Effects of naloxone and electroacupuncture treatment on plasma concentrations of LH in sheep

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ABSTRACT

Mature ewes were injected intravenously with the opioid antagonist naloxone (1-1 mg/kg) during the breeding season. Ewes with luteal phase concentrations of plasma progesterone responded with a significant ($P<0.05$) increase in plasma LH 14–23 min after naloxone injection. In contrast, non-luteal ewes with low plasma progesterone did not respond to injection of naloxone with an LH increase. Similar treatment of castrated males (wethers) with this dosage of naloxone failed to increase plasma LH. Electroacupuncture (EA) treatment of luteal phase ewes prevented the ability of exogenous naloxone to increase plasma LH. Treatment of wethers by EA decreased significantly ($P<0.01$) their high basal concentrations of plasma LH, but similar EA treatment of intact ewes did not change their low basal concentrations of LH.


INTRODUCTION

Endogenous opioid peptides appear to inhibit release of luteinizing hormone (LH) because administration of the opioid antagonist naloxone has been shown in many experiments to increase LH concentrations in blood (Quigley & Yen, 1980; Van Vught & Meites, 1980; Cox & Baizman, 1982). Acute LH responses to naloxone injection constitute one essential criterion (Sawynok, Pinsky & LaBella, 1979; Holaday & Loh, 1981) in establishing that LH release is being tonically inhibited by unidentified endogenous opioid peptides. Additional evidence is provided by the ability of endogenous opioid peptides, administered intravenously or intracerebrally, to decrease plasma LH concentrations (Parvizi & Ellendorff, 1980; Reid, Hoff, Yen & Li, 1981; Kinoshita, Nakai, Katakami & Imura, 1982).

Release of LH from the anterior pituitary gland is acutely regulated by the hypothalamic peptide, LH releasing hormone (LHRH). Therefore, inhibitory effects of endogenous opioid peptides on LHRH release could lead indirectly to decreased LH release. Drouva, Epelbaum, Tapia-Arancibia et al. (1981) quantified the release of LHRH from superfused tissue of the medial basal hypothalamus (MBH) and observed that in-vitro administration of endogenous opioid peptides decreased potassium-induced release of LHRH. In addition, Wilkes & Yen (1981) reported that in-vitro antagonism of endogenous opioid action by naloxone treatment augmented the release of LHRH from superfused MBH tissue. Therefore, it seems likely that endogenous opioid peptides decrease LH release indirectly by inhibiting the hypothalamic release of LHRH.

Electrostimulation through acupuncture loci (electroacupuncture, EA) or of non-specific sites (stress) produces significant cutaneous analgesia some of which can be diminished by naloxone antagonism of endogenous opioid peptides (Han & Terenius, 1982; Watkins & Mayer, 1982). Such observations suggest that EA and stress can activate endogenous opioid systems. An experiment was conducted in sheep to examine the cutaneous analgesia produced by EA and these results are published elsewhere (Bossut, Stromberg & Malven, 1983). Samples of blood plasma collected during this experiment were available for quantification of LH, and these data constitute the present paper. The specific objectives were to determine (1) whether EA treatment affects plasma LH, (2) whether exogenous naloxone increases plasma LH in...
sheep as it does in other species, and (3) whether gonadal hormones influence naloxone-induced release of LH in sheep.

MATERIALS AND METHODS

Experiments were conducted during October, utilizing the following sheep: 11 Rambouillet wethers (castrated males), five intact Suffolk ewes, four intact Rambouillet ewes and four intact crossbred ewes. Body weights averaged 38 ± 1 kg for wethers and 57 ± 3 kg for ewes. Experimental animals were maintained outside under ambient conditions. All 13 ewes were mature and multiparous, and they were assumed to be cyclic in October.

Each sheep was subjected sequentially to four trials designated A to D, allowing 2–7 days between successive trials. Just before each trial, a BD Longdwell catheter (Becton, Dickinson & Co., Rutherford, New Jersey, U.S.A.) was inserted into the jugular vein. Blood samples were collected at sequential intervals of 8–10 min with the first sample being collected before any treatment was applied. Trial A consisted of collecting a total of six blood samples. Trial B consisted of quantifying the cutaneous sensitivity to tactile stimuli (i.e., sensitivity test) followed by an i.v. injection (1·1 mg/kg) of naloxone (Endo Laboratories, Garden City, New York, U.S.A.) and then a second cutaneous sensitivity test. Six blood samples were collected; three before and three after naloxone. Beginning at about the time of the second blood sample, cutaneous sensitivity tests were begun. They consisted of applying each tactile stimulus (variable force pin-prick or pinch) sequentially to seven body areas and quantifying the behavioural reaction of the animal to the stimulus. The stimulus was terminated as soon as the animal reacted.

Trials C and D were similar (nine blood samples each) and involved EA but at different loci. Immediately after collection of the first sample, stainless steel EA needles were inserted (loci described later) and, when electrostimulation commenced, physical restraint of the animal was often required. Stimulating voltage and frequency from a Chinese-made stimulator (Model SB 71-2, Peoples Wireless Electronics Factory of TIAN JIN, China) were increased gradually to levels just below those at which the animal reacted adversely to the electrostimulation. Stimulating parameters were continued at this level throughout the trial. Approximately 32 min from the onset of stimulation, the first cutaneous sensitivity test was performed. After completion of the sensitivity test, naloxone (1·1 mg/kg) was injected i.v. and a second sensitivity test performed. Because of variation in the time necessary to reach final stimulating parameters, the first sensitivity test and the subsequent injection of naloxone occurred anywhere between the fifth and seventh blood sample.

Loci of EA for trial C were named as a group YAO PANG (Chinese) and consisted of one 8 cm needle inserted at acupuncture point BAI HUI between spinous processes of the lumbosacral junction until the point of the needle contacted the floor of the spinal canal. Two other 15 cm needles were inserted symmetrically through the skin at point YAO PANG, located at the level of the extremity of the fourth lumbar vertebrae. Both needles were directed toward the body of the last thoracic vertebrae passing ventral to the transverse processes of the third, second and first lumbar vertebrae.

Loci of EA for trial D were named as a group SAN YANG LU (Chinese) and consisted of two needles in the left thoracic limb. One needle (5 cm long) penetrated the skin (at acupuncture point QUIANG FENG) perpendicularly in the intermuscular depression felt through the skin between the muscle deltoideus, long head of the triceps brachii, and lateral head of the triceps brachii. The full length of the needle was inserted so that its tip was near the root of the radial nerve in the brachial plexus. After flexing the limb, a second needle (15 cm long) penetrated the skin at acupuncture point SAN YANG LU located on the lateral side of the upper foreleg about one-third of the distance from the elbow joint to the knee. The needle was directed distally and medially passing between the lateral extensor muscle and external carpi radialis muscle, through the deep flex of the digit muscle, and caudal to the radius and ulna bones. The insertion ceased when the tip of the needle could be felt in subcutaneous tissue of an area on the medial side of the leg corresponding to the chestnut of the horse (acupuncture point YE YAN), located about two-thirds of the distance from elbow joint to knee. All animals tolerated well the insertion of needles and physical restraint was only necessary when electrostimulation commenced.

Blood samples (about 8 ml) were drawn into heparinized syringes and placed into centrifuge tubes. Plasma was obtained by centrifugation within 60 min of collection and frozen (−20 °C) for later hormone analysis. Plasma LH was quantified by radioimmunoassay as described previously (Rasmussen & Malven, 1982). Since LH concentrations were lower in ewes than in wethers, duplicate 200 µl aliquots of ewe plasma and duplicate 50 µl aliquots of wether plasma were assayed. When samples of ewe plasma contained less LH than the minimum detectable concentrations (0·2 µg/l), these samples were assigned this value for statistical analysis.

To determine whether ewes were undergoing oestrous cycles, plasma concentrations of progest-
erone were quantified by radioimmunoassay in the first sample of every trial for all ewes (Niswender, 1973). Based upon the plasma progesterone concentration, each ewe in a trial was classified as luteal (i.e. plasma progesterone > 3-2 nmol/l) or as non-luteal (i.e. plasma progesterone < 1-6 nmol/l). Every ewe was found to have one or more trials in which she was classified as luteal. All but one ewe had one or more trials in which she was classified as non-luteal. This exception was found later to be pregnant (days 20–40) during the trials so she was always classified as luteal.

RESULTS

Effects of naloxone without EA

 Plasma LH fluctuated episodically in wethers during sequential sampling of trials A and B. The data in Table 1 from trial B show that naloxone injection failed to increase plasma LH concentrations. In contrast, there was a tendency for a slight decrease in plasma LH immediately after naloxone especially when expressed as a percentage of the pre-naloxone average for each wether. However, this tendency was highly variable and probably not important.

 Plasma LH in ewes during trials A and B was much lower than that in wethers and episodic fluctuations were less evident. Table 2 presents the average LH concentration for all samples in trials A and B after classification of ewes into luteal and non-luteal categories. Plasma progesterone averaged 8-9 and 7-6 nmol/l for luteal ewes in trials A and B respectively.

Average progesterone was uniformly low (0-3 nmol/l) in the non-luteal ewes because several ewes with intermediate concentrations of progesterone (between 1-6 and 3-2 nmol/l) were excluded from analysis. Average LH concentrations in trial A never exceeded 1-0 μg/l. Naloxone treatment of non-luteal ewes failed to raise the average LH concentration above 1-0 μg/l. However, naloxone treatment of luteal ewes occurring between samples nos 3 and 4 caused plasma LH to be increased significantly in sample no. 5 (1-9 μg/l) and no. 6 (5-7 μg/l) which were drawn 14 and 23 min after naloxone respectively. In five out of seven luteal ewes, plasma LH rose from non-detectable levels (0-2 μg/l) to peak concentrations ranging from 3-0 to 13-9 μg/l. However, plasma LH remained at non-detectable levels in one naloxone-treated luteal ewe.

<table>
<thead>
<tr>
<th>Time of sample (relative to naloxone)</th>
<th>Plasma LH (μg/l)</th>
<th>Plasma LH as % of pre-naloxone value for each wether (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample just before</td>
<td>11-2±2-6</td>
<td>100</td>
</tr>
<tr>
<td>First sample after (4 ± 1 min)</td>
<td>9-1±1-5</td>
<td>80±6</td>
</tr>
<tr>
<td>Second sample after (12 ± 1 min)</td>
<td>9-7±1-5</td>
<td>84±8</td>
</tr>
<tr>
<td>Third sample after (20 ± 1 min)</td>
<td>10-3±2-0</td>
<td>91±10</td>
</tr>
</tbody>
</table>

Table 2. Plasma progesterone concentrations and plasma LH concentrations before and after naloxone injections given between samples no. 3 and no. 4 in ewes classified as luteal and non-luteal. Values are means ± S.E.M.; numbers of ewes are shown in parentheses.

**P<0-05, **P<0-01 compared with the sample just before naloxone injection (t-test for unpaired observations with unequal variance).
Effects of EA

In wethers, overall plasma LH concentrations were reduced in trials C (8.8 ± 0.7 µg/l) and D (7.9 ± 0.6 µg/l) when compared with trials A (10.8 ± 0.5 µg/l) and B (10.2 ± 0.5 µg/l). The relationship between time after onset of sampling and plasma LH was quantified by curvilinear regression for each type of trial. This relationship was not significant in trials A and B indicating that neither repetitive sampling nor sensitivity testing affected LH concentrations. The decrease in plasma LH during EA treatments of trials C and D was found to be related (P < 0.01) to time from onset of the trial. Although quadratic and cubic functions were examined, only the linear function of time was significant and the regression coefficients (± S.E.M.) were −0.09 ± 0.01 ng/min and −0.07 ± 0.02 ng/min for trials C and D respectively. There was no significant difference between trials C and D in plasma LH concentrations or in the linear regression of plasma LH on time from onset of EA treatment. Administration of naloxone to wethers during EA treatment in trials C and D was not followed by any increase in mean plasma LH (not shown) but, rather, the steady EA-induced decline in LH concentrations appeared to continue at the same rate as before naloxone.

Since LH data for EA-treated ewes were not significantly different in trials C and D, the data have been combined for presentation in Table 3. However, EA-treated ewes were categorized as luteal or non-luteal since this classification aided interpretation of naloxone effects in untreated ewes. Although luteal ewes had overall lower plasma LH concentrations than non-luteal ewes, neither group of ewes showed any EA-induced LH decrease as was noted in wethers. When EA-treated ewes were injected with naloxone, no significant increase in plasma LH was observed (Table 3).

DISCUSSION

Exogenous naloxone failed to increase plasma LH in wethers which had been orchiectomized since early in life (Table 1). Since episodic release of LH from the pituitary gland of these wethers was already large (Riggs & Malven, 1974), our brief antagonism of endogenous opioid action by this dosage of naloxone may have been insufficient to trigger additional release of LH. However, chronic infusion of naloxone in large dosages (200 mg/animal per h) did increase LH release in wethers (Schanbacher, 1982). In orchiectomized rats, bolus injections of naloxone increased serum LH in some (Cicero, Wilcox, Bell & Meyer, 1980) but not all studies (Bhanot & Wilkinson, 1983).

![Image](https://example.com/figure.png)

**Table 3.** Plasma progesterone concentrations and plasma LH concentrations in luteal and non-luteal ewes receiving electroacupuncture (EA) treatment followed by injection of naloxone. Values are means ± S.E.M.; numbers of ewes are shown in parentheses

<table>
<thead>
<tr>
<th>EA Treatment (trials C and D)</th>
<th>Luteal</th>
<th>Non-luteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma progesterone (nmol/l)</td>
<td>8.9 ± 1.3</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>Plasma LH (µg/l)</td>
<td>Sample numbers during EA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>Sample just before naloxone</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>First sample after (6 ± 1 min) naloxone</td>
<td>0.4 ± 0.1</td>
<td>0.8 ± 0.4</td>
</tr>
<tr>
<td>Second sample after (14 ± 1 min) naloxone</td>
<td>1.0 ± 0.5</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>Third sample after (21 ± 1 min) naloxone*</td>
<td>0.7 ± 0.3</td>
<td>—</td>
</tr>
</tbody>
</table>

*Because of variation in sampling times relative to injection of naloxone, the number of observations was reduced to 2 for non-luteal ewes (data omitted) and to 8 for luteal ewes.

Although cutaneous sensitivity tests were administered to ewes in trial B before and after naloxone, plasma LH only increased 14–23 min after naloxone and only in those ewes with raised plasma progesterone (Table 2). An interaction between naloxone and the sensitivity testing cannot be ruled out from the present results. However, other studies in ewes not subjected to cutaneous sensitivity tests have confirmed that naloxone alone can increase plasma LH (W. E. Trout and P. V. Malven, unpublished observations).

The apparent progesterone requirement in ewes for naloxone to increase LH release is consistent with data obtained in women exhibiting normal menstrual cycles (Quigley & Yen, 1980). Intravenous infusion of naloxone (1.6 mg/subject per h) to women in their mid-luteal phase (serum progesterone > 16 nmol/l) increased serum LH to an initial peak at about 1 h after the start of infusion (Quigley & Yen, 1980; Ropert, Quigley & Yen, 1981). In contrast, the same infusion of naloxone during days 2–4 of the early follicular phase of the cycle (serum progesterone < 3.2 nmol/l) failed to increase plasma LH in normal subjects (Quigley & Yen, 1980). Although these results suggest a relationship between corpus luteum function and the ability of naloxone to increase LH release, raised serum proges-
terone does not appear to be required for all naloxone-induced increases in serum LH in women since responses can be obtained in some subjects with amenorrhoea (Quigley, Sheehan, Casper & Yen, 1980; Lightman, Jacobs, Maguire et al. 1981).

In female rats naloxone-induced increases in LH have varied among experiments. In cyclic females, Gabriel, Simpkins & Kalra (1982), but not Blank, Panerai & Friesen (1979), observed significant naloxone-induced increases in serum LH. However, the magnitude of naloxone-induced increases in LH were similar on different days of the oestrous cycle (Gabriel et al. 1982, 1983). After ovariectomy, there seems to be general agreement that treatment with oestrogen and progesterone to inhibit spontaneous LH release increases the magnitude of naloxone-induced LH increases (Gabriel et al. 1982; Sylvester, Van Vugt, Aylsworth et al. 1982; Bhanot & Wilkinson, 1983).

Treatment by EA of luteal ewes prevented the naloxone-induced LH increases which had been observed in untreated luteal ewes (Tables 2 and 3). This effect is consistent with one or more of the following possible LH-inhibitory actions of EA treatment: (1) increased activity of endogenous opioid peptides acting through naloxone-sensitive sites, (2) stimulation of endogenous opioid peptides which act at sites which are not sensitive to this dosage of naloxone or (3) activation of non-opioid neurotransmitters with LH-inhibiting potency. Even though EA treatment has been shown to activate endogenous opioid-mediated analgetic systems (Han & Terenius, 1982), stressful electric shock can also activate such systems (Watkins & Mayer, 1982). Therefore, we cannot know for certain whether the blockage of naloxone-induced LH increases in luteal ewes reflects a specific effect of EA or a non-specific stress associated with the imposition of the EA treatment. Gabriel et al. (1982) observed that immobilization stress inhibited naloxone-induced LH increases in ovariectomized rats. Pontiroli, Bai, Stella et al. (1982) reported that 12 women exhibiting menstrual cycles (stages not known) failed to have LH increases following naloxone during surgical stress. At least some of these women would have been in the luteal phase of the menstrual cycle and probably would have responded to naloxone without the surgical stress. Perhaps stressors such as immobilization and surgery act in one or more of the ways suggested above for EA treatment to decrease the effectiveness of naloxone.

Treatment by EA decreased the raised concentrations of plasma LH in wethers but did not affect the already low concentrations in ewes (Table 3). Because EA treatment and the physical restraint necessary to impose it may stress the wethers, we cannot determine whether or not the observed LH inhibition was specific to EA stimulation. However, it has been demonstrated that non-specific stress of confinement can inhibit the raised concentrations of LH in ovariectomized ewes (Rasmussen & Malven, 1983).

The differences between ewes and wethers in the effects of naloxone and of EA treatment on plasma LH contrasted with the analgesia data (Bossut et al. 1983). Treatment by EA produced cutaneous analgesia equally in ewes and wethers. However, analgesia induced in trial C by EA of the YAO PANG loci was partially reversed by naloxone whereas that induced in trial D by EA of the SAN YANG LU loci was not significantly affected by naloxone.

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REFERENCES


