EB). The results were compared with those observed in male rats to determine a possible sexual dimorphism in the LHRH effects.

METHOD

A total of 37 male and 204 female Sprague–Dawley rats, weighing 180–200 g, were used in the experiments. They were housed in groups of six per cage under a 12 L:12 D cycle (lights on from 0800 to 2000 h) with free access to food and tap water. Female rats were submitted daily to vaginal smears for determination of different stages of estrous cycle. Only rats exhibiting three or more consistent 4-day cycles were used. One hundred thirty-six cycling rats were assigned to four groups according to the phase of the cycle: diestrus, proestrus, estrus, and metestrus. Another group of 68 females was bilaterally ovariectomized under light ether anesthesia. Fourteen days after surgical removal of the ovaries, animals were randomly divided into two groups that received either 0.2 ml of corn oil (OVX + OIL) or 10 μg/kg of estradiol benzoate (OVX + EB), injected SC in the dorsal region of the neck 48 h prior to the administration of LHRH. Before EB administration, the vaginal smears were invariably diestrus, thus confirming the completeness of ovariectomy. The dose of EB used is the minimum amount needed to induce vaginal smears similar to that observed at proestrus and estrus, with latencies of 48 and 72 h, respectively. Each of the experimental groups was SC injected with either synthetic LHRH (Sigma Chemical Co.) dissolved in 2% benzyl alcohol at doses of 6.25, 25, and 50 μg/kg or the vehicle (0.2 ml). The behavioral tests began 60 min after LHRH treatment. Each animal was tested only once between 1000 and 1400 h, by using a fixed design:spontaneous motility measures followed by conditioned avoidance training. These behavioral tests took place in different testing rooms.

Spontaneous Motor Activity

Each animal was individually placed in a Plexiglas cage (30 × 30 × 30 cm), placed inside a sound-attenuated testing chamber. The floor of the cage was an activity platform (Lafayette Instrument Co.) connected to an electromechanical counter. Motor activity was monitored for 30 min and simultaneously the following responses were recorded: number of times each animal reared, number of headshakes, and the time spent in grooming behavior. Each animal was observed continuously for the 30-min observation period, via a video camera connected to a VHS tape recorder. Scores were made from the live picture, and the video sequences were used for some subsequent reanalysis.

Active Avoidance Conditioning

The conditioning experiments were carried in a sound-attenuated testing room by using a two-way shuttle box (Lafayette Inst. Co.) composed of two stainless steel modular testing units, which were equipped with a 18-bar insulated shock grid floor, two 28-V DC lights, and a tone generator (Mallory Sonalert 2800 Hz). Electric shocks were provided to the grid floor by a Master shock supply (Lafayette Inst. Co.). Immediately after the spontaneous motility test, the rats were individually placed in the shuttle box and were trained over 50 trials, after a 5-min period of habituation. Each trial consisted of the presentation of a tone (conditioned stimulus) which, after 5 s, was combined with a 0.2-mA foot shock until the animal escaped to the opposite chamber. The maximum shock duration was 10 s. The trials were separated by 15-s intertrial intervals. A conditioned avoidance response (CAR) was defined as a crossing within the first 5 s (tone alone). If the rat did not escape by crossing the box during the shock it was considered as an escape failure.

Statistics

For each experimental group (n = 8–13 rats) the results are expressed as mean ± SEM. The data were analyzed by

Student's *t*-test and two-way analysis of variance (ANOVA) followed by post hoc Newman–Keuls's multiple comparison test. Differences were always considered significant when p was equal to or less than 0.05.

RESULTS

Active Avoidance Conditioning

Normal intact rats. The results of the active avoidance experiment are shown in Fig. 1. Two-way ANOVA revealed significant effects of hormonal status, $F(4, 153) = 3.27$, $p < 0.05$, and LHRH treatment, $F(3, 153) = 58.82$, $p < 0.01$, on the acquisition of CARs. Post hoc analysis indicated that the avoidance conditioning in solvent injected rats was similar in diestrous and male rats but decreased in proestrous and was almost abolished in estrous and metestrous rats. The significant interaction between LHRH treatment and hormonal status, $F(12, 153) = 13.27$, $p < 0.01$, suggests that the effects of LHRH are dependent upon the estrous cycle and the gender of the rat. Administration of LHRH induced both stimulatory and inhibitory effects on the conditioning performance. LHRH-6.25 significantly improved the acquisition of CARs in the female rats at proestrus, estrus, and metestrus; LHRH-25 moderately increased the response at proestrus and estrus but impaired it at diestrus; and LHRH-50 markedly reduced the acquisition at diestrus and at proestrus. In males, all three doses of LHRH impaired this behavior. No significant differences were observed regarding the foot shock thresholds among the experimental groups. The effects of the interaction between LHRH and estrous cycle on the learning rate, expressed as the percent of CARs by blocks of 10 successive trials, are presented in Fig. 2. LHRH-6.25 increased acquisition rate and LHRH-50 decreased it independently of the stage of the estrous cycle.

Figure 3 shows that the impairment in the acquisition of CARs is accompanied with increased escape failures. Two-way ANOVA revealed significant effects of hormonal status, $F(4, 153) = 2.70$, $p < 0.05$, and LHRH, $F(3, 153) = 13.96$, $p < 0.01$, on the percent of escape failures. The interaction between these two factors was also significant, $F(12, 153) = 2.07$, $p < 0.05$. Post hoc analysis demonstrates that proestrous, estrous, and metestrous rats failed significantly more than diestrous and male rats. All three doses of LHRH increased escape failures in diestrous and male rats. It is interesting that LHRH-50 markedly increased escape failures in the five experimental groups, whereas LHRH-25 reverted the escape failures observed in proestrous, estrous, and metestrous rats.

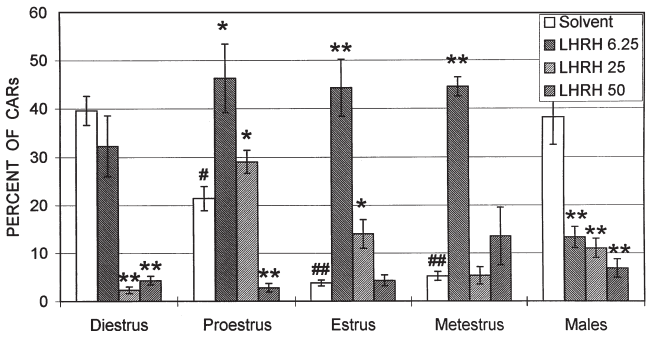

FIG. 1. Effects of several doses of LHRH on the acquisition of conditioned avoidance responses (CARs) in normal cycling female and male rats. Percent of CARs out of 50 trials. Comparisons were made by using two-way ANOVA followed by post hoc Newman–Keuls test: $*p < 0.05$; $*p < 0.0005$ compared with its solvent control group, and $\#p < 0.05$; $\#\#p < 0.0005$ compared with diestrus plus solvent. The number of animals in each group was 8–13.

OVX rats (Fig. 4). Two-way ANOVA indicates significant effects of EB priming, $F(1, 60) = 109.41$, $p < 0.01$, and LHRH, $F(3, 60) = 8.80$, $p < 0.01$, on avoidance conditioning in OVX animals. Post hoc comparisons show that LHRH-25 and -50 significantly impaired acquisition of CARs in OVX rats treated with corn oil. Priming with EB markedly reduced the acquisition of CARs and, in these conditions, further impairment was observed after LHRH-50. Because the interaction between these two factors was significant, $F(3, 60) = 3.23$, $p < 0.05$, the effect of LHRH seems to be dependent upon the hormonal status of the rat.

Figure 5 shows significant effects of LHRH, $F(3, 60) = 21.06$, $p < 0.01$, and EB, $F(1, 60) = 2.87$, $p < 0.05$, on escape failures in OVX rats. LHRH-50 significantly increased escape failures in OVX + OIL rats. Pretreatment with EB markedly increased escape failures; but, in this condition, no significant effect of LHRH was observed.

Spontaneous Motor Responses

Normal intact rats. The overall effects of hormonal status and LHRH on motor behaviors are summarized in Table 1. Data analysis revealed that hormonal status induced significant modifications on motor activity, $F(4, 153) = 5.17$, $p < 0.001$, and head shakes, $F(4, 153) = 7.93$, $p < 0.0001$. Subsequent Newman–Keuls tests indicated that in saline-injected rats, motor activity was lower at proestrus and estrus than at diestrus, while the number of headshakes was lower at estrus and in males but was increased at metestrus. There were significant effects of LHRH treatment on rearings, $F(3, 153) = 4.28$, $p < 0.01$, head shakes, $F(3, 153) = 13.88$, $p < 0.0001$, and grooming behavior, $F(3, 153) = 6.30$, $p < 0.001$. Post hoc comparisons showed that LHRH significantly decreased the number of rearing at diestrus, but increased it during proestrus, estrus, and in males. A significant decrease in the number of headshakes was observed during diestrus after LHRH, but LHRH markedly increased this behavior in the other experimental groups. Finally, LHRH decreased the time spent in grooming behavior, particularly at diestrus and males. The interaction between treatment and hormonal condition was significant on motor activity, $F(12, 153) = 3.37$, $p < 0.001$, rearings, $F(12, 153) = 2.33$, $p < 0.01$, head shakes, $F(12, 153) = 3.15$, $p < 0.001$, and grooming behavior, $F(12, 153) = 3.35$, $p < 0.001$. This suggests that the effects of LHRH on spontaneous motility are dependent upon the hormonal status of the rat.

OVX rats (Table 2). Two-way ANOVA showed significant effects of LHRH on the number of rearing, $F(3, 60) = 5.35$, $p < 0.0$, head shakes, $F(3, 60) = 6.58$, $p < 0.01$, and grooming behavior, $F(3, 60) = 4.60$, $p < 0.01$. Rearings and headshakes were increased in a dose-unrelated way by LHRH treatment in both groups. Grooming behavior was increased only in OVX rats primed with EB.

DISCUSSION

The present study confirms previous observations that the peripheral injection of LHRH induces potent behavioral effects in the rat (24,26,27). Some of these effects are dose dependent and some are modified by the gender and the stage of the estrous cycle in the female. Males were more sensitive to the impairing effects of LHRH upon the acquisition of the conditioned avoidance response (CAR) than female rats. A

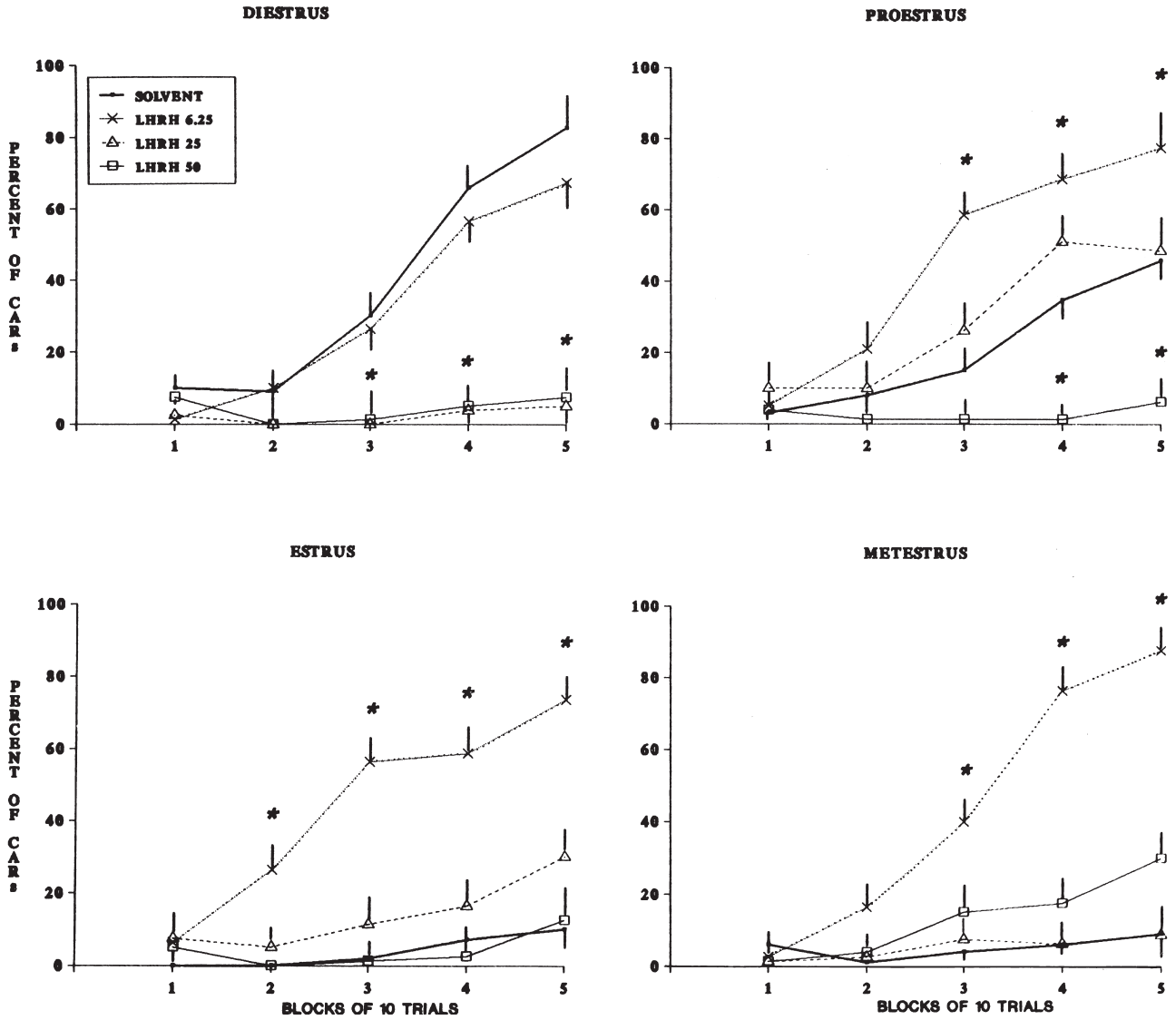

FIG. 2. Effects of different doses of LHRH on the acquisition of CARs across the estrous cycle of the female rat. Each point of the curves represents the mean ± SEM of the percent of CARs by blocks of 10 successive trials. Comparisons were made by using Student's *t*-test: $*p < 0.05$ compared with its solvent control group. The number of animals in each group was 8–13.

low dose of LHRH (6.25 μg/kg) clearly induced a sexual dimorphic effect on this behavior. We demonstrated, in a previous study (26), that both LHRH and testosterone induced a similar impairment in the acquisition of CARs in male rats. The effect of LHRH was apparently not mediated through changing levels of circulating testosterone because the peptide was also active in castrated rats with or without testosterone replacement (26).

In the female rat, the acquisition of CARs is facilitated during diestrus, decreased at proestrus, and almost suppressed at estrus and metestrus (8). Ovariectomy improves the acquisition performance, while priming with estradiol induces a severe disruption of the response, similar to that observed in the intact rat at estrus (9). As this effect takes at least 48 h to appear, estradiol priming might act probably via genomic receptors in neural sites. It is known that estradiol can have both suppressive and inductive effects on hypothalamic LHRH secretion according to the dose and time of administration. Small amounts of estradiol decrease secretion of LHRH, especially by altering the frequency of LHRH pulses (18). On the other hand, the peak in plasma estradiol observed at noon of proestrus induces the preovulatory LH surge, which is accompanied by an increase in pulsatile LHRH release (38). Further, estradiol enhances the pituitary response to LHRH increasing the number of LHRH receptors at the time of LH surge (4). These evidence led us to suggest that the changes in the acquisition of CARs across the estrous cycle are mediated by changes in LHRH activity which, in turn, are triggered by fluctuations in the estradiol level. A physiological role of LHRH in the performance of conditioned responses in female rats was previously postulated (31). We demonstrated that the administration of an anovulatory dose of a synthetic LHRH antagonist at noon during proestrus prevented the impairment of the acquisition pre-

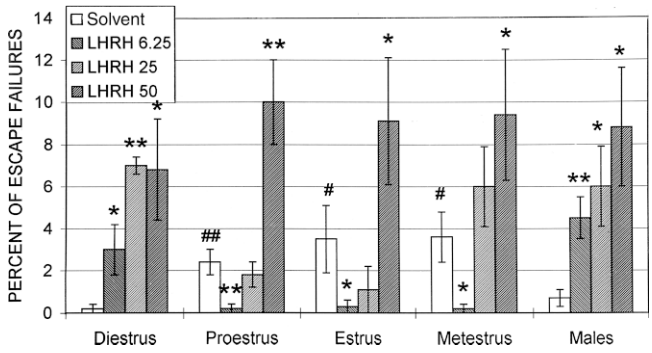

FIG. 3. Effects of LHRH on two-way shuttle-box escape responding in normal cycling female and male rats. The bars represent the mean ± SEM of the percent of trials on which they failed to escape the shock. Comparisons were made by using two-way ANOVA followed by post hoc Newman–Keuls test: $*p < 0.05$; $*p < 0.0005$ compared with its solvent control group, and $\#p < 0.05$; $\#\#p < 0.0005$ compared with diestrus plus solvent. The number of animals in each group was 8–13.

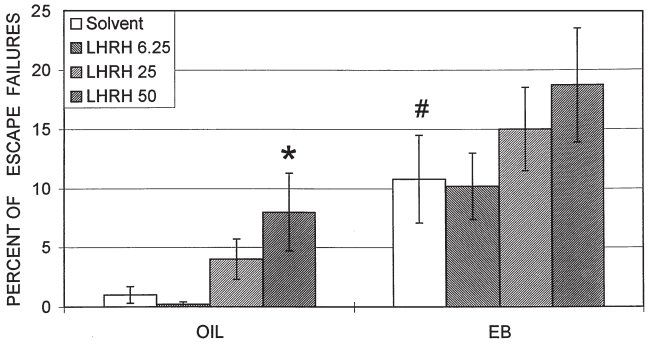

FIG. 5. Effects of LHRH on two-way shuttle-box escape responding in OVX and OVX+;EB rats. The bars represent the mean ± SEM of the percent of trials on which they failed to escape the shock. Comparisons were made by using two-way ANOVA followed by post hoc Newman–Keuls test: $*p < 0.05$ compared with its solvent control group and $\#p < 0.05$ compared with oil-treated rats. The number of animals in each group was 8–13.

sented at estrus and metestrus. In addition, the inhibitory effects of EB in OVX females were also prevented by the LHRH antagonist injected 24 h before the test (31).

The data presented here indicate that different doses of peripheral injected LHRH exert both stimulatory and inhibitory effects on the acquisition of CARs in the intact female rat. Low doses of LHRH improved the conditioned response during proestrus, estrus, and metestrus. Thus, this treatment abolished the changes in the acquisition of CARs observed across the estrous cycle, suggesting that LHRH may act as a buffer against behavioral consequences of fluctuations of ovarian steroid levels. In contrast, high doses of LHRH inhibited the acquisition of CARs in normally cycling females and males. The effects of the intermediate dose of LHRH (25 μg/kg) were different according to the hormonal status. It impaired the conditioning task during diestrus, whereas improved it in proestrous and estrous rats. These findings support the hypothesis that changes in the avoidance conditioning occurring across the estrous cycle of the female rat could be mediated by fluctuations of endogenous LHRH in the brain. They also suggest a biphasic modulator role of this neuropeptide on the acquisition of conditioned responses in the female rat.

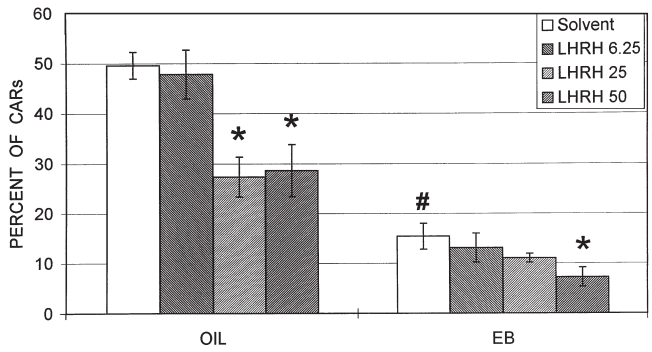

FIG. 4. Effects of several doses of LHRH on the acquisition of conditioned avoidance responses (CARs) in OVX rats and OVX primed with estradiol (EB, 10 μg/kg). The bars represent the mean ± SEM of the percentage of CARs out of 50 trials. Comparisons were made by using two-way ANOVA followed by post hoc Newman–Keuls test: $*p < 0.05$ compared with its solvent control group and $\#p < 0.05$ compared with oil plus solvent. The number of animals in each group was 8–13.

The present study also supports the idea that estradiol and progesterone could regulate the acquisition of CARs in female rats and influence the effects of LHRH on this behavior. Ovariectomy facilitated the acquisition of the response, while estradiol priming markedly impaired it. On the other hand, LHRH induced moderate inhibitory effects in OVX not primed with estradiol. These effects were less potent than those observed in normally cycling females. Nevertheless, LHRH 50 μg/kg potentiated the impairment induced by estradiol priming. These results suggest that the inhibitory effects of LHRH on this behavior could be partially estrogen dependant. Contrary to the observed in intact females, LHRH-6.25 μg/kg failed to improve acquisition of CARs in OVX rats treated either with oil or estradiol. This could be due to the lack of progesterone, which could play a facilitatory role on the acquisition of CARs and then modulate the effects of LHRH. This is supported by a study in which we demonstrate that progesterone is able to antagonize the impairment in conditioning observed in the female at estrus and in the OVX primed with EB (10). The study of the interaction between LHRH and progesterone is currently in course.

Two-way active avoidance is most often used to assess hormone or drug effects on learning (acquisition) or on memory processes. In other occasions this paradigm have been used to assess aspects that are not directly related to learning and memory per se, such as anxiety and learned helplessness (3). Because LHRH was administered prior to acquisition training, it is possible that LHRH may alter avoidance performance without acting specifically to alter learning processes, by altering noncognitive factors necessary for avoidance performance, such as motor activity, shock sensitivity, sensory processing, emotionality, motivation, and so on (40). Treatment with LHRH did not modify global motility in both intact and OVX rats. The LHRH effects on other motor responses were either stimulatory or inhibitory, according to the hormonal status and the dose administered. During diestrus, higher doses of LHRH induced depressant effects on rearing, head shaking, and grooming behavior. The dose-unrelated in-

TABLE 1

EFFECTS OF LHRH ON SPONTANEOUS MOTOR RESPONSES IN INTACT RATS UNDER DIFFERENT HORMONAL CONDITIONS

Hormonal Condition	*n*	Motor Activity (Counts)	Rearing (No.)	Head Shaking (No.)	Grooming (s)
Diestrus					
Solvent	10	608.7 ± 67.6	32.1 ± 3.7	10.7 ± 1.3	351.4 ± 23.1
LHRH 6.25 μg/kg	8	757.3 ± 43.1	28.1 ± 3.4	15.4 ± 3.5	549.9 ± 51.4*
LHRH 25 μg/kg	8	509.3 ± 69.9	23.4 ± 2.7*	7.3 ± 1.5*	226.5 ± 30.6*
LHRH 50 μg/kg	8	372.1 ± 57.7	14.5 ± 2.8*	6.8 ± 1.4*	187.0 ± 17.9*
Proestrus					
Solvent	10	370.5 ± 37.1†	21.9 ± 2.0	13.7 ± 2.3	335.9 ± 41.9
LHRH 6.25 μg/kg	8	372.4 ± 19.7	28.3 ± 1.6*	21.0 ± 2.0*	252.6 ± 12.9*
LHRH 25 μg/kg	8	476.0 ± 74.9	23.8 ± 2.9	20.6 ± 2.8*	251.6 ± 50.4
LHRH 50 μg/kg	8	504.5 ± 52.7	21.3 ± 2.8	16.3 ± 3.6	244.4 ± 32.0
Estrus					
Solvent	10	449.7 ± 55.5†	22.3 ± 3.4	4.3 ± 0.7†	315.7 ± 49.8
LHRH 6.25 μg/kg	8	490.3 ± 54.5	28.8 ± 2.4	18.3 ± 3.5*	339.3 ± 66.8
LHRH 25 μg/kg	8	452.5 ± 40.4	31.9 ± 1.9*	19.1 ± 4.3*	299.6 ± 58.8
LHRH 50 μg/kg	8	478.9 ± 50.7	23.0 ± 2.4	11.3 ± 1.7*	307.8 ± 56.9
Metestrus					
Solvent	10	799.2 ± 124.8	27.4 ± 2.2	17.7 ± 3.0†	535.0 ± 104.4
LHRH 6.25 μg/kg	8	401.8 ± 33.6	25.4 ± 2.2	18.9 ± 2.7	211.1 ± 25.8*
LHRH 25 μg/kg	8	643.4 ± 101.2	29.6 ± 2.7	26.5 ± 5.6*	329.1 ± 59.5
LHRH 50 μg/kg	8	626.9 ± 44.1	27.4 ± 3.5	14.6 ± 2.3	389.3 ± 64.9
Males					
Solvent	13	493.0 ± 44.8	20.7 ± 1.9	3.9 ± 0.9†	442.4 ± 40.8
LHRH 6.25 μg/kg	8	486.3 ± 26.5	31.0 ± 3.7*	23.3 ± 1.3*	353.0 ± 24.7*
LHRH 25 μg/kg	8	546.3 ± 41.1	23.5 ± 2.2	18.4 ± 2.2*	259.6 ± 18.2*
LHRH 50 μg/kg	8	536.1 ± 30.0	22.4 ± 2.6	20.3 ± 1.6*	280.3 ± 31.1*

Values are expressed as mean ± SEM. Comparisons were made by using two-way ANOVA followed by post hoc Newman–Keuls test.

*$p < 0.05$ when compared to its solvent control group; †$p < 0.05$ when compared to diestrus group.

crease in the number of headshakes was probably the most consistent motor effect of LHRH during the other stages of the cycle. LHRH-6.25 μg/kg increased grooming behavior in the rat at diestrus but decreased it during proestrus, metestrus, and in males. LHRH increased rearings and headshakes in OVX with or without estradiol priming. Finally, LHRH increased grooming behavior in the OVX primed with estradiol. These results suggest that, in general, the effects of LHRH on avoidance performance cannot be correlated with impairing effects on spontaneous motility.

TABLE 2

EFFECTS OF LHRH ON SPONTANEOUS MOTOR RESPONSES IN OVARIECTOMIZED (OVX) RATS

Hormonal Condition	*n*	Motor Activity (Counts)	Rearing (No.)	Head Shaking (No.)	Grooming (s)
OVX + OIL					
Solvent	10	662.1 ± 32.8	26.8 ± 3.0	16.1 ± 1.8	411.1 ± 22.9
LHRH 6.25 μg/kg	8	808.3 ± 76.7	51.8 ± 8.2*	26.9 ± 3.3*	474.7 ± 46.4
LHRH 25 μg/kg	8	600.1 ± 55.5	33.9 ± 1.9*	27.9 ± 1.9*	504.1 ± 76.7
LHRH 50 μg/kg	8	674.5 ± 82.2	37.9 ± 3.8*	26.1 ± 2.8*	461.5 ± 35.2
OVX + EB 2 μg					
Solvent	10	572.4 ± 57.5	31.4 ± 3.6	21.7 ± 3.6	327.5 ± 15.8
LHRH 6.25 μg/kg	8	633.1 ± 75.4	44.0 ± 4.8*	40.8 ± 4.8*	540.1 ± 20.5*
LHRH 25 μg/kg	8	625.0 ± 48.0	38.0 ± 3.4*	33.1 ± 4.1*	518.6 ± 29.8*
LHRH 50 μg/kg	8	614.6 ± 21.6	41.5 ± 3.5*	36.9 ± 6.9*	522.3 ± 56.4*

Values are expressed as mean ± SEM. Comparisons were made by using two-way ANOVA followed by post hoc Newman–Keuls test: *$p < 0.05$ when compared to its solvent control group.

The effects of LHRH on avoidance performance could be secondary to influences on emotional or motivational aspects. In our study, low doses of LHRH reversed escape deficits in female rats at proestrus, estrus, and metestrus, whereas high doses increased escape failures. Then, LHRH could determine changes in the emotional reactivity to the foot shock. Some studies have demonstrated that LHRH is able to exert sedative–anxiolytics properties in mice (16). In addition, LHRH exerts antidepressant-like properties, similar to those of tricyclic antidepressants, in the learned helplessness behavioral model of depression in intact male rats (22). Recently it was suggested that the chronic treatment with amitriptyline may promote transcription and translation in LHRH cells, and that the peptide may be involved in depression, subserving a role as a mediator in the action of antidepressant drugs (14).

The mechanisms underlying the effects of LHRH upon behavior are still not clear. The interaction of LHRH with the dopaminergic neurotransmission has been widely investigated. Pretreatment with L-DOPA antagonized the inhibitory effects of LHRH on active avoidance behavior and also attenuated the LHRH antagonism of improved performance induced by amphetamine in the same test (28). In vitro biochemical studies demonstrated an inhibition of dopamine synthesis and release in rat striatal tissue, and a negative feedback action of LHRH on dopaminergic neurons was postulated (29,41). The study of the effects of the superactive agonist analog D-Trp-6-LHRH in several pharmacological tests in male mice (16) indicates that this peptide exerts behavioral effects similar to those of neuroleptic or dopamine antagonists, like induction of a cataleptic-like state, antagonism of apomorphine-induced cage climbing, and inhibition of open-field activity. These effects can be antagonized by the pretreatment with a potent LHRH antagonist designed to block pituitary LHRH receptors (17), indicating that the actions of the LHRH agonist are mediated through specific LHRH receptors. The site of action of peripherally injected LHRH is most probably central, because LHRH injected intracerebrally caused potent and rapid behavioral effects similar to those demonstrated after SC administration (30). There is evidence supporting the notion that the primary LHRH function may be by an intracerebral action and that its behavioral effects do not require pituitary LH release. For instance, LHRH and LHRH analogue peptides facilitate mating behavior via an extrapituitary action because they are effective in hypophysectomized animals (12,36). Furthermore, LHRH and its behaviorally active fragment, Ac-LHRH(5–10), modify the electric properties of CA1 neurons, suggesting that LHRH may have modulator actions on the hippocampus (2). These data support possible roles for LHRH on learning and memory processes and in the modulation of mood and behavior. The behavioral effects of LHRH seem to be modulated by the fluctuations of ovarian steroid levels. The population of LHRH neurons is reduced after gonadectomy in rats (39), whereas an increase in the levels of steroids during proestrus is responsible for the increase in the number of LHRH neurons and the stimulation of LHRH secretion (19). Changes in steroid levels affect behavior, and the properties of LHRH might be relevant in determining the behavior related to fluctuations in ovarian hormone levels. This could be either directly, modifying neuronal activity through an interaction with LHRH neurons in the limbic system, or indirectly, by stimulating or inhibiting the release or activity of other neuroendocrine agents. Because LHRH neurons are devoid of steroid receptors (13,39), it has been suggested that the action of steroids on LHRH could be mediated by some other neuronal system possessing these receptors and having contact with LHRH neurons (39). Immunocytochemical studies have revealed opposition of fibers containing GABA (21), β-endorphin (1), and dopamine (13) to LHRH neurons. These neurons are also found to contain steroid receptors (13,21).

In conclusion, the present study suggests that behavioral changes accompanying the estrous cycle in female rats are associated with fluctuations in LHRH levels which, in turn, are regulated by fluctuations in circulating ovarian steroids. Increasing levels of LHRH could be responsive of the impaired acquisition of CARs during some stages of the cycle. On the other hand, low levels of LHRH seem to facilitate the acquisition of this behavior, perhaps enabling the animal to cope with the stress induced by the experimental situation. Further, the injection of a low dose of LHRH demonstrated to be able to prevent the impaired behavior during the estrous cycle. This finding would be interesting to our understanding of the etiology and treatment of premenstrual syndrome in women. Some studies have demonstrated striking improvement among patients given LHRH agonists together with ovarian steroids (32).

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