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Evidence that Estrogens Inhibit LH Secretion through Opioids in Postmenopausal Women Using Naloxone

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Abstract. To evaluate whether ovarian steroid environment may modify endogenous opioid activity at hypothalamic-pituitary level, the effects of naloxone infusion (1.2 mg/h for 4 h) on gonadotropin secretion were studied in 5 postmenopausal women who had natural menopause 3–5 years before the study. In addition, naloxone infusion was repeated in the same subjects after chronic oral treatment with conjugated estrogens (1.25 mg/day in two divided doses for 20 days). Before treatment, both the circulating levels of estrogens and plasma gonadotropins were in the normal range for postmenopausal women and naloxone infusion did not induce any significant modification of gonadotropin secretion. In contrast, after estrogen therapy, and the consequent rise in estrogen plasma levels, naloxone infusion induced a significant LH increase ($p < 0.01$) starting during the last hour of treatment. These findings seem to confirm that endogenous opioid peptides may modulate the inhibitory effect exerted by estrogens on LH secretion, in humans.

Endogenous opioid peptides seem to be involved in the modulation of a wide variety of physiological and behavioral functions [3, 13]. The observation that these peptides are present in the hypothalamus and the pituitary [11, 22] strongly suggests that they may also participate in the control of pituitary hormone secretion. It has been reported that endogenous opioids differentially affect prolactin, growth hormone and corticotrophin secretion, both in animals and in humans. These neuroendocrine effects seem to be partially mediated through interactions with other neurotransmitter systems [15, 18]. However, there is general agreement that opioid peptides may exert a tonic inhibitory control on gonadotropin secretion [15, 18]. Treatment with morphine has been reported to inhibit ovulation in the rat [1]. Acute injection with morphine [5, 21], met- or leu-enkephalin [4, 8], β-endorphin [8] or enkephalin analogues [12, 26] has been shown to reduce luteinizing hormone (LH) and, in some instances, follicle-stimulating hormone (FSH) release. Moreover, the opiate receptor-blocking agent naloxone (NAL) has been reported either to counteract the inhibitory effect of exogenous opioids [14, 20, 27] or to stimulate gonadotropin secretion [2, 7, 17]. Recently, *Quigley and Yen* [24] demonstrated that the LH response to NAL infusion is different during various phases of the menstrual cycle in normal women, suggesting that ovarian steroids may modulate the functional activity of endogenous opioid peptides in the hypothalamic-pituitary system.

The aim of the present investigation was then to evaluate the effects of NAL infusion on gonadotropin secretion in postmenopausal women both before and after chronic estrogen treatment.

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Materials and Methods

The study was performed in 5 volunteer women, aged 51–53 years. All subjects had natural menopause 3–5 years before the study and were in good health. Their mean (± SE) body weight was 61.8 ± 3.7 kg. They had not received estrogens or other treatments for at least 6 months prior to the study. After an overnight fast, an intravenous polyethylene catheter was inserted, at 08.00 h, in an antecubital vein of each arm and kept open by the slow infusion of saline solution. The first catheter was used for a 4 h infusion with NAL (1.2 mg/h) and the other one for blood sampling. The subjects remained in bed and were not permitted to eat, drink, smoke or sleep during the experiment. Their pulse rate and blood pressure were evaluated during the study. Heparinized blood samples were collected every 15 min throughout the experiment (for 2 h before, during NAL infusion for 4 h and finally for another 2 h during saline alone). The same experiment was repeated in each subject after

Table I. Mean (± SE) plasma concentrations of E_2 and E_1 measured at 08.00 and 16.00 h in 5 postmenopausal women during NAL studies (1.2 mg/h for 4 h) before and after treatment with conjugated estrogens (1.25 mg/day for 20 days)

	Clock hours	
	08.00 h	16.00 h
	Before estrogen administration	
E_2, pg/ml	15.4 ± 3.5	14.7 ± 4.2
E_1, pg/ml	24.0 ± 4.3	26.2 ± 4.7
	After estrogen administration	
E_2, pg/ml	60.5* ± 10.8	61.9* ± 11.8
E_1, pg/ml	120.6* ± 18.3	122.1* ± 15.2

* $p < 0.001$ vs. values measured before estrogen treatment, by Student's t test.

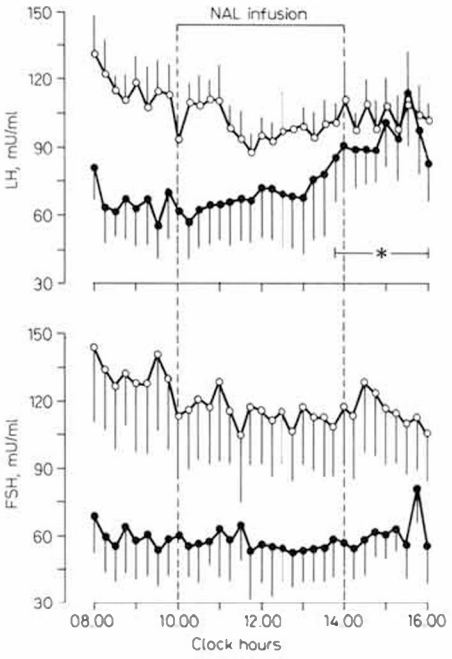

Fig. 1. Effects of NAL infusion on gonadotropin secretion in 5 postmenopausal women, before (○) and after (●) chronic treatment with conjugated estrogens. Estrogens induced the expected significant fall ($p < 0.001$, by analysis of variance) in gonadotropin plasma levels. The upper pannel shows the patterns of LH secretion: no significant effects of NAL infusion were observed before estrogens; after estrogen treatment a significant increase (* = $p < 0.01$ vs. preinfusion values, by analysis of variance) in LH concentrations was observed starting during the last hour of NAL infusion. The lower pannel shows the patterns of FSH secretion: no significant effects of NAL infusion were observed both before and after estrogen treatment. Doses were: NAL = 1.2 mg/h for 4 h; conjugated estrogens = 1.25 mg/day for 20 days (see Materials and Methods for more details).

chronic oral treatment with conjugated estrogens (1.25 mg/day, in two divided doses) for 20 days. The dose of 1.25 mg/day is the one commonly used to control the symptoms associated with the menopause. The duration of treatment (20 days) was chosen because chronic estrogen replacement for 3 weeks has been reported to restore hypothalamic β-endorphin release into portal vessels that was abolished by ovariectomy of female monkeys [29].

Blood samples were immediately centrifuged and the plasma frozen until assayed. Plasma concentrations of LH, FSH, 17β-estradiol (E_2) and estrone (E_1) were determined by radioimmunoassay [9, 16]. LH and FSH levels were measured in each sample, while E_2 and E_1 were measured in the samples collected at 08.00 and 16.00 h.

Statistical analysis of the results was performed by Student's t test and analysis of variance, as appropriate. To evaluate the integrated hormone secretion for each 1 h interval, the areas under the curves were determined. The observed concentrations were connected by straight-line segments, and the area under these segments was calculated by the method of triangulation. The areas were expressed in mU/ml × 60 min and compared using analysis of variance.

Results

All the results are reported as the mean ± SE. Before estrogen treatment both plasma gonadotropin and estrogen levels were in the normal range for postmenopausal women in our laboratory (table I, fig. 1). Chronic estrogen treatment was followed by an expected significant increase ($p < 0.001$) in plasma estrogen levels (table I) and by a significant decrease ($p < 0.001$) in basal gonadotropin concentrations (fig. 1). Both before and after estrogen treatment, plasma E_2 and E_1 concentrations measured at 16.00 h were not different from those measured at 08.00 h (table I).

Before chronic estrogen treatment NAL infusion did not induce any significant change in the mean gonadotropin levels (fig. 1). In fact, the slight decrease in mean plasma LH levels observed between 11.00 and 13.00 h (2nd and 3rd hour of NAL infusion) did not reach statistical significance (fig. 1). The lack of any effect of NAL infusion on LH secretion in untreated postmenopausal women was confirmed by the analysis of integrated LH secretion (fig. 2). In contrast, after chronic estrogen treatment, although mean FSH levels failed to show any variation, NAL infusion induced a significant progressive increase ($p < 0.01$, by analysis of variance) in mean LH concentrations (fig. 1). The onset of LH increase

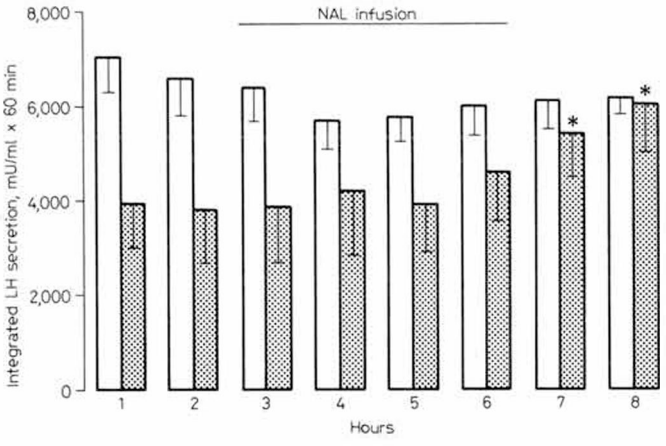

Fig. 2. Effects of NAL infusion on integrated LH secretion measured at each 1 hour interval, before (□) and after (▨) estrogen treatment. Estrogen induced a significant fall ($p < 0.001$, by analysis of variance) in integrated basal LH secretion. No effects of NAL infusion were observed before estrogens, while after estrogen administration the integrated LH secretion was significantly increased (* = $p < 0.01$ vs. preinfusion values, by analysis of variance) (see Materials and Methods and legend to figure 1 for more details).

was delayed and started during the last hour of the infusion. The highest LH levels were reached 90 min after the end of the infusion with NAL (fig. 1). The rise in mean LH levels was associated with a significant increase ($p < 0.01$, by analysis of variance) in integrated LH secretion (fig. 2).

Discussion

The present data give further evidence that ovarian steroid environments may modulate LH sensitivity to NAL administration in human beings. In fact, a lack of LH response to NAL infusion was observed in postmenopausal women with low circulating estrogen levels. Conversely, NAL infusion stimulated LH secretion after the increase of plasma estrogen levels induced by chronic estrogen treatment in the same subjects. These findings agree with the results reported by *Quigley and Yen* [24] in fertile women, showing that NAL infusion (1.6 mg/h for 4 h) had no effects on LH secretion during the low estrogen phase of the menstrual cycle (early follicular), while it stimulated LH release during the high estrogen phases (late follicular and midluteal).

Several studies suggest that gonadal steroids may modify endogenous opioid activity at hypothalamic-pituitary level. *Petraglia* et al. [23] observed a reduction of immunoreactive β-endorphin in both anterior and neurointermediate pituitary lobes after gonadectomy in rats. Estrogen replacement was able to restore pituitary β-endorphin concentrations [23]. *Wardlaw* et al. [29] and *Weherenberg* et al. [30] demonstrated in female monkeys that β-endorphin levels were undetectable in hypophyseal portal blood both during menstruation and 4–12 months after ovariectomy, while high β-endorphin concentrations were measured during late follicular and luteal phases of the menstrual cycle. Chronic estradiol replacement for 3 weeks reversed the effect of ovariectomy in monkeys restoring hypothalamic β-endorphin release in portal vessels [29]. *Genazzani* et al. [10] reported a decrease in endogenous opioid levels in postmenopausal subjects in comparison with normal cycling women.

All these findings seem to suggest that NAL failed to affect LH secretion in postmenopausal women because of the reduction of endogenous opioid activity due to the low estrogen environment. By contrast, chronic estrogen treatment was probably followed by the induction of the opioid inhibitory effect on LH release and NAL was therefore able to stimulate LH secretion by exerting its opioid antagonistic action.

These data may provide indirect evidence that, as demonstrated in rats [6, 28], endogenous opioids may be involved in the mechanisms of steroid negative feedback on LH secretion in humans. However, the modes and the sites through which NAL exerts these effects appear unclear.

NAL and opioid peptides failed to show any significant effect on gonadotropin release from anterior pituitary in vitro [6, 15]. Moreover, NAL significantly increases LHRH efflux from superfused rat [31] and human [25] hypothalami.

These findings and the delayed LH response to NAL infusion [24] suggest that the stimulatory effect of NAL is mediated through the increase of LHRH. Thus, the interactions between estrogens and NAL on LH release are probably mediated via the hypothalamus.

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