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Diurnal Influences on Serum Luteinizing Hormone Responses to Opiate Receptor Blockade with Naloxone or to Luteinizing Hormone-Releasing Hormone in the Immature Female Rat[1] (41283)

MICHAEL S. BLANK[2] AND DAVID R. MANN*

*Yerkes Regional Primate Research Center of Emory University, Atlanta, Georgia 30322, and *Department of Physiology, School of Medicine at Morehouse College, Atlanta, Georgia 30314*

Abstract. The major objective of these studies was to determine whether there is a temporal pattern in the gonadotropin response of immature rats to luteinizing hormone-releasing hormone (LHRH) or the opiate antagonist, naloxone. Thirty-day-old female rats were injected at 3-hr intervals over a 24-hr period with either naloxone (2.5 mg/kg body wt) or LHRH (8 ng/100 g body wt). Animals were decapitated 15 min later and serum samples were assayed for luteinizing hormone (LH) by radioimmunoassay. The serum LH response to naloxone and LHRH varied significantly with the time of day. Naloxone administration had no statistically significant ($P > 0.05$) effect on levels of serum LH at 1500 and 1800 hr compared to levels in saline-injected controls, but induced a significant rise in serum LH at all other times. Naloxone had its greatest effect during the late evening and early morning hours (2100 to 0900 hr). A similar, but not identical, pattern of LH responsiveness to LHRH was observed, with the two rhythms being truly divergent only during the late afternoon when LH sensitivity to LHRH was high but low to naloxone. These data indicate that there is a diurnal pattern of pituitary sensitivity to both naloxone and LHRH in the immature rat and suggest, for the most part, that temporal variations in the LH response to opiate antagonists may result from altered pituitary sensitivity to endogenous LHRH. However, the enhanced response of the pituitary to LHRH during the late afternoon, when opioid inhibition of hypothalamic LHRH secretion appears to be at a nadir could provide a mechanism in the immature rat whereby adult-like LH surges can be stimulated. The early afternoon LH response to various doses of naloxone was examined in intact and ovariectomized 30-day-old rats. Intacts displayed a lower absolute but higher percentage increase above basal values of LH than did ovariectomized animals. These latter findings contrast with those previously found in adult female rats.

Opiate receptor blockade with naloxone elevates serum concentrations of luteinizing hormone (LH) in rats (1–3) and humans (4) under a variety of physiological circumstances (5–11). The effect on LH secretion of sole administration of naloxone is opposite to that elicited by morphine (12–15) or exogenous opiate peptides (1). Therefore, naloxone appears to antagonize an inhibition of LH secretion which is mediated by opiate receptors. However, the serum LH response to naloxone is absent or minimal at certain stages of the human menstrual cycle (5, 10, 11) or at specific ages, such as 30 days, in immature rats (2, 9).

Several factors including ovarian steroids may be responsible for modulating opiate regulation of LH secretion. It is known, for example, that injections of estradiol block naloxone stimulation of LH secretion in immature and ovariectomized, adult rats (2, 7). Intact adult female rats are unresponsive to naloxone (7), but following ovariectomy an LH response to naloxone can be demonstrated (2). Other important determinants of the endocrine effects of opiate receptor blockade with naloxone include sex (2), age (2, 9), reproductive status (7, 11), and, perhaps, the time of day at which naloxone

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[2] To whom all correspondence and requests for reprints should be addressed.

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is administered (7, 26). To more clearly understand why female rats of certain ages fail to exhibit a clear-cut serum LH response to opiate receptor blockade, we studied the effects of phase of the diurnal cycle and reproductive status on the LH response of immature rats to naloxone. We also compared pituitary sensitivity to exogenous luteinizing hormone-releasing hormone (LHRH) with the serum LH response to naloxone in intact animals.

Materials and Methods. *Animals and experimental protocols.* Female Sprague–Dawley rats (Charles River Breeding Laboratories, Wilmington, Mass.) were shipped to our animal facility at 21 days of age. Upon arrival, they were exposed to a daily light cycle of 14-hr light:10-hr darkness (lights on at 0700 hr). The importance of the time of day at which naloxone was administered to the subsequent rise in serum LH was studied in 30-day-old rats. Female rats at this age were previously shown to be less responsive to the LH-elevating effects of early afternoon naloxone injections than prepubertal rats of other ages (2). Groups of rats 7–10 were randomly selected at 3 hr intervals between midnight and 2100 hr to receive subcutaneous (sc) injections of naloxone–HCl (2.5 mg/kg body wt) or saline. This dose of naloxone produces robust and highly reproducible elevations of LH in serum of immature female rats (12) but is well below doses at which narcotic agonist effects of naloxone have been reported (13). Other groups of 9 or 10 rats received sc injections of LHRH (8 ng/100 g body wt) at 2 or 3-hr intervals throughout the day. In our hands, this dose of LHRH produces a uniform but submaximal release of LH of approximately the same magnitude as naloxone (14). Each animal was decapitated 15 min following the naloxone, LHRH, or saline injection. During periods of darkness, injections and decapitations were performed in dim red light (25-W bulb). In a separate experiment, the effects of ovariectomy on the dose–response characteristics of naloxone-induced LH secretion were studied in 30-day-old rats. Ovaries were removed on Day 24 under ether anesthesia. Six days after ovariectomy, each animal received a single subcutaneous injection of naloxone–HCl (0.05–10.0 mg/kg body wt) or saline between 1300 and 1330 hr, followed by decapitation 15 min later. For comparison, intact 30-day-olds were challenged with saline or identical doses of naloxone at the same time of day and bled by decapitation 15 min later. This time of day was chosen to be consistent with that reported in previous experiments (2, 7). Sham-ovariectomized rats were not deemed necessary for this study because of the long delay (6 days) between surgery and the test of LH sensitivity to naloxone.

Serum LH measurements. Trunk blood was collected at the time of decapitation and allowed to clot at 4° overnight. The time of storage at 4° for each group of blood samples was not kept constant. The serum was then separated by centrifugation and stored at −22° until assayed for LH by RIA (15). The elapsed times between decapitation and storage of serum at −22° did not exceed 24 hr. The NIAMDD rat LH RP-1 standard was used as the reference preparation and serum values are expressed in nanograms of RP-1 per milliliter of serum. Rat LH RP-1 has a biological potency equivalent to 0.03 × NIH-LH-S1. The between- and within-assay coefficients of variation at $B/B_0 = 0.60$ were 15.6 and 11.9%, respectively ($n = 10$). All samples from an individual experiment were analyzed in the same assay.

Materials. The naloxone-HCl used was obtained from Endo Laboratories, Garden City, New York. Luteinizing hormone-releasing hormone for these experiments was purchased from Sigma Chemical Company, St. Louis, Missouri.

Statistical analyses. Data were analyzed by one or two-way ANOVA followed by post hoc testing. The Student–Newman–Keuls test was utilized for multiple comparisons of treatment means following one-way ANOVA (16). Following two-way ANOVA (17), treatment means for time-matched groups were compared by partitioning the treatment sum of squares and multiple comparisons of treatment means were analyzed by orthogonal contrasts (18).

Results. The administration of naloxone (2.5 mg/kg body wt) resulted in significant ($P < 0.05$ at 1200 hr; $P < 0.01$ at other times) elevations in serum LH above saline-injected controls throughout the 24-hr period except at 1500 and 1800 hr (Fig. 1a). The magnitude of the increase in serum LH varied with the time of opiate antagonist administration ($P < 0.025$ for drug × time-of-day interaction by two-way ANOVA). Naloxone-stimulated levels of LH were significantly ($P < 0.05$) higher during the late evening (2100 hr) and morning (0000–0900 hr) than during the afternoon (1500 and 1800 hr). The dramatic decline in naloxone sensitivity which was evident between 0900 and 1200 hr reached significance ($P < 0.01$) by 1500 hr. Basal levels of serum LH in saline controls did not differ significantly ($P > 0.05$) between time periods (Fig. 1b).

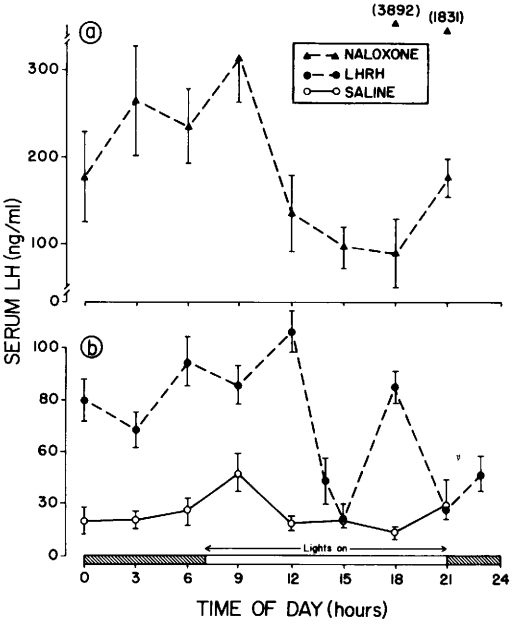

FIG. 1. Mean levels of serum LH in 30-day-old female rats after receiving subcutaneous injections of (a) naloxone (2.5 mg/kg body wt) (▲) or (b) LHRH (8 ng/100 g body wt) (●) or saline (○) at various times during a 24-hr period. Decapitations were performed 15 min after the injections. Hatched areas on abscissa correspond to periods of darkness. Vertical brackets represent ± 1 SEM, $n = 7-10$ group. LH levels in one rat treated with naloxone at 1800 hr and another at 2100 hr were >1800 ng/ml. These values were not included in the statistical analyses and they are plotted separately in a (▲)

The temporal pattern of the LH response to LHRH (Fig. 1b) was similar but not identical to naloxone. Injections of LHRH significantly ($P < 0.01$) elevated levels of LH in serum at most time points, with the exception of 1500 and 2100 hr. Pituitary sensitivity to LHRH was elevated during the morning hours (0000–1200 hr), but dropped sharply between 1200 and 1400 hr ($P < 0.01$) before rising again between 1500 and 1800 hr ($P < 0.01$). At 2100 and 2300 hr the LH response to LHRH was again lower than morning values ($P < 0.01$) with one exception: the 2300 and 0300-hr responses were not significantly different. Replication of key time points substantiated the fall and subsequent rise in LHRH sensitivity during the afternoon but not the falloff in sensitivity after 1800 hr. Therefore, except for the increased sensitivity to LHRH at 1800 hr (when the naloxone response was low), the temporal pattern of sensitivity to LHRH or naloxone was quite similar.

The naloxone dose–response curves for intact and ovariectomized rats were quite similar (Fig. 2). Once a minimally effective dose of naloxone was attained (2.5 mg/kg body wt in intacts, 5 mg/kg body wt in ovariectomized) further increases in naloxone dosage led to no further increase in serum levels of LH. Intacts showed significant ($P < 0.05$) elevations in LH at doses of naloxone between 2.5 and 10.0 mg/kg body wt (Fig. 2a) and ovariectomized at 5.0 and 10.0 mg/kg body wt (Fig. 2b). When the LH responses for intact and ovariectomized rats were compared at equivalent doses of naloxone, ovariectomized rats exhibited a greater absolute but lower percentage increase in serum LH at each dose (Table I).

Discussion. These studies have shown that there is a diurnal rhythm of sensitivity in the serum LH response to the opiate antagonist, naloxone, in immature female rats, with the response being more prominent in the late evening and morning hours than in the afternoon. This diurnal pattern of LH responsiveness to naloxone is the reciprocal of the diurnal rhythm for the

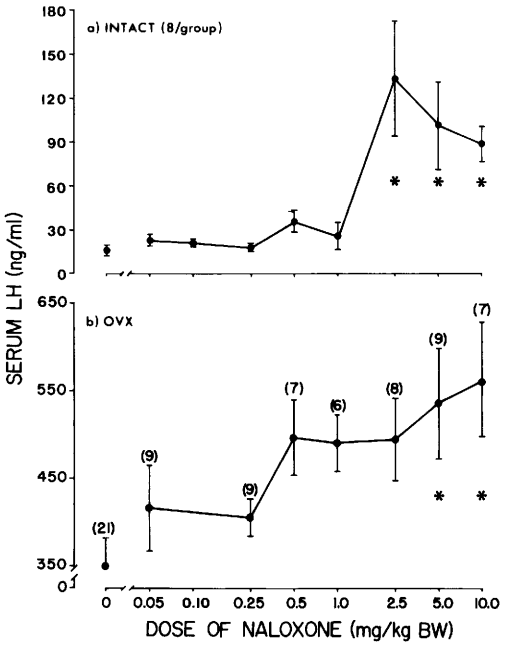

FIG. 2. Naloxone dose–response potentiation of LH secretion in 30-day-old (a) intact or (b) ovariectomized rats injected with naloxone between 1300 and 1330 hr. Mean levels of serum LH are plotted on the ordinate and the logarithm of the naloxone dose on the abscissa. Zero dose corresponds to saline-injected controls. Vertical brackets represent ± 1 SEM. The numbers of ovariectomized animals per group are in parentheses in b. *$P < 0.05$ compared with saline-injected controls.

hyperalgesic effects of this drug reported in mice (19). The hyperalgesia associated with injections of naloxone in mice is greatest during the afternoon and lowest during the morning. Species differences cannot be ruled out as accounting for this discrepancy in temporal response patterns to naloxone. However, we might also infer from this difference that to the extent neuroendocrine rhythms (21, 22) participate in opiate regulation of gonadotropin secretion, they are probably distinct from endogenous rhythms which mediate pain (20, 27).

Enhanced physiological responses to naloxone have been associated, by inference, with increased endogenous opiate receptor activity (10, 19) and correlated directly with elevations in levels of brain opioids (23, 24). Conversely, a diminished response to opiate antagonists could indicate lower opiate receptor activity. The diminution of the LH response to naloxone which occurred during the early afternoon could signal the removal of a tonic opiate inhibition of gonadotropin release. However, basal LH levels did not rise at this time as would be predicted if gonadotropin secretion were actually disinhibited. Thus opiate neuronal systems may not be the primary final determinant of LH release during this phase of the diurnal cycle.

Since injections of naloxone probably stimulate LH secretion by effecting endogenous LHRH release (2, 3), temporal shifts in the naloxone-induced serum LH pattern could be accounted for by time-dependent changes in pituitary sensitivity to LHRH. In fact, the temporal pattern of the LH response to naloxone and LHRH were very similar. Thus, the diurnal rhythm of sen-

TABLE I. COMPARISON OF THE LH ELEVATING EFFECTS OF NALOXONE IN INTACT AND OVARIECTOMIZED 30-DAY-OLD FEMALE RATS

Reproductive status	Change in serum LH[a]	Doses of naloxone resulting in significant elevations of serum LH in both intact and ovariectomized rats	
		5 mg/kg body wt	10 mg/kg body wt
Intact	Absolute	84.6	72.2
	Percentage	+637.6[b]	+558.8[b]
Ovariectomized	Absolute	186.0[c]	213.0[c]
	Percentage	+153.0	+160.8

[a] Expressed as the mean increase (Absolute) or the mean fractional increase × 100 (Percentage) in levels of serum LH above those found in saline-injected controls.

[b] $P < 0.05$ vs percentage change in serum LH in ovariectomized group (two-way analysis of variance: means compared by orthogonal contrasts).

[c] $P < 0.05$ vs absolute changes in serum LH in intacts (two-way analysis of variance: means compared by orthogonal contrasts).

sitivity to naloxone may, for the most part, be explained on the basis of altered changes in the response to naloxone-released endogenous LHRH. At 1800 hr, however, the two rhythms diverged: the sensitivity to LHRH was elevated while there was a decreased LH response to naloxone. This finding has now been replicated on several occasions in our laboratory, suggesting it may serve a physiological function. One possibility is that a decreased opiate inhibition of endogenous (probably hypothalamic) LHRH secretion during the afternoon in concert with an increased pituitary sensitivity to LHRH could provide the basis in the immature rat for an adult-like competency for surge release of LH.

The plateau found in the naloxone dose–response curve for LH in intact 30-day-old female rats, is in agreement with studies in 25-day-old females (12). However, in 25-day-olds, as little as 0.5 mg/kg body wt of naloxone stimulates LH secretion (12). The shift of the dose–response curve to the right in 30-day-olds conforms with the finding of lowered sensitivity to naloxone in rats of this age (2).

Studies in adult female rats (2, 7) have indicated that the ovary exerts a strong negative influence on the release of LH which is provoked by opiate receptor blockade. That is, ovariectomized adults display a pronounced LH response to naloxone which is absent in the intact animal (2) or the ovariectomized rat treated with estrogen (7). In contrast to the situation in the adult, immature rats in the present study were less sensitive to the LH elevating effects of naloxone following ovariectomy. This conclusion is based on the observation that ovariectomized rats showed a lower fractional increment in LH than their intact counterparts. It could be argued that the higher absolute elevations in serum LH in ovariectomized rats indicate enhanced sensitivity to naloxone. Yet the already elevated circulating levels of LH in castrates make this comparison less useful. Thus, the present findings do not agree with studies in adult rats which have shown that the hormonal effects of opiate antagonists (7) and brain levels of opiate receptors (25) are quite sensitive to gonadectomy. The reasons for these differences between adult and immature rats is unclear but may be due to either qualitative and/or quantitative differences in steroid output from the ovaries of mature animals or to discrepancies in steroid target sensitivity.

In summary, we have shown that the serum LH response to naloxone administration varies in a diurnal pattern. The response was maximal during the morning hours and declined to insignificant levels during the afternoon. On the basis of parallel changes in pituitary sensitivity to LHRH, we postulate that, in general, the amount of endogenous LHRH released by naloxone does not vary, but that the amount of LH discharged by the pituitary in response to the same bolus of endogenous LHRH varies with the time of day. However the late afternoon return of LHRH sensitivity to preafternoon levels, at a time when opiate inhibition of LH is diminished, may signify a potential for surge discharge of LH in the immature animal. We have also demonstrated that in contrast to adult rats, ovariectomy in immature rats does not significantly potentiate the LH response to naloxone.

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