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Opiatergic Inhibition of Pulsatile Luteinizing Hormone Release during the Menstrual Cycle of Rhesus Macaques[1] (42485)

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Abstract. The endogenous opioid peptides (EOPs) may inhibit the rate of hypothalamic gonadotropin-releasing hormone (GnRH) release and hence the frequency of pulsatile luteinizing hormone (LH) release, particularly in the luteal phase of the menstrual cycle. Our objectives were to compare the effects of an opiate antagonist, naloxone (NAL), on the patterns of LH, estradiol-17β (E_2), and progesterone (P_4) secretion during the follicular and luteal phases of the macaque menstrual cycle. Plasma levels of E_2, P_4, and bioactive LH were measured in serial, 15-min blood samples during 8-hr infusions of NAL (2 mg/hr) or saline, either on Days 5 or 6 of the follicular phase (FN and FS, n = 5 and 4, respectively) or on Days 8, 9, or 10 of the luteal phase (LN and LS, n = 5 each) of a menstrual cycle. The pulsatile parameters of each hormone were determined by PULSAR analysis and the correspondence of steroid pulses with those of LH were analyzed for each cycle stage in each animal. As expected, LH mean levels and pulse frequencies in LS monkeys were only about one-third of those values in FS animals. NAL had no effects on pulsatile LH, E_2, or P_4 release during the follicular phase. In contrast, luteal phase NAL infusions increased both LH mean levels and pulse frequencies to values which were indistinguishable from those in FS animals. LH pulse amplitudes did not differ among the four groups. Mean levels and pulse frequencies of P_4 secretion in LS monkeys were about 4- and 14-fold greater than those values in FS animals. Mean levels and pulse amplitudes of P_4 release in LN animals were greater than those values in all other groups. LH and E_2 pulses were not closely correlated in follicular phase animals, and this pulse association was not altered by NAL. In FS monkeys, LH and P_4 pulses were not correlated; however, NAL increased this LH-p_4 pulse correspondence. LH and P_4 pulses were closely correlated in luteal phase animals and this association was not affected by NAL. Our data suggest that the EOPs inhibit the frequency of pulsatile LH secretion in the presence of luteal phase levels of P_4. During the midfollicular phase when LH pulses occur every 60 to 90 min, the opioid antagonist NAL alters neither the pulsatile pattern of LH release nor E_2 secretion, but NAL may directly affect P_4-secreting cells.

In rhesus monkeys and women, luteinizing hormone (LH) release is characterized by low amplitude pulses occurring every 1 to 2 hr in the follicular phase (1–3), and by high amplitude pulses every 3 to 8 hr during the luteal phase of the menstrual cycle (1–7).

A substantial amount of data now indicates that the endogenous opioid peptides (EOPs), presumably of hypothalamic origin, participate in the tonic inhibition of LH secretion (3, 4, 8). However, the inhibitory influence of EOPs on LH secretion varies markedly with the prevailing ovarian steroid milieu. In women, for example, intravenous (iv) infusions of the opiate antagonist naloxone (NAL) do not alter plasma LH titers during the early follicular phase (4, 9, 10) when peripheral levels of ovarian steroids are low. In the late follicular phase, when estrogen (E_2) titers are elevated, NAL infusions result in slow, progressive increases in plasma LH concentrations (9, 10) while infusions of NAL during the midluteal phase, when progesterone (P_4) levels are elevated, increase both the frequency and amplitude of LH pulses (4, 9). In rhesus monkeys, acute iv injections of NAL increase serum LH levels on most days of the luteal phase, but are unable to stimulate LH release during the follicular phase of the menstrual cycle (8, 11). Continuous NAL infusions into monkeys during the luteal phase increase the frequency of LH pulses, whereas LH pulse amplitudes are not altered by NAL (3). The data from these studies in women and monkeys suggest

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that the EOPs mediate the negative feedback actions by P_4 (3, 11), or E_2 and P_4 (9, 10) on the hypothalamic–pituitary–LH axis, and that the decreased frequency of LH pulses during the luteal phase of the menstrual cycle is the result of an increased activity of hypothalamic opiatergic neurons (3, 4, 9).

To more clearly elucidate the role of the EOPs in the tonic inhibition of LH release, we compared the influence of NAL infusions on the parameters of pulsatile LH secretion during precise intervals of both the follicular and luteal phases of the macaque menstrual cycle. Our specific aims were to ascertain whether the EOPs are responsible for suppressing the amplitude of LH pulses during the follicular phase, as well as for reducing the frequency of LH pulses during the luteal phase. Since our laboratory recently reported (7) that all LH pulses are followed by P_4 pulses during the late stages of the luteal phase, we also examined, as a second objective, the association between LH and P_4 pulses during the follicular and luteal phases, and that of LH and E_2 pulses during the follicular phase with and without NAL infusions.

Materials and Methods. *Animals.* Ten adult female rhesus monkeys (*Macaca mulatta*) were used for these studies, each with several consecutive menstrual cycles of 26 ± 2 days. All animals were caged individually and maintained on a diet of monkey chow and fresh fruit, and had free access to tap water. Lights were on from 0600 to 1800 hr daily.

To examine the parameters of pulsatile LH, E_2, and P_4 release during specific stages of the menstrual cycle, each animal was fitted with an indwelling jugular or femoral vein catheter. The jugular catheter was passed subcutaneously (sc) to the top of the head and exteriorized via a mobile tether asembly attached to the calavarium as previously described (5). The femoral catheter was passed sc to the back and exteriorized via a tether assembly attached to a protective primate vest. The catheters were kept filled with heparin (1000 IU/ml; Upjohn Co., Kalamazoo, MI) to maintain their patency. With the use of these devices, frequent blood samples were taken without restraint of the animals. All monkeys had menstrual cycles of normal duration throughout the experimental period.

To determine the day of the preovulatory E_2 peak, daily blood samples (3 ml) were collected at 0800–0900 hr from each monkey throughout the menstrual cycle, and E_2 was then measured with the use of a rapid E_2 assay. In rhesus monkeys, peak E_2 and LH levels prior to ovulation nearly always occur within the same 24-hr interval (12). Therefore, the day after peak E_2 levels was designated as Day 1 of the luteal phase, while the first day of menses was designated as Day 1 of the follicular phase.

Monkeys received continuous, 8-hr infusions of the opiate antagonist naloxone (2 mg/hr, iv) either on Day 5 or 6 of the follicular phase (FN, $n = 5$), or on Day 8, 9, or 10 of the luteal phase (LN, $n = 5$) of a menstrual cycle. Most animals served as their own controls, and therefore received 8-hr infusions of saline (2 ml/hr) on identical days of the follicular (FS, $n = 4$) or luteal phase (LS, $n = 5$) of successive menstrual cycles. The order of saline and NAL infusions for each monkey in each cycle was random. Animals never received infusions during successive phases of the same menstrual cycle. Prior to the 8-hr experimental period, the patency of catheters was maintained by a 2-hr infusion (0.6 ml/hr) of heparinized saline (5 IU/ml). Blood samples (1.5 ml) were collected at 15-min intervals during the entire 10-hr infusion period (0600–1600 hr). The plasma were harvested and stored at −20°C until analyzed for E_2, P_4, and bioactive LH.

Assays. Biologically active LH was measured in duplicate in 1- to 10-μl aliquots of plasma using a mouse Leydig cell bioassay, as previously described (13). The assay standard was partially purified cynomolgous pituitary gonadotropin, RP-1. The lower limit of assay detection was 25 pg/tube or 2.5 ng/ml (using 10 μl of plasma). The average intraassay and interassay coefficients of variation (CVs) for all assays were 10 and 16%, respectively. The E_2 and P_4 were measured by radioimmunoassay as described previously (14, 15). Intra- and interassay CVs for the E_2 assays were 8 and 13%, respectively, while those for the P_4 assays were 7 and 10%, respectively. Distilled water and ovariectomized (OVX) serum blanks did not exceed 12 pg for E_2 or 35 pg for P_4, and recoveries after extraction of plasma samples were greater than 92% in all assays. Bioactive LH and immunoreactive P_4 were measured in

serial samples during both the follicular and luteal phases, while E_2 was measured in serial samples only during the follicular phase.

Analysis of data. The LH, E_2, and P_4 pulses were identified and the parameters of each, i.e., mean levels, pulse amplitudes, and pulse frequencies, were determined in each monkey with the use of the PULSAR algorithm (16). Mean values for all three parameters of each hormone were then calculated by average of the values from all animals within the same treatment group, with "*n*" equal to the number of monkeys in that group. Undetectable levels were assigned a value of the assay sensitivity (LH = 2.5 ng/ml; E_2 = 5.0 pg/ml; P_4 = 0.1 ng/ml). Differences among groups for mean levels, pulse amplitudes, and pulse frequencies for all three hormones were determined by analysis of variance (ANOVA) followed by Duncan's multiple-range test (17).

The correspondences of steroid pulses with LH pulses were analyzed in each animal by counting the number of E_2 or P_4 pulses which occurred within 45 min after each LH pulse. The data from individual animals were treated by Arcsine $\sqrt{\text{percentage}}$ transformation (17), and the mean percentage (%) correlations of E_2 and P_4 pulses with LH pulses were calculated by average of the values from all animals within the same treatment group. Differences among groups for %LH-E_2 and %LH-P_4 pulse correspondences were determined by Student's *t*-test and by ANOVA and Duncan's test (17), respectively.

Results. Figure 1 contains the LH data from three representative monkeys which received 8-hr infusions of saline (FS) and NAL (FN) on Day 5 or 6 of the follicular phase. Figure 2 shows the LH data from these same animals which received 8-hr saline (LS) and NAL (LN) infusions on Day 8, 9, or 10 of the luteal phase. It is apparent from comparisons of the data in individual animals in these figures that the pulsatile nature of LH secretion in LS animals differed markedly from that observed in FS monkeys; these group differences in LH release are more clearly depicted in Table I. Mean plasma LH levels in FS monkeys were nearly threefold greater ($P < 0.01$) than those in LS animals, in part because LH pulse frequencies in FS animals were greater ($P < 0.05$) than those in LS monkeys (0.63 pulses/hr vs 0.18 pulses/hr, respectively). The difference in mean LH pulse amplitudes between FS and LS animals was not significant, although higher LH amplitudes tended to occur in LS monkeys with slower pulse frequencies.

It is also apparent from the individual data shown in Fig. 1 and from the mean data presented in Table I that NAL had no effect on either mean levels, pulse amplitudes, or pulse frequencies of LH release in follicular phase monkeys (FS vs FN, $P > 0.05$). Furthermore, NAL did not alter any of the parameters of pulsatile P_4 or E_2 secretion (Table I) in follicular phase animals (FS vs FN, $P > 0.05$).

In marked contrast to those results observed in follicular phase monkeys, NAL infusions

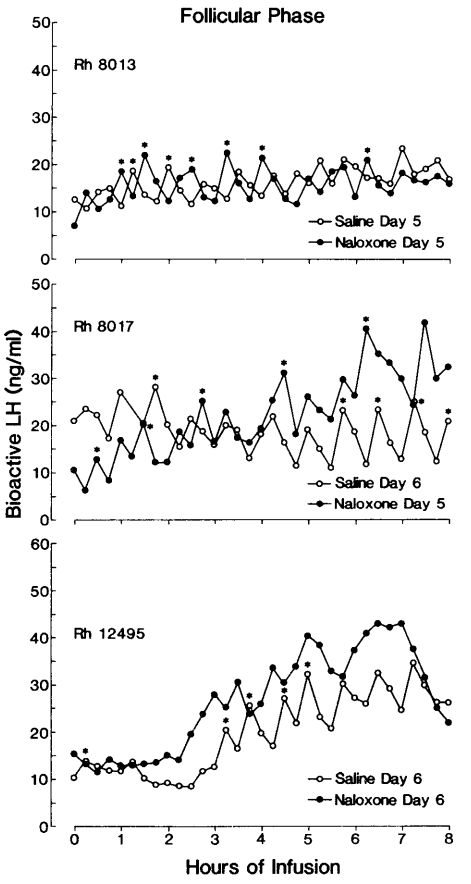

FIG. 1. Effects of naloxone (2 mg/hr, iv) on pulsatile LH release in three representative female rhesus monkeys. Animals received 8-hr infusions of either naloxone (2 mg/hr, closed circles) or saline (2 ml/hr, open circles) on Day 5 or 6 of the follicular phase of successive menstrual cycles. Asterisks indicate pulses of LH.

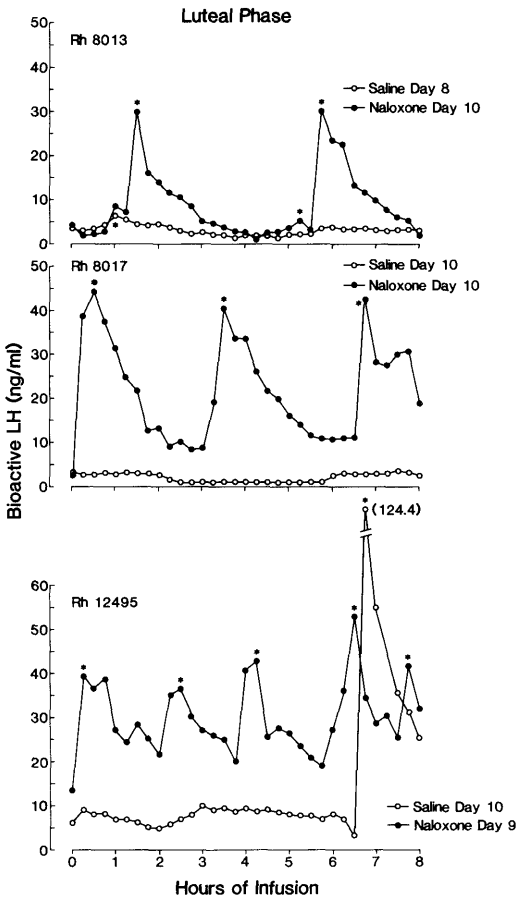

FIG. 2. Effects of naloxone (2 mg/hr, iv) on pulsatile LH release in three representative female rhesus monkeys. Animals received 8-hr infusions of either naloxone or saline on Day 8, 9, or 10 of the luteal phase of successive menstrual cycles. For further details see legend of Fig. 1.

during the luteal phase had a profound influence on pulsatile LH secretion. As demonstrated in Fig. 2 and Table I, NAL caused nearly a threefold increase ($P < 0.05$) in LH pulse frequencies and a comparable increase in mean plasma LH titers (LS vs LN, $P < 0.01$). Therefore, NAL elevated LH mean levels and pulse frequencies in luteal phase animals to values which were indistinguishable from those observed in FS control monkeys (FS vs LN, $P > 0.05$). Although it appears from Fig. 2 that NAL also increased the amplitudes of LH pulses, LS monkeys occasionally showed endogenous, high-amplitude LH pulses which were expected to occur during an 8-hr sampling interval. Thus, there were no significant differences in LH pulse amplitudes between LS and LN monkeys (Table I).

The frequencies of P_4 pulses did not differ significantly between LS and LN monkeys (Table I); however, P_4 pulse amplitudes in LN monkeys were nearly threefold greater ($P < 0.001$) than those in LS animals, resulting in higher mean P_4 concentrations (LS vs LN, $P < 0.001$). Interestingly, both P_4 mean levels and pulse amplitudes in LN animals were greater ($P < 0.001$) than those observed in all other treatment groups.

Figure 3 compares the relationships between the pulsatile releases of LH and E_2 in two representative follicular phase monkeys which received 8-hr infusions of either saline or NAL. As suggested in this figure, and more clearly demonstrated in Table II, E_2 pulses were not closely associated with those of LH in FS animals. The correspondence of LH and E_2 pulses did not differ between FS and FN groups (Table II, $P > 0.05$).

The relationships between the pulsatile releases of LH and P_4 are also shown in representative follicular (Fig. 3) and luteal phase (Fig. 4) monkeys which received 8-hr infusions of either saline or NAL. As demonstrated in Fig. 3, and more clearly depicted in Table II, P_4 pulses were not associated with LH pulses in FS animals. Interestingly, NAL increased the correspondence of LH and P_4 pulses during the follicular phase (FS vs FN, $P < 0.001$). Pulses of LH and P_4 were highly associated in both LS and LN monkeys (Fig. 4), and NAL did not alter this pulse correspondence during the luteal phase (Table II, LS vs LN, $P > 0.05$).

Discussion. The results of this study have led us to several conclusions. First, the EOPs are involved in tonically inhibiting the frequency of LH pulses during the luteal phase of the macaque menstrual cycle. Second, LH and P_4 pulses are tightly associated during the luteal phase, but are not closely associated during the follicular phase. Third, NAL markedly enhances the correspondence of LH and P_4 pulses during the follicular phase, but does not do so in the luteal phase. Lastly, follicular phase LH and E_2 pulses are not closely associated and are not affected by NAL.

Our results and those of previous studies (1–7) demonstrate that the frequency of LH pulses is slower and the amplitude of these pulses is higher in the luteal phase than those

TABLE I. EFFECTS OF NALOXONE ON PULSATILE LH, P_4, AND E_2 RELEASE DURING THE RHESUS MENSTRUAL CYCLE[a]

Hormone	Group	(n)[b]	Mean levels	Mean pulse amplitudes	(n')[c]	Mean pulse frequencies
			(ng/ml)	(ng/ml)		(pulses/hr)
LH	FS	4	20.57 ± 2.37[d]	11.20 ± 3.25[d]		0.63 ± 0.15[d]
	FN	5	22.02 ± 2.16[d]	8.89 ± 1.42[d]		0.50 ± 0.11[d]
	LS	5	7.62 ± 2.57[e]	39.53 ± 26.89[d]	(4)	0.18 ± 0.06[e]
	LN	5	19.12 ± 3.64[d]	19.16 ± 4.20[d]		0.48 ± 0.05[d]
P_4	FS	4	0.12 ± 0.02[d]	0.23 ± 0.02[d]	(2)	0.13 ± 0.09[d]
	FN	5	0.14 ± 0.01[d]	0.29 ± 0.12[d]		0.38 ± 0.08[de]
	LS	5	1.66 ± 0.32[e]	1.75 ± 0.65[d]		0.50 ± 0.10[e]
	LN	5	6.93 ± 0.84[f]	4.57 ± 0.95[e]		0.43 ± 0.12[de]
			(pg/ml)	(pg/ml)		
E_2	FS	4	53.82 ± 11.60[d]	24.65 ± 1.61[d]	(3)	0.34 ± 0.16[d]
	FN	5	47.23 ± 6.39[d]	24.60 ± 2.28[d]		0.33 ± 0.08[d]

Note. FS, follicular/saline; FN, follicular/naloxone; LS, luteal/saline; LN, luteal/naloxone. Values (means ± SEM) in each column with different superscripts are significantly different at $P < 0.05$ or better.

[a] Monkeys received 8-hr intravenous infusions of either naloxone (2 mg/hr) or saline (2 ml/hr) on Day 5 or 6 of the follicular phase and on Day 8, 9, or 10 of the luteal phase of a menstrual cycle.

[b] Number of animals in each group.

[c] Number of animals used for statistical analysis in each group; the difference between (n) and (n') is due to zero pulse frequency in one or more animals.

in the follicular phase of the menstrual cycle. These LH parameters are regulated, in part, by the pulsatile release of hypothalamic gonadotropin-releasing hormone (GnRH) (18–23) although the ovarian steroids also modify these LH profiles (21, 24). For example, the administration of P_4 to follicular phase women (6) and to ovariectomized monkeys (25) slowed the frequency of LH pulses and reduced mean plasma LH levels. It has been postulated that this modulatory action of P_4 on LH secretion results from alterations in the hypothalamic GnRH pulse generator (26). The presence of P_4 receptors in monkey hypothalamus (27) is consistent with a central site of P_4 action. Furthermore, recent findings in the monkey (28, 29) suggest that ovarian steroids may act to enhance the secretion of EOPs. The present results showed that infusions of NAL into monkeys on Days 8 to 10 of the luteal phase increased both LH mean levels and pulse frequencies to values which were indistinguishable from those observed in saline-infused, follicular phase animals; NAL did not alter either the LH pulse amplitudes or the association between LH and P_4 pulses at this stage of the menstrual cycle (see Table II). These effects of NAL on the pattern of LH secretion are consistent with those after acute NAL injections into rhesus monkeys (11). In contrast, NAL infusions into midluteal phase women increased not only the frequency of LH pulses, but also the LH pulse amplitudes (4, 9).

The majority of data have indicated that the EOPs modulate LH secretion primarily via hypothalamic mechanisms. In primates, high concentrations of opioid peptides and opiate receptors (30, 31) have been localized in proximity to GnRH-containing neurons in the mediobasal hypothalamus-median eminence (MBH-ME) region (32). Direct evidence has been reported that opiate agonists inhibited GnRH release from the rat hypothalamus both *in vivo* (33) and *in vitro* (34), while NAL increased GnRH release from superfused rat (35) and human fetal (36) hypothalamic tissue. Also, we have found that intrahypothalamic perfusion of NAL in the female rabbit stimulates hypothalamic GnRH release into push–pull perfusates *in vivo* (unpublished observations). Whatever the specific mechanism, our data are consistent with the hypothesis of a hypothalamic site of EOP/P_4 interaction in the

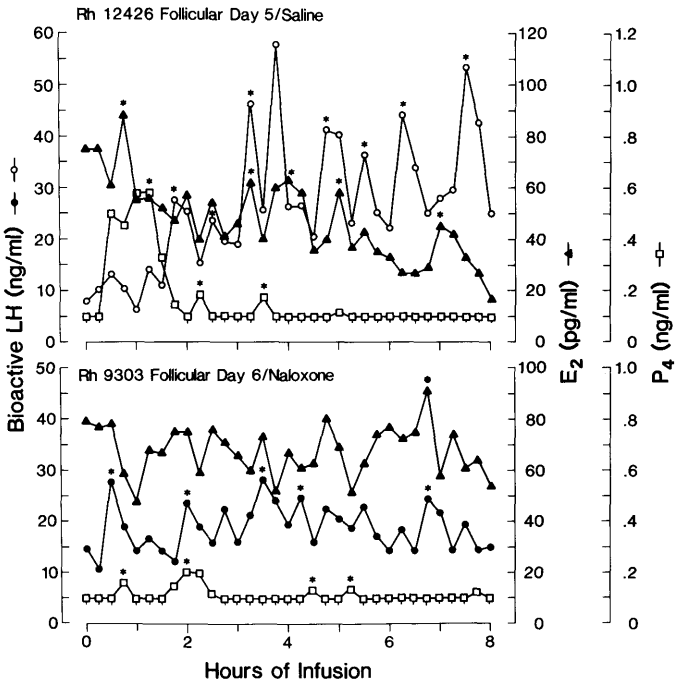

FIG. 3. Effects of naloxone (2 mg/hr, iv) on the correspondence of LH (circles), E_2 (triangles), and P_4 (squares) pulses in two representative female rhesus monkeys. Animals received 8-hr infusions of either naloxone or saline on Day 5 or 6 of the follicular phase of the menstrual cycle. Asterisks indicate pulses of LH, E_2, and P_4. For further details, see legend of Fig. 1.

TABLE II. EFFECTS OF NALOXONE ON THE CORRESPONDENCE OF LH PULSES WITH E_2 AND P_4 PULSES DURING THE RHESUS MENSTRUAL CYCLE[a]

Group	(n)[b]	% LH-E_2 pulse correspondence[c]
FS	4	27.50 ± 16.01[d]
FN	5	44.80 ± 16.65[d]
		% LH-P_4 pulse correspondence[c]
FS	4	6.25 ± 6.25[d]
FN	5	56.00 ± 13.96[e]
LS	5	79.25 ± 12.47[e]
LN	5	75.40 ± 10.40[e]

Note. FS, follicular/saline; FN, follicular/naloxone; LS, luteal/saline; LN, luteal/naloxone. Values (means ± SEM) with different superscripts are significantly different at $P < 0.001$.

[a] Monkeys received 8-hr intravenous infusions of either naloxone (2 mg/hr) or saline (2 ml/hr) on Day 5 or 6 of the follicular phase and on Day 8, 9, or 10 of the luteal phase of a menstrual cycle.

[b] Number of animals in each group.

[c] Statistics were performed on data after arcsine percentage transformation.

regulation of LH pulse patterns in the macaque luteal phase.

A final aspect of the present investigation was related to the association of ovarian steroid pulses with LH pulses during the follicular phase of the menstrual cycle. In follicular phase monkeys, E_2 pulses were not closely associated with those of LH, and NAL did not alter the association of LH and E_2 pulses. In contrast, NAL increased the association between LH and P_4 pulses during the follicular phase. Since P_4 was nondetectable in many samples from individual follicular phase animals, mean P_4 concentrations in both FS and FN groups were only slightly greater than the sensitivity of the assay (0.1 ng/ml). However, pulses of P_4 were detected in two of four FS animals and in all five FN monkeys. Since a viable corpus luteum is not present in follicular phase animals, one can only speculate that NAL may act directly on the ovary to increase the sensitivity to LH, or that the P_4 is derived from some tissue other than the ovary (e.g., the adrenal gland).

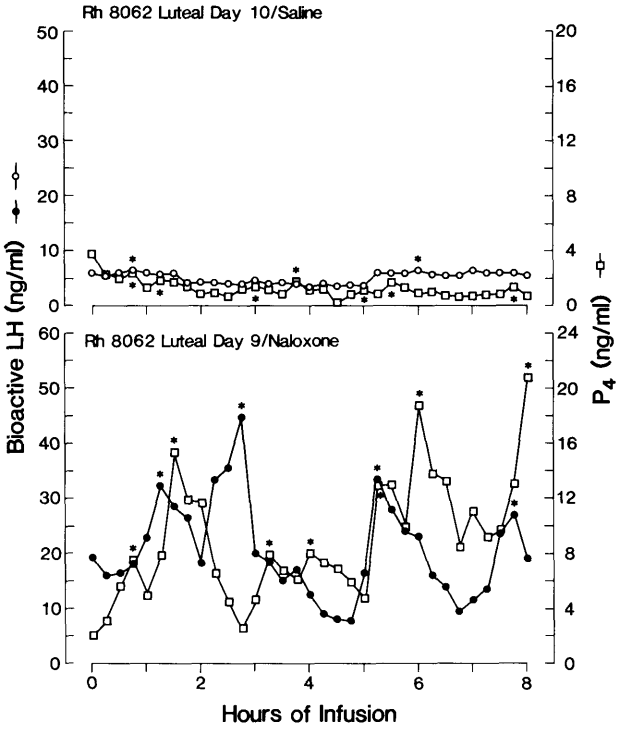

FIG. 4. Effects of naloxone (2 mg/hr, iv) on the correspondence of LH (circles) and P_4 (squares) pulses in two representative female rhesus monkeys. Animals received 8-hr infusions of either naloxone or saline on Day 9 or 10 of the luteal phase of the menstrual cycle. Asterisks indicate pulses of LH and P_4. For further details see legend of Fig. 1.

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