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Hypothalamo–pituitary portal blood concentrations of β-endorphin during suckling in the ewe*

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Summary. Matched hypothalamo–pituitary portal and jugular blood samples were collected over about 6 h from 7 lactating Corriedale ewes penned with their lambs, and a careful record was kept of ewe/lamb behaviour. Hypothalamo–pituitary portal blood concentrations of β-endorphin were measured by radioimmunoassay and the secretion rates were calculated; these were related to peripheral plasma prolactin and LH concentrations, and the sucking bouts of the lambs.

Basal LH concentrations remained <1 ng/ml with 0–2 pulses of 1·5–3·5 ng/ml amplitude per 6-h collection period. Prolactin secretion was episodic with individual baselines varying from 24 to 286 ng/ml, and peak concentrations of 50–631 ng/ml. Portal β-endorphin was secreted in an episodic pattern with individual baseline secretion rates varying from 0·125 to 0·495 ng/min, and peak secretion rates of 0·768 to 3·216 ng/min. A close correlation was seen between sucking bouts and the secretion of portal β-endorphin and peripheral prolactin; 86% of sucking bouts resulted in a significant release of β-endorphin, and 46% of sucking bouts resulted in a significant release of prolactin.

These results show that hypothalamic β-endorphin is released in response to the sucking stimulus. This provides support for the hypothesis that, during lactation, β-endorphin acts within the hypothalamus to reduce GnRH release and hence depress pituitary gonadotrophin secretion.

Introduction

Many mammals exhibit a period of suckling-induced inhibition of reproduction which ensures adequate spacing between successive births (Short, 1976, 1983). In sheep, lactation prolongs the duration of post-partum anoestrus in ewes lambing early in the breeding season, although seasonal anoestrus often obscures this lactational inhibition in ewes lambing late in the breeding season (Hunter, 1968; Mallampati *et al.*, 1971).

Lactational anoestrus is a result of the sucking activity of the lamb on the teat. Denervation of the mammary gland abolishes this inhibitory effect of lactation and also prevents the suckling-induced discharge of prolactin from the ewe's pituitary gland (Kann & Martinet, 1975), whilst still permitting sufficient milk secretion for normal lamb growth (Denamur & Martinet, 1960).

Some authors have concluded that lactational anoestrus is caused by the suckling-induced hyperprolactinaemia (Lamming *et al.*, 1974; Kann *et al.*, 1978). Suckling blunts the positive feedback effect of oestradiol on LH release in women (McNeilly, 1979) and ewes (Kann *et al.*, 1976;

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Wright *et al.*, 1980). It has also been suggested that hyperprolactinaemia may interfere with steroid production by the ovary (McNatty *et al.*, 1974; Dorrington & Gore-Langton, 1981; Demura *et al.*, 1982) and cause increased hypothalamo–pituitary sensitivity to the negative feedback effects of oestradiol (Wright *et al.*, 1981). The ultimate cause of the lactational infertility is undoubtedly a reduced hypothalamic secretion of GnRH; appropriate GnRH replacement therapy of lactating ewes can restore normal oestrous cycles (Wright *et al.*, 1984). The chief objection to the hypothesis that prolactin is the endocrine mediator of lactational infertility is that suppression of lactational hyperprolactinaemia with bromocriptine does not completely abolish the inhibitory effect of suckling in rhesus monkeys (Schallenberger *et al.*, 1981), or rats (Sirinathsinghji & Martini, 1984).

An attractive alternative hypothesis is that hypothalamic β-endorphin may be released during suckling and inhibit GnRH release (Short, 1984). Morphine or β-endorphin given intravenously or into the third ventricle decreases the secretion of LH (Reid *et al.*, 1981; Kubo *et al.*, 1983) and FSH (Ferin *et al.*, 1983) and increases prolactin secretion (Rivier *et al.*, 1977; Van Loon *et al.*, 1980a; Wehrenberg *et al.*, 1981). In women with hyperprolactinaemic amenorrhoea as a result of a pituitary adenoma, infusions of the opiate antagonist naloxone can restore the normal pulsatile pattern of LH secretion without affecting the hyperprolactinaemia (Quigley *et al.*, 1980). It has been suggested that brain opiates may cause the suckling-induced hyperprolactinaemia of rats (Ferland *et al.*, 1978), and it is established that suckling induces increased peripheral concentrations of β-endorphin (Riskind *et al.*, 1984). In the pig, naloxone injection reduces the hyperprolactinaemia characteristic of early lactation and blunts the acute prolactin response to suckling. This is associated with an increase in LH pulsatility (Mattioli *et al.*, 1986).

We have made use of the technique developed by Clarke & Cummins (1982) for the collection of hypothalamo–pituitary portal blood from conscious sheep to see whether the concentrations of β-endorphin in the portal blood of lactating ewes were related to suckling bouts.

Materials and Methods

Animals and surgery. Seven mature Corriedale ewes at the Animal Research Institute, Victorian Department of Agriculture, Werribee, Victoria, were used for this study. The ewes had all given birth to single or twin lambs after a normal gestation. At 2–7 weeks after parturition the ewes were anaesthetized and surgically prepared for subsequent hypothalamo–pituitary portal blood collection; this involved the insertion of two metal 12-gauge cannulae through the right nasal bone and cavity into the top and bottom of an artificial sinus created around the pituitary fossa (see Fig. 1) as described previously (Clarke & Cummins, 1985). After recovering from the anaesthetic, jugular cannulae were inserted and the ewes were penned with their lambs overnight. During subsequent experimental procedures ewes with twins were only allowed to suckle one lamb in order to simplify the observations.

Experimental procedure. On the day of experimentation the ewes were premedicated with an intravenous injection of 25 000 i.u. heparin and thereafter infused (30 drops/min) with a drip system containing 75 000 i.u. heparin/l saline (9 g NaCl/l) for the duration of the experiment. About 1 h after the injection of heparin, some of the portal vessels were cut by introducing a 16-gauge stilette through the top metal cannula to a previously determined distance and rotating its sharp point through a predetermined arc calculated at the time of surgery to cut the portal vessels. The stilette was then withdrawn and replaced with a polythene cannula (PE205; Clay Adams, Parsippany, NJ, U.S.A.) which was introduced through the lower 12-gauge needle so that the end just protruded into the artificial sinus created at the time of surgery. By applying continuous gentle suction portal blood could be continuously withdrawn from the sinus, and collected into siliconized glass tubes containing 500 µl preservative (5 mM-Bacitracin [Sigma, St Louis, MO, U.S.A.], 1·2% (w/v) glutathione and 0·04% (w/v) salicylic acid acetate) on ice. The tubes were changed after ~2 ml blood had been collected (about every 2–6 min), the time was carefully recorded, and a 6-ml sample of blood was aspirated by syringe from the jugular catheter and placed in a plastic tube containing 1 ml of the preservative. A small aliquant of all blood samples was taken into capillary tubes for the measurement of packed cell volume, so as to detect any possible contamination of the portal samples with cerebrospinal fluid, which in practice occurred in <1% of samples. The volumes of portal samples were recorded to allow blood flow measurements to be equated with plasma concentrations. The blood samples were then centrifuged at 2500 *g* at 4°C for 15 min. The plasma was separated, frozen, and stored at −20°C until assayed.

Careful records of the behaviour of the lambs and ewes were kept throughout the experiments and the number, time and duration of suckling bouts occurring during each sampling period was recorded.

Group 1 ewes were penned so that their lambs had free access to them throughout the sampling period. Group 2 ewes were similarly penned except that the lambs were temporarily excluded by a wiremesh gate. This was done in an attempt to establish a baseline β-endorphin release rate before the onset of a series of suckling bouts.

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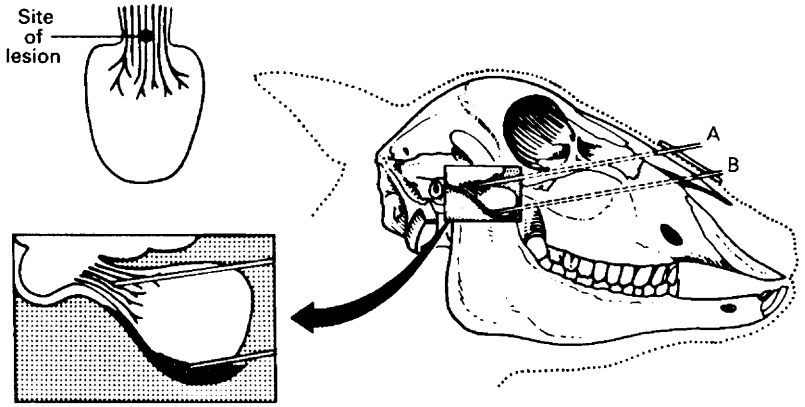

Fig. 1. Surgical approach for collection of pituitary portal blood in the conscious ewe. A sharp stilette of predetermined length is passed through the metal cannula A and rotated through a predetermined arc to cut some of the portal vessels on the anterior surface of the pituitary. Blood from these cut vessels then collects in the artificial sinus created around the pituitary at the time of operation and can be collected by gentle suction through a polythene tube passed through the metal cannula B.

Radioimmunoassays. β-Endorphin-like immunoassayable material (β-EP-LI) was measured in hypothalamo–pituitary portal plasma by a modification of the method of Lim *et al.* (1982), using their rabbit anti-ovine β-endorphin antiserum (R56 30/5/79) at an initial dilution of 1:4000. This gave a standard curve with a sensitivity of <50 pg/ml. Inter- and intra-assay coefficients of variation ($n = 10$) were respectively 11 and 8% at 124 pg/ml, 7 and 12% at 231 pg/ml and 10 and 12% at 277 pg/ml.

Briefly, the assay procedure was as follows. All dilutions were carried out with 0·01 M-sodium chloride, 0·5% (w/v) BSA, 0·02 M-EDTA, 0·1% sodium azide, hereafter referred to as assay buffer. Portal plasma, 100 µl diluted 1/10 or 1/20 with assay buffer or standard (camel β-endorphin: Sigma), was added to 10 × 75 mm glass tubes in duplicate. To this was added 100 µl antiserum (at 1/4000 dilution), and 400 µl assay buffer. Tubes were then vortexed and left to incubate at 4°C overnight, before the addition of 100 µl of iodinated human β-endorphin (Sigma) tracer (5000 c.p.m./100 µl) prepared by a mild chloramine-T iodination (Greenwood *et al.*, 1963). Tubes were then incubated for a further 2 days before 100 µl normal rabbit serum (NRS), obtained by bleeding normal rabbits (1/800 dilution), and 100 µl donkey anti-rabbit serum (ARS) (1/40 dilution) (RD17, Wellcome Foundation Ltd, Dartford, U.K.) were added; this was left for a further 8–16 h and then 1 ml 6% polyethylene-glycol (PEG) solution was added. The tubes were then centrifuged at 2500 *g* for 45 min (Beckman J6-B centrifuge). The supernatant was then aspirated, and the pellets counted for 10 min in a gamma counter (Wallac LKB 1260).

Peripheral concentrations of plasma prolactin were measured by the radioimmunoassay of Clarke *et al.* (1982) using NIH-P-S15 as standard. Assay sensitivity was $1{\cdot}4 \pm 0{\cdot}1$ ng/ml. Intra-assay CV was $<10\%$ over the range of 4·0–135·0 ng/ml and between-assay CV was 12·6% at 90 ng/ml, and 8·4% at 60 ng/ml.

Peripheral concentrations of plasma LH were measured by the radioimmunoassay of Lee *et al.* (1976) using NIH-LH-S18 as standard. Assay sensitivity was 0·3–0·5 ng/ml. Inter- and intra-assay coefficients of variation ($n = 7$) were respectively 8·4 and 22% at 1·2 ng/ml, 6·3% and 6·8% at 3·6 ng/ml and 5 and 6·1% at 4·6 ng/ml.

Definition of a pulse. A pulse was defined as having occurred when the assay value of the sample in question exceeded that of the previous sample by at least 25% ($\times 2$ greatest s.d. of assay). If two or more significant rises were seen in successive samples they were taken as being part of the same pulse and measured accordingly.

Amplitude of a pulse. The amplitude of a pulse was taken to be the difference between the value for the sample immediately preceding the first significant rise and the maximum value obtained during that pulse (as defined above).

Correlation of pulses with suckling bouts. A pulse was assumed to have been associated with a suckling bout if the initiation of the pulse occurred during the collection of the sample coincident with the suckle, or the next sample thereafter.

Verification of the origin of β-endorphin. Since β-endorphin is synthesized in the pituitary gland as well as the hypothalamus and since the collection of portal blood involved stabbing vessels on the anterior face of the pituitary gland, we were concerned that the portal blood samples may have contained some β-endorphin of pituitary origin. To control for this we measured the FSH and α-*N*-acetyl β-endorphin concentrations in pooled samples of portal and peripheral blood. Our rationale was that these pituitary-derived hormones would be substantially higher in portal blood samples than in jugular blood samples if significant retrograde flow was occurring from the pituitary gland into

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the portal blood; thus any substantial increase in portal/jugular ratios above unity would indicate pituitary contribution to the portal samples. On the other hand, if portal/jugular ratios for these hormones approached unity then we would be able to assume that the portal blood was being derived from an arterial source without passage through the pituitary gland, and that no retrograde flow was occurring. The choice of FSH as a 'monitor' hormone was made because peripheral plasma concentrations in sheep do not fluctuate dramatically (Baird *et al.*, 1981). FSH was assayed by the radioimmunoassay method of Bremner *et al.* (1980); assay sensitivity was 11 ng/ml and the intra-assay coefficient of variation was 5·7%. We also assayed α-*N*-acetyl β-endorphin because this hormone is not found in the sheep hypothalamus but is found in the pituitary gland (Cheng *et al.*, 1986; Smith *et al.*, 1986); it was measured by the radioimmunoassay of Cheng *et al.* (1985).

Data analysis. The plasma concentrations of β-endorphin in the portal samples were calculated after correcting for the dilution of the sample by enzyme inhibitor and possible contamination of the sample by CSF, using the formula of Clarke & Cummins (1985). Since portal blood flow rates change during the collection of hypothalamo–pituitary portal blood (Clarke & Cummins, 1985), measurements of β-endorphin expressed in terms of concentration alone probably do not accurately reflect the efflux of β-endorphin from the hypothalamus. We have therefore expressed the results as concentrations and secretion rates (ng/min). The secretion rates calculated are of course only a proportion of the total secretion rate as only some of the portal vessels are lesioned. Peripheral plasma concentrations of prolactin and LH were corrected for dilution by the enzyme inhibitor. Baseline levels of β-endorphin and prolactin were taken as the means of the 5 lowest concentrations of samples for each animal.

Autopsy. After blood collection each ewe was killed, and the top of the skull was removed and the brain reflected dorsally to allow inspection of the hypothalamo–pituitary area. The positioning of the lesioning needle(s) was then checked and the area of lesioned portal vessels was examined to confirm that the lesions were superficial.

Results

Peripheral LH concentrations during the 6-h sampling period

Basal LH concentrations were <1 ng/ml, with about 1 pulse (amplitude 1·5–3·5 ng/ml) per 6-h collection period (data not shown). This is characteristic of ewes in lactational anoestrus (Clarke *et al.*, 1984).

Peripheral prolactin concentrations

Prolactin concentrations showed episodic peaks, rising from a variable individual baseline of 24–286 ng/ml to peak values of 50–631 ng/ml. Mean concentrations ($\pm$s.e.m.) for individual animals varied from $33{\cdot}0 \pm 1{\cdot}6$ to $354 \pm 8{\cdot}5$ ng/ml. The number of prolactin pulses occurring during each collection period varied from 1 to 8. Mean pulse amplitudes varied from 15·3 to 167 ng/ml (see Table 1).

Portal blood flow

Portal blood flow rates fluctuated up to 10-fold during the collection periods (see Figs 2–4). However, this did not appear to influence the secretion rate of β-endorphin since pulses were usually present in the concentration and secretion rate profiles (Figs 2–4).

Secretion of β-endorphin

Portal β-endorphin secretion was characterized by an episodic pattern of release, with basal rates of 0·125–0·495 ng/min, and individual peak values of 0·768–3·216 ng/min. The number of β-endorphin pulses occurring during individual collection periods varied from 6 to 14. The mean amplitude of the β-endorphin pulses varied from 0·242 to 0·677 ng/min (Table 1).

Correlation of β-endorphin secretion and prolactin concentrations with suckling

For each individual sheep the profiles of β-endorphin and prolactin were plotted on the same time axes. Figure 2 shows characteristic profiles from 2 individual sheep in Group 1, in which lambs

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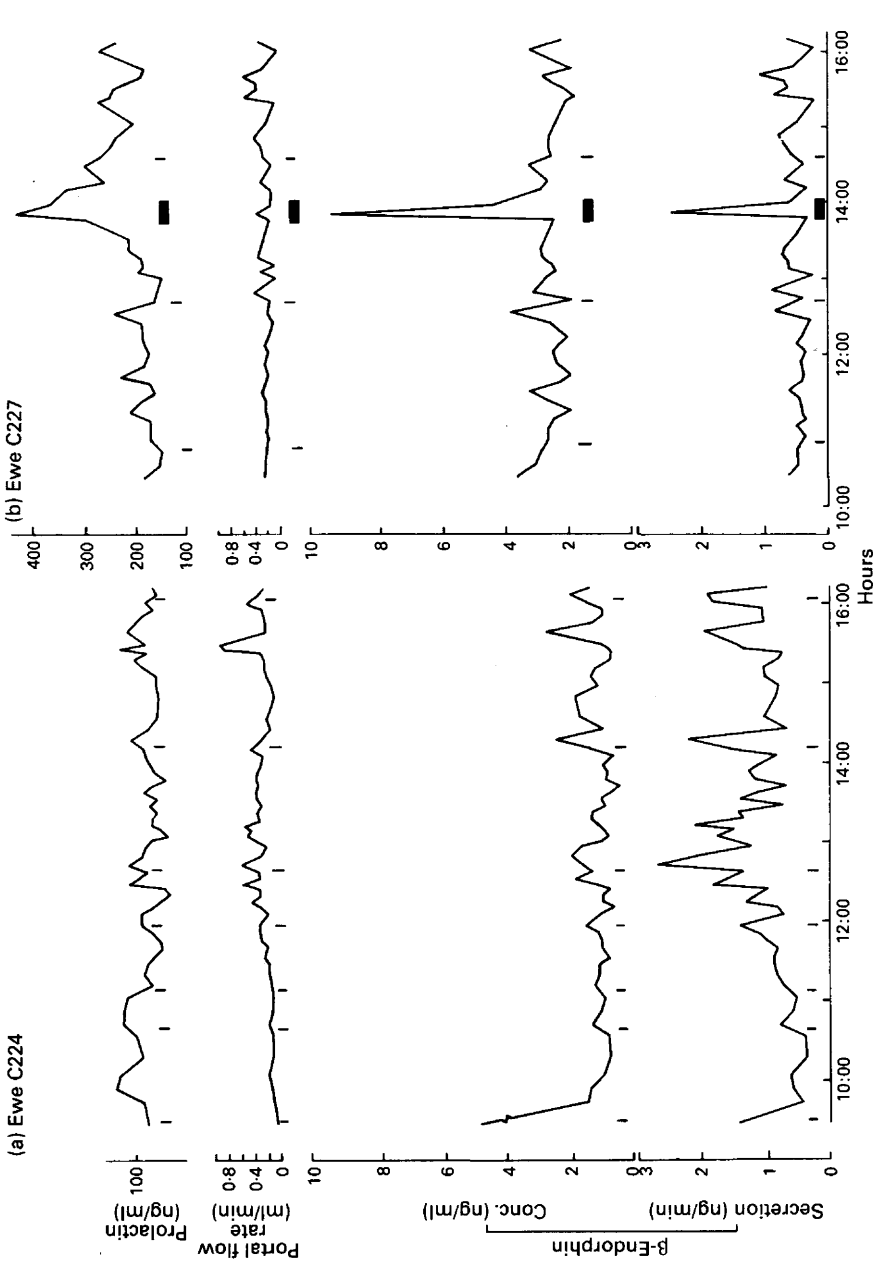

Fig. 2. Peripheral prolactin concentration, portal blood flow rate, β-endorphin concentration and β-endorphin secretion rate in Group 1 lactating ewes (a, No. C224; b, No. C227). The black bars (some of which appear as thin vertical bars) indicate time and duration of suckling bouts occurring during the collection period.

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Table 1. Basal values and number and amplitude of secretory episodes of jugular prolactin and β-endorphin and their association with the suckling stimulus

	Group 1			Group 2			
	Ewe C224	Ewe C225	Ewe C227	Ewe C249	Ewe C251	Ewe C252	Ewe C256
Duration of exp. (min)	425	130	363	409	369	358	271
Suckling bouts	7	3	4	7	7	3	1
Jugular prolactin							
Basal conc. (ng/ml)*	57	286	157	118	42	118	24
Highest peak (ng/ml)	132	447	440	281	221	631	50
Mean amplitude of pulses (ng/ml)	36·6	98	99	90	167	153	15
No. of pulses/exp.	8	1	5	5	3	5	3
Pulses associated with suckling bout	1	0	2	3	3	3	1
β-Endorphin							
Basal secretion (ng/min)*	0·495	0·171	0·218	0·175	0·281	0·313	0·125
Highest peak (ng/min)	2·646	2·646	2·455	1·620	1·812	3·216	0·768
Mean amplitude of pulses (ng/min)	0·677	0·600	0·526	0·416	0·535	0·659	0·242
No. of pulses/exp.	14	10	12	9	8	13	6
Pulses associated with suckling bout	5	2	4	6	3	3	1

*Defined as mean of 5 lowest values/animal.

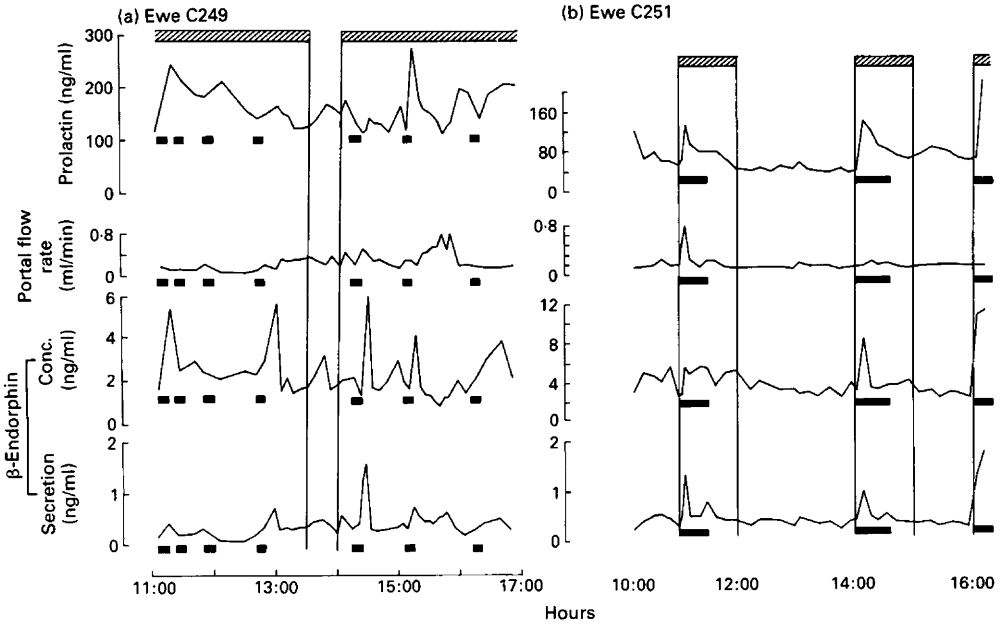

Fig. 3. Peripheral prolactin concentration, portal blood flow rate, β-endorphin concentration and β-endorphin secretion rate in Group 2 lactating ewes (a, No. C249; b, No. C251). The black bars indicate time and duration of suckling bouts occurring during the collection period; ▨, indicates the period during which the lamb had access to the ewe.

had unrestricted access to their dams. From a visual examination of the data the episodic natures of β-endorphin and prolactin are apparent and there was a close correlation between suckling bouts and peaks of β-endorphin and prolactin; however, there were also a number of significant peaks not apparently associated with the suckling bouts. Figure 3 shows the profiles of 2 representative ewes from Group 2 in which the lambs were given only restricted access to their dams. The episodic

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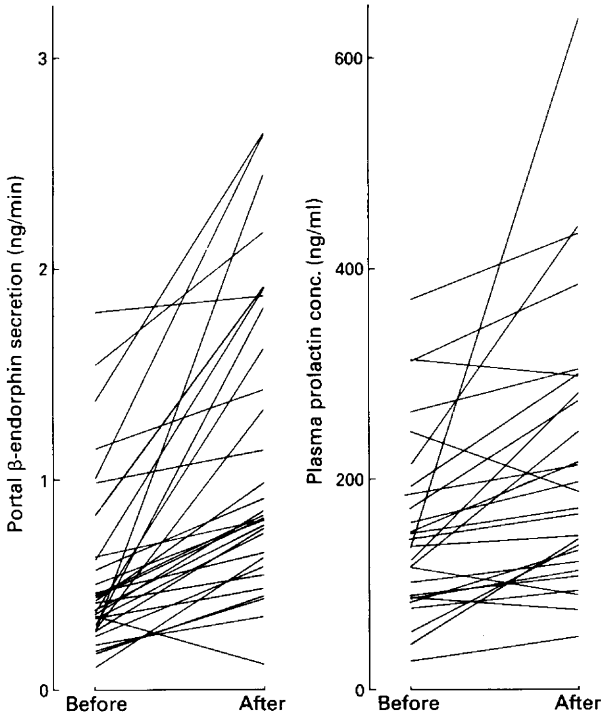

Fig. 4. A before and after the event plot of the peripheral prolactin concentrations and the portal β-endorphin secretion rates showing the general trend for both values to be elevated by suckling.

Table 2. Ratios of concentrations of *N*-acetyl-β-endorphin and FSH in pooled samples of jugular and portal blood

		N-Acetyl-β-endorphin conc. (pg/ml)			Plasma FSH conc. (ng/ml)		
Group	Animal no.	Portal (P)	Jugular (J)	Ratio P:J	Portal (P)	Jugular (J)	Ratio P:J
1	C224	1273	519	2·45	14·6	4·9	3·0
	C225	705	1155	0·61	5·9	4·6	1·28
	C227	961	1127	0·85	18·6	6·7	2·78
2	C249	1440	1282	1·12	12·8	4·9	2·61
	C251	982	839	1·17	10·9	6·7	1·63
	C252	522	745	0·70	6·2	4·9	1·27
	C256	950	1263	0·75	9·7	6·3	1·54

nature of both β-endorphin and prolactin is again apparent, although in this group there is less variability in the baseline values of both hormones, with the large peaks occurring when the lambs were given access to their dams and promptly initiated a vigorous suckling bout.

Taking each suckling bout as an individual event (pooling data from both groups), a before and after the event plot of prolactin and β-endorphin concentrations/secretion rates (Fig. 4) shows that 86% of suckling bouts resulted in a significant rise in β-endorphin secretion rates into the portal circulation and 46% of suckling bouts resulted in significant rises in peripheral prolactin concentrations.

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Verification of the origin of β-endorphin

Pooled samples of portal and jugular blood assayed for FSH and α-*N*-acetyl-β-endorphin had mean ± s.e.m. portal/jugular ratios of 2·02 ± 0·28 and 1·09 ± 0·24 respectively (Table 2).

Discussion

Many studies utilizing opiates or the opiate receptor blocking agent naloxone have suggested that endogenous opiate peptides inhibit GnRH production in the hypothalamus (Quigley & Yen, 1980; Reid *et al.*, 1981; Ellingboe *et al.*, 1982; Kubo *et al.*, 1983; Van Vugt *et al.*, 1983; Ferin *et al.*, 1984). Our study provides the first direct measurement of concentrations of β-endorphin in hypothalamo–pituitary portal blood on a minute-to-minute basis in conscious suckling animals. We have shown that during lactation the secretion of β-endorphin increases in response to the sucking stimulus of the lamb, thus providing strong support for the hypothesis that suckling-induced β-endorphin release in the hypothalamus is responsible for the inhibition of GnRH secretion in the lactating ewe.

There is some anatomical evidence that pituitary secretions might also be able to pass up the pituitary stalk to reach the hypothalamus (Bergland & Page, 1978). If this were the case we would have expected the portal/jugular ratios of FSH and α-*N*-acetyl-β-endorphin to have been much greater than unity. The mean portal/jugular ratios of α-*N*-acetyl-β-endorphin were indistinguishable from unity, and the mean FSH ratios of portal/jugular blood concentrations were only slightly higher than unity, suggesting that there can have been little contamination of portal blood with pituitary venous blood. We can therefore be confident that the portal β-endorphin we are measuring is of hypothalamic origin. Further support comes from HPLC analyses of portal samples collected by our method, which show a profile of endorphin-like peptides characteristic of the hypothalamic area rather than of the pituitary (K. Prince, R. Horton & I. J. Clarke, unpublished observations).

The variability in β-endorphin secretion rates seen between animals did not appear to be related to the age of the lamb, and probably reflects a regional variation in sampling sites. For example, the concentration of dopamine in blood from portal vessels is higher from median portal vessels than in lateral portal vessels (Reymond *et al.*, 1983). It is also important to remember that this technique samples only a proportion of the total portal blood flow depending on the number of vessels cut, which is likely to have varied between individuals.

The presence of high concentrations of β-endorphin in the hypothalamo–pituitary portal blood may reflect merely the 'leakage' of excess β-endorphin from the hypothalamus, or could indicate a possible pituitary site of action. Such a pituitary effect has been reported by Lien *et al.* (1976). However, Rivier *et al.* (1977) failed to find any in-vitro effect of β-endorphin or morphine on pituitary prolactin or growth hormone secretion. Also, there are no opiate receptors in the anterior pituitary of the sheep (Boublik & Clarke, 1984), so a pituitary effect is unlikely in this species.

An hypothalamic action of opiates on GnRH secretion is supported by the fact that morphine does not inhibit pituitary LH responsiveness to exogenous GnRH in stalk-sectioned rhesus monkeys, whereas it does inhibit LH secretion in intact animals (Ferin *et al.*, 1982). Naloxone perfusions increase GnRH release from rat (Wilkes & Yen, 1981) and fetal human (Rasmussen *et al.*, 1983) medial basal hypothalami in culture; a pulse of β-endorphin given midway through the naloxone infusion results in a sharp reduction in GnRH output (Wilkes & Yen, 1981; Rasmussen *et al.*, 1983). GnRH competitive antagonists block the stimulatory effects of naloxone *in vivo* on LH release in male rats (Cicero *et al.*, 1985).

The stimulatory effect of opiates on prolactin secretion is also thought to be hypothalamic in origin. For example, morphine and β-endorphin fail to stimulate prolactin release in pituitary-stalk sectioned monkeys (Wardlaw *et al.*, 1980) though they are active in intact animals (Gold *et al.*,

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1979; Wehrenberg *et al.*, 1981). In the rat, β-endorphin is thought to reduce dopamine release into the hypothalamo–pituitary portal circulation (Gudelsky & Porter, 1979; Van Loon *et al.*, 1980a, b; Wilkes & Yen, 1980).

Gonadal steroids are known to have an important feedback effect on hypothalamic β-endorphin production, with increased concentrations of β-endorphin in the portal blood of rhesus and cynomolgus monkeys during the luteal phase of the menstrual cycle (Wardlaw *et al.*, 1982; Wehrenberg *et al.*, 1982; Ferin *et al.*, 1984). The concentration of β-endorphin in monkey stalk blood falls after ovariectomy (Wehrenberg *et al.*, 1982). Oestradiol and progesterone replacement results in a return to pre-ovariectomy levels (Wardlaw *et al.*, 1982). Daily naloxone treatment produces larger LH responses during the luteal phase than in the follicular phase of the cycle, reflecting the level of endogenous opiate involvement at these times (Van Vugt *et al.*, 1983). In the rhesus monkey β-endorphin is thought to act during the luteal phase to reduce the frequency of LH pulses (Ferin *et al.*, 1984). We are now suggesting that a similar situation arises during lactation, when suckling-induced release of β-endorphin acts to reduce GnRH output and hence suppress FSH and LH concentrations.

Many increases in β-endorphin secretion coincided with peaks in peripheral prolactin concentrations, suggesting that the two events may be causally linked. This is supported by the results of Mattioli *et al.* (1986) who showed that prior administration of naloxone to pigs reduces the suckling-induced elevation of prolactin. Ferland *et al.* (1978), Miki *et al.* (1981) and Selmanoff & Gregerson (1986) also found a reduced prolactin response to suckling in rats after naloxone pretreatment, although Riskind *et al.* (1984) could not confirm this. No such inhibitory effect of naloxone on suckling-induced prolactin secretion has been found in women (Lodico *et al.*, 1983; Cholst *et al.*, 1984).

Table 3. Frequency and durations of suckling bouts occurring during sample collections

Animal no.	Frequency of suckling (bouts/h)	Mean duration of individual suckle (sec)	Age of lamb (weeks)
C224	1·04	86	6–7
C225	1·5	15	6–7
C227	0·7	251	6–7
C249	1·31	458	2–3
C251	1·38	1380	2–3
C252	1	698	2–3
C256	1	120	3–4

Our experimental technique is limited in that the method of sampling requires the ewe to be closely tethered. This makes it impossible for her to terminate a suckling bout, which is her normal practice (K. Gordon & M. Siegmann, unpublished observations). Such restriction probably resulted in the prolonged suckling bouts seen in this study (see Table 3) as compared with the natural condition in the field (K. Gordon & M. Siegmann, unpublished observations). When the lambs had restricted access there was less random fluctuation in β-endorphin and prolactin, with clear elevations occurring shortly after introduction of the lamb and initiation of the vigorous sucking that inevitably followed.

In conclusion, our studies strongly suggest that suckling causes an increased release of β-endorphin in the hypothalamus; this we suggest results both in the inhibition of GnRH release, and hence depressed pituitary gonadotrophin secretion, and depressed dopamine production, leading to elevated pituitary prolactin secretion. Prolactin should therefore perhaps be viewed as an index of the degree of hypothalamic inhibition provided by lactation, rather than as the primary cause of the ovulatory inhibition.

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