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Behavioral Effects of a LHRH Antagonist in Intact and Ovariectomized Rats

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MORA, S., F. URRESTA, N. DUSSAUBAT, F. BENAVENTE, R. BAEZA AND G. DÍAZ-VÉLIZ. *Behavioral effects of a LHRH antagonist in intact and ovariectomized rats.* PHARMACOL BIOCHEM BEHAV 46(3) 673–677, 1993.— The effects of the LHRH antagonism on the acquisition of conditioned responses (CARs) and spontaneous motility were studied in intact and ovariectomized rats. A synthetic antagonist of LHRH, [*N*-acetyl-D-*p*-chloro-Phe^{1,2},D-Trp^{3},D-Arg^{6},D-Ala^{10}]-LHRH, was injected in a single dose (10 μg/rat, SC) at noon on the day of proestrus in the normally cycling rat, and behavioral experiments were carried out on the morning of estrus or metestrus. Two procedures were followed in the ovariectomized rats: in the first, the antagonist was injected 1 h before estradiol, and in the second, at noon on the day after estradiol replacement. The experiments were carried out 24 and 48 h after estradiol, respectively. The LHRH antagonist facilitated the acquisition of CARs in both experimental groups, thus reversing the impairments observed during estrus and metestrus and those induced by estradiol replacement. The antagonist decreased the number of head shakes during estrus, whereas it induced an increase in total motility and rears in ovariectomized control animals. On the other hand, the antagonist increased the number of rears and reversed the decrease in grooming behavior induced by estradiol. The results led to the idea of a role of LHRH in behaviors not apparently related to sex, which could explain the behavioral changes observed accross the estrous cycle and those induced by estradiol replacement in ovariectomized rats.

LHRH LHRH antagonism Estradiol Estrous cycle Ovariectomy Conditioned avoidance responses Grooming

SOME evidence has suggested that the acquisition of conditioned avoidance responses (CARs) is influenced by the hormonal changes occurring during the estrous cycle of the female rat (8,26). The response is facilitated at diestrus, impaired at proestrus, and practically abolished at estrus and metestrus. These effects have been associated with fluctuations in the serum estradiol levels across the cycle (26). Considering that ovariectomy enhances acquisition performance (8), we have postulated an inhibitory effect of estradiol upon this behavior. This is supported by recent evidence that shows that the administration of a single dose of estradiol benzoate (2 μg per rat) in ovariectomized rats induces a marked impairment in the acquisition of CARs, which is observed 48 h after injection (9). It has been postulated that the increase in estradiol levels, occurring during proestrus or after exogenous administration, could trigger behavioral changes that are evident in the presence of low serum levels of estradiol; that is, during estrus or 48 h after estradiol replacement in the ovariectomized rat (9). Thus, the behavioral effects of estradiol could be mediated, at least in part, by the release of other neuroendocrine agents.

It is known that the peak of estradiol at noon of proestrus is able to trigger several neuroendocrine events in the night of estrus, including secretion of LHRH and gonadotropins as well as ovulation (1). These events are suppressed by the administration of antagonistic analogs of LHRH, which inhibit ovulation in several species by eliminating the preovulatory surge of gonadotropins (10). The LHRH antagonist [*N*-acetyl-D-*p*-chloro-Phe^{1,2},D-Trp^{3},D-Arg^{6},D-Ala^{10}]-LHRH has a potent antiovulatory effect in the rat (4). As little as 5 μg of this peptide completely inhibited ovulation in 4-day cycling rats when injected SC on the day of proestrus (20). In addition, we postulate that the increase of LHRH activity could be responsible for at least some of the behavioral changes observed at estrus and metestrus, or induced by estradiol replacement in the ovariectomized rat, and that these changes could also be prevented by LHRH antagonists.

There is pharmacological evidence suggesting that LHRH, like many other neuropeptides, is able to induce behavioral changes that are not apparently related with its neuroendocrine properties. Administration of LHRH in male rats, either SC (16,17) or into several brain areas (18), induced a dose-dependent impairment in the acquisition of avoidance conditioning. Besides, LHRH injected during the diestrous stage of

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the cycle induced a disruption in the conditioning similar to that observed at estrus (7).

The present work was designed to investigate the role of endogenous LHRH in conditioning and other behavioral changes described in normal cycling rats and in ovariectomized rats treated with estradiol. The study was carried out by administering a LHRH antagonist at noon on the day before the behavioral experiments.

METHOD

Animals

Female Sprague-Dawley rats, weighing 180–200 g, were used in the experiments. They were maintained housed in groups of six per cage under a 12L : 12D cycle (lights on from 0800 to 2000 h) with free access to food and water. Females were submitted daily to vaginal smears for determination of different stages of estrous cycle. Only rats exhibiting consistent 4-day estrous cycles were utilized. Half of these animals were bilaterally ovariectomized under light ether anesthesia. Fourteen days after surgical removal of the ovaries they received corn oil vehicle (0.2 ml/rat) or estradiol benzoate (2 μg/rat), injected SC in the dorsal region of the neck (2). Vaginal smears were taken for at least 4 days before commencement of estradiol administration; they were invariably found to be diestrus, confirming the completeness of ovariectomy.

The LHRH antagonist [*N*-acetyl-D-*p*-chloro-Phe^{1,2},D-Trp^{3}, D-Arg^{6},D-Ala^{10}]-LHRH (Sigma Chemical Co.) was dissolved in 40% propylene glycol in saline. Normally cycling rats were treated SC with a single dose of 10 μg of the peptide or 0.2 ml of the solvent at noon on the day of proestrus, and behavioral experiments were carried out on the morning of estrus or metestrus. Two separate procedures were used with different sequences of injections and testing with ovariectomized rats. In the first (procedure 1), the LHRH antagonist, or its vehicle, was injected 1 h before estradiol, or corn oil, and behavioral tests were applied 24 h after estradiol replacement. In the second (procedure 2), tests were conducted 48 h after estradiol and 24 h after LHRH antagonist injection. Each animal was tested only once.

Spontaneous Motor Activity

The animals were individually placed into a Plexiglas cage (30 × 30 × 30 cm) contained in a sound-attenuated chamber. The floor of the cage was an activity platform (Lafayette Instrument Co.) connected to an electromechanical counter. Spontaneous motility was recorded automatically for a 30-min period and simultaneously the following responses were recorded: number of times each animal reared; number of head shakes; and the time in seconds spent in grooming behavior. Each animal was monitored via a video camera connected to a VHS tape recorder. Scores were made from the live picture and the video sequences were used for a subsequent reanalysis.

Active Avoidance Conditioning

The conditioning experiments were carried out with a two-way shuttle box (Lafayette Instrument Co.) composed of two stainless steel modular testing units, which were equipped with an 18-bar insulated shock grid floor, two 28-V DC lights, and a tone generator (Mallory Sonalert 2800 Hz). Electric shocks were delivered through the grid floor by a master shock supply (Lafayette Instrument Co.). Immediately after the spontaneous motility test, the rats were placed into the shuttle box and, after a 5-min period of habituation, trained over 50 trials. Each trial consisted of the presentation of a tone that, after 5 s, was overlapped with a 0.20-mA foot shock until the animal escaped to the opposite chamber. A conditioned avoidance response (CAR) was defined as a crossing within the first 5 s (tone).

Statistics

Two-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison procedure, and Student's *t*-test for individual comparisons, were applied to evaluate the statistical significance of the results. In all cases, differences were considered to be significant when $p \leq 0.05$.

RESULTS

Effects of the LHRH Antagonist in Normal Cycling Females

Figure 1 shows that the single administration of the antagonist during the proestrous stage of the cycle facilitates the acquisition of CARs at estrus and metestrus ($p < 0.01$ and $p < 0.005$, respectively), thus reversing the impairments observed in the controls. No significant change was evident in spontaneous motility, number of rearings, or grooming behavior, but a significant decrease in the number of head shakes was induced by the antagonist during estrus (Table 1).

Effects of the LHRH Antagonist on the Acquisition of CARs in Ovariectomized Rats (Fig. 2)

In procedure 1, the estradiol injection induced a significant depression of CARs, while pretreatment with the LHRH antagonist fully reversed that effect. One-way ANOVA indicates a significant effect of the LHRH antagonist, $F(3, 33) = 3.18$, $p < 0.05$. In procedure 2, the depression of CARs induced by estradiol was severe, but this effect was antagonized by the LHRH antagonist injection. One-way ANOVA again indicates a significant effect of the antagonist, $F(3, 36) = 10.22$, $p < 0.01$. Dunnett's test indicates that, when the antagonist

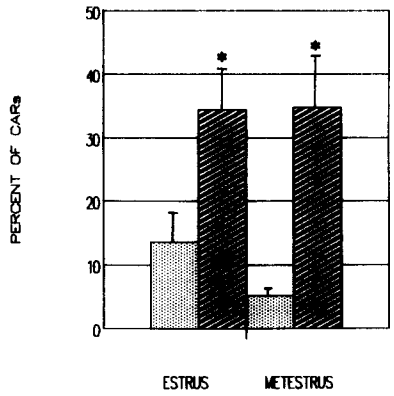

FIG. 1. Effects of the sc injection of the LHRH antagonist, [Ac-D-p-Cl-Phe^{1,2},D-Trp^{3},D-Arg^{6},D-Ala^{10}]-LHRH (10 μg/rat), on the acquisition of conditioned avoidance responses (CARs) in normal cycling female rats. The bars represent the mean ± SEM of the percentage of CARs out of 50 trials. The antagonist (hatched bars) or its vehicle (stippled bars) was injected at noon on the day of proestrus. Comparisons were made during estrus or metestrus by using Student's *t*-test (*significantly different from its control group, $p < 0.01$). The number of animals in each group was 8–10.

TABLE 1

EFFECTS OF A LHRH ANTAGONIST ON SPONTANEOUS MOTOR RESPONSES IN NORMALLY CYCLING RATS

Treatment	N	Motor Activity (counts)	Rearing (number)	Head Shaking (number)	Grooming (s)
SOL_2-E	9	731.7 ± 79.4	67.1 ± 8.9	36.0 ± 7.3	526.4 ± 57.0
ANT-E	10	703.0 ± 60.3	63.8 ± 4.4	17.0 ± 3.4*	471.6 ± 32.7
SOL_2-M	10	799.2 ± 131.5	67.4 ± 6.3	17.7 ± 3.2	535.0 ± 110.0
ANT-M	8	787.8 ± 98.1	74.1 ± 7.5	27.3 ± 6.2	465.9 ± 56.1

Values are expressed as mean ± SEM. Animals were injected with ANT [Ac-D-pCl-Phe^{1,2}, D-Trp^3, D-Arg^6, D = Ala^{10}]-LHRH) (10 μg/rat) or SOL_2 (vehicle) at noon during proestrus. Experiments were performed at estrus (E) or metestrus (M).

*$p < 0.05$ compared with its SOL_2 control.

is added to estradiol, the CAR acquisition is not significantly different from that of animals not treated with estradiol. In both procedures, the peptide did not modify the CAR performance in animals not treated with estradiol.

Effects of the LHRH Antagonist on Spontaneous Motor Activity in Ovariectomized Rats (Table 2)

The LHRH antagonist significantly increased spontaneous motility and rearing behavior in the ovariectomized rats not treated with estradiol. Estradiol enhanced the number of rears, but it decreased grooming behavior in both experimental procedures. In procedure 1, LHRH antagonist injection significantly increased the number of rears and antagonized the decrease in grooming behavior induced by estradiol. In procedure 2, the antagonist increased the number of rears and head shakes in the rats treated with estradiol, while the decrease in grooming behavior induced by estradiol was fully reversed.

DISCUSSION

The present study supports the idea that at least some of the behavioral changes occurring across the estrous cycle of the normal intact rat and those observed in the ovariectomized rat, after estradiol replacement, could be mediated by an increase in LHRH activity. In fact, the disruptions in the acquisition of CARs observed during the stages of estrus and metestrus were prevented by administering an LHRH antagonist at noon during proestrus. In addition, the CAR impairment induced by estradiol replacement in ovariectomized rats was also prevented by the LHRH antagonist. This evidence sug-

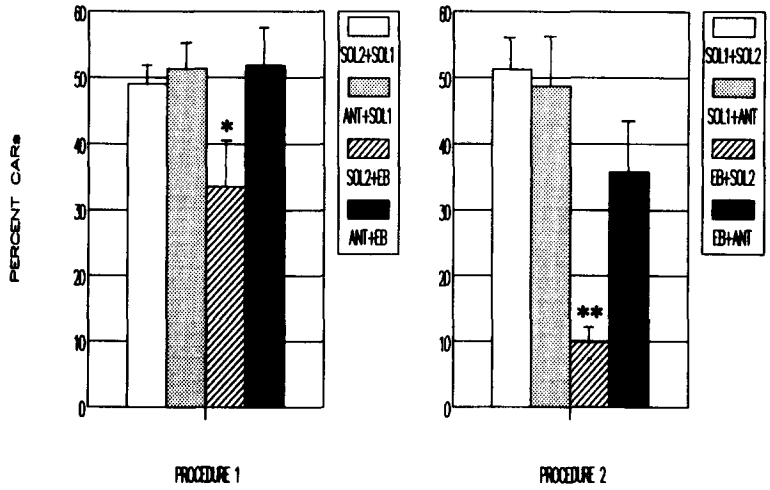

FIG. 2. Effects of the SC injection of the LHRH antagonist, [Ac-D-pCl-Phe^{1,2},D-Trp^3,D-Arg^6,D-Ala^{10}]/-LHRH (10 μg/rat), on the acquisition of conditioned avoidance responses (CARs) in ovariectomized rats and ovariectomized with estradiol replacement (EB, 2 μg/rat). The bars represent the mean ± SEM of the percentage of CARs out of 50 trials. In procedure 1, tests were conducted 24 h after EB or corn oil (SOL1) and 25 h after LHRH antagonist (ANT) or its vehicle (SOL2) injection. In procedure 2, tests were conducted 48 h after EB or SOL1 and 24 h after ANT or SOL2 injection. Comparisons were made by using two-way ANOVA, followed by Dunnett's test (*$p < 0.05$ and **$p < 0.01$ compared with SOL2 + SOL1 for procedure 1, or SOL1 + SOL2 for procedure 2). The number of animals in each group was 8–11.

TABLE 2

EFFECTS OF A LHRH ANTAGONIST ON SPONTANEOUS MOTOR RESPONSES IN OVARIECTOMIZED RATS

Treatment	N	Motor Activity (counts)	Rearing (number)	Head Shaking (number)	Grooming (s)
Procedure 1					
SOL_2 + SOL_1	10	646.2 ± 35.8	26.0 ± 2.9	18.5 ± 2.8	358.4 ± 17.9
ANT + SOL_1	10	750.0 ± 43.2*	52.1 ± 3.7*	18.6 ± 3.6	375.1 ± 22.9
SOL_2 + EB	9	485.0 ± 40.1*	37.4 ± 1.8*	23.7 ± 6.7	249.4 ± 29.5*
ANT + EB	8	576.4 ± 82.8	58.2 ± 6.4*	19.5 ± 5.1	373.5 ± 44.0
Procedure 2					
SOL_1 + SOL_2	10	516.6 ± 9.7	27.9 ± 2.9	18.9 ± 1.3	554.3 ± 11.9
SOL_1 + ANT	9	739.3 ± 86.6†	67.4 ± 4.5†	20.3 ± 5.6	446.6 ± 43.0
EB + SOL_2	10	460.1 ± 35.8	37.6 ± 3.7†	18.6 ± 3.6	375.1 ± 22.9†
EB + ANT	11	480.5 ± 51.0	44.6 ± 3.8†	32.5 ± 5.4†	531.3 ± 54.6

Values are expressed as mean ± SEM. EB, estradiol benzoate (2 μg/rat); ANT, [Ac-D-pCl-$Phe^{1,2}$, D-Trp^3, D-Arg^6, D-Ala^{10}]-LHRH (10 μg/rat); SOL_1 and SOL_2, vehicles of EB and ANT, respectively.

*$p < 0.05$ compared with SOL_2 + SOL_1 control.

†$p < 0.05$ compared with SOL_1 + SOL_2. See more details in Fig. 2.

gests that the mechanisms underlying inhibition of CARs could be similar to the neuroendocrine events mediated by LHRH, such as LH and FSH release, as well as ovulation. It is known that LHRH and many of its synthetic analogs increase pituitary secretion of gonadotropins, which, in turn, stimulate the ovary function (24). In contrast, several antagonistic analogs of LHRH inhibit ovulation in several species by suppressing the preovulatory surge of gonadotropins (10). The LHRH antagonist used in our experiments has a potent antiovulatory effect in the rat. As little as 5 μg of the peptide completely inhibited ovulation in 4-day cycling rats when injected SC at noon on the day of proestrus (20). Furthermore, the analog is also able to suppress LH levels in ovariectomized rats (20). As is shown in this study, the LHRH antagonist is also able to suppress behavioral consequences of LHRH activation. The behavioral role of LHRH is suggested by several experiments demostrating that LHRH, injected either subcutaneously (16,17) or intracerebrally (18) in male rats, induces a very potent depressant effect in the acquisition of CARs. Preliminary results obtained in female rats demonstrate that the SC administration of LHRH during the stage of diestrus induces a severe impairment in CAR performance, similar to that observed at estrus, along with a moderate decrease in grooming behavior (7).

Estradiol level fluctuations seem to be of great importance in triggering the neuroendocrine and behavioral events during the estrous cycle (6,19). The peak in plasma estradiol level has been observed at noon of proestrus and the lowest levels have been found between midnight of estrus and 1500 h of metestrus (1). Estradiol can have both inductive and suppressive effects on gonadotropin secretion, and the nature of these effects is time and dose dependent (11,12). The positive feedback response seems to involve multiple sites within the hypothalamo-hypophyseal axis. One site of action is the anterior pituitary gland, where estradiol enhances the response to LHRH, likely reflecting changes in both gonadotropin biosynthesis (3) and the number of LHRH receptors (5,15,21). Just prior to, and during, the LH surge in sheep there is an increase in pituitary content of mRNA coding for gonadotropic hormones (13). This increase can also be induced by acute exposure to estradiol (13). Further, the number of receptors for LHRH on pituitary cells increases at the time of LH surge (5). Another site is the hypothalamus, where estradiol stimulates LHRH secretion (14,22). In the rat, the acute increase in LHRH during the afternoon of proestrus seems to be essential for initiating the preovulatory LH surge (25). According to our results, estradiol replacement in ovariectomized rats triggers disruptions in the acquisition of CARs, assessed 24 and 48 h later. The fact that these disruptions are reversed by the LHRH antagonist suggests that they could also be mediated by LHRH. It is interesting that the analog does not exert significant effects on conditioning when it is injected in ovariectomized rats not treated with estradiol, thus demostrating a lack of direct effects on this behavior.

Spontaneous motor responses were not modified by the LHRH antagonist in the normally cycling rat. Nevertheless, total motility and rears were stimulated by the peptide in the ovariectomized controls. Estradiol replacement induced different motor responses in these animals: there was a decrease in total motility in procedure 1 and, while rears were increased by estradiol, there was a decrease in the time spent in grooming behavior in both procedures 1 and 2. The LHRH antagonist seems to interact with estradiol but in different ways. In fact, it potentiated the effects of estradiol on rears but fully reversed the depressant effect of estradiol on grooming behavior in both procedures 1 and 2. Head shakes were also potentiated, but only in procedure 2. Previously (8), we have observed that ovariectomy failed to induce significant changes on total motility, rearing, and head shakes, although it enhances acquisition of CARs as well as grooming behavior. The present results demonstrate that both conditioning and grooming are depressed by estradiol replacement and that the LHRH antagonist prevents these effects, suggesting an inhibitory role of estradiol on both behaviors, which could be mediated, in turn, by LHRH activation.

In conclusion, this study supports a behavioral role of LHRH in the female rat that is estradiol dependent and could explain some of the changes observed accross the estrous cy-

cle. Although the physiological role of LHRH on behavior has not been clearly established, there is evidence supporting a neurotransmitter or neuromodulator role of LHRH in the CNS. Besides, it is possible that the effects of LHRH could be mediated by its interaction with neurochemical systems more involved in adaptative behavior. Nevertheless, since the neuroendocrine events triggered by estradiol in the hypothalamus-pituitary-gonadal axis are numerous and complex, we cannot rule out the participation of other endogenous agents, such as LH, progesterone, prolactin, etc.

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REFERENCES

1. 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.
2. Cheng, H. C.; Jonhson, D. C. Temporal changes in serum estradiol and prolactin in immature female rats given a single injection of estradiol benzoate. Steroids 24:657–664; 1974.
3. Clarke, I. J.; Cummins, J. T. Direct pituitary effects of estrogen and progesterone on gonadotropin secretion in the ovariectomized ewe. Neuroendocrinology 39:267–274; 1984.
4. Coy, D. H.; Horvath, A.; Nekola, M. V.; Coy, E. J.; Echergyi, J.; Schally, A. V. Peptide antagonists of LHRH: Large increases in anovulatory activities produced by basic D-amino acids in the six position. Endocrinology 110:1445–1447; 1982.
5. Crowder, M. E.; Nett, T. M. Pituitary contents of gonadotropins and receptors for gonadotropin-releasing hormone (GnRH) and hypothalamic content of GnRH during the preovulatory period of the ewe. Endocrinology 114:234–239; 1984.
6. Davidson, J. M.; Smith, E. R.; Rodgers, C. H.; Bloch, G. J. Relative thresholds of behavioral and somatic responses to estrogen. Physiol. Behav. 3:227–229; 1968.
7. Díaz-Véliz, G.; Afani, A.; Kusanovic, R.; Soto, V.; Mora, S. Estudio comparativo de los efectos conductuales de LHRH en ratas machos y hembras. Arch. Biol. Med. Exp. 21:R505; 1988.
8. 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.
9. 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.
10. Horvath, A.; Coy, D. H.; Nekola, M. V.; Coy, E. J.; Schally, A. V.; Teplan, I. Synthesis and biological activity of LH-RH antagonists modified in position 1. Peptides 3:969–971; 1982.
11. Karsch, F. J. Central actions of ovarian steroids in the feedback regulation of pulsatile secretion of luteinizing hormone. Annu. Rev. Physiol. 49:365–382; 1987.
12. Knobil, E. The neuroendocrine control of the menstrual cycle. Recent Prog. Horm. Res. 36:53–88; 1981.
13. Landefeld, T.; Kepa, J.; Karsch, F. J. Estradiol feedback effects on the α-subunit mRNA in the sheep pituitary gland: Correlation with serum and pituitary luteinizing hormone concentration. Proc. Natl. Acad. Sci. USA 81:1322–1326; 1984.
14. Levine, J. E.; Ramírez, V. D. Luteinizing hormone-releasing hormone release during the rat estrous cycle and after ovariectomy, as estimated with push-pull cannulae. Endocrinology 111:1439–1455; 1982.
15. Libertun, C.; Orias, R.; McCann, S. M. Biphasic effect of estrogen on the sensitivity of the pituitary to luteinizing hormone-releasing factor (LRF). Endocrinology 94:1094–1100; 1974.
16. Mora, S.; Nasello, A. G.; Mandelli-Lopes, M.; Díaz-Véliz, G. LHRH and rat avoidance behavior: Influence of castration and testosterone. Physiol. Behav. 30:19–22; 1983.
17. Mora, S.; Caro, F.; Cardenas, G.; Espinoza, M.; Díaz-Véliz, G. Dose-dependent and time-dependent effects of luteinizing hormone releasing hormone on active avoidance behavior in rats. IRCS Med. Sci. 11:1108–1109; 1983.
18. Mora, S.; Afani, A.; Kusanovic, R.; Tapia, C.; Díaz-Véliz, G. Behavioral effects of intracerebral administration of luteinizing hormone releasing hormone (LHRH) in rats. Pharmacol. Biochem. Behav. 38:705–709; 1991.
19. Moss, R. L.; Dudley, C. A. Differential effects of a luteinizing-hormone-releasing hormone (LHRH) antagonist analogue on lordosis behavior induced by LHRH and the LHRH fragment Ac-LHRH$_{5-10}$. Neuroendocrinology 52:138–142; 1990.
20. Nekola, M. V.; Horvath, A.; Ge, J.; Coy, D, H.; Schally, A. V. Suppression of ovulation in the rat by an orally active antagonist of luteinizing hormone-releasing hormone. Science 218:160–162; 1982.
21. Reeves, J. J.; Arimura, A.; Schally, A. V. Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH) in anestrous ewes pretreated with estradiol benzoate. Biol. Reprod. 4:88–92; 1971.
22. Sarkar, D. K.; Chiappa, S. A.; Fink, G. Gonadotropin-releasing hormone surge in pro-oestrus rats. Nature 264:461–463; 1976.
23. Savoy-Moore, R. T.; Schwartz, N. B.; Duncan, J. A.; Marshall, J. C. Pituitary gonadotropin-releasing hormone receptors on proestrus: Effect on pentobarbital blockade of ovulation in the rat. Endocrinology 109:1360–1364; 1981.
24. Schally, A. V.; Coy, D. H. Stimulatory and inhibitory analogs of luteinizing hormone releasing hormone (LHRH). In: Porter, J. C., ed. Hypothalamic peptide hormones and pituitary regulation. New York: Plenum Press; 1977:99–121.
25. Schwartz, N. B. A model for the regulation of ovulation in the rat. Recent Prog. Horm. Res. 25:1–55; 1969.
26. Sfikakis, A.; Spyraki, C.; Sitaras, N.; Vanoros, D. Implications of the estrous cycle on conditioned avoidance behavior in the rat. Physiol. Behav. 21:441–446; 1978.