Luteinizing Hormone Secretion as a Response to a Second Naltrexone Administration (43105)

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Abstract. **Previous studies with naltrexone (Nalt), a "long-lasting" opioid antagonist, demonstrated a rapid increase in luteinizing hormone (LH) secretion which gradually declined, reaching baseline values after 1 hr. A second Nalt challenge, 120 min later, caused only a blunted response. This poor reaction has been shown in this study not to be due to lack of pituitary responsiveness, because LH-releasing hormone treatment revealed a normal response. A time-response study was carried out in order to establish the refractory period length, by administering a second Nalt injection at 0 hr (immediately after the first injection) and at 2, 4, 8, 16, and 24 hr after the first bolus.**
Partial responsiveness could be achieved 2 and 4 hr after the first challenge. However, only after 8 hr was a full response recorded. The diurnal changes in serum LH (nadir at 18.00 hr) did not affect the response to Nalt challenge. It is suggested that in the presence of a Nalt blockade, nonopioid systems are able to "normalize" LH blood levels. However, when Nalt blood levels have fallen sufficiently to allow the endogenous opioid system to take primary control again, then a second Nalt injection will provoke a renewed response. [P.S.E.B.M. 1990, Vol 195]

Opioid peptides exert a tonic inhibitory effect on luteinizing hormone (LH) secretion (1). They participate in the negative androgen-induced feedback control of the release of this hormone (2). Extended earlier observations have shown that blocking the hypothalamic opiate receptor causes a transient increase in LH levels (3). This effect was mediated by catecholamine secretion, and thus naloxone stimulation of LH release could be blocked by α-adrenergic receptor blockers (4).

Naltrexone (Nalt), a long-acting opiate receptor blocker (5), caused LH secretion within 15 min of administration as a bolus to a rat. However, a gradual decrease to baseline levels occurred 60 min later (6). Two hours after the injection, LH levels were found to be even lower than the baseline, and a further injection of Nalt at this time evoked only a slight increase in serum LH (6, 7). In a previous study it was found that tolerance to a second naloxone injection lasted for 4 to 6 hr (8). In comparison to naloxone, Nalt has a longer duration of action and greater potency (5). The biologic half-life is about 4.6 hr compared with 40 min of naloxone (5). The half-time of blockade of brain μ-opiate receptors by Nalt ranges from 72 to 108 hr (9), whereas no differences were found in the amount of naloxone present in the brains of animals pretreated 2 hr earlier with naloxone or saline (8). The purpose of this study, therefore, was to monitor serum LH levels after a second injection of Nalt at different intervals following the first bolus in order to elucidate its influence.

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Materials and Methods

Male Wistar rats weighing 350–450 g were used in the study. The animals were housed under constant temperature (23 ± 2°C) on a 14:10-hr light:dark cycle (lights on −05:00–19:00 hr).

Naltrexone hydrochloride (Du Pont Pharmaceuticals, Geneva, Switzerland), 1 mg/rat (about 2.5 mg/kg), was dissolved in 0.5 ml of saline and injected intravenously. LH-releasing hormone (LHRH) (Ferring) was given intravenously as a bolus, 200 ng/0.5 ml of saline/rat.

Blood for LH analysis (0.8 ml/sample) was collected from an indwelling cannula that had been inserted into the right atrium as described previously (10).

When sampling occurred during the dark phase, the room was illuminated by a 25-W red light bulb. Blood samples were centrifuged, and the plasma was collected and stored at −20°C. Blood cells were resuspended in saline and returned to the animals via the cannula after subsequent bleeding. When the same rats were used for more than one treatment, plasma from donor rats (the same volume withdrawn by the bleeding) was returned to the experimental animals after the last bleeding of the day.

Experiment 1. An initial injection of Nalt was given at 10.00 hr, and the LH response to a second 1-mg injection was evaluated at 0, 2, 4, 8, 12, 16, or 24 hr after the first injection. Blood samples were collected shortly before the first and second injection and at 15, 30, and 60 min following the second Nalt administration.

Experiment 2. In order to examine the possibility of different hypothalamic pituitary sensitivity to Nalt administration due to different times of the day, LH surge 15 min after Nalt injection at 10:00, 14:00, 18:00, 22:00, and 02:00 hr was determined. These hours were selected to serve as controls for the second Nalt injection in Experiment 1.

Experiment 3. Pituitary LH reserves were measured in rats following 2-hr pretreatment with Nalt, by administration of 200 ng of LHRH or its vehicle (saline). The same rats served for both control (saline) and experiment (LHRH) with a 4-day hiatus. Blood samples were taken immediately before and 15, 120, 135, and 180 min after Nalt injection.

LH Assay and Statistical Analyses. LH levels were determined by using NIADDK radioimmunoassay kit. NIADDK rat LH-RP-2 was used as reference preparation. The intra- and interassay coefficients of variation were 7.5 and 14%, respectively. Samples from each experiment were estimated in the same assay to avoid interassay variation. Statistical analysis of results was performed, using analysis of variance for repeated measurements followed by Duncan's multiple range test and paired t test.

Results

The length of the refractory period of the pituitary to a second challenge of Nalt was studied by monitoring LH levels (Fig. 1). A second injection of Nalt, 2 hr following the first, caused a significant increase in serum LH within 15 min ($P < 0.05$) (second open bar in Fig. 1), but this increase did not exceed the baseline level as was measured immediately before the first Nalt injection. The response was increased 4 hr later, but not to a statistically significant level. Eight hours or more after the first injection the response was sustained and became normal (Fig. 1).

In order to evaluate whether the response to a second Nalt injection was influenced only by the first injection rather than by diurnal changes, LH response to Nalt was recorded every 4 hr throughout the day at the above-mentioned hours. Baseline levels of serum LH fluctuated during the day with a significantly decreased level at 18:00 hr ($P < 0.05$). Nevertheless, there was a 2- to 3-fold increase in LH levels 15 min following Nalt injection, at all times examined (Fig. 2).

To eliminate the possibility of the refractoriness to the second injection of Nalt being due to the absence of "readily releasable" LH reserves in the anterior pituitary, LHRH was administered to rats preinjected with Nalt 2 hr earlier. A significant increase in serum LH compared with LH level after saline injection was registered: 2.8 ± 0.16 and 0.3 ± 0.07 ng/ml (mean ± SE) 15 min after LHRH and saline administration, respectively ($P < 0.001$), which lasted for at least 1 hr (Fig. 3). The small increase in LH level as a response to saline injection was found to be not statistically significant.

Discussion

This study revealed a diurnal variation in basal serum LH levels of adult male rats. However, Nalt caused the same marked increase in LH secretion, enhancing the baseline level by 2- to 3-fold at different hours of the day. Diurnal variations in the level of immunoreactive β-endorphine in male rats were found in plasma and in the hypothalamus when the highest levels were measured during the first hours of dark (11). This might explain the lowest LH levels which were found in this study at around that time.

Previous studies with Nalt have shown that although it has a relatively long biologic half-life, as was measured in the plasma (5) and the brain (9), its effect on LH secretion *in vivo* lasts for less than 1 hr. The fact that the attenuation in LH secretion is not due to Nalt disappearance was also shown indirectly by morphine administration to rats preinjected 2 hr earlier with Nalt. Morphine normally causes a significant release of PRL for at least 1 hr. When this treatment was given to Nalt-pretreated rats, no response was detected for at least 2 hr (6). In the present study the Nalt-preinjected rats responded less intensively to a second challenge of Nalt if given within 2–4 hr after the first injection. The rats regained normal responsiveness starting 8 hr following the first administration of Nalt. This interval length is similar in duration to that found for restoration of morphine analgesia after removal of the Nalt pellet (5). This is also about the same period required (6 hr) for naloxone to produce the maximal degree of enhanced sensitivity to morphine as judged by serum LH depression (12). It is interesting to note that although Nalt is accepted as a long-acting opioid antagonist, with a terminal elimination half-life of 4.6 hr, it was found to have about the same period of refractoriness to a second, identical injection as did naloxone (8).

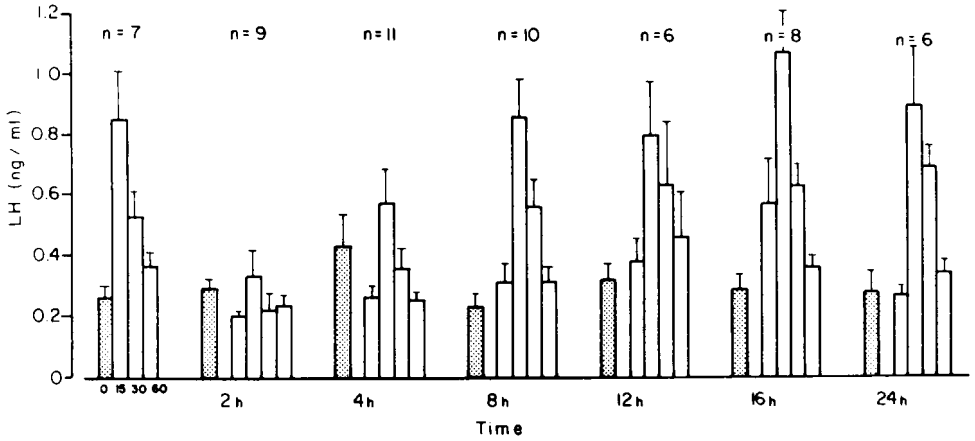

Figure 1. Serum LH levels before first naltrexone injection and at 0, 15, 30, and 60 min after the second naltrexone administration. Second dose was given at different intervals after the first one. The first set of bars depicts serum LH time response to a bolus of two doses (5 mg/kg) of Nalt. Baseline levels immediately before the first naltrexone injection are depicted by dotted columns. Numbers are mean ± SE, *n*, number of animals.

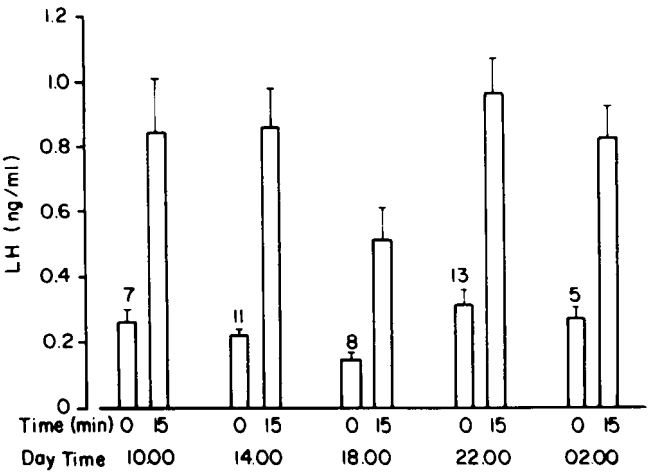

Figure 2. Plasma LH levels (mean ± SE) 15 min after naltrexone injection every 4 hr throughout the day. Number of animals used at each time of examination is given above the first column.

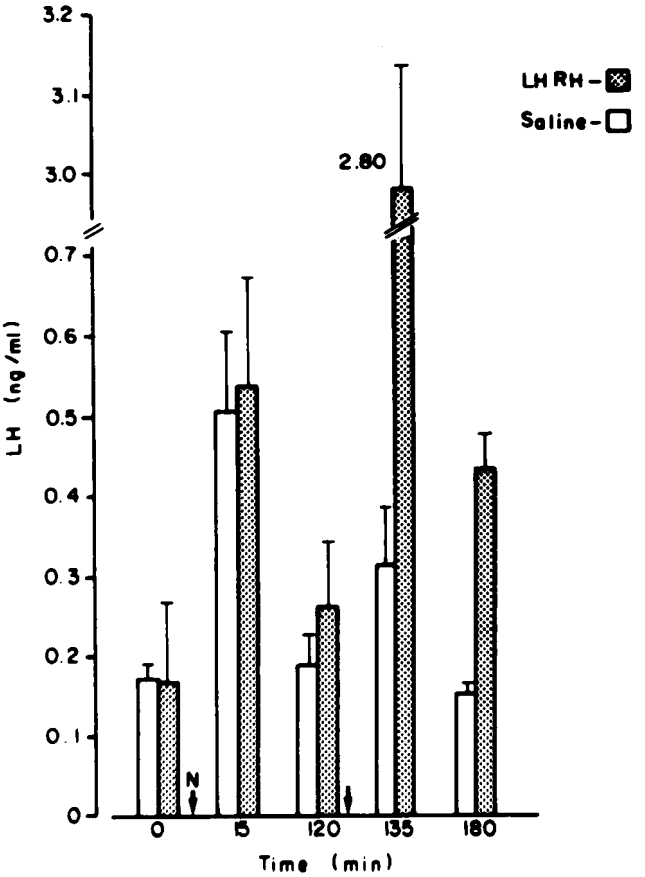

Figure 3. Effect of LHRH or saline on LH levels in rats pretreated 2 hr earlier with naltrexone (N). Each column represents mean ± SE of nine rats.

This refractoriness could not be attributed to decreased pituitary readily releasable LH reserve, since a bolus of LHRH given 2 hr after Nalt administration caused a significant increase in LH secretion. This was also shown in naloxone-pretreated rats, where no effect of naloxone on the responsiveness of the pituitary to LHRH was observed (12).

Naloxone was effective in stimulating LH and testosterone secretion when it was given three times daily (13). However, when it was administered constantly by means of a pellet, a moderately enhanced initial rate of increase in LH secretion in the ovariectomized, estradiol-treated rats was observed, followed by low LH levels at 3 and 7 days postimplantation (14). It would therefore appear that the antagonist level has to be low in order to achieve a second, enhanced LH secretion. It might be that in the presence of a Nalt blockade, nonopioid systems are able to "normalize" LH blood levels until Nalt blood levels have fallen sufficiently to allow the endogenous opioid system to take primary control again and the system again becomes sensitive to Nalt.

Another possibility for the refractory period of LH following the first Nalt injection is that Nalt undergoes

metabolism. In particular, a major pathway of Nalt metabolism in the rat is the hydroxylation to 6β-hydroxynaltrexone which has mixed agonist/antagonist properties (15), thus interfering with the efficacy of the second Nalt injection. This may also explain why the LH level 2 hr post-Nalt is even lower than baseline.

When nalmefene, another opiate receptor blocker, was tested at high doses (25 and 50 mg/kg), a loss of effectiveness in terms of induced LH secretion was observed, when compared with lower doses of the same drug. This was in contrast to the stimulatory effect of naloxone, both in low and high doses. The authors suggested different effects of the antagonists due to binding to different subtypes of receptors (13). The occupation of different classes of opioid receptors may cause an agonistic effect in addition to its antagonistic action, depending on the experimental conditions (dose, duration, etc.). Available data are consistent with the view that the sensitivity of the opioid receptors involved in LHRH release becomes markedly increased by either opioid agonist or antagonist pretreatment (12, 16). Since no changes in the binding capacity of the receptors were shown, an alternative approach of selective binding to opioid receptor subtypes becomes logical. Thus, it is possible that the moderate refractoriness or insensitivity in Nalt-pretreated rats is due to the binding to different subtype opioid receptors with different characteristics, causing only partial stimulation of LHRH release from the hypothalamus. The dual effect of Nalt remains to be proven by further studies on the occupation of Nalt by different subtypes of the opioid receptors.

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