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Differential Gonadotropin Responses to *N-Methyl*-D,L-aspartate in Metestrous, Proestrous, and Ovariectomized Rats[1]

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ABSTRACT

Peripheral administration of *N*-methyl-D,L-aspartate (NMA), an analogue of the excitatory amino acid aspartate, elicits LH and prolactin (PRL) release in rats, most likely by increasing endogenous releasing-hormone secretion. These experiments were carried out to assess the degree to which NMA stimulates FSH and to analyze the relationship between endocrine status and responsiveness to NMA in female rats, in contrast to male rats, as described in the companion paper [Biol Reprod 48:000–000]. In experiment 1, estrous rats (n = 10) and diestrous rats (n = 10) and in experiment 2, estrous rats (n = 11) and rats ovariectomized (OVX) 8 days previously (n = 10) were fitted with atrial catheters and injected s.c. with 100 μg of an LHRH antagonist or vehicle at 2100 h. Starting at 0900 h the next day (metestrus, proestrus, or Day 9 post-OVX), blood was withdrawn every 10 min for 3 h. Each animal received i.v. 5 mg NMA after the first hour and i.v. 500 ng LHRH after the second hour. NMA significantly increased LH in metestrous and proestrous females, and LHRH antagonist blunted the increases. In OVX females, LH decreased after NMA. FSH was not affected by NMA in any group. PRL increased after NMA in proestrous and metestrous animals. LHRH caused surge-like LH and small FSH increases in vehicle groups; these increases did not differ in amplitude between intact and OVX animals and were blunted by pretreatment with LHRH antagonist. In experiment 3, 10 diestrous rats were fitted with atrial catheters and were serially bled at 2-h intervals from 1200 h on the following day (proestrus) until 0600 h on estrus morning. After the first sample the animals were injected s.c. with 0.2 mg/kg MK801, a noncompetitive NMA receptor antagonist, or with saline. Four of the 5 saline-treated animals exhibited surges of LH and FSH as well as elevated progesterone levels, with LH and progesterone peaking at 2000 h. Five of 5 MK801-treated animals failed to have elevated LH, FSH, or progesterone levels at any time point. These data demonstrate that LHRH mediates the LH response to NMA in rats and that endogenous NMA receptor binding may be necessary for the preovulatory gonadotropin surges. The lack of FSH responses to NMA during periods of low-level gonadotropin secretion suggests that physiological increments in endogenous LHRH secretion sufficient to induce a pulse of LH are insufficient to stimulate pulse-like FSH release. Comparison of metestrous and proestrous NMA responses suggests that elevated proestrous estradiol levels do not enhance the releasability of LHRH by NMA, while the suppression of LH levels following NMA in OVX rats suggests that in the absence of ovarian feedback the inhibitory effects of NMA on LHRH release predominate over its stimulatory effects.

INTRODUCTION

Peripheral administration of *N*-methyl-D,L-aspartate (NMA), an analogue of the excitatory amino acid aspartate, has been shown to elicit LH secretion in rats [1–4], monkeys [5, 6], and sheep [7]. In a companion to this study we [8] have shown that in intact, but not castrated, male rats NMA induces the release of LH and that this effect is fully antagonized by prior LHRH antagonist administration. We have also discussed the other convincing evidence that the induction of LH secretion by NMA is secondary to stimulation of LHRH secretion.

The effects of NMA on FSH secretion have not been as well studied as its effects on LH secretion; however, the limited data suggest that in monkeys [5, 6], but not in rats [2], NMA elicits FSH secretion that is blocked by pretreatment with an LHRH antagonist. In the companion study [8] we have substantiated that in intact and castrated male rats, NMA-stimulated LHRH secretion does not elicit FSH release. One aim of the following experiments, therefore, was to examine the dependence of LH and FSH secretion on pulsatile LHRH release after NMA administration in female rats.

A second major aim of these experiments was to analyze the relationship between endocrine status and LH and FSH responsiveness to NMA in cycling and ovariectomized female rats. We chose three different steroidal milieus: proestrus morning with its high estrogen and low progesterone levels [9]; metestrus with its low estrogen and moderately elevated progesterone levels [9]; and 9 days after ovariectomy, when levels of all gonadal hormones are extremely low. Previous work has shown that pulsatile LH release does not differ greatly, or at all, between metestrus and proestrus mornings [10, 11], while pituitary LHRH receptor levels [9] and in vivo [12] and in vitro [13] LH and FSH release in response to LHRH administration are increased on proestrus. If NMA administration were to elicit a larger LH pulse in proestrous than in metestrous animals, the source of this difference could be either the pituitary's greater sensitivity to LHRH stimulation and/or the greater sensitivity of the hypothalamus to NMA stimulation on proestrus. To distinguish between these possibilities, an LHRH challenge was given at the end of the experiment. Quite a different situation exists in the ovariectomized rat. LH pulse amplitude

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and frequency [14], plasma FSH levels [15], pituitary LHRH receptor content [16], and LH and FSH content [15] are all markedly higher by 9 days after ovariectomy than during metestrus. Since mean LHRH release in the median eminence is low after ovariectomy [17], conclusive analysis of LHRH pulse amplitude and frequency after ovariectomy has not been possible. However, the frequent low-amplitude fluctuations of LHRH (noted in [17]) together with the observed increase in LH pulse frequency [14] suggest that LHRH pulse frequency may also be increased. In contrast, hypothalamic LHRH content [18] decreases after ovariectomy. The decrease in LHRH content, in light of increased gonadotropin secretion and content and a possible increase in LHRH pulse frequency after ovariectomy, may represent a depletion of the readily releasable pool of LHRH. If this hypothesis were true a given dose of NMA would result in a smaller release of LH in ovariectomized versus intact rats, as has been seen in male rats [8].

Finally, we used a noncompetitive NMA receptor antagonist to assess the importance of endogenous NMA receptor binding on LH and FSH secretion on the afternoon of proestrus, a time when FSH secretion is clearly dependent on LHRH stimulation [19].

MATERIALS AND METHODS

Animals

Adult female Charles River (Portage, MI) Sprague-Dawley rats (200–300 g) were housed 3 or 4 to a cage in a temperature-controlled room with lights-on from 0500 to 1900 h. Animals had free access to tap water and standard laboratory rat chow. Only animals that had displayed at least two consecutive 4-day estrous cycles as determined by daily examination of vaginal cytology were used for experimentation.

Experimental Procedures

Experiment 1: Effect of NMA in proestrous versus metestrous animals. Between 2000 and 2200 h on estrus (n = 10) or diestrus (n = 10), rats were anesthetized with methoxyflurane and fitted with right atrial catheters, which were inserted via the external jugular vein. Catheterization at this time has been found to be least disruptive of estrous cyclicity between diestrus and proestrus [20]. At 2100 h, half the rats from each of the groups received s.c. 100 μg LHRH antagonist ([Ac-B(2)-D-NAL′4-Fd-Phe^2-D-Trp^3-D-Arg^6]-LHRH; Wyeth Laboratories, Philadelphia, PA) in 250 μl sesame oil, while the other half received oil vehicle. The antagonist was injected at 2100 h because previous work with this compound showed FSH suppression to be maximal at 12 h after administration [21]. Beginning at 0900 h the next morning (metestrus or proestrus), 0.5-ml blood samples were withdrawn from the catheter every 10 min for 3 h. Blood samples were dispensed into sample tubes for centrifugation; plasma was then stored at −20°C until subsequent assay for LH, FSH, and prolactin (PRL) by RIA. After each blood sample was taken, an equal volume of a blood replacement mixture [22], consisting of erythrocytes from female donor rats reconstituted with human plasma protein fraction (Plasmanate; Cutter Laboratories, Berkeley, CA), was slowly injected through the sampling catheter. The mixture contains no detectable immunoreactive LHRH, LH, or FSH. Immediately after the first hour of blood sampling, each animal received i.v. 5 mg of NMA (*N*-methyl-D,L-aspartate; Sigma Chemical, St. Louis, MO) in 0.5 ml of 0.9% saline. This dose of NMA was chosen because it elicits physiologically proportioned LH pulses in estrous rats [23]. One hour later each rat received i.v. 500 ng of LHRH (Sigma) in 0.5 ml saline. This large dose of LHRH was chosen because unpublished studies in our laboratory have shown that FSH in females is not significantly increased after administration of as much as 250 ng of LHRH. Autopsies were performed at 1800 h. Trunk blood was collected for LH, FSH, and PRL RIA, and uterine and ovarian weights were obtained. Of 6 diestrous rats cannulated and injected with vehicle, 5 were found to be in proestrus on the next day as determined by vaginal cytology, presence of ballooned uteri, and presence of LH and FSH surges at 1800 h. The sixth rat was not included in the statistical analysis.

Experiment 2: Effect of NMA in metestrous versus ovariectomized animals. The described protocol was repeated in intact metestrous rats and in rats ovariectomized on metestrus. One group of metestrous (n = 10) rats were bilaterally ovariectomized under methoxyflurane anesthesia at 0900 h. Eight days later between 0900 and 1200 h, these rats, plus a second group of intact estrous (n = 11) females, were fitted with atrial catheters. LHRH antagonist pretreatment, serial blood sampling, and NMA and LHRH treatment were conducted as in experiment 1.

Experiment 3: Effect of MK801 on the preovulatory LH and FSH surges. Between 2000 and 2300 h on diestrus (n = 10), rats were cannulated as in the first two experiments. Beginning at 1200 h on the next day (proestrus) and continuing every 2 h until 0400 h on estrus, 0.7 ml of blood was withdrawn through the sampling catheter. After each sample was removed, an equal volume of blood replacement mixture was administered. Immediately after the first sample was withdrawn, half of the animals received s.c. 0.2 mg/kg body weight of the NMA receptor antagonist, MK801 (Merck, Sharpe and Dohme, Rahway, NJ) dissolved in saline (1 mg MK801/5 ml saline), while the other half received saline alone. MK801 has been shown to bind only to the activated state of the NMA receptor [24, 25]. At 0600 h on estrus the animals were decapitated, trunk blood was collected, uterine weights were obtained, and ovaries were processed for histology. LH, FSH, and progesterone levels were subsequently determined in each sample by RIA.

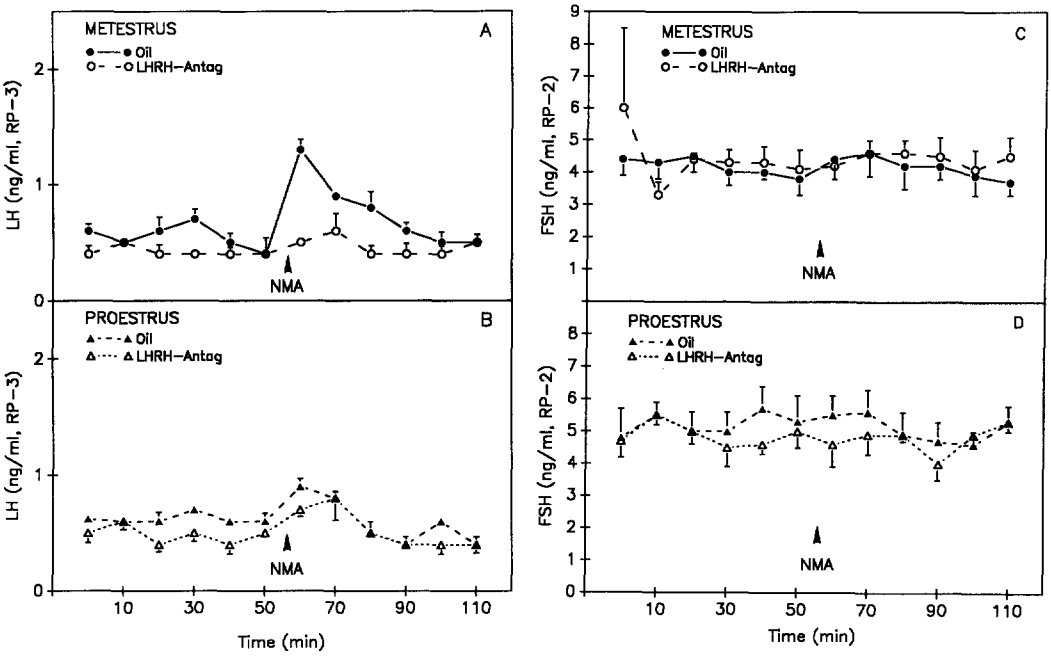

FIG. 1. Plasma LH (A, B) and FSH (C, D) levels in oil- and LHRH antagonist-treated metestrous (A, C) and proestrous (B, D) rats at 10-min intervals 1 h before and 1 h after i.v. injection of 5 mg NMA (arrow). Each symbol in this and the other figures represents the mean ± SE hormone level for all 5 animals within a group (or 6 for the oil-treated metestrous group in experiment 1) at a particular time point.

Hormone Assays

Levels of LH, FSH, and PRL in blood samples were determined by RIA through use of materials supplied by NIDDK (Bethesda, MD). The standards used in these assays were rLH-RP-3, rFSH-RP-2, and rPRL-RP-3. Plasma progesterone levels were measured by means of a commercially available progesterone kit (ICN Biomedicals, Carson, CA). The levels of sensitivity for the first experiment were 14 pg/tube for LH, 144 pg/tube for FSH, and 73 pg/tube for PRL as defined by 90%, 90%, and 80% binding, respectively. The intraassay coefficients of variation were 16.7% at 134 pg/tube for LH, 4% at 1017 pg/tube for FSH, and 12.9% at 644 pg/tube for PRL. For the second experiment the levels of sensitivity were 30 pg/tube for LH, 400 pg/tube for FSH, and 160 pg/tube for PRL as defined by 84.9%, 80.7% and 80% binding, respectively. The intraassay coefficients of variation were 14.2% at 227 pg/tube for LH, 9.7% at 741 pg/tube for FSH, and 6.1% at 231 pg/tube for PRL. For the third experiment the S25 LH standard was used. We have verified that this standard is equivalent to the RP-2 standard. The levels of sensitivity were 30 pg/tube for LH, 250 pg/tube for FSH, and 2 ng/ml for progesterone at 80% binding. The intraassay coefficients of variation were 4% at 175 pg/tube for LH, 4% at 410 pg/tube for FSH, and 11% for progesterone at 20.8 ng/ml.

Statistical Analysis

All results are expressed as the mean ± SE. Analysis of variance (ANOVA) with repeated measures was used to assess differences in LH, FSH, and PRL secretion between and within groups. The between-subjects variables tested were treatment (vehicle vs. LHRH antagonist) and cycle stage or surgery (intact vs. ovariectomy). The within-subjects variable was time of blood sampling. Post hoc comparisons between mean values at each time point were made using Newman-Keuls test. Results were considered significant if $p < 0.05$. All statistics were computed using the CRUNCH statistical software package (CRUNCH Software, San Francisco, CA).

RESULTS

Experiment 1: Effect of NMA in Metestrous Versus Proestrous Animals

Effect of LHRH antagonist on LH and FSH secretion. Mean plasma LH (Fig. 1, A and B) and FSH (Fig. 1, C and D) levels during the first hour of blood sampling did not differ between metestrous and proestrous females treated with oil. LHRH antagonist pretreatment significantly ($p < 0.004$) suppressed LH, but not FSH, secretion. LH and FSH levels in the trunk blood samples that were obtained at 1800 h from metestrous animals did not differ from those during serial sampling; however, in the proestrous females the gonadotropins were clearly surging at 1800 h (data not shown in figures), verifying that the surgery and blood sampling did not disrupt the surge mechanism. LH and FSH at 1800 h on proestrus were reduced from 9.4 ± 1.1 ng/ml and 20.2 ± 2.1 ng/ml in the oil-treated animals to 0.50 ± 0.04

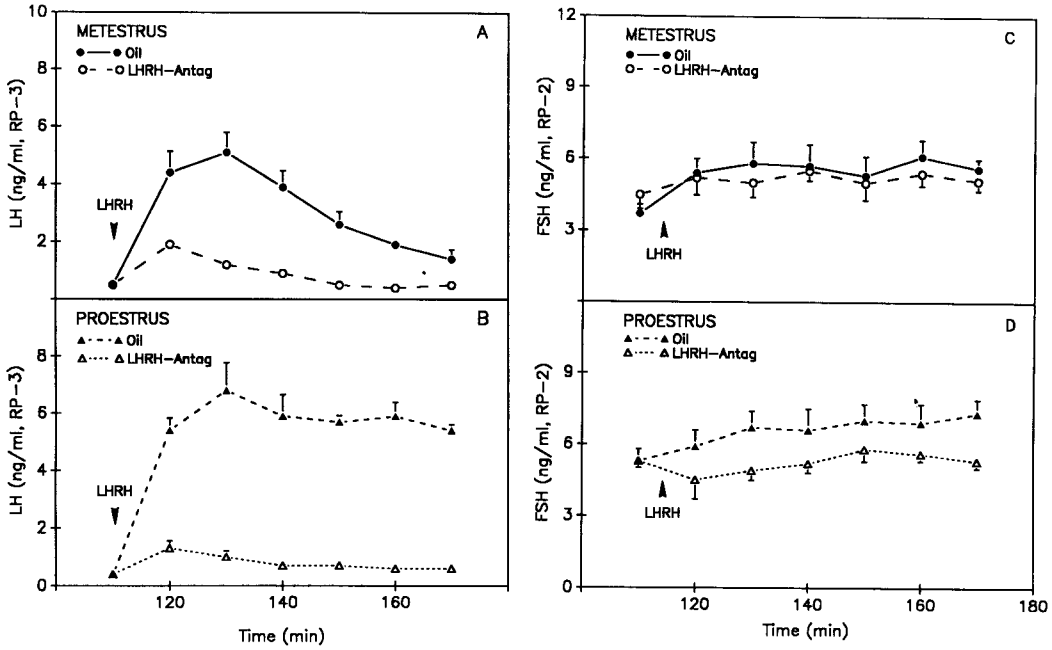

FIG. 2. Plasma LH (A, B) and FSH (C, D) levels in oil- and LHRH antagonist-treated metestrous (A, C) and proestrous (B, D) rats at 10-min intervals 10 min before and 60 min after i.v. injection of 500 ng LHRH (arrow).

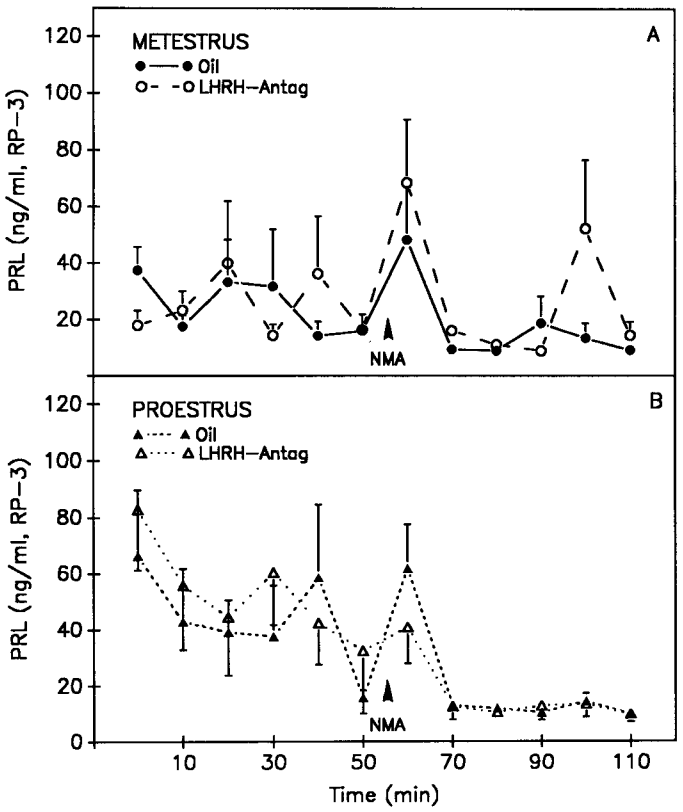

FIG. 3. Plasma PRL levels in oil- and LHRH antagonist-treated metestrous (A) and proestrous (B) rats at 10-min intervals 1 h before and 1 h after i.v. injection of 5 mg NMA (arrow).

ng/ml and 6.1 ± 0.4 ng/ml in the LHRH antagonist-treated animals.

Effect of NMA on LH and FSH secretion. Mean plasma LH and FSH levels during the hour before and the hour after i.v. administration of 5 mg NMA are depicted in Figure 1. LH levels increased significantly ($p < 0.01$) by 10 min after NMA injection, reaching 3-fold pre-injection values in metestrous animals (Fig. 1A) and 1.5-fold pre-injection values in proestrous animals (Fig. 1B). LH levels remained significantly elevated in the metestrous group at 20 min after NMA injection ($p < 0.05$). The NMA-induced LH increase was completely blocked in metestrous rats and was partially blocked in proestrous animals by prior LHRH antagonist treatment (Fig. 1). There was a significant ($p < 0.005$) interaction between cycle stage and treatment during the hour following NMA administration because the LH increase in response to NMA was higher in the metestrous than the proestrous oil-treated animals. Conversely, the LH increase was higher in the proestrous than in the metestrous antagonist-treated animals. FSH failed to increase significantly in either metestrous or proestrous females following NMA (Fig. 1, C and D).

Effect of LHRH on LH and FSH secretion. Mean plasma LH and FSH secretion before and after i.v. administration of 500 ng LHRH in metestrous and proestrous females is depicted in Figure 2. Within 20 min after LHRH injection, 10-fold and 15-fold increases in metestrous (Fig. 2A) and proestrous (Fig. 2B) LH levels occurred ($p < 0.01$). Me-

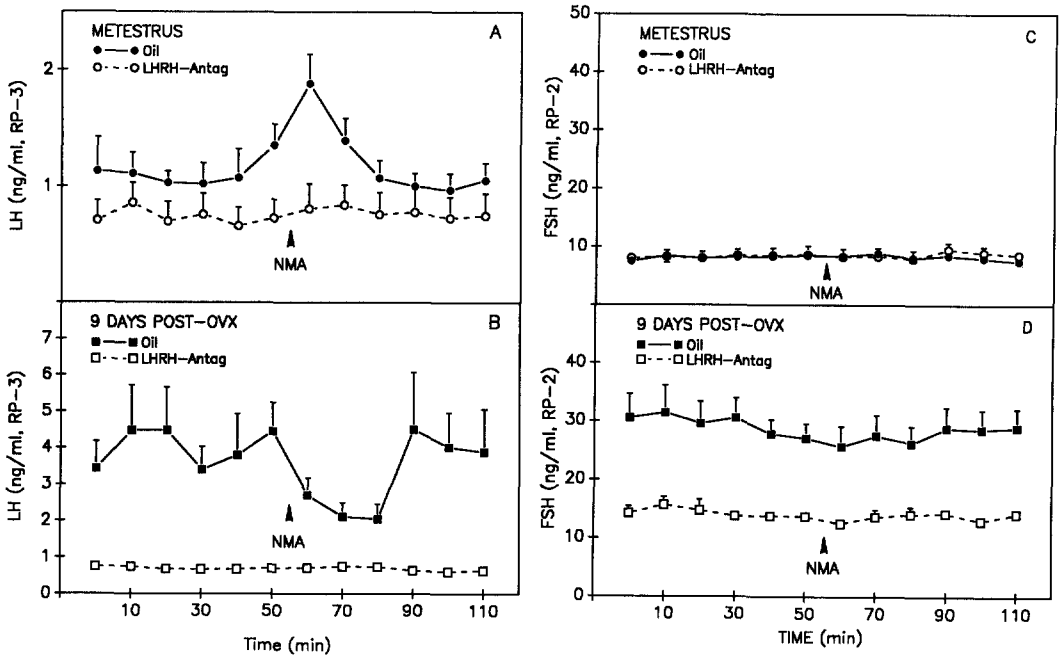

FIG. 4. Plasma LH (A, B) and FSH (C, D) in oil- and LHRH antagonist-treated metestrous (A, C) and 9-day ovariectomized (B, D) rats at 10-min intervals 1 h before and 1 h after i.v. injection of 5 mg NMA (arrow). Note that some of the symbols in graph C overlap.

testrous LH levels then declined, while proestrous LH levels remained elevated through the end of the sampling period ($p < 0.002$, effect of cycle stage). LHRH antagonist pretreatment considerably blunted LH responses to LHRH ($p < 0.001$). FSH (Fig. 2, C and D) increased 1.6-fold by 50 min after LHRH injection and 1.4-fold by 60 min after LHRH injection in the metestrous and proestrous oil-treated rats ($p < 0.01$). There were no differences in FSH levels be-

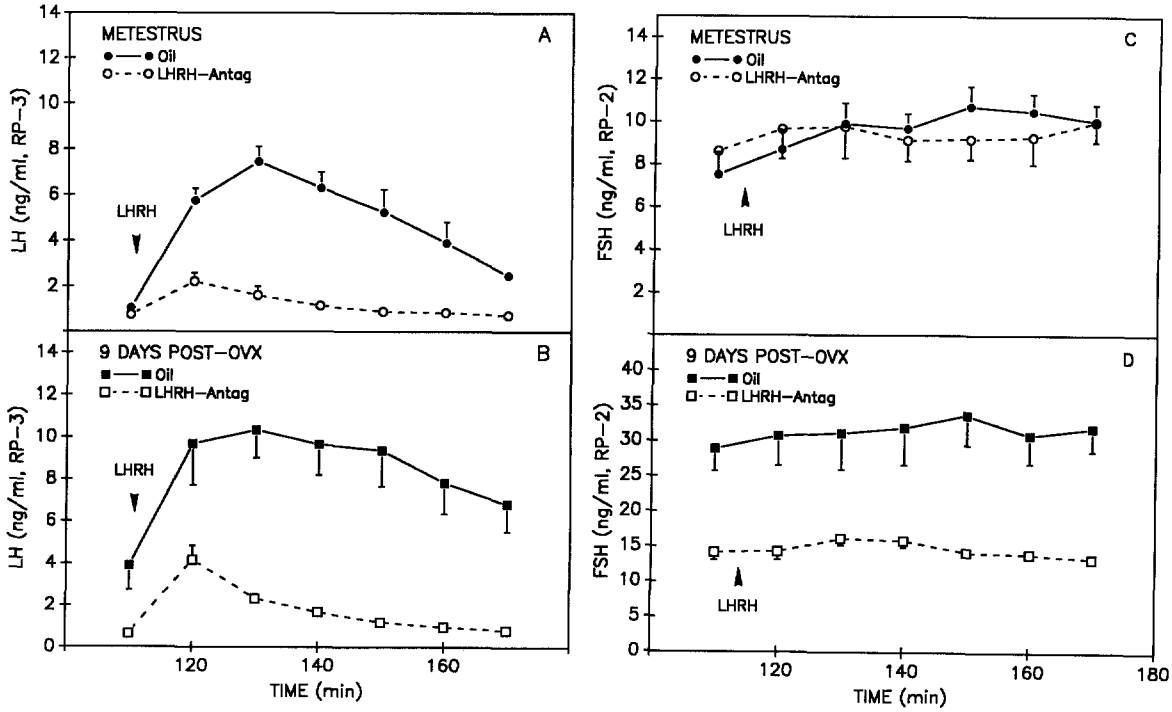

FIG. 5. Plasma LH (A, B) and FSH (C, D) levels in oil- and LHRH antagonist-treated metestrous (A, C) and 9-day ovariectomized (B, D) rats at 10-min intervals 10 min before and 60 min after i.v. injection of 500 ng LHRH (arrow).

tween samples taken before and after LHRH administration in the LHRH antagonist-treated animals.

Effect of NMA on PRL secretion Mean plasma PRL levels in metestrous and proestrous oil-treated and LHRH antagonist-treated females are shown in Figure 3. Mean plasma PRL levels during the first hour of blood sampling were higher than during the third hour (not shown). The mean levels in metestrous animals declined from 24.9 ± 4.4 ng/ml during the first hour to 10.7 ± 1.9 ng/ml during the third hour, while in proestrous animals they declined from 47.8 ± 10.7 ng/ml to 14.6 ± 3.9 ng/ml. PRL levels in metestrous animals (Fig. 3A) increased significantly ($p < 0.05$) from 16.0 ± 2.2 ng/ml 10 min before, to 48.3 ± 18.7 ng/ml 10 min after, NMA injection. The PRL elevation in proestrous animals (Fig. 3B) failed to reach significance by Newman-Keuls post hoc test; however, mean levels did increase from 15.7 ± 2.7 ng/ml 10 min before NMA injection to 61.8 ± 15.7 ng/ml 10 min after NMA in the oil-treated animals, and from 32 ± 22.4 ng/ml to 40.8 ± 12.8 ng/ml in the antagonist-treated animals. Both LHRH (not shown) and LHRH antagonist failed to have any effect on plasma PRL levels in animals of either cycle stage.

Experiment 2: Effect of NMA in Metestrous Versus Ovariectomized Animals

Effect of LHRH antagonist on LH and FSH secretion. As expected, mean plasma LH levels (Fig. 4, A and B) for the first hour of blood sampling were significantly ($p < 0.0001$) higher in ovariectomized than in metestrous females, as were mean plasma FSH levels (Fig. 4, C and D). In the ovariectomized rats LHRH antagonist significantly ($p < 0.007$) reduced LH release by 81% to levels observed in the metestrous females, but FSH was significantly ($p < 0.002$) reduced by only 51%, to about 1.6 times the metestrous levels.

Effect of NMA on LH and FSH secretion. Mean plasma LH and FSH concentrations during the hour before and the hour after i.v. injection of 5 mg NMA in the oil-treated and LHRH antagonist-treated metestrous rats are depicted in Figure 4. Metestrous LH and FSH responses to NMA were similar to those in experiment 1 with LH, but not FSH, increasing. This NMA-induced LH increase was blocked by LHRH antagonist pretreatment. In the oil-treated ovariectomized animals (Fig. 4B), NMA administration resulted in a 40% decrease in LH levels by 10 min after injection; this was sustained for three sampling intervals. Two-way ANOVA comparing the mean of LH levels in the six samples taken during the first hour for each animal with the corresponding mean for the second hour revealed that in the oil-treated animals the mean hourly LH was significantly lower ($p < 0.004$) after NMA (3.24 ± 0.75 ng/ml) than before (4.00 ± 0.83 ng/ml). In the ovariectomized animals pretreated with LHRH antagonist, the LH levels were suppressed to the limit of detectability of the assay throughout the first 2 h of bleeding (Fig. 4B). NMA failed to alter FSH secretion in any of the groups (Fig. 4, C and D).

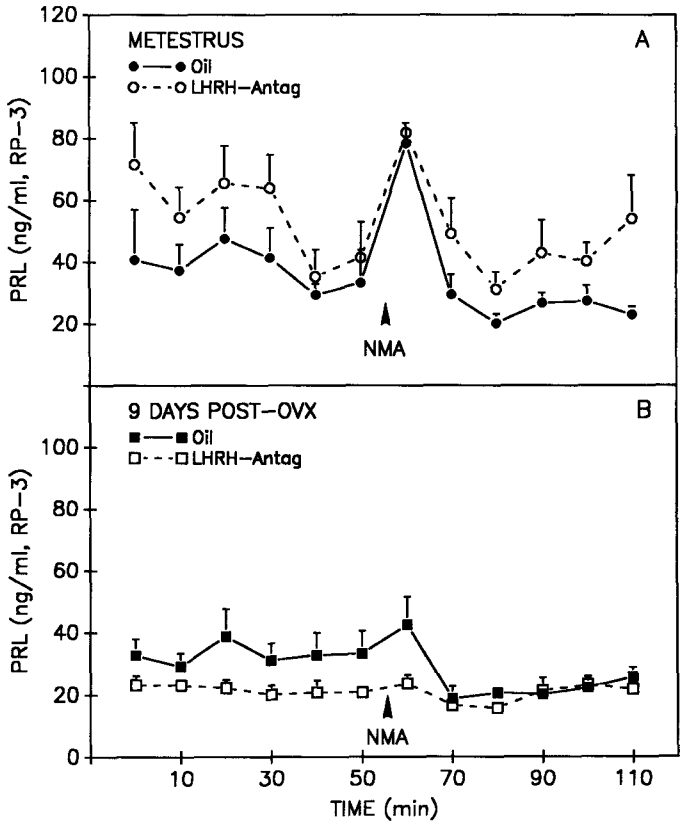

FIG. 6. Plasma PRL levels in oil- and LHRH antagonist-treated metestrous (A) and 9-day ovariectomized (B) rats at 10-min intervals during the hour before and the hour after i.v. injection of 5 mg NMA.

Effect of LHRH on LH and FSH secretion. Figure 5 depicts mean plasma LH and FSH concentrations before and after the i.v. injection of 500 ng LHRH in metestrous and 9-day ovariectomized rats pretreated with oil or LHRH antagonist. LH levels rose 5.5-fold in intact (Fig. 5A) and 2.3-fold in ovariectomized (Fig. 5B) females within 20 min of LHRH injection ($p < 0.01$), although the amplitude of the increase (about 6 ng/ml at maximum) was identical in the two groups. There was a significant blunting of the LH ($p < 0.001$) response to LHRH in the LHRH antagonist-pretreated animals. LHRH injection also significantly ($p < 0.05$) increased FSH levels by 26% in the oil-treated metestrous rats, but not in the oil-treated ovariectomized or intact or the antagonist-treated ovariectomized rats (Fig. 5, C and D).

Effect of NMA on PRL secretion. Circulating plasma PRL levels in metestrous rats (45.1 ± 5.3 ng/ml, Fig. 6A) were approximately 2-fold higher than PRL levels in ovariectomized animals (27.3 ± 2.9 ng/ml, Fig. 6B) during the first hour of blood sampling ($p < 0.005$). As in experiment 1, by the sixth sample PRL concentrations in most of the animals had fallen from initially elevated levels (37.0 ± 3.9 ng/ml was the mean for the sixth sample in intact animals).

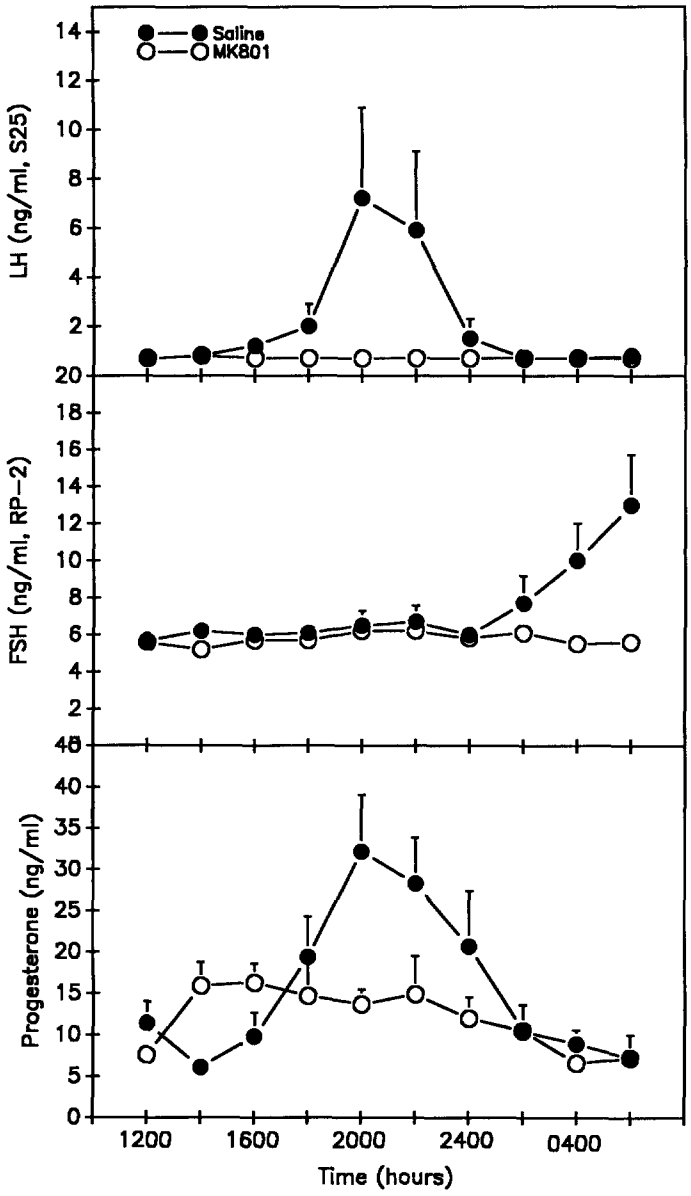

FIG. 7. Plasma LH (top), FSH (middle), and progesterone (bottom) levels at 2-h sampling intervals beginning at 1200 h on proestrus or metestrus in rats treated with saline or MK801 immediately after the first blood sample was withdrawn.

PRL is known to increase in response to stress; therefore, it is likely that the variable plasma PRL levels in intact females during the first hour were due to stress from the initiation of the blood sampling procedure in a subset of animals. There was no significant effect of LHRH antagonist treatment on PRL levels in intact or ovariectomized rats (Fig. 6). NMA injection again resulted in significant ($p < 0.001$) 2-fold increases in PRL levels in both the oil- and antagonist-treated metestrous animals (Fig. 6A), but not in antagonist- and oil-treated ovariectomized animals (Fig. 6B). This difference between the PRL responses of intact and ovariectomized animals to NMA was significant ($p < 0.001$).

Experiment 3: Effect of MK801 on the Preovulatory LH and FSH Surges

Four out of 5 rats that received saline injections at 1200 h on the expected day of proestrus had LH and FSH surges and elevated progesterone levels that evening (Fig. 7). The fifth rat was eliminated from the statistical analysis because its hormone levels indicated that it was not in proestrus. Peak LH and progesterone levels occurred at 2000 h. FSH levels had not yet begun to decline by 0600 h on estrus. Injection of 0.2 mg/kg MK801 at 1200 h on proestrus blocked the LH ($p < 0.001$, interaction of treatment and sampling time), FSH ($p < 0.001$), and progesterone ($p < 0.002$) increases in 5 out of 5 proestrous rats (Fig. 7). Examination of ovarian histology in the saline-treated proestrous rats revealed stimulated follicles in 3 of the 4 animals and ova in the oviduct of the fourth. No stimulated follicles or ova were found in the ovaries of the MK801-treated rats.

DISCUSSION

The results of this and the companion study [8] provide further evidence that NMA stimulates LH release in intact female and male rats by increasing LHRH secretion, and that LHRH by itself is a sufficient stimulus for LH secretion. The LH pulses elicited by NMA injection in metestrous and proestrous rats were similar in amplitude and duration to endogenous LH pulses that have been reported by others in intact females during these cycle stages [10, 11], as well as to the LH pulses induced by NMA administration in estrous rats [23] and intact male rats [8]. Moreover, the ability of LHRH antagonist to block the NMA-induced LH secretion provides further evidence that it is an NMA-elicited elevation in LHRH release that stimulates LH secretion in the rat. In addition, we have found that NMA receptor binding by endogenous ligands is necessary for the stimulation of the proestrous LH surge. NMA receptor binding had previously been shown to be necessary for the occurrence of LH surges in estrogen-primed ovariectomized rats [26] and at a single time point in proestrous rats [27].

The inability of NMA injection to stimulate the acute release of FSH, combined with the less rapid and less pronounced FSH responses to LHRH administration or antagonism in female and in male rats [8], suggests that the acute release of FSH is less dependent upon LHRH stimulation than is LH secretion. These results are consistent with those of other studies in the rat showing that FSH secretion is less dependent minute to minute than LH secretion upon LHRH stimulation [21, 28]. Thus, in both male and female rats, LHRH appears to have more slowly developing effects on FSH than on LH secretion; this is consistent with a mechanism of action affecting synthesis of the hormone [8]. In contrast to low-level gonadotropin secretion during periods of the rat estrous cycle, LHRH stimulation is clearly necessary for FSH secretion during the primary preovulatory FSH surge [29, 30]. We have verified previous findings

that pretreatment with LHRH antagonist completely blocks the primary proestrous FSH surge [29, 30]. We have also shown for the first time that NMA receptor antagonist administration on proestrus prior to the time at which LHRH secretion rises [17] blocks the preovulatory primary and secondary FSH surges; this suggests that NMA receptor binding may play an important role in regulating FSH secretion at this time during the estrous cycle when FSH secretion is dependent upon LHRH stimulation. Brann and Mahesh [27] recently found that MK801 administration blocks the FSH surge in estrogen-primed, progesterone-treated rats and in eCG-treated immature rats. They did not, however, observe a significant attenuation of the primary FSH surge in intact proestrous rats, possibly because they examined a single time point at which FSH levels may not yet have peaked [27].

Others [28] have argued that the relative lack of responsiveness of FSH to LHRH antisera or antibodies provides evidence for the existence of a separate FSH-releasing factor. The failure of NMA administration to elicit FSH release may be consistent with this hypothesis. It may be that a separate FSH releasing factor exists, but that excitatory amino acid receptors do not play a role in its release. However, the release of PRL in response to NMA injection in both our male [8] and female studies, as well as the ability of NMA to elicit growth hormone release [2, 6], argues against this possibility by demonstrating that at least two other hypothalamic releasing factors can be stimulated by NMA. Alternately, FSH release patterns may be fully explained by partial dependence upon LHRH stimulation plus dependence upon gonadal feedback factors such as inhibin, activin, and steroids. Unlike LHRH antagonist alone, inhibin plus LHRH antagonist [31] or plus the sex-appropriate gonadal steroid [32, 33] can suppress serum FSH levels in gonadectomized rats to intact levels. Moreover, inhibin also is capable of suppressing FSH mRNA synthesis [34, 35], and activin is a powerful stimulator of FSH synthesis and secretion [35, 36]. Finally, specific patterns of LHRH can selectively induce FSH without LH release. In the female rat, slow constant infusion of LHRH or low-intensity, low-frequency electrical stimulation of the medial preoptic area favors FSH over LH secretion [37]. The LH responses in our experiments suggest that the NMA stimulus used probably elicited a single, short, low-amplitude pulse of LHRH. This type of LHRH stimulus appears to be a poor signal for FSH stimulation [37].

In contrast to gonadectomized male rats, in which there was no NMA-evoked LH response [8], ovariectomized female rats showed a decline in LH levels after NMA administration. Suppression of LH secretion by NMA has also been reported in ovariectomized monkeys [38]. Moreover, steroid replacement reversed this inhibitory effect of NMA on LH secretion in monkeys [39]. In contrast, in ovariectomized ewes there was no effect of NMA administration on LH secretion [7]. NMA did, however, elicit LH elevations in these ewes when LH release was first suppressed by estradiol replacement [7]. The effect of steroid replacement on NMA-induced LH release in rats remains to be studied. The lack of an LH increase in response to NMA after ovariectomy might be due to a loss of pituitary sensitivity to LHRH. This possibility, however, is refuted by previous studies in which LHRH injection resulted in significant increases in LH and FSH secretion in ovariectomized rats [12, 13], and in the present studies by the fact that LHRH induced LH and FSH elevations of similar amplitude in the ovariectomized and the intact rats. The absence of an increase in LH secretion after NMA injection may instead be due to a smaller releasable pool of LHRH in the hypothalamus of ovariectomized rats. Evidence for this includes the observation that median-eminence LHRH content in female rats declines after gonadectomy [18], as does LHRH release induced by electrical stimulation of the median eminence in vitro [40]. An apparently increased LHRH pulse frequency after ovariectomy [14, 17] may be responsible for a depletion of LHRH releasable stores. A reduction in releasable LHRH in ovariectomized animals may permit a decline in LH levels after NMA injection to occur if, in addition to stimulating LHRH secretion, NMA also stimulates secretion of a factor(s) inhibitory to LHRH and/or LH secretion. This hypothesis is supported by evidence that in monkeys corticotropin-releasing hormone and endogenous opiates may mediate the inhibitory effect of NMA on LH secretion [38]. The lack of a suppressive effect of NMA on LH secretion in castrated male rats [8] may indicate that in these animals NMA does not exert inhibitory effects on LHRH and/or LH secretion or that the stimulatory effects of NMA on LHRH and/or LH cancel the inhibitory effects.

Another interesting finding was the lack of differences in the LH and FSH responses to NMA injections between metestrus and proestrus. Although the LHRH signal to the pituitary is known to be amplified by the pituitary's increased sensitivity to LHRH on proestrus [12, 13], this enhanced pituitary sensitivity is manifested only after several priming pulses when low doses of LHRH are administered in vivo [12]. Accordingly, the LH responses to a single injection of NMA were not greater in proestrous than in metestrous animals in the present study. There was, however, an enhanced LH response to the dose of LHRH in that LH levels of the proestrous rats remained elevated longer after LHRH administration. Since in the current experiments differences in pituitary sensitivity to LHRH did not play an important role in determining the LH responses to NMA, the similar LH responses to NMA suggest that the releasability of LHRH is not greater in the presence of the high proestrous morning estradiol levels than it is on metestrous morning. Previous studies have shown that in vivo [17, 41] LHRH secretion and hypothalamic electrical activity [42, 43] are elevated during the afternoon of proestrus. Although in vivo LHRH secretion has not been examined on the mornings of proestrus versus metestrus, morning LH secretion

has been found to be similar on the two days [11] or slightly greater [10] on metestrus compared to proestrus. In addition, hypothalamic electrical activity [43] has not been found to differ significantly between metestrous and proestrous mornings. Since experiments using ovariectomized rats have shown that both estradiol [40] and progesterone [44] enhance in vitro LHRH release, it may be that both the elevated metestrous morning progesterone levels and the elevated proestrous morning estradiol levels [9] are stimulatory to LHRH release. This conclusion is supported by observations that LHRH secretion is lowest during estrus and after ovariectomy, when circulating levels of estradiol and progesterone are both low [17, 41].

Our data also show that i.v. administration of NMA stimulates secretion of PRL in metestrous and proestrous rats, as it does in estrous rats [23], male rats [8, 23], and monkeys [5]. This action of NMA is probably mediated through the release of a hypothalamic PRL-releasing factor (PRF). Substances that stimulate PRL secretion and are candidate PRFs include thyrotropin-releasing hormone, vasointestinal peptide, and a number of other endogenously occurring compounds [45]. Interestingly, we also observed a post-gonadectomy difference in PRL release: the PRL response to NMA was significantly blunted by ovariectomy. This suggests that NMA-induced release of PRF and/or PRL is enhanced in the presence of ovarian feedback. A stimulatory role for ovarian steroids on PRL secretion has been previously described [46].

In summary, in the present study we have used the LHRH secretagogue, NMA, to provide evidence that 1) endogenous LHRH pulses, which stimulate physiologically proportioned LH pulses in intact female rats, are not capable of stimulating FSH pulses; 2) LH secretion in ovariectomized rats decreases following NMA administration, suggesting diminished LHRH response to the secretagogue and/or stimulation by NMA of an inhibitor to LHRH secretion; 3) LHRH responses to a secretagogue are not increased on the morning of proestrus compared to metestrus; and 4) NMA receptors may play a role in the regulation of LH and FSH secretion on the afternoon of proestrus.

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