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Effects of Morphine and Naloxone on Phasic Release of Luteinizing Hormone and Follicle-Stimulating Hormone[1] (40849)

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Several recent studies have indicated that the endogenous opioid peptides (EOP) can influence the secretion of gonadotropic and other pituitary hormones. Acute injections of morphine (MOR) or methionine-enkephalin were reported to inhibit, whereas naloxone (NAL), a specific opiate receptor antagonist, stimulated LH and FSH release (1, 2). The EOP also have been implicated in the regulation of the proestrous LH surge in cycling female rats (3) and in LH release in prepubertal rats (4). MOR and EOP were shown to block ovulation and the preovulatory gonadotropin surge on the afternoon of proestrus (5, 6) and these effects were reversed by NAL (7). The rise in LH produced by castration of male rats was partially prevented by MOR and enhanced by NAL (8).

It previously was demonstrated that ovariectomized rats treated with estradiol benzoate (EB) showed a daily proestrous-like surge of LH, and that estrogen is the stimulus which "turns-on" the daily neural signal of LH release (9, 10). It also was shown that injection of progesterone (P) to ovariectomized EB-primed rats enhanced the LH surge on the day of P injection, but abolished subsequent LH surges (11, 12). It is believed that P "turns-off" the expression of the daily LH surge induced by estrogen. The purpose of the present investigation was to examine the effects of MOR and NAL on the EB induced daily surge signal for LH and FSH release in ovariectomized rats, and their effects on the ability of P to block expression of these daily neural signals.

Materials and methods. Animals. Female Sprague–Dawley rats (Harlan Industries, Cumberland, Ind.) weighing 250–300 g, were housed under a 14-hr light/10-hr dark schedule (lights on 0500–1900 hr), and fed food pellets (Ralston Purina Co., St. Louis, Mo.), and water *ad libitum*. All rats were ovariectomized for at least 4 weeks before treatment with ovarian steroids to induce daily gonadotropin surges.

Drugs. Morphine sulfate (MS, Mallinkrodt Labs., St. Louis, Mo.), and naloxone hydrochloride (NAL, Endo Laboratories, Garden City, N.Y.), were dissolved in 0.87% NaCl solution (SAL). Estradiol benzoate (EB) and progesterone (P, Sigma Chemical Co., St. Louis, Mo.) were dissolved in corn oil. Synthetic gonadotropin-releasing hormone (LHRH) was kindly provided by Dr. K. Folkers (Institute for Biomedical Research, University of Texas, Austin), and was dissolved in saline. All injections were given subcutaneously (sc).

Experiments. In experiment 1, 24 ovariectomized rats were given two injections of 20 μg EB at 1000 hr with an interval of 72 hr. On the day following the second injection, the animals were divided into three groups and given four injections of either MOR (5 mg/kg), NAL (0.2 mg/kg), or SAL, at 1300, 1500, 1700, and 1900 hr. Blood was collected via orbital sinus puncture under light ether anesthesia at 1000, 1700, and 2000 hr on the day of drug treatment (Day 1) and on the following day (Day 2).

In experiment 2, 24 rats were treated with EB and drugs in the same manner as in experiment 1. However, starting at 1500 hr on

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Day 1, the animals were given six consecutive injections of either SAL or LHRH (50 ng/100 g body wt) sc every 30 min. Blood was collected at 1000, 1800, and 2000 hr for 2 days, as in experiment 1.

In experiment 3, 24 rats were injected first with EB, followed 72 hr later by a 2.5-mg P injection at 1100 hr. On the day of P injection, drug treatments and blood sampling were performed in a manner similar to experiment 1. Experiment 4 was conducted in the same manner as experiment 3, except that a 10-mg dose of P was used. Blood was taken at 1000 and 1700 hr on Days 1 and 2.

Hormone assays and statistical analysis. Serum levels of LH and FSH were assayed by standard RIA procedures with NIAMDD kits, kindly provided by Dr. A. F. Parlow, using the double antibody method of Niswender *et al.* (13) for LH and the method for FSH given in the kit. The LH results were expressed as micrograms per milliliter in Figs. 1 and 2 and nanograms per milliliter in Table 1, in terms of the respective reference preparation. Analysis of variance and Student–Newman–Keuls test for multiple comparison among groups were used to analyze the data for the significance of differences among means.

Results. Effects of MOR and NAL on the LH and FSH surges in EB–EB-treated ovariectomized rats. EB treatment of control (SAL) rats induced an afternoon surge of LH on Days 1 and 2 (Fig. 1). The LH surge was blocked by MOR on Day 1, whereas the NAL-treated group showed a significantly greater LH surge than the SAL-treated controls on Day 1. On the next day, the trend was reversed, with the MOR group showing a large rebound LH surge and the NAL group showing no significant surge. Serum FSH showed a surge in the control rats similar to that of LH. MOR blocked, but NAL had no significant effect on the FSH surge on Day 1. On Day 2, MOR-treated rats showed no effect, whereas NAL treatment suppressed the FSH surge.

Effects of LHRH on the LH and FSH release in MOR and NAL-treated ovariectomized rats given EB–EB. Table 1 shows that the SAL and SAL controls displayed a surge of LH on Days 1 and 2. The MOR and SAL group showed a block of the surge on Day 1 and a large rebound surge on Day 2. The rats given MOR and LHRH showed a large LH surge on Day 1 equal to that of the SAL- and LHRH-treated group, and also showed a large surge on Day 2. The SAL and LHRH group showed a very large LH surge on Day 1 and a Day 2 surge equal to that of SAL and SAL-treated controls. The NAL-treated rats showed a characteristic potentiated LH surge on Day 1, and on Day 2, again showed a loss of the LH surge. The

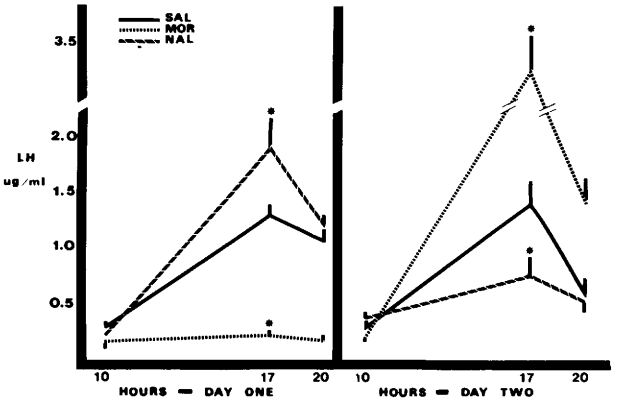

FIG. 1. Serum LH concentration in EB-primed ovariectomized rats at 10, 17, and 20 hr on Days 1 and 2, given four injections of morphine (MOR), naloxone (NAL), or physiological saline at 13, 15, 17, and 19 hr on Day 1. Each point represents the mean and vertical bars represent the SE. $^*P < 0.05$, as compared to SAL controls.

TABLE I. EFFECT OF MORPHINE (MOR) AND NALOXONE (NAL) ON RELEASE OF LH IN RESPONSE TO LHRH IN EB-TREATED OVARIECTOMIZED RATS[a]

Treatment	Day 1			Day 2		
	1000 hr	1800 hr	2000 hr	1000 hr	1800 hr	2000 hr
SAL + SAL	201 ± 44[a]	1277 ± 160	1112 ± 80	261 ± 24	1170 ± 188	440 ± 77
MOR + SAL	156 ± 32	281 ± 66*	319 ± 77	264 ± 34	3242 ± 477*	1367 ± 337
MOR + LHRH	129 ± 25	6496 ± 834*	1254 ± 195	396 ± 74	2735 ± 379*	1271 ± 276
NAL + SAL	227 ± 48	1677 ± 129	1358 ± 268	246 ± 32	588 ± 117*	336 ± 61
SAL + LHRH	203 ± 40	6393 ± 488*	2375 ± 311	216 ± 38	1210 ± 280	503 ± 100

[a] Number of rats = 8 per group.
* $P < 0.05$ as compared to controls. Values expressed as ng/ml (mean ± SEM).

FSH response to the different treatments produced similar trends to that of LH. However, the effects of the treatments on serum FSH were of lesser magnitude than on LH.

Effects of MOR and NAL on the LH and FSH surges in EB–P-treated ovariectomized rats. The LH surge on Day 1 in EB–P-treated controls reached a peak about three times as high as in the EB–EB-treated rats (Fig. 2). MOR blocked the LH surge on Day 1, but NAL had no effect on the LH surge. On Day 2, the NAL- and SAL-treated groups showed no LH surges in the EB–P-treated rats. Surprisingly, however, the MOR-treated group showed a large LH surge on Day 2. Similar trends were observed on serum FSH after treatment with MOR or NAL.

To determine if the LH surge on Day 2 in MOR-treated rats could be blocked by a higher dose of P, 10 mg/rat was given in experiment 4. The NAL- and SAL-treated groups showed similar surges of LH on Day 1, whereas the LH surge in the MOR group was blocked. On Day 2, the LH surges were blocked in all groups. FSH responded similarly to LH.

Discussion. These observations show that MOR and NAL can alter expression of the EB-induced daily surge signal in ovariectomized rats, not only on the day of drug treatment, but also on the next day. Previous observations demonstrated that MOR could inhibit the preovulatory surge of LH and ovulation in cycling rats (5, 6), but subsequent events were not followed. In EB–EB-treated rats, MOR blockade of

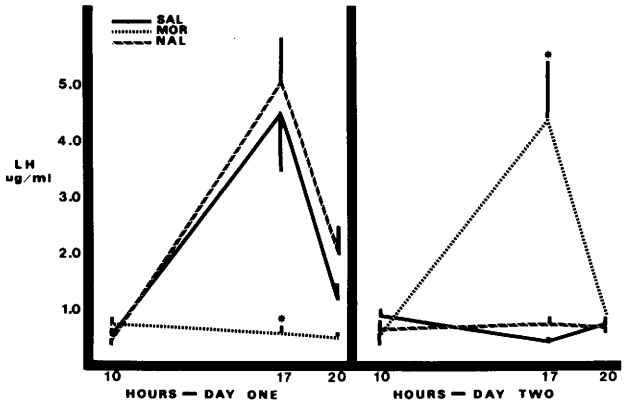

FIG. 2. Serum LH concentration in EB–progesterone (2.5 ng) primed ovariectomized rats at 10, 17, and 20 hr on Days 1 and 2, given four injections of MOR, NAL, or saline at 13, 15, 17, and 19 hr on Day 1. Each point represents the mean and vertical bars represent the SE. *$P < 0.05$ as compared to SAL controls.

the gonadotropin surge on Day 1 resulted in a large rebound surge of LH on the afternoon of Day 2. In contrast, NAL treatment potentiated the gonadotropin surge on Day 1, but inhibited expression of the daily surge signal on Day 2.

It seemed unlikely that the unique effects of MOR and NAL on LH release on Day 2 were due simply to the amount of hormones available for release by the pituitary. MOR blockade of the LH surge on Day 1 could have permitted a buildup of gonadotropin stores so that a rebound surge was seen on Day 2. However, since rats given MOR and LHRH showed large increases of LH on Day 1 and demonstrated the same rebound surge of LH on Day 2, it is reasonable to conclude that these surges seen on Day 2 in MOR-treated rats were not due merely to an increase in releasable gonadotropin stores on Day 1. Likewise, the NAL potentiated surge on Day 1 could have depleted the stores of gonadotropin and rendered the pituitary unable to respond with a normal gonadotropic surge on Day 2. However, rats given SAL and LHRH did not show a suppression of the LH surge on Day 2, as in the NAL- and SAL-treated rats, even though the Day 1 surge in the SAL- and LHRH-treated rats was many times larger than that of the NAL- and SAL-treated rats. Thus, the action of MOR and NAL on the gonadotropin surge are not believed to be due to alterations in capacity of the pituitary to secrete hormone, but rather to their central effects on the daily surge signal.

The EB–P induced LH surge in ovariectomized rats differs from that of EB–EB-treated animals, as previously reported, in that the surge was many times greater, and a subsequent LH surge did not occur. Estrogen is believed to turn on the daily surge signal, whereas P potentiates the LH surge on Day 1, but turns it off subsequently (11). Evidence also suggests that P may not inhibit the neural signal for the estrogen-induced LH surge, but rather render the hypothalamus unable to respond to the signal with sufficient LHRH release to induce a gonadotropin surge (14). NAL had no effect on the high LH surge in the EB–P-treated animals on Day 1 nor on the blockade of the gonadotropin surge on Day 2. However, MOR blocked the EB–P-induced LH surges on Day 1, but a large surge of LH was seen on the afternoon of Day 2. This LH surge on Day 2 in MOR-treated rats is in direct contrast to control rats in which P exposure inhibited the LH surge on Day 2. Nembutal blockage of the Day 1 LH surge has also recently been shown to result in a large LH surge on Day 2 in EB–P-treated ovariectomized rats (14). These results show that MOR injection on Day 1 can antagonize the inhibitory effect of P on the gonadotropin surge induced by EB on Day 2. This antagonism was found to be dose-related, since a higher dose of P (10 mg/rat) overcame the central action of MOR and prevented an LH surge on Day 2.

The response of FSH to MOR and NAL in both the EB–EB- and EB–P-treated rats showed trends similar to that of LH. Hence the data for FSH were not shown here.

The neural signal for the preovulatory gonadotropin surge in rats originates in the preoptic–anterior hypothalamic area (15), where LHRH-containing neurons have been found (16). Under the appropriate estrogenic conditions, this signal results in the discharge of LHRH into the portal circulation (17, 18). Estrogen also enhances the preoptic area stimulated release of LHRH (19) and increases the firing rate of the preoptic neurons (20). Stimulation of the preoptic area by estrogen is believed to "turn-on" the daily preovulatory surge signal, whereas P decreases the firing rate of these neurons (20) to possibly "turn-off" the surge signal.

Localization of enkephalin-containing neurons has been investigated immunohistochemically, and the distribution of these terminals were found to be adjacent to the cell bodies of the steroid-concentrating neurons in the preoptic and other areas of the hypothalamus (21). Also the content of methionine-enkephalin in the preoptic–anterior hypothalamic area was shown to be high on the morning of proestrus, but declined on the afternoon of proestrus and estrus (22). These observations suggest that the actions of MOR and NAL occur at the preoptic–anterior hypothalamic areas of the brain, and that the opiates modulate

steroid regulation of LHRH release. An alternate explanation is that MOR and NAL act via other neurotransmitters in the brain to regulate LHRH release. There is some evidence that brain opiates can alter catecholamine and serotonin activity in the hypothalamus (23). This latter possibility is currently being investigated in our laboratory.

Summary. In ovariectomized rats given estradiol benzoate (EB), followed 3 days later by EB or progesterone, morphine, and naloxone altered the LH and FSH surges on the day of treatment (Day 1) and on the next day (Day 2). In the EB–EB-treated rats, morphine prevented, whereas naloxone enhanced the surge of LH and FSH on Day 1. On Day 2, the morphine-treated rats showed a large rebound surge of LH and FSH, whereas the naloxone-treated rats showed no surge. In the EB–progesterone-treated rats, morphine blocked, whereas naloxone had no effect on the LH and FSH surge on Day 1. On Day 2, the morphine-treated rats showed a large LH and FSH surge, whereas the naloxone-treated rats showed no surge. The effects of these drugs on the Day 2 surge of LH and FSH were found not to be the result of a build up or depletion of releasable stores of LH in the pituitary on Day 1. These results suggest a possible role for the endogenous opioid peptides in modulating steroid regulation of the neural surge signal for LH and FSH.

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