Opioids and suckling in relation to inhibition of oestradiol-induced LH secretion in *postpartum* ewes

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The effects of time *postpartum* and suckling status on the oestradiol-induced LH surge in ewes and some of the possible mechanisms involved were investigated. Lambs were removed from nonsuckled ewes at parturition (day 0) and the ewes hand-milked 12 and 48 h later to prevent discomfort. Nonsuckled ewes on day 1, 7, 14 or 28, and suckled ewes on day 7 or 28 *postpartum* were injected i.m. with 50 µg oestradiol benzoate (hour 0). A further eight suckled ewes were given oestradiol on day 1 (as above), and four were also given naloxone ($1 \text{ mg kg}^{-1} \text{ h}^{-1}$) from 10 until 14 h after administration of oestradiol benzoate. There was no LH surge in response to oestradiol on day 1. In nonsuckled ewes the onset of the LH surge was significantly ($P < 0.05$) delayed when oestradiol was administered on day 7 ($17.4 \pm 1.5 \text{ h}$), compared with when it was injected on days 14 and 28 *postpartum* (14.4 ± 1.7 and $14.0 \pm 1.4 \text{ h}$). The LH surge was also delayed ($P < 0.05$) when oestradiol was administered on day 7 ($18.0 \pm 2.8 \text{ h}$) in suckled ewes. There was no difference in the duration or amplitude of the LH surge with time *postpartum* in nonsuckled ewes. Suckling significantly ($P < 0.05$) reduced the amplitude of the LH surge on both day 7 (21.0 ± 2.4 versus $26.4 \pm 2.3 \text{ ng ml}^{-1}$) and day 28 (23.6 ± 3.2 versus $28.8 \pm 2.9 \text{ ng ml}^{-1}$), but had no effect on the onset or duration of the surge. There was no difference in the timing or the amplitude of the oestradiol peak following the oestradiol benzoate challenge with time *postpartum*. Naloxone had no effect on the mean LH concentration (control, 2.3 ± 1.6 ; naloxone-treated, $2.2 \pm 1.5 \text{ ng ml}^{-1}$) or on the pattern of LH secretion on day 1. In conclusion, time *postpartum* influenced the timing of the onset, while suckling reduced the amplitude, of the oestradiol-induced LH surge. The lack of an oestradiol-induced LH surge on day 1 *postpartum* in the ewe is due to a nonopioidergic mechanism.

Introduction

After parturition, the endocrine mechanisms controlling ovulation are disrupted, leading to *postpartum* anoestrus in many species, including sheep and cows (Nett, 1987). In dairy cows during this period, the time taken for oestradiol benzoate to elicit an LH surge decreases as time *postpartum* increases (Alam and Dobson, 1987). In sheep, the percentage of ewes responding to oestradiol benzoate with an LH surge increases with time *postpartum*, with no ewes responding before day 3 *postpartum* (Wright and Findlay, 1977; Wright *et al.*, 1980), although precise parameters of the LH surges were not determined.

Lactation has also been associated with *postpartum* anoestrus (Peters and Lamming, 1990). In sheep, suckling delays the return to cyclicity in some breeds (Call *et al.*, 1976) but not in others (Moss *et al.*, 1980). Furthermore, this effect of suckling on *postpartum* anoestrus also varies within a breed, depending upon whether lambing occurred in the spring or in the autumn (Pope *et al.*, 1989). Further studies on the mechanism by which

such effects could be mediated revealed that suckling inhibits tonic LH secretion in sheep (Schirar *et al.*, 1990), as in cows (Convey *et al.*, 1982) and rats (Taya and Sasamoto, 1989).

Parturition is both a painful and stressful experience, with associated high peripheral concentrations of opioids, which have been linked to the suppression of gonadotrophin secretion and to *postpartum* acyclicity (Millan and Herz, 1985; Haynes *et al.*, 1989). Administration of the opioid antagonist naloxone (1 mg kg^{-1}) increases tonic LH secretion in *postpartum* (Malven and Hudgens, 1987) and cyclic (Gregg *et al.*, 1983) ewes. Naloxone also increases tonic LH secretion in cows (Whisnant *et al.*, 1986).

In ewes there is some evidence to suggest a link between opioids and oestradiol positive feedback (Currie and Rawlings, 1987; Knight *et al.*, 1990). Such a link has been established in rats, in which naloxone increases the LH surge response to oestradiol (Gabriel *et al.*, 1983).

The aim of the present study was to investigate whether time *postpartum* or suckling or both influences the parameters of the oestradiol-induced LH surge in ewes, and what role opioids play in the inability of oestradiol to induce an LH surge in the early *postpartum* period.

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Materials and Methods

Animals and collection of blood samples

Mature, spring-lambing Welsh Mountain ewes, of approximately 35 kg body mass, were housed 2 weeks before parturition in the same room with natural lighting and ambient temperature. After lambing, the ewes were kept in pairs in straw-bedded pens with water and hay freely available.

An indwelling jugular venous catheter was inserted under local anaesthesia (2 ml Lignocaine 0.5%, Intervet; Crewe) the day before each experiment, and patency was maintained by filling with heparinized saline (50 iu ml⁻¹). Blood samples (7 ml) were taken into heparinized syringes (50 iu ml⁻¹), immediately centrifuged at 1000 g for 10 min and the plasma was stored at -20°C.

Experimental design

At parturition (day 0) the ewes were randomly divided into two groups: suckled (one lamb per ewe) and nonsuckled. Lambs were removed from the ewes in the nonsuckling group on day 0, and the ewes hand-milked twice only, 12 and 48 h after birth, to prevent discomfort.

The LH response to oestradiol was examined in different groups of nonsuckled ewes on days 1 (*n* = 5), 7 (*n* = 7), 14 (*n* = 5) or 28 (*n* = 5), and in suckled ewes on day 7 (*n* = 7) or 28 (*n* = 8). On each occasion the ewes were given 50 µg oestradiol benzoate in 2 ml arachis oil i.m. (hour 0), and blood samples were collected every 2 h from 8 h until 24 h after oestradiol benzoate injection.

The effect of naloxone on the LH response to oestradiol on day 1 *postpartum* was examined in an additional eight suckled ewes given 50 µg oestradiol benzoate, as above (hour 0). Half of the ewes (*n* = 4) were also given 1 mg naloxone kg⁻¹ (Sigma, Poole) in 2 ml 0.9% saline via the catheter once per hour from 10 h until 14 h after oestradiol benzoate injection, while the other four ewes received 2 ml saline alone. This dose schedule has previously been used in sheep to modify LH secretion (Gregg *et al.*, 1983). Blood samples were collected from all ewes at 8 h, 9 h and then every 15 min until 15 h, followed by every 2 h until 23 h after oestradiol benzoate injection.

Hormone analysis

Plasma samples were analysed for oestradiol by a radioimmunoassay characterized and verified by Dobson and Dean (1974). All samples were measured in one assay, with an intra-assay coefficient of variation of 9.9%. The minimum detectable quantity was 1.5 pg ml⁻¹.

Plasma samples were analysed for LH by a radioimmunoassay characterized and verified by Dobson and Ward (1977). In the present study, a specific ovine LH antiserum (Cunningham LH 07/08) and iodinated purified ovine LH (LER 1056 C2) (both donated by F. Cunningham, Reading) were used with 0.1 ml plasma samples, and results were expressed as ng equivalents of NIH-LH-S21 ml⁻¹ plasma. Contemporary intra- and interassay coefficients of variation were 9.7% and

10.4%, respectively. The minimum detectable quantity was 0.5 ng ml⁻¹.

Statistical analysis

Results are mean values ± SD. An LH surge was defined as two or more consecutive samples containing > 10 ng LH ml⁻¹. The onset of the surge was defined as the time of the first sample with LH > 10 ng ml⁻¹. Duration of the LH surge was defined as the time LH was > 10 ng ml⁻¹. Amplitudes were defined as peak height. LH pulsatility was defined using the criteria of Webb *et al.* (1985). Briefly, an LH pulse was considered to have occurred if the lower 95% confidence limit of the peak (maximum turning point) was greater in value than twice the upper 95% confidence limit of the previous nadir (minimum turning point). Data were compared where appropriate by Student's *t* test, following ANOVA where *P* < 0.05.

Results

There was no difference in either the timing (11 ± 2.3, 13 ± 0 and 12 ± 2 h, respectively) or amplitude (63.5 ± 11.3, 64.8 ± 3.8 and 63.7 ± 12.6 pg ml⁻¹, respectively) of the oestradiol peak following i.m. injection of oestradiol benzoate between days 1, 7 and 14 *postpartum* in nonsuckled ewes.

There was no LH surge in response to oestradiol on day 1 in any ewe. However, on days 7, 14 and 28 all ewes responded to the oestradiol challenge with an LH surge.

In nonsuckled ewes the onset of the oestradiol-induced LH surge was significantly (*P* < 0.05) delayed when oestradiol was injected on day 7, compared with results from ewes injected on days 14 and 28 (Table 1). However, there was no difference in LH surge duration or amplitude with time *postpartum* (Table 1).

In suckled ewes injected with oestradiol on day 7, the onset of the oestradiol-induced LH surge was significantly (*P* < 0.05) delayed compared with results from ewes injected on day 28 and the surge duration was significantly (*P* < 0.01) reduced, but there was no difference in surge amplitude (Table 1).

In suckled ewes compared with nonsuckled ewes, the amplitude of the oestradiol-induced LH surge was significantly (*P* < 0.05) lower on both day 7 and day 28. However, suckling had no effect on the onset or duration of the LH surge on either day (Table 1).

On day 1 *postpartum* in the suckled ewes there was no LH surge in response to oestradiol in either the control or the naloxone-treated groups. Naloxone had no effect on the overall mean LH concentration (control, 2.3 ± 1.6; naloxone-treated, 2.2 ± 1.5 ng ml⁻¹), or on the overall LH profiles (Fig. 1). The LH pulse frequency over the period 10–15 h after oestradiol benzoate treatment was 0.9 ± 0.1 pulses h⁻¹ for both control and naloxone-treated ewes.

Discussion

The lack of an oestradiol-induced LH surge on day 1 *postpartum* in the study reported here is in agreement with the findings in suckled ewes on day 3 *postpartum* (Wright and Findlay, 1977)

Table 1. Mean ($\pm$ SD) characteristics of the LH surge in suckled (S) and non-suckled (NS) ewes pretreated with 50 μ g oestradiol benzoate i.m. (hour 0)

Ewes	Onset time (h)	Duration (h)	Amplitude (ng ml ⁻¹)
NS, day 7 (n = 7)	17.4 $\pm$ 1.5 ^a	6.8 $\pm$ 1.0 ^{a,b}	26.4 $\pm$ 2.3 ^a
NS, day 14 (n = 5)	14.4 $\pm$ 1.7 ^b	7.6 $\pm$ 0.9 ^a	27.3 $\pm$ 2.6 ^a
NS, day 28 (n = 5)	14.0 $\pm$ 1.4 ^b	7.6 $\pm$ 0.9 ^a	28.8 $\pm$ 2.9 ^a
S, day 7 (n = 7)	18.0 $\pm$ 2.8 ^a	6.0 $\pm$ 0.0 ^b	21.0 $\pm$ 2.4 ^b
S, day 28 (n = 8)	14.5 $\pm$ 1.4 ^b	7.8 $\pm$ 0.7 ^a	23.6 $\pm$ 3.2 ^b

Within a column, groups without a common superscript are significantly different ($P < 0.05$).

Fig. 1. A typical LH response to the administration of 50 μ g oestradiol benzoate i.m. (hour 0) (■) with and (□) without naloxone treatment (1 mg kg⁻¹ h⁻¹ from 10 to 14 h after oestradiol benzoate treatment) in day 1 postpartum ewes (arrows indicate time of naloxone or saline injections).

and in day 1 postpartum cows (Alam and Dobson, 1987). On days 1–3 postpartum in ewes not pretreated with oestradiol, there were fewer GnRH and oestrogen receptors in the pituitary (Crowder *et al.*, 1982; Clarke *et al.*, 1984), and a reduced GnRH pulsatility (Wise, 1990). Thus, oestradiol positive feedback would be expected to be impaired at both hypothalamic and pituitary levels. However, the LH pulsatility detected in oestradiol-pretreated ewes in the current investigation was much greater than that reported for day 3 postpartum ewes, which had not been given oestradiol (Wise, 1990). Indeed, the pulsatility of LH in the present study was similar to that found in ewes in the late follicular phase (Domanski *et al.*, 1989), suggesting that oestradiol positive feedback was occurring on day 1, but that some mechanism was preventing the triggering of the LH surge. This must be a nonopioidergic mechanism because, in the current investi-

gation, naloxone could not overcome this inhibition of the LH surge on day 1. The failure of oestradiol to induce an LH surge in day 1 postpartum ewes could have been due to a lack of LH, particularly in the readily releasable pool, since total LH stores have been found to be depleted in ewes on days 1–3 postpartum (Wise *et al.*, 1986).

In the present study all of the ewes from day 7 postpartum onwards, in both the suckled and nonsuckled groups, displayed an LH surge in response to oestradiol. This percentage (100%) was much greater than that (50%) reported for Merino ewes (Wright *et al.*, 1980). This discrepancy was probably due to the lower dose (0.7 μ g kg⁻¹ compared with 1.42 μ g kg⁻¹) of oestradiol benzoate used in the previous study. Other studies have shown a negative correlation between the dose of oestradiol and the ease with which the ensuing LH response was disrupted (Yuthasastrakosol *et al.*, 1974).

The onset of the oestradiol-induced LH surge was delayed on day 7 compared with days 14 and 28 in both the suckling and nonsuckling ewes. A similar delay has been reported for dairy cows (Alam and Dobson, 1987).

Both the hypothalamic and pituitary actions of oestradiol are necessary to induce an LH surge (Kaynard *et al.*, 1988). However, in early *postpartum* ewes GnRH pulsatility is reduced (Wise, 1990), as is the number of hypothalamic oestradiol receptors (Wise *et al.*, 1988). In addition, pituitary responsiveness to GnRH is suppressed in the early (Crowder *et al.*, 1982) but not the late (Wright *et al.*, 1983) *postpartum* ewe. Furthermore, there are fewer oestradiol receptors in the pituitary in the early *postpartum* ewe (Clarke *et al.*, 1984). Oestradiol positive feedback would therefore be impaired at both the hypothalamic and pituitary levels, and this could lead to the delay of the oestradiol-induced LH surge in the day 7 *postpartum* ewe. This could also explain the shortened duration of the LH surge in the suckled ewe on day 7 *postpartum*.

The amplitude of the oestradiol-induced LH surge did not vary with time *postpartum* in the present study, confirming previous findings for ewes (Restall *et al.*, 1977).

There was no difference in either the timing or the amplitude of the oestradiol peak with day *postpartum* in nonsuckling ewes, a finding previously reported for endogenous oestradiol secretion in suckling ewes (Mandiki *et al.*, 1990). Altered oestradiol metabolism cannot therefore be responsible for the delayed oestradiol-induced LH surge on day 7.

Suckling *per se* had no effect on the onset or duration of the oestradiol-induced LH surge. However, suckling did inhibit the amplitude of the oestradiol-induced LH surge to a similar degree in both early and late stage *postpartum* ewes. This reduction could not be due to an altered rate of oestradiol metabolism, as this was not affected by suckling in sheep (Mandiki *et al.*, 1990). Another possible mechanism is the reduction of the pituitary responsiveness to GnRH. However, although suckling has been reported to inhibit GnRH-induced LH secretion in sheep (Newton *et al.*, 1988), this has been disputed (Moss *et al.*, 1980). There are no changes in numbers of GnRH receptors in suckled ewes (Peters and Lamming, 1990). In cows there is also no change in GnRH-induced LH secretion or number of GnRH receptors with suckling (Parfet *et al.*, 1986). Alternatively, suckling could inhibit LH secretion by suppressing GnRH pulsatility. Opioids modulate GnRH secretion (Haynes *et al.*, 1989) and have been implicated in the inhibition of LH secretion by suckling (Gregg *et al.*, 1983).

In conclusion, the onset of the oestradiol-induced LH surge was delayed in the early (day 7), but not in the late (day 14 or more), *postpartum* ewe. Furthermore, suckling reduced the amplitude of the oestradiol-induced LH surge in both early and late *postpartum* ewes. In addition, the lack of an LH surge response to oestradiol on day 1 *postpartum* is due to a non-opioidergic mechanism.

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