Effect of naloxone on gonadotrophin secretion at various stages of development in the ewe lamb

N. C. Rawlings and I. J. Churchill

Department of Veterinary Physiological Sciences, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 0W0

Summary. Spring-born crossbred ewe lambs were raised in a natural photoperiod and saline (N = 6) or naloxone (1 mg/kg) in saline (N = 6) was injected (i.m.) every 2 h for 6 h at 5, 10 and 15 weeks of age and for 8 h at 20, 25 and 30 weeks of age. Blood samples were taken every 12 min during treatment periods.

Naloxone had no effect on time to first oestrus (controls 235 ± 6 days, naloxone 242 ± 7 days). Mean serum LH concentrations and LH pulse frequency were elevated by naloxone in ewe lambs at 20, 25, and 30 weeks of age (P < 0.05). The only FSH response to naloxone was a depression of mean serum concentrations at 30 weeks of age (P < 0.05). LH pulse amplitude was elevated at 5 weeks of age in all ewe lambs and declined thereafter to a nadir at 30 weeks of age in control, but not in naloxone-treated animals (P < 0.05). LH pulse frequency was elevated at 10 weeks of age in control ewe lambs and in all animals at 30 weeks of age (P < 0.05). FSH pulse frequency declined from 5 weeks of age in control ewe lambs (P < 0.05), with very few pulses noted in 25- and 30-week-old animals. We conclude that (1) opioidergic suppression of LH, but not FSH, secretion developed at 20 weeks of age in the growing ewe lambs used in the present study, with no obvious change in suppression before the onset of first oestrus: (2) pulsatile FSH secretion occurred in the young ewe lamb but was lost as the lamb matured: (3) attainment of sexual maturity was preceded by an elevation in LH pulse frequency.

Keywords: ewe lamb; naloxone; LH; FSH; sexual maturation

Introduction


Naloxone, an opioid antagonist, causes an elevation of LH secretion in immature female rats as early as 5 days of age (Ieiri et al., 1979). Naloxone stimulation of LH secretion may be increased or decreased as sexual maturity approaches (Kamberi et al., 1980; Cicero et al., 1986). Naloxone was found to increase LH pulse frequency in prepubertal intact, and ovariectomized ewe lambs (Ebbling et al., 1989). Opioid agonists appear to suppress LH secretion in immature female rats and it has been suggested that the response to such agonists is diminished before sexual maturity (Bhanot & Wilkinson, 1983; Cicero et al., 1985). Endogenous opioids may therefore diminish LH secretion in the immature female rat and a release of this inhibition could be involved in sexual maturation. Treatment of immature female rats with opioid antagonists has had both stimulatory and inhibitory effects on their rate of sexual maturation (Zagon & McLaughlin, 1984; Sirinathsinghji et al., 1985).
The purpose of the present study was to investigate the effects of naloxone on LH and FSH secretion in the ewe from 5 to 30 weeks of age, to see whether changes in opioidergic effects on gonadotrophin secretion have significance in the process of sexual maturation.

Materials and Methods

Six weight- and age-matched pairs of spring-born ewe lambs of mixed breeding were housed in sheltered dry lots. Creep feed was available from 1 week of age. The lambs were weaned at 7 weeks of age and housed in a separate pen, but in close proximity to ram lambs. Commercial mixed feeds were fed according to NRC recommendations (National Research Council, 1968); mixed minerals, salt, water and alfalfa-brome hay were fed ad libitum.

Six lambs received sterile physiological saline (control) and 6 were treated with naloxone (1 mg/kg; E. I. DuPont de Nemours & Co., Inc., Garden City, NY, USA) in saline (i.m.) every 2 h for 6 at 5, 10 and 15 weeks of age and for 8 h at 20, 25 and 30 weeks of age. The dose of naloxone had been shown previously to elevate serum LH concentrations in cyclic ewes (Currie & Rawlings, 1987). Blood samples (3 ml) were taken from the ewe lambs every 12 min during the treatment period, by indwelling jugular catheters (vinyl tubing, SV-70, i.d. 1-00 mm, o.d. 1-50 mm; Dural Plastics and Engineering, Dural, New South Wales, Australia) inserted the day before treatment. Blood samples were allowed to clot at 20°C, clots were removed and serum stored at −20°C until analysed. LH and FSH concentrations were measured in all samples and oestradiol-17β in a pool of serum comprised of samples taken every hour at each treatment.

To determine the time of sexual maturity 3 vasectomized rams, wearing colour-marker harnesses, were introduced to the ewe lambs at 20 weeks of age. The ewe lambs were checked daily for collogue marks and the ram harness colour was changed every 15 days. The time of sexual maturity was taken as the first of at least 2 markings occurring with a regular spacing of 17–21 days.

Serum concentrations of LH and FSH were determined by radioimmunoassay and are expressed in terms of NIAMDD-oLH-24 and NIAMDD-oFSH-RP1, respectively (Currie & Rawlings, 1989). Sensitivities of the assays, defined as the concentrations of the lowest standard different from zero (P < 0.05), were 0-06 ng LH/ml serum and 0-08 ng FSH/ml serum. Intra- (n = 13) and inter- (n = 91) assay coefficients of variation for LH were 6-3% and 8-1% respectively for a reference serum (mean = 1-21 ng/ml) replicated in every assay. Intra- (n = 13) and inter- (n = 91) assay coefficients of variation for FSH were 4-8% and 13-1%, respectively for a reference serum (mean = 2-00 ng/ml).

Serum concentrations of oestradiol-17β were determined by radioimmunoassay (Currie & Rawlings, 1989). Assay sensitivity was 1-0 pg oestradiol/ml serum. The assay blank was not different from zero. All samples were analysed in one assay with an intra-assay coefficient of variation of 16-1% (n = 6).

LH and FSH pulses were detected as values above basal by the method of Goodman & Karsch (1980). LH and FSH data are expressed as mean concentrations and as pulse amplitude and frequency. Oestradiol-17β concentrations are expressed as means for each treatment period. Data were analysed by analysis of variance and Student-Newman-Keul’s procedure.

Results

Naloxone treatment did not affect time to first oestrus (controls 235 ± 6 days; naloxone 242 ± 7 days). Administration of naloxone to ewe lambs did not influence LH secretion at 5, 10 or 15 weeks of age. Naloxone elevated mean serum LH concentrations and LH pulse frequency in ewe lambs at 20, 25 and 30 weeks of age (P < 0.05, Table 1). The increase of mean LH concentrations in response to naloxone was 100%, 125% and 240% at 20, 25 and 30 weeks of age respectively. This pattern of increasing response with age was not noted for LH pulse frequency (91%, 126%, 78% at 20, 25 and 30 weeks respectively) (Fig. 1). LH pulse amplitude was not affected by naloxone administration (P > 0.1). The only FSH response to naloxone was a depression of mean serum concentrations at 30 weeks of age (P < 0.005, Table 2).

Trends in LH pulse amplitude and frequency and FSH pulse frequency were noted with age. LH pulse amplitude was elevated at 5 weeks of age in all ewe lambs and declined thereafter to a low at 30 weeks of age in control (P < 0.05, Table 1), but not in naloxone-treated animals. LH pulse frequency was elevated at 10 weeks of age (P < 0.05, Table 1) in control ewe lambs and in all ewe lambs at 30 weeks of age. There was no significant trend in mean serum concentrations of LH with increasing age. The only significant trend with increasing age in the FSH values was the decline in FSH pulse frequency in control ewe lambs (P < 0.05): FSH pulses were noted in 10 of 12 ewe lambs at 5 weeks of age, none at 25 weeks of age and in only 2 of 12 at 30 weeks of age (Table 2; Fig. 2).
Table 1. Mean serum concentrations of LH and LH pulse amplitude and frequency in ewe lambs blood sampled every 12 min for 6 h at 5, 10 and 15 weeks of age and for 8 h at 20, 25 and 30 weeks of age and the effects of naloxone (1 mg/kg; i.m.) administered at the onset of and every 2 h during each sampling period

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Mean LH (ng/ml)</th>
<th>LH pulse amplitude (ng/ml)</th>
<th>LH pulse frequency (pulses/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Naloxone</td>
<td>Control</td>
</tr>
<tr>
<td>5</td>
<td>0.67 ± 0.24</td>
<td>0.70 ± 0.40</td>
<td>3.65 ± 0.75a</td>
</tr>
<tr>
<td>10</td>
<td>0.35 ± 0.08</td>
<td>0.28 ± 0.07</td>
<td>1.09 ± 0.26b</td>
</tr>
<tr>
<td>15</td>
<td>0.29 ± 0.05</td>
<td>0.41 ± 0.05</td>
<td>1.58 ± 0.26b</td>
</tr>
<tr>
<td>20</td>
<td>0.34 ± 0.05</td>
<td>0.68 ± 0.12*</td>
<td>1.52 ± 0.21b</td>
</tr>
<tr>
<td>25</td>
<td>0.28 ± 0.03</td>
<td>0.63 ± 0.09**</td>
<td>1.59 ± 0.20b</td>
</tr>
<tr>
<td>30</td>
<td>0.33 ± 0.06</td>
<td>1.12 ± 0.31*</td>
<td>0.96 ± 0.18c</td>
</tr>
</tbody>
</table>

Means with different superscripts within columns are significantly different, *P < 0.05. Differences between treatments were significant, *P < 0.05, **P < 0.01.

Fig. 1. Pulsatile LH secretion in control (■) and naloxone-treated (□) ewe lambs. Blood samples were taken every 12 min for 6 h at 5 weeks of age and for 8 h at 20, 25 and 30 weeks of age. Pulses are marked by arrows. Details of the naloxone treatment are given in the text.
Table 2. Mean serum concentrations of FSH and FSH pulse amplitude and frequency, and number of lambs exhibiting pulses in ewe lambs blood sampled every 12 min for 6 h at 5, 10 and 15 weeks of age and for 8 h at 20, 25 and 30 weeks of age and the effects of naloxone (1 mg/kg; i.m.) administered at the onset of and every 2 h during each sampling period.

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>Mean FSH (ng/ml)</th>
<th>FSH pulse amplitude (ng/ml)</th>
<th>FSH pulse frequency (pulses/h)</th>
<th>Lambs with pulses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Naloxone</td>
<td>Control</td>
<td>Naloxone</td>
</tr>
<tr>
<td>5</td>
<td>3.11 ± 1.14</td>
<td>3.68 ± 1.48</td>
<td>3.79 ± 0.25</td>
<td>3.63 ± 0.17</td>
</tr>
<tr>
<td>10</td>
<td>1.84 ± 0.25</td>
<td>1.48 ± 0.18</td>
<td>2.00 ± 0.11</td>
<td>2.37 ± 0.08</td>
</tr>
<tr>
<td>15</td>
<td>1.93 ± 0.17</td>
<td>2.08 ± 0.11</td>
<td>1.99 ± 0.08</td>
<td>3.51 ± 0.06</td>
</tr>
<tr>
<td>20</td>
<td>1.96 ± 0.15</td>
<td>2.29 ± 0.16</td>
<td>2.49 ± 0.16</td>
<td>2.73 ± 0.04</td>
</tr>
<tr>
<td>25</td>
<td>1.49 ± 0.12</td>
<td>1.52 ± 0.16</td>
<td>2.02 ± 0.19</td>
<td>1.33 ± 0.09*</td>
</tr>
<tr>
<td>30</td>
<td>1.94 ± 0.19</td>
<td>1.33 ± 0.09*</td>
<td>2.02 ± 0.02</td>
<td>2.08 ± 0.02</td>
</tr>
</tbody>
</table>

Means with different superscripts within columns are significantly different, *P < 0.05.
Differences between treatments were significant, *P < 0.05.

Fig. 2. Pulsatile FSH secretion in 2 control (■) and 2 naloxone-treated (□) ewe lambs at 5 weeks of age. Blood samples were taken every 12 min for 6 h. Pulses are marked by arrows. Details of the naloxone treatment are given in the text.

Serum oestradiol-17β concentrations were not influenced by age or by treatment (controls 5.0 ± 0.7 pg/ml; naloxone treatment 5.0 ± 0.7 pg/ml).

Discussion

Apart from an apparent early decline in the control ewe lambs, serum concentrations of FSH did not follow any obvious pattern with age in the present study. Previous reports have presented a similar picture, with the exception of serum FSH concentrations being influenced by season, and
elevated during the summer months (Fitzgerald & Butler, 1982; Foster et al., 1975). In the present study, there were no changes in serum FSH or oestradiol-17β concentrations indicative of the onset of ovulatory cycles. Kennedy et al. (1974) found that ovarian weight did not change from 12 to 33 weeks of age in ewe lambs, and follicular growth and turnover were relatively stable in this period. The relatively constant serum concentrations of oestradiol-17β seen in the present study would support this observation of a period of stable follicular growth and turnover. While changes in FSH and oestradiol-17β secretion immediately before first ovulation may not have been adequately reflected in the present study, no major long-term changes in secretion of these two hormones preceded the onset of ovulatory cycles in the ewe lamb.

In the adult cyclic ewe, FSH secretion is not regarded as being pulsatile (Currie & Rawlings, 1987). In the present study, FSH secretion was clearly pulsatile in ewe lambs that were 5–10 weeks old. Pulsatility was more sporadic or absent in older animals. Such pulsatility was suggested in a previous report (Huffman et al., 1987). In the ewe lambs in the present study pulsatile secretion of FSH appeared at a time when ovarian follicular growth has been shown to be high (Kennedy et al., 1974). The reason for pulsatile FSH secretion in the young ewe lamb is unclear. It is possible that sensitivity to pulsatile GnRH secretion changes as the ewe lamb matures or that the clearance rate of FSH changes, masking pulsatility. In all ewe lambs, FSH pulse frequency was always less than the pulse frequency for LH.

As with FSH, the changes in serum concentrations of LH with age were not dramatic. In previous reports (Foster et al., 1975; Fitzgerald & Butler, 1982; Ebling et al., 1989) the temporal pattern of serum concentrations of LH with age in ewe lambs were quite variable, with an indication of an increase in mean LH concentrations at the time of sexual maturity. As shown in the present study, LH pulse frequency increased and pulse amplitude declined just before the onset of ovulatory cycles (Huffman et al., 1987). These changes contributed to the equivocal changes in mean serum LH concentrations. An increase in LH pulse frequency is an important component of the endocrine cascade leading to ovulation in the mature cyclic ewe (Karsch, 1984).

In the present study, LH pulse amplitude was high and LH pulse frequency was low in the 5-week-old ewe lambs. This is at a time when serum FSH concentrations were high and ovarian folliculogenesis was high. This change in the LH secretory pattern, concomitant with an elevation of folliculogenesis, is qualitatively opposite to that occurring immediately before the onset of ovulatory cycles (i.e. an increase in pulse frequency, but a decrease in amplitude). An increase in LH pulse frequency is critical to the endocrine cascade resulting in ovulation (Karsch, 1984), but the qualitative changes in LH secretion may be of less importance for the support of the earlier stages of folliculogenesis. The changes in LH and FSH secretion seen in the 5–10-week-old ewe lamb may represent early maturation of the hypothalamic–pituitary axis, followed by the establishment of gonadal steroid negative feedback control of gonadotrophin secretion (Rawlings et al., 1978).

Administration of naloxone did not affect FSH secretion in the present study, apart from a depression of mean serum concentrations of FSH in 30-week-old ewe lambs. In the mature ewe we have failed to demonstrate effects of naloxone or morphine on FSH secretion (Currie & Rawlings, 1987, 1989).

In the adult cyclic ewe, naloxone administration causes an elevation of LH pulse frequency and amplitude (Brooks et al., 1986; Malven, 1986; Currie & Rawlings, 1989). In the present study naloxone administration caused an increase in LH pulse frequency and mean serum concentrations of LH in ewe lambs 20 weeks of age and older, agreeing with results from previous studies (Ebling et al., 1989). In the adult ewe opioidergic suppression of LH secretion largely reflects a mediation of progesterone negative feedback on LH secretion (Brooks et al., 1986; Malven, 1986). The age at which naloxone became effective in the ewe lambs in this study was probably not correlated with changes in secretion of ovarian steroids, as serum concentrations of oestradiol-17β did not change across the course of this study and progesterone will not be produced until ovulatory cycles commence. Previous studies have found that naloxone, in doses of 1 mg/kg or greater, increased LH
pulse frequency in all but neonatal ewe lambs, under both oestrogen and progesterone treatment, and in the absence of ovarian steroids (Ebling et al., 1989).

The final process of sexual maturation in the ewe lamb is clearly influenced by season. Spring-born lambs exhibit ovulatory cycles late in the breeding season of mature ewes (November in study of Foster & Ryan, 1979). This, as in the adult, is preceded by an elevation in LH secretion, which is due largely to a decrease in the negative feedback sensitivity to oestradiol-17β (Foster & Ryan, 1979). The period of influence of naloxone on LH secretion seen in the present study would not appear to be a seasonal phenomenon. Naloxone affected LH secretion in ewe lambs from 20 to 30 weeks of age, a period longer than that over which the changes in LH responsiveness to oestradiol-17β occur preceding the onset of ovulatory cycles (Foster & Ryan, 1979). In the present study the period of responsiveness to naloxone appeared after the summer solstice and during the period of decreasing daylength. Decreasing daylength is stimulatory to ovarian cyclicity in the ewe (Hammond, 1944).

In this study, the development of the stimulatory effects of naloxone on LH secretion in the ewe lamb were delayed compared to the very early development of effectiveness in the female rat (Ieiri et al., 1979). It has been suggested that a diminished opioidergic suppression of LH secretion may be involved in the initiation of ovarian cyclicity in the prepubertal female rat (Sirinathsinghji et al., 1985). There was no evidence of this in the present study. It is possible that opioidergic suppression of LH secretion may decline immediately before the onset of ovulatory cycles in the ewe lamb; this may not have been readily detected in the present study. In the luteal phase of the adult cyclic ewe, the effects of naloxone on LH pulse frequency have ranged from no effect to an 80% increase and, on pulse amplitude, from no effect to a 165% increase (Brooks et al., 1986; Currie & Rawlings, 1987, 1989). Compared with the effects noted in the present study, it is difficult to conclude that a major shift in opioidergic suppression of LH pulse frequency occurs as a prerequisite to sexual maturity in the ewe lamb. Naloxone did not influence LH pulse amplitude in the present study as it does in the mature ewe, but upward shifts in pulse frequency appear to be critical in initiating ovulatory cyclicity (Huffman et al., 1987; Ebling et al., 1989).

Naloxone was effective in influencing LH secretion in the ewe lamb when secretion of oestradiol-17β was low and constant and progesterone was not secreted. In the mature cyclic ewe endogenous opioids probably mediate steroid negative feedback control of LH secretion, although some steroid-independent effects have been suggested (Malven et al., 1984; Schillo et al., 1985; Brooks et al., 1986; Currie & Rawlings, 1987). This suggests that a steroid-independent component of opioidergic suppression of LH secretion may be important in the immature ewe lamb and that this may diminish as ovulatory cyclicity begins.

In the immature ram lamb opioidergic suppression of LH secretion did not occur until the ram approached sexual maturity and as circulating concentrations of testosterone rose (unpublished observations). This is in contrast to the ewe lamb in which naloxone caused an elevation in LH secretion some time before first oestrus and against relatively constant circulating concentrations of oestradiol-17β. In the ram and ewe a decrease in opioidergic suppression of LH did not appear to be a major component of reproductive endocrine maturation. However, the significant effects of naloxone on LH pulse frequency, but not amplitude, agree with a central site of action of opioids (Ebling et al., 1989) and support the hypothesis that opioid mechanisms inhibit LH secretion in the prepubertal period through regulation of the frequency of episodic LH secretion (Blank et al., 1979; Ieiri et al., 1979).

We thank David Currie, Ingrid Joseph and Susan Cook for assistance with the experiments; and Margot Buckley and her staff for assistance with animal care. This work was supported by a grant from NSERC. Naloxone was generously provided by DuPont Pharmaceuticals. Standards for the LH assays and the FSH assay system were provided by NIAMDD. LH for iodinations was provided by Dr L. E. Riechert Jr.
References


Received 25 September 1989