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Estrogen augments hypothalamic β-endorphin secretion and activates an inhibitory β-endorphin short-loop feedback system

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The role of estrogen in the regulation of hypothalamic β-endorphin hormone secretion is studied by determining β-endorphin concentration in pituitary portal plasma of ovariectomized rats in the presence or absence of this steroid and/or the opioid antagonist naloxone. Twenty-six hours following s.c. injection of 10 μg estradiol benzoate (estrogen) or oil, rats anesthetized with Saffan (alphaxolone/alphadolone) underwent pituitary stalk exposure and hypophysectomy, after which pituitary portal blood was continuously collected and stored in 15 min aliquots from 1100–1400 h. At 1100 h, animals were given an initial bolus iv injection of naloxone or saline (naloxone, 2 mg/kg, or saline, 0.1 ml) and then infused (iv) continuously with naloxone (2 mg/kg/h) or saline (0.8 ml/h) until 1400 h. Plasma samples were extracted and assayed by radioimmunoassay for β-endorphin. Treatment with estrogen increased the mean β-endorphin levels twofold as compared to oil-treated controls. Naloxone potentiated estrogen action of β-endorphin secretion, but did not affect basal β-endorphin secretion. These results suggest that estrogen enhanced β-endorphin secretion from the hypothalamus. Furthermore, the hypersecretion of β-endorphin induced by naloxone with, but not without, estrogen supports the existence of an estrogen-activated short-loop negative feedback mechanism regulating β-endorphin secretion.

Keywords: Hypothalamic β-endorphin secretion; estrogen; pituitary portal blood; aphadolone/alphaxolone anesthesia; ovariectomized rats; short-loop feedback

Introduction

Considerable evidence suggests a physiological role for hypothalamic β-endorphin in the regulation of LH and LHRH secretion during the ovulatory cycle and ovulation in laboratory animals and humans (Barraclough & Sawyer, 1955; Packman & Rothchild, 1976; Koves *et al.*, 1981; Kalra & Kalra, 1983; Bicknell, 1985; Sarkar & Yen, 1985). Since administration of opiate antagonists affects LH or LHRH secretion differentially depending on the day of the estrous cycle (Piva *et al.*, 1985; Petraglia *et al.*, 1986) or on the steroidal environment (Bhanot & Wilkinson, 1984; Petraglia *et al.*, 1984; Gabriel *et al.*, 1986; Nickolarakis *et al.*, 1986; Allen *et al.*, 1988; Lustig *et al.*, 1988), a role for steroids in endogenous opioid regulation or in mediation of LHRH secretion has been suggested. Immunohistochemical studies have shown that a substantial number of hypothalamic β-endorphin neurons accumulate estrogen (Morrell *et al.*, 1985; Jirikowski *et al.*, 1986). In the monkey, estrogen moderately increases and ovariectomy decreases the concentration of β-endorphin in pituitary portal blood, suggesting a possible stimulatory role of estrogen on β-endorphin secretion (Ferin *et al.*, 1984). In the rat, the effect of estrogen on β-endorphin secretion is not established. Like in the monkey, ovariectomy lowers β-endorphin in pituitary portal blood in the rat (Sarkar & Yen, 1985). However, replacement with estrogen reduces proopiomelanocortin (POMC) mRNA levels (Roberts *et al.*, 1986) and β-endorphin concentrations in the hypothalamus (Knuth *et al.*, 1983; Wardlaw *et al.*, 1985), which suggests a decreased synthesis, and possibly a release, of β-endorphin. The present study was conducted to determine the effect of estrogen on β-endorphin release into pituitary portal blood in ovariectomized rats. Since estrogen appears to have a time-dependent biphasic action on LHRH (Fink, 1979), we chose to examine the period (1100–1400 h) during which some of the events leading up to the LHRH surge are being established. We also examined the role of estrogen in the autocrine regulation of β-endorphin secretion using the opiate antagonist naloxone.

Results

Effects of estrogen and naloxone on β-endorphin secretion

As the β-endorphin levels in pituitary portal plasma fluctuated due to pulsatile hormone release (Frautschy & Sarkar, 1988; Minami *et al.*, 1992), the hormone levels during a 3 h period were first averaged for each rat, in order to reduce the variation of β-endorphin levels during this time period within an animal. The data presented in the Figure 1 are the mean levels of hormone secretion during a 3 h period. As shown in this figure, the level of β-endorphin in portal plasma was elevated in animal treated with estrogen as compared with oil vehicle-treated animals. Two-way analysis of variance showed that estrogen and naloxone had significant interaction in causing an increase in β-endorphin release in portal plasma ($P<0.001$). The significant interaction could be explained because the effect of naloxone on β-endorphin required the presence of estrogen ($P<0.005$).

Discussion

The data presented in this report suggest that estrogen stimulates hypothalamic β-endorphin secretion in pituitary portal plasma. In the presence of estrogen, treatment with naloxone markedly stimulated β-endorphin secretion; such a response strongly suggests a feedback inhibitory loop. Since the stimulatory effect of naloxone on β-endorphin secretion is not observed in the absence of estrogen treatment, estrogen appears to activate this short-loop feedback system. *In vitro* studies have also suggested that the magnitude of β-endorphin secretion may be partly determined by an inhibitory feedback loop system which involves a decrease both in secretion (Almeida *et al.*, 1986) and synthesis (Mocchetti & Costa, 1987). The present results have confirmed the existence of this inhibitory feedback system of hypothalamic β-endorphin neurons *in vivo* and further suggested that this system is activated by estrogen.

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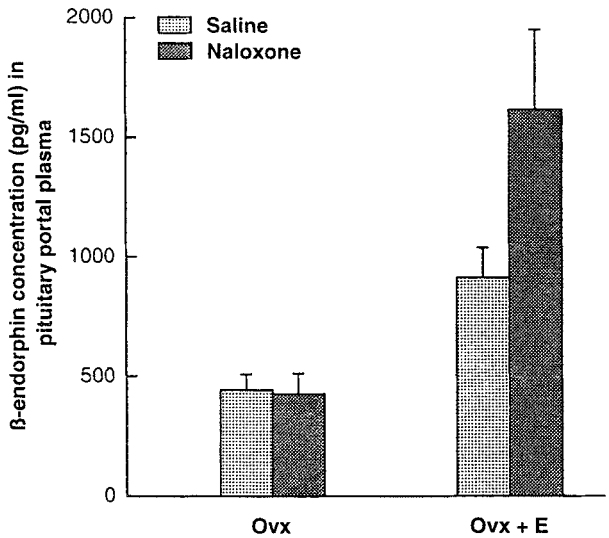

Figure 1 Effect of estrogen and naloxone on β-endorphin secretion in pituitary portal plasma in ovariectomized rats. Estradiol benzoate (E) (10 μg/rat in 0.1 ml oil, sc) or oil (0.1 ml; sc) was administered at 0800 h the day before the experiment. Twenty-six hours after this treatment, animals were anesthetized and pituitary stalk was exposed. These animals were injected first with a bolus injection of naloxone (2 mg/kg in 0.1 ml saline, iv) or saline (0.1 ml) at 1100 h, and then naloxone or saline was infused iv at a rate of 2 mg/kg/hr/0.8 ml between 1100–1400 h. The levels of β-endorphin in portal plasma collected during 1100–1400 h were measured by radioimmunoassay. Data are mean ± of 8–12 rats. [a], $P<0.05$, significantly different from the rest of the groups. [b], $P<0.01$, significantly different from the rest of the groups

The mechanism underlying the obligatory role of estrogen in activating the short-loop feedback system of β-endorphin neurons is unclear. Neuronal responsiveness to a secretagogue could be altered by the known ability of estrogen to influence either synaptic remodeling and neuronal ultrastructure in the adult hypothalamus (Carrer & Aoki, 1982; Matsumoto *et al.*, 1985; Garcia-Segura *et al.*, 1987) or affinity and number of specific receptors (LaBella, 1985; Hammer & Bridges, 1987). Alternatively, an estrogen-increased GABA or noradrenergic neurotransmission could mediate our observed estrogen-induced enhanced responsiveness to naloxone. GABAergic and noradrenergic neurons often concentrate estrogen (Sar & Stumph, 1981; Maggi & Perez, 1984; Leranth *et al.*, 1985) and have been shown to be involved in naloxone-induced hypersecretion of LH under steroidal influence (Akabori & Barraclough, 1986; Masotto & Negro-Vilar, 1987).

Since in the presence of estrogen, naloxone increases both β-endorphin (this study) and LH/LHRH secretion (Piva *et al.*, 1985; Petraglia *et al.*, 1986; Allen *et al.*, 1988; Lustig *et al.*, 1988), the action of naloxone appears to depend on antagonism of opiate receptors on at least two separate populations of neurons: (1) blockade of opiate receptors on neurons involved in the negative feedback loop of β-endorphin neurons, and (2) blockade of opiate receptors on neurons involved in control of LHRH secretion. Therefore, the anticipated hypersecretion of β-endorphin following naloxone would not be able to alter LHRH secretion due to naloxone blockade of opiate receptors on LHRH neurons. In this regard, it is interesting to note that in addition to well known inhibitory action of β-endorphin, some studies have shown a stimulatory action of β-endorphin. The β-endorphin feedback system may explain the paradoxical stimulatory effect of opioids on LH (Pang *et al.*, 1955; Piva *et al.*, 1984; Gabriel *et al.*, 1987) or LHRH (Rasmussen *et al.*, 1988) and why chronic morphine potentiates both positive and negative feedback actions of estrogen on LH (Gabriel *et al.*, 1987).

The highest and lowest levels of β-endorphin in pituitary portal blood during the rat estrous cycle occur consecutively on the afternoon of proestrous with a short time period preceding and coinciding with the ovulatory surge of LHRH (Sarkar & Yen, 1985). The divergent action which estrogen appears to exert on β-endorphin activity (Ferin *et al.*, 1984; Wardlaw *et al.*, 1985; Roberts *et al.*, 1986) could be explained by a biphasic effect of estrogen. Together these results raise the possibility that the initial hypersecretion of β-endorphin observed during proestrous could not only be triggered by the estrogen surge, but also may be sufficient to trigger the short-loop feedback inhibition of β-endorphin secretion, which would explain the suppression of β-endorphin during the LHRH surge. Whether this estrogen-activated β-endorphin short-loop feedback participates in removing opioid-inhibitory tone on LHRH neurons and facilitates the generation of preovulatory LHRH surge is an interesting possibility but remains to be established.

Materials and methods

Animals

Female Sprague-Dawley rats (Charles Rivers Lab, Wilmington, MA, USA), exhibiting normal estrous cycles prior to ovariectomy and weighing 250–300 g, were used 2–3 weeks after surgery. They were housed under controlled lighting (lights on from 0500–1900 h) and temperature (25°C) conditions. Food and water were given *ad libitum*. Animal surgery and care were in accordance with institutional guidelines and complied with the NIH policy governed by The Principles for Use of Animals and The Guide for the Care and Use of Laboratory Animals.

Surgery and blood collection

Animals were anesthetized with Saffan (alphaxolone and alphadolone acetate; 0.4–0.6 ml/100 g ip; a gift of Glaxo, Inc., Research Triangle Park, NC), and 0.2–0.4 ml/h was injected as needed to maintain anesthesia throughout the experiment. The pituitary stalk and median eminence of the rat were exposed transpharyngeally and the pituitary aspirated with the aid of an operating microscope using the surgical procedure described previously (Sarkar & Yen, 1985). Samples were collected continuously in 15 min aliquots between 1100–1400 h and stored on ice until centrifugation and extraction.

Treatment

Animals received a single dose of estrogen (10 μg/animal, sc; Sigma) in oil or oil alone (0.1 ml) at 0800 h on the day preceding the experiment. This treatment initiates a surge of LHRH and LH 36 h after estrogen injection in the presence of Saffan anesthesia (Petraglia *et al.*, 1987). Pituitary stalk plasma collection was initiated at 1000 h on the day after estrogen treatment. After collection of the fourth sample (1100 h), animals were injected iv with naloxone (2 mg/kg; Sigma) in saline or saline (0.9%, 0.1 ml) alone and then continuously infused iv with naloxone (2 mg/kg/h) or saline (0.8 ml/h). There were 9, 6, 12 and 8 animals in vehicle-, naloxone-, estrogen- and estrogen + naloxone-treated groups, respectively. The purpose of collecting plasma samples between 1000 and 1100 h was to determine if baseline levels prior to naloxone injection had to be normalized. However, they were not significantly different from the corresponding controls at the corresponding time interval.

Radioimmunoassay

β-Endorphin was extracted from pituitary portal plasma using 6 × vol. of methanol as previously described (Sarkar,

1988). Recovery for β-endorphin was 74%. Plasma β-endorphin levels were measured by radioimmunoassay using the antiserum R no. 10 (provided by Dr S.S.C. Yen, University of California, San Diego, CA) and by the method described by this laboratory previously (Sarkar & Yen, 1985). The sensitivity of this assay was 5.8 pg/tube. The intra- and inter-assay CV were 14.7%, respectively. All samples from each animal were measured together. Each assay also included samples from all four treatments.

Statistical analysis

The mean levels of portal β-endorphin for each animal were first calculated. These data were used to determine treatment effects on hormone secretion. Mean and SE of these data are presented in the figure and the text. Data are logarithmically transformed to established normal distribution and then statistically analysed using two-way ANOVA. A value of $P < 0.05$ was considered significant.

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