Pharmacology Biochemistry & Behavior, Vol. 22, pp. 967–972, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

LHRH and Mating Behavior: Sexual Receptivity Versus Sexual Preference[1]

CAROL A. DUDLEY AND ROBERT L. MOSS

Department of Physiology, The University of Texas Health Science Center at Dallas Southwestern Medical School, 5323 Harry Hines Boulevard, Dallas, TX 75235

Received 6 September 1984

DUDLEY, C. A. AND R. L. MOSS. *LHRH and mating behavior: Sexual receptivity versus sexual preference.* PHARMACOL BIOCHEM BEHAV 22(6) 967–972, 1985.—Sexual behavior and sexual preference measurements were obtained from ovariectomized female rats treated with estrogen, estrogen and progesterone, or estrogen followed by third ventricular infusion of luteinizing hormone releasing hormone (LHRH) or saline. The lordosis-to-mount ratio and the occurrence of receptive and proceptive behaviors were scored to assess total sexual receptivity. Sexual preference was determined by placing the test female in the center of a four-winged choice box apparatus in which each of the outer wings contained one of the following incentive animals: a sexually active male (SM), a castrate male (CM), a female in proestrus (PF), and an ovariectomized female (OF). Time spent in close proximity to the incentive animals was measured as an index of sexual preference. Estrogen and progesterone treatment resulted in high sexual receptivity and a marked preference for SM. Estrogen alone or in combination with saline also produced a significant preference for SM. Animals treated with estrogen and LHRH exhibited high levels of sexual receptivity compared to estrogen saline treated controls, but no enhancement of preference for SM was detected. The results indicate that fractionation of sexual receptivity and sexual motivation occurs following estrogen-LHRH treatment.

LHRH Estrogen Sexual receptivity Resistive behavior Proceptive behavior Choice box Sexual preference Sexual motivation

SYSTEMIC administration of luteinizing hormone-releasing hormone (LHRH) has been demonstrated to potentiate lordosis behavior in ovariectomized, estrogen-primed female rats [13, 14, 16, 21]. Infusion of the decapeptide into the medial preoptic area [1, 4, 5], arcuate-ventromedial area [4,5], midbrain central gray [18, 19], and the spinal subarachnoid space [20] also results in elevated lordotic responding. The quality of the female behavioral response was noted in initial investigations to be unlike that normally occurring during spontaneous heat or following estrogen, progesterone (P)-priming in that the LHRH treated animals displayed a large number of resistive behaviors and a reduced number of proceptive behaviors [13,16]. Proceptive behaviors are those actions displayed by the female which indicate a propensity to establish and maintain sexual contact with the male whereas resistive behaviors are those actions which indicate a desire to avoid sexual contact with the male. Subsequent studies also observed a lack of proceptive behavior and/or increased resistivity during mating behavior tests in estrogen, LHRH treated animals [11, 12, 15, 20]. In one study, the receptive and proceptive behavioral patterns of estrogen LHRH primed animals and estrogen-P animals were compared [21]. The level of sexual receptivity (lordosis-to-mount ratio) produced by P was significantly higher than that obtained with LHRH. In addition, P facilitated proceptive behaviors (hopping, darting, and ear wiggling), whereas LHRH did not. Thus, it appears that LHRH and P have different effects on both the quality of female sexual behavior and the level of lordotic responding.

Another aspect of female copulatory behavior that appears to be hormonally controlled is sexual motivation. This behavior has most commonly been measured in terms of sexual preference. Meyerson and Lindstrom [10] demonstrated that preference for a sexually active male varied over the estrous cycle. Time spent in proximity to a sexually active male as well as willingness to cross an electrified grid in order to obtain contact with a sexually active male were highest in the proestrus phase of the estrous cycle, a time in which sexual receptivity also peaks. In a runway choice apparatus, a significant preference for the sexually active male was observed during the proestrus/estrus phase of the cycle when the female was separated from the incentive animals by a wire mesh screen [3], as well as when direct copulatory contact was permitted [7]. Estradiol benzoate given to ovariectomized rats also increased preference for the sexually active male [10].

The copulatory behavioral pattern induced by estrogen and LHRH, characterized by resistive behavior, lack of proceptive behavior, and varying levels of receptivity, is suggestive of reduced sexual drive. In the present investigation, sexual preference of estrogen-LHRH treated female rats was examined using an expanded version of the two-

[1]This work was supported by NIH Research Grant HD-11814 awarded to Robert L. Moss.

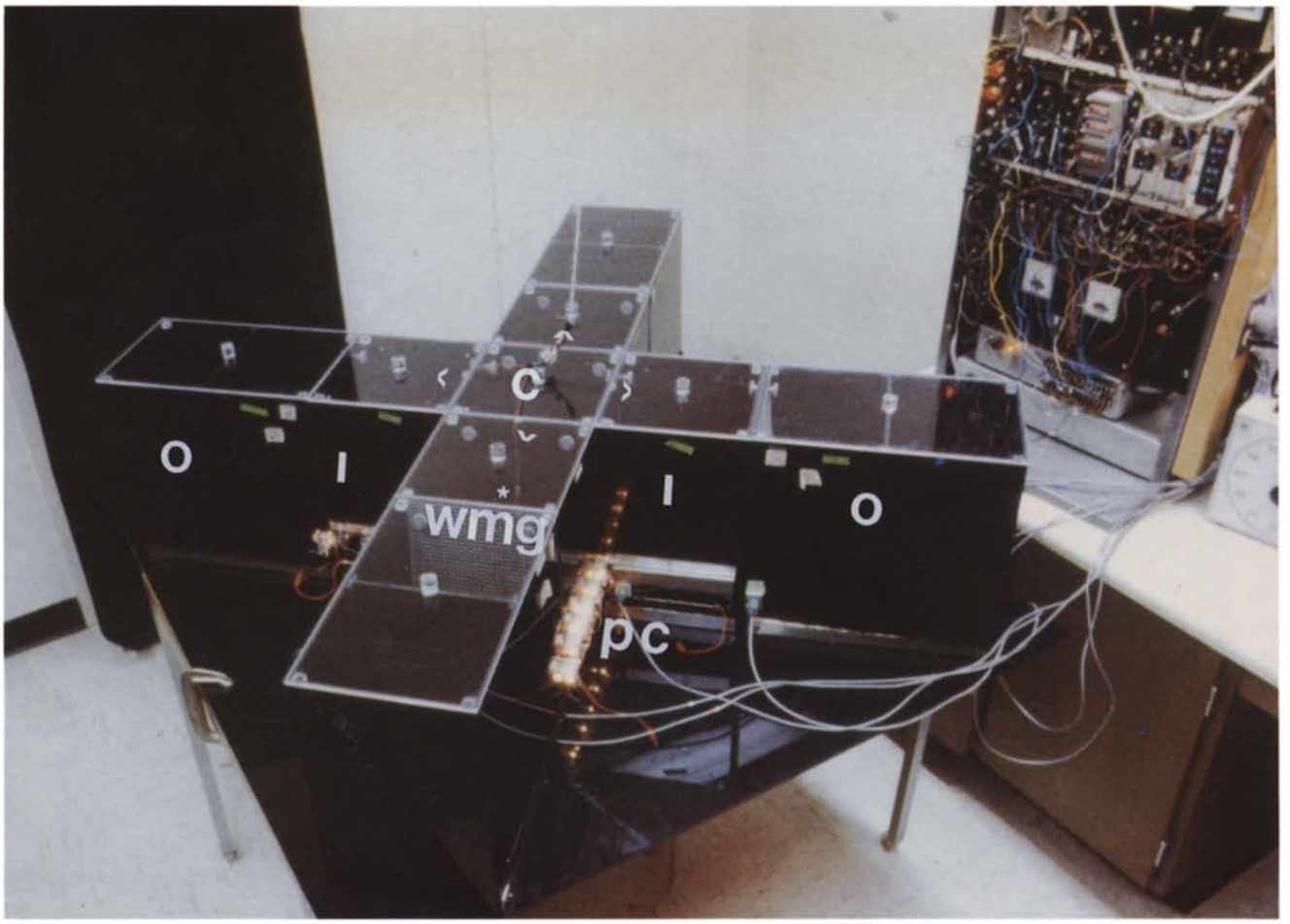

FIG. 1. Photograph of the four-winged choice box apparatus. The test female was placed in the center box (c) to begin a sexual preference test. After a two minute adaptation period, the four doors on each side of the center box (arrows) were opened and the test female was then allowed to roam freely through the inner boxes (i) to the outer boxes (o) containing the incentive animals. The wall between the inner and outer boxes contained an arched opening through which the test animal could poke her head. Incentive animals were restricted to the outer boxes by a piece of wire mesh grid (wmg). Inner boxes as well as the partial entry area to the outer boxes were lined with photocells (pc) which automatically recorded the frequency of entrance into each inner and outer box as well as the time spent in partial entry into the outer boxes.

winged runway choice apparatus. A four-way choice box apparatus was constructed to allow preference measurements on the basis of simultaneous presentation of four incentive animals, one of which was a sexually active male. Three other incentive animals with no known particular sexual attractiveness to the test female (an ovariectomized female, a female in proestrus, and a castrate male) were presented in order to insure that preference for a specifically sexual stimulus could be discriminated from the tendency to establish social contact. In addition to measuring sexual preference, sexual receptivity, resistive behavior, and proceptive behavior were scored to provide a complete characterization of the nature of estrogen-LHRH induced copulatory behavior. Preliminary results of this investigation were presented at the 7th International Congress of Endocrinology in Quebec City, Canada; Abstract No. 859, pg. 690, 1984.

METHOD

Sprague-Dawley female rats (Simonsen Labs, Gilroy, CA) weighing 220–240 g were individually housed in a temperature controlled room with a 14:10 light/dark cycle (lights off at 1100 hr) and given Purina Lab Chow and water ad lib. The animals (n=20) were ovariectomized under ether anesthesia. Approximately two weeks later, the experiment was begun.

Sexual Preference Testing

The choice box apparatus was constructed of black Plexiglas and consisted of four inner boxes which formed alleys leading from a center box (Fig. 1). At the end of each alley was an outer box which held one of the four incentive animals. Inner boxes were separated from outer boxes by a piece of wire mesh grid angled towards the outer box so that test animals could partially enter the outer boxes but could not make contact with the incentive animals. Frequency of entrance into the inner alleys, frequency of entrance into the outer boxes, as well as time spent in close proximity to the incentive animals (time spent in partial entry into the outer boxes) were recorded automatically by interruption of photocell beams lining the alleys. The incentive animals

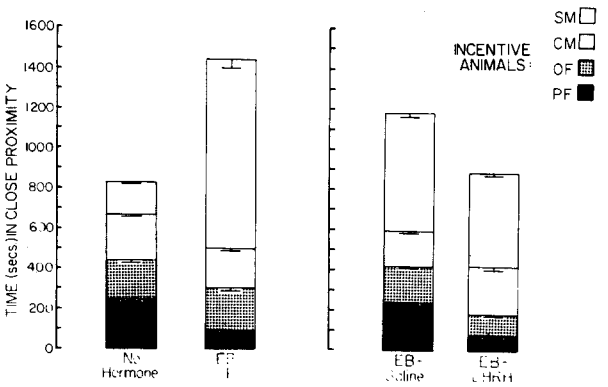

FIG. 2. Bar graph depicting the results of sexual preference testing in Group I animals (n=7). The height of each treatment bar indicates the mean total time spent in close proximity to all incentive animals. The shaded and open portions of each bar indicate the mean amount of time spent in close proximity to specific incentive animals. SM=sexually active male; CM=castrate male; OF=ovariectomized female; PF=proestrus female; ⊥=standard error of the mean.

were a proestrus female (PF), an ovariectomized female (OF), a sexually active male (SM), and a castrated male (CM). The incentive animals were placed in the outer boxes 15 to 30 min prior to the start of a preference test and remained in the same outer box until preference testing for the day was complete. The four incentive animals were rotated to a different box for the next preference test.

Two weeks after ovariectomy, the female rats were individually placed in the choice box apparatus for a 15-minute period on two successive days. During these adaptation trials, no incentive animals were present. Measurements of inner and outer box frequency as well as time in close proximity to the outer boxes were obtained to insure that no preference occurred in the absence of incentive animals.

A total of four preference tests were conducted on each test female, so incentive animals were in a different box for each test. A sexual preference test was begun by placing the test female into the center box. After two minutes, the four sliding doors separating the center box from the inner boxes were simultaneously lifted and the test animal was allowed to roam the four alleys for a fifteen minute period. At the end of this period, the center box doors were closed, the test female removed, the data was recorded, and the center and inner boxes were wiped with an alcohol sponge before placing the next test female into the center box. All sexual preference tests were conducted during the dark phase of the light/dark cycle.

Mating Behavior Tests

Sexual receptivity tests consisted of placing the female rat into a semicircular Plexiglas mating arena containing two sexually active male rats. Presence or absence of lordotic posture in response to male copulatory contacts (mounts, intromissions, or intromissions with ejaculation) was scored for 15 minutes or until a total of 15 copulatory contacts were achieved. Results were expressed in terms of the lordosis-to-mount ratio (L/M: number of lordotic postures/number of male copulatory contacts) and used as an index of sexual receptivity. The occurrence of the following preceptive behaviors were noted: spontaneous lordosis, soliciting (nudging the male, usually with the nose, and then quickly turning to assume a presenting posture), hopping and darting, and ear wiggling. The occurrence of the following resistive behaviors were noted: squealing, hindkicking, and fending off the male (rearing as the male attempted to mount and pushing the male away with the forelegs). All mating behavior tests were conducted during the dark phase of the light/dark cycle.

Testing Procedures

Three weeks after ovariectomy, the first sexual preference test was conducted. No hormones were administered to the animals prior to this test. One day later, all animals were injected with 5 μg estradiol benzoate (EB). Forty-two hours later, half of the animals received 2.5 mg progesterone (P) (Group I) while the other half were not treated at this time (Group II). At 48 hours, sexual preference tests were conducted. Animals in Group I were tested for mating behavior following sexual preference testing. The following week all animals received implants of 23 gauge stainless steel cannulae stereotaxically positioned in the third ventricle under Equithesin type anesthesia. The animals were allowed a one-week recovery period and were then injected with 5 μg EB. LHRH (100 ng/1 μl) or saline (1 μl) was infused under ether anesthesia 46.5 hours after EB treatment. Sexual preference testing was performed 1.5 hours after infusion. Immediately after the 15 minute sexual preference test, mating behavior tests were conducted. Two weeks later, sexual preference and mating behavior tests were repeated following EB priming and infusion of LHRH or saline in a counterbalanced fashion.

The treatment protocol can be summarized as follows: Group I—sexual preference test with no hormone priming, sexual preference and mating behavior test following EB-P priming, sexual preference and mating behavior test following saline infusion, and sexual preference and mating behavior tests following LHRH infusion. Experimental protocol for Group II—sexual preference test with no hormone priming, a sexual preference test following EB priming, a sexual preference and mating behavior test following saline infusion, and a sexual preference and mating behavior test following LHRH infusion. Three animals from Group I and two animals from Group II died following the cannulation procedure. Their data was excluded from the analysis.

Data Analysis

Preliminary analysis of the sexual preference data revealed a high correlation between frequency of entrance into a given inner box, frequency of partial entrance into the associated outer box, and time spent in close proximity to the incentive animal located in the associated outer box. Due to this high correlation and the assumption that time spent in close proximity to a given incentive animal was the most precise measurement of the motivational state of the animal, the proximity times were chosen for statistical assessment instead of frequency scores.

Sexual preference scores (i.e., time spent in close proximity to the incentive animals) were analyzed for each group by a two-way (a×b×s) within subjects ANOVA. Preference scores obtained with no hormone therapy versus those obtained following EB only or EB-P were analysed separately from preference scores obtained following infusion of LHRH

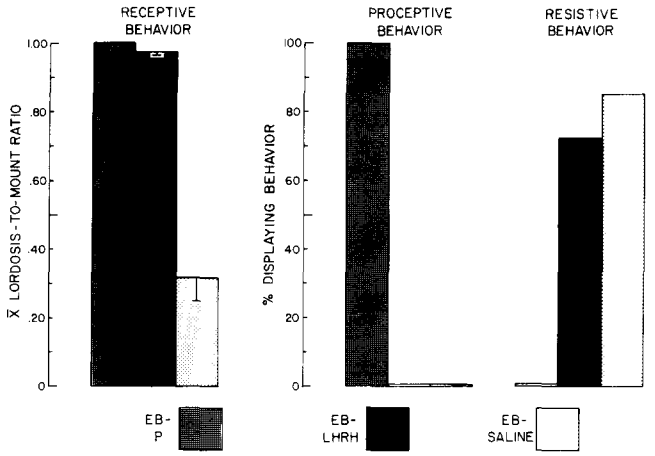

FIG. 3. Bar graphs summarizing results of sexual behavior testing in Group I animals. Sexual receptivity scores are presented in the left hand portion of the graph and the percent of animals displaying proceptive and resistive behaviors is presented in the right hand portion.

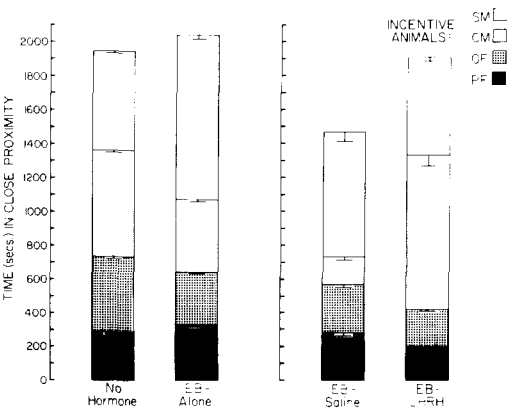

FIG. 4. Bar graph summarizing the results of sexual preference testing in Group II animals (n=8). The height of each treatment bar indicates the mean total time spent in close proximity to all incentive animals. The shaded and open portions of each bar indicate the mean amount of time spent in close proximity to specific incentive animals. SM=sexually active male; CM=castrate male; OF=ovariectomized female; PF=proestrus female; ±=standard error of the mean.

or saline. The lordosis-to-mount ratios in each group were statistically compared using Student's t test.

RESULTS

Sexual preference test results for animals in Group I are presented in Fig. 2. The ANOVA comparing results obtained from non-primed animals with those from EB-P primed animals (Fig. 2; left panel) yielded a significant overall choice effect, $F(3,21)=6.53$, $p<0.01$, as well as a significant choice × drug interaction, $F(3,21)=7.60$, $p<0.01$. Tukey's test for pairwise comparisons of the choice effect revealed that significantly more time was spent with SM than any other incentive animal. In addition, time spent with CM was significantly greater than time spent with PF. No difference between OF and CM was detected. When Tukey's test was applied to the choice × drug interaction, it was found that EB-P primed animals exhibited a highly significant preference for SM, whereas non-primed animals spent significantly less time in proximity to SM than PF or CM. Thus, without hormone treatment, these animals exhibited a tendency to avoid SM, whereas, following EB-P priming, a dramatic preference for SM was obtained. After infusion of LHRH or saline, the overall effect for choice was again significant $F(3,18)=12.48$, $p<0.005$ (Fig. 2; right panel). Subsequent examination revealed that both LHRH and saline infused animals displayed a preference for SM. The choice × drug interaction was not significant. Thus, LHRH infused and saline infused animals did not differ with regard to time spent in close proximity to the incentive animals. Both treatments produced a preference for SM.

Mating behavior results from this group of animals are presented in Fig. 3. Receptive behavior in EB-P primed animals was high (mean L/M=1.00) as expected. Lordotic behavior was significantly higher following LHRH infusion than saline infusion, $t(6)=10.1$, $p<0.005$. Although the L/M ratios were high in EB-LHRH treated animals, (mean=0.97), none of them displayed proceptive behavior. EB-saline treated animals exhibited poor receptive behavior (mean

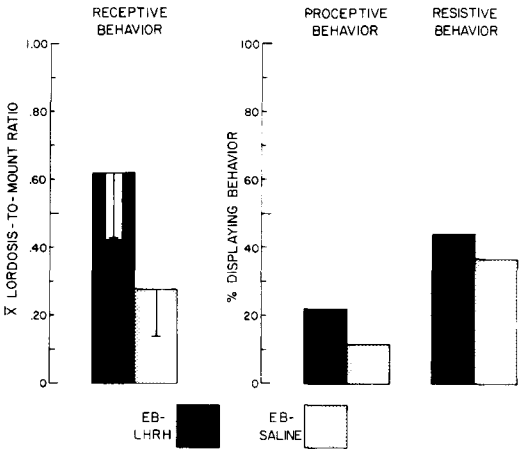

FIG. 5. Bar graph summarizing results of sexual behavior testing in Group II animals. Although LHRH treatment produced a higher lordosis to mount ratio than saline treatment, both treatments resulted in a similar percentage of animals displaying proceptive and resistive behaviors.

L/M=0.31) and a lack of proceptive behavior. In contrast, proceptive behavior was observed in all of the animals primed with EB-P and none of the EB-P primed animals displayed resistive behavior. At least one instance of resistive behavior was observed in 71.4% of EB-LHRH treated animals and in 85.7% of EB-saline treated animals. Thus, resistive, proceptive, and sexual preference measurements were similar in EB-LHRH and EB-saline conditions. The level of receptivity was comparable in EB-P and EB-LHRH treated animals and a preference for SM was found in both cases although it was much more pronounced after EB-P treatment.

Sexual preference results from animals in Group II are presented in Fig. 4. The first two preference tests in this

group of animals were conducted following no hormone therapy and EB only. No overall choice or drug effect was significant, however, the ANOVA revealed a significant choice × drug interaction, $F(3,24)=3.64$, $p<0.05$. When animals were primed with EB, more time was spent in proximity to SM and less time was spent in proximity to OF and CM than in the non-primed condition. Analysis of sexual preference after infusion of saline or LHRH revealed no significant overall choice effect, drug effect, or choice × drug interaction. Examination of the data for all four treatments revealed that EB only and EB-saline yielded similar incentive choice profiles in which SM was the preferred incentive animal. In this group of animals, EB-LHRH did not result in preference for SM. The mating behavior data for Group II animals is presented in Fig. 5. The L/M scores were higher in EB-LHRH treated animals than in EB-saline treated animals, however, this difference failed to attain statistical significance at the 0.05 level, $t(7)=1.79$, $p<0.10$.

Proceptive behavior was obtained in a small percentage of animals following both treatments and resistive behavior was displayed in a slightly larger percentage of animals.

The receptive behavior of Group I animals primed with EB-LHRH was higher than that of Group II animals similarly treated. Group I animals also displayed a preference for SM when treated with EB-P, EB-LHRH, or EB-saline. Preference for SM was detected in Group II animals after EB only and EB-saline treatment but not in the non-primed or EB-LHRH condition. Thus, preference for SM was obtained with EB only. Progesterone in combination with EB enhanced preference for SM but LHRH in combination with EB did not.

DISCUSSION

Ovariectomized animals primed with EB or primed with EB and infused intraventricularly with saline displayed a significant preference for SM over the three other incentive animals. This data is in agreement with previous investigations in which administration of EB to ovariectomized females increased the preference for sexually active males as opposed to a sexually active female in the runway choice apparatus [9,10] and increased preference for a sexually active male over a sexually inactive castrate male [2]. No such preference was obtained in ovariectomized, non-primed animals. Without hormone priming, Pfeifle and Edwards [17] found no evidence of preference for a sexually active male over a castrate male. During pregnancy, a time in which a very low amount of estrogen is present, preference for a sexually active male in the runway choice apparatus was significantly decreased [3]. Thus preference for a sexually active male appears to be estrogen dependent. Discrimination for SM over three other incentive animals in the present investigation is a powerful indication that the preference was specifically sexual in nature.

When progesterone was given to EB primed animals in a sequence mimicking natural heat, the strength of the preference for SM was increased. Preference for a sexually active male during the proestrus/estrus phase of the estrus cycle and/or following estrogen-progesterone priming has been reported previously [2, 6, 7, 9, 10]. In agreement with the present study is a report that progesterone increased the preference for a sexually active male, as opposed to a castrate male, over that obtained in animals treated with estrogen only [2].

Administration of LHRH did not elevate preference for SM over the level obtained in EB treated animals. Even in Group I animals, who displayed high levels of lordotic responding after LHRH treatment, preference for SM was the same following infusion of saline as following infusion of LHRH. Proceptive behaviors were decreased and resistive behaviors increased in LHRH treated animals compared to P treated animals. Thus, both in terms of sexual preference and the display of proceptive and receptive behavior, the effects produced by P were different than those produced by LHRH. In Group II animals, LHRH tended to elevate lordotic responding, but the difference between LHRH and saline treatment failed to attain significance. Variability in responsiveness to LHRH has been noted before [8, 11, 12] and has been attributed to individual differences in estrogen sensitivity [12]. This may have been the case in the present study, however, prior to LHRH infusion, Group I animals had been injected with EB and P and had been tested for mating behavior whereas Group II animals received EB only and no mating behavior testing. Prior exposure to P as well as prior mating experience may have sensitized the relevant neural substrate to the subsequent injection of EB and/or infusion of LHRH, thus rendering equivalent doses of EB and/or LHRH slightly more effective in elevating L/M in Group I animals than in Group II animals. Thus, prior P exposure and prior mating experience may partially account for the difference in L/M between the two groups following LHRH infusion. No preference for SM was observed in Group II animals after LHRH treatment. In fact, Group II animals treated with LHRH spent the largest amount of time in proximity to CM, although the saline control animals did choose the SM.

In the present study, LHRH increased sexual receptivity without increasing preference for the sexually active male. This finding indicates that the neural systems mediating lordotic behavior are different from those mediating the motivational aspects of sexual behavior. Alternatively, perhaps the effect exerted by LHRH at any particular brain site is not sufficiently strong to recruit all aspects of female sexual behavior. In support of the former suggestion is the demonstration that lesions of the midbrain peripeduncular region eliminated lordotic responding and proceptive behavior but did not destroy preference for a sexually active male over a castrate male [17]. In support of the later possibility is the repeated observation that LHRH facilitation of lordotic behavior is not as consistently obtainable as is progesterone facilitation of the behavior. The issue may be resolved in a systematic fashion by measuring lordotic behavior and sexual preference following LHRH infusion into brain sites known to be involved in the mediation of sexual behavior and then infusing the decapeptide into a combination of those sites simultaneously. In this manner, it would be possible to determine if a neural site exists in which LHRH is capable of activating all components of sexual behavior.

ACKNOWLEDGEMENTS

The authors would like to express their appreciation to Deborah Aldridge and June Roman for excellent technical assistance and to Anita Gomez and Susan Sauter for aid in preparation of the manuscript. Luteinizing hormone releasing hormone (Gonadorelin) was a gift from Ayerst Laboratories, Rouses Point, NY.

REFERENCES

1. Cooper, R. L., M. Seppala and M. Linnoila. Effect of luteinizing hormone-releasing hormone antiserum on sexual behavior in the female rat. *Pharmacol Biochem Behav* **20:** 527–530, 1984.
2. Edwards, D. A. and J. K. Pfeifle. Hormonal control of receptivity, proceptivity, and sexual motivation. *Physiol Behav* **30:** 437–443, 1983.
3. Eliasson, M. and B. J. Meyerson. Sexual preference in female rats during estrous cycle, pregnancy and lactation. *Physiol Behav* **14:** 705–710, 1975.
4. Foreman, M. M. and R. L. Moss. Effects of subcutaneous injection and intrahypothalamic infusion of releasing hormones upon lordotic response to repetitive coital stimulation. *Horm Behav* **8:** 219–234, 1977.
5. Foreman, M. M. and R. L. Moss. Roles of gonadotropins and releasing hormones in hypothalamic control of lordotic behavior in ovariectomized, estrogen-primed rats. *J Comp Physiol Psychol* **93:** 556–565, 1979.
6. French, D., D. Fitzpatrick and O. T. Law. Operant investigations of mating preferences in female rats. *J Comp Physiol Psychol* **81:** 226–232, 1972.
7. Gilman, D. P. and W. H. Westbrook. Mating preference and sexual reinforcement in female rats. *Physiol Behav* **20:** 11–14, 1978.
8. Kastin, A. J., D. H. Coy, A. V. Schally and J. E. Zadina. Dissociation of the effects of LH-RH analogs on pituitary regulation and reproductive behavior. *Pharmacol Biochem Behav* **13:** 913–914, 1980.
9. Meyerson, B. J. Interactions between steroid hormones, neurotransmitters and socio-sexual behaviors. *Prog Brain Res* **61:** 1984–1985, in press.
10. Meyerson, B. J. and L. H. Lindstrom. Sexual motivation in the female rat—a methodological study applied to the investigation of the effect of estradiol benzoate. *Acta Physiol Scand (Suppl)* **389:** 1–80, 1973.
11. Moss, R. L. and C. A. Dudley. Luteinizing hormone-releasing hormone (LHRH): A role in extrapituitary function. In: *The Role of Peptides in Neuronal Function*, edited by J. L. Barker and T. G. Smith, Jr. New York: Marcel Dekker, 1980, pp. 456–478.
12. Moss, R. L. and C. A. Dudley. The challenge of studying the behavioral effects of neuropeptides. In: *Handbook of Psychopharmacology*, vol 18, edited by L. L. Iversen, S. D. Iversen and S. H. Snyder. New York and London: Plenum Publishing Corporation, 1984, pp. 397–454.
13. Moss, R. L. and S. M. McCann. Induction of mating behavior in rats by luteinizing hormone-releasing factor. *Science* **181:** 177–179, 1973.
14. Moss, R. L. and S. M. McCann. Action of luteinizing hormone-releasing factor (LRF) in the initiation of lordosis behavior in the estrone-primed ovariectomized female rat. *Neuroendocrinology* **17:** 309–318, 1975.
15. Moss, R. L., P. Riskind and C. A. Dudley. Effects of LH-RH on sexual activities in animals and man. In: *Central Nervous Systems Effects of Hypothalamic Hormones and Other Peptides*, edited by R. Collu, A. Barbeau, J. R. Ducharme and J. G. Rochefort. New York: Raven Press, 1979, pp. 345–365.
16. Pfaff, D. W. Luteinizing hormone releasing factor (LRF) potentiates lordosis behavior in hypophysectomized ovariectomized female rats. *Science* **182:** 1148–1149, 1973.
17. Pfeifle, J. K. and D. A. Edwards. Midbrain lesions eliminate sexual receptivity but spare sexual motivation in female rats. *Physiol Behav* **31:** 385–389, 1983.
18. Riskind, P. and R. L. Moss. Midbrain central gray: LHRH infusion enhances lordotic behavior in estrogen-primed ovariectomized rats. *Brain Res Bull* **4:** 203–205, 1979.
19. Sakuma, Y. and D. W. Pfaff. Modulation of the lordosis reflex of female rats by LHRH, its antiserum and analogs in the mesencephalic central gray. *Neuroendocrinology* **36:** 218–224, 1983.
20. Sirinathsinghji, D. J. S. GnRH in the spinal subarachnoid space potentiates lordosis behavior in the female rat. *Physiol Behav* **31:** 717–723, 1983.
21. Tennent, B. J., E. R. Smith and D. M. Dorsa. Comparison of some CNS effects of luteinizing hormone-releasing hormone and progesterone. *Horm Behav* **16:** 76–86, 1982.