Continuous infusion of GnRH reduces the LH response to an intravenous GnRH injection but does not inhibit endogenous LH secretion in cows

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Summary. Plasma LH concentrations were monitored in 6 Hereford × Friesian suckled cows at about 80 days *post partum*, before and during a 14-day period of continuous s.c. infusion of GnRH (20 µg/h). Blood samples were collected at 10-min intervals on Days –2, –1, 1, 2, 3, 4, 7, 10, 13 and 14 (Day 1 = start of infusion). Plasma LH concentrations rose from mean pretreatment levels of 1.3 ± 0.20 ng/ml to a maximum of 17.1 ± 3.09 ng/ml within the first 8 h of GnRH infusion, but returned to pretreatment levels by Day 2 or 3. In 4/6 animals, the initial increase was of a magnitude characteristic of the preovulatory LH surge. In all animals, an i.v. injection of 10 µg GnRH, given before the start and again on the 14th day of continuous infusion, induced an increase in LH concentrations but the increase to the second injection was significantly ($P < 0.01$) less (mean max. conc. 6.4 ± 0.76 and 2.3 ± 0.19 ng/ml). Mean LH concentrations (1.0 ± 0.08 , 1.1 ± 0.08 and 0.9 ± 0.06 ng/ml) and LH episode frequencies (3.3, 4.3 and 3.2 episodes/6 h) did not differ significantly on Days –2, 7 and 13. However, the mean amplitude of LH episodes was significantly lower ($P < 0.05$) on Day 13 (1.3 ± 0.10 ng/ml) than on Day –2 (1.8 ± 0.16 ng/ml). Therefore, although the elevation in plasma LH concentrations that occurs in response to continuous administration of GnRH is short-lived and LH levels return to pre-infusion values within 48 h of the start of infusion, these results show that the pituitary is still capable of responding to exogenous GnRH, although the LH response to an i.v. bolus injection of GnRH is reduced. In addition, this change in pituitary sensitivity is not fully reflected in endogenous patterns of episodic LH secretion.

Keywords: GnRH infusion; pituitary sensitivity; LH; cows

Introduction

Administration of exogenous GnRH can be used to induce ovulation in a number of acyclic mammals. Repeated injection of GnRH results in ovulation in prepubertal monkeys (Wildt *et al.*, 1980) and heifers (McLeod *et al.*, 1985), post-partum acyclic cows (Riley *et al.*, 1981) and ewes (Wright *et al.*, 1983b), seasonally anoestrous ewes (McLeod *et al.*, 1982) and hypogonadotrophic women (Crowley & McArthur, 1980; Leyendecker *et al.*, 1980). Each injection of GnRH induces episodic release of LH and the sustained elevation in mean plasma gonadotrophin concentrations that results from continued treatment promotes follicular development that eventually culminates in ovulation. In contrast, when GnRH is administered continuously, the elevation in plasma LH concentrations is short-lived, and although of sufficient duration to promote preovulatory follicle development in seasonally anoestrous ewes (McLeod *et al.*, 1983; Wright *et al.*, 1983a), it does not

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consistently induce ovulation in the post-partum cow (Jagger *et al.*, 1987). Knobil (1980) reported that continuous administration of GnRH in monkeys ultimately resulted in desensitization of the pituitary, which was then unable to respond to further stimulation. However, recent work with sheep in this laboratory suggests that, during continuous low-level GnRH infusion, episodic LH secretion continues even after the initial stimulatory response to infusion of exogenous GnRH has ceased (M. Khalid, B. J., McLeod & W. Haresign, unpublished data). Therefore, in this study pituitary function in terms of LH secretion was assessed in animals exposed to continuous administration of GnRH. Endogenous patterns of LH secretion, and the response to an i.v. bolus injection of GnRH, were monitored in cows both before and during an extended period of continuous infusion of GnRH. A high rate of infusion (20 µg GnRH/h) was chosen, in comparison to the 2.5 µg/h dose shown to be effective by Jagger *et al.* (1987), to maximize the possibility of pituitary desensitization.

Materials and Methods

Animals and management. Six multiparous, suckled Hereford × Friesian cows (mean liveweight 502.1 ± 11.0 kg, 84.0 ± 3.5 days *post partum*), penned as a single group, were housed indoors under conditions of natural daylight and temperature. During periods of intensive blood sampling, which were carried out during daylight hours, the cows were restrained individually.

Treatment and blood sampling. All animals received GnRH (Lutal: Fabwerke Hoechst A.G., Frankfurt, West Germany) as a continuous s.c. infusion, delivered by osmotic minipump (Alzet; Model 2ML2, Alza Corp., Palo Alto, CA, U.S.A.) at the rate of 20 µg/h in 5 µl sterile saline (0.9% NaCl w/v) for 14 days. The minipumps, which had been preincubated in saline at 37°C for 10 h, were inserted subcutaneously in the axilla region, under local anaesthesia of lignocaine hydrochloride (Lignavet: C-Vet Ltd, Bury St. Edmunds, Suffolk). To confirm their effectiveness, minipump performance was tested after the in-vivo treatment period, by incubation *in vitro* (saline at 37°C) for 15 h immediately following pump removal. A sample of the GnRH stock solution was also incubated at 37°C at this time, and this, along with the infusates from in-vitro incubations, was assayed for GnRH content by the method of Jeffcoate *et al.* (1974). Bolus i.v. injections of GnRH (10 µg in 2 ml saline) were given via an indwelling jugular vein catheter, 1 day before the start and again on the 14th day of GnRH infusion.

Blood samples (2 ml) for the determination of plasma LH concentrations were taken via the indwelling catheter at 10-min intervals. Samples were collected for an 8-h period on Days -2, 7 and 13 to assess episodic patterns of LH secretion, for a 10-h period on Days -1, 1 and 14 to monitor the LH response to i.v. bolus injections of GnRH and the initial response to GnRH infusion, and for a 2-h period on Days 2, 3, 4 and 10 for estimation of mean LH concentrations. On each of these sampling days, an additional blood sample (10 ml) was taken for progesterone determination.

LH assay. Plasma LH concentrations were measured by the specific double-antibody method of Webb *et al.* (1977). Within this study the limit of sensitivity was 0.4 ng NIH-LH-B9 equiv./ml plasma and the inter- and intra-assay coefficients of variation were 12.1% ($n = 3$) and 6.7% ($n = 50$) respectively.

Progesterone assay. Plasma progesterone concentrations were determined by the radioimmunoassay method previously described by Webb *et al.* (1977). The limit of sensitivity was 0.2 ng/ml, the intra-assay coefficient of variation was 7.1% ($n = 50$) and the mean extraction efficiency was 72.3%.

Analysis of data. An LH episode was defined as (i) an increase in plasma LH concentrations of at least 4 times the coefficient of variation between duplicate pairs, in which (ii) the peak concentration occurred within 2 sample points of the preceding baseline, (iii) there were at least 3 sample points from the peak to the succeeding trough and (iv) the rate of decay was within the limits of the known half-life of LH.

Differences in plasma LH concentrations, and in patterns of endogenous LH secretion, were assessed by one-way analysis of variance in which the sources of variation included period (day of sampling), animals within period, and animal by period interactions. Differences in LH episode frequency were compared by χ^2 analysis, and in the LH responses to i.v. GnRH injections by paired *t* test.

All values are expressed as mean ± s.e.m.

Results

LH concentrations

Profiles of plasma LH concentrations throughout the period of intensive blood sampling, are shown for 2 animals in Fig. 1. The endogenous patterns of LH release and the response to i.v.

Fig. 1. Plasma LH concentrations in post-partum beef cows (Cows 4 and 1) before and in response to i.v. injections of 10 µg GnRH, given the day before and on the 14th day of a period of continuous infusion of GnRH (20 µg/h). The times of the GnRH injections and of the start of GnRH infusion are shown. Blood samples for LH determination were taken at 10-min intervals.

injections of GnRH observed in these animals are representative of all cows, and the response to continuous s.c. infusion shown by Cow 4 is typical of 5/6 cows. In the 6th animal (Cow 1, Fig. 1) the initial response was low, but LH concentrations continued to increase throughout the period of blood-sampling on the day of pump insertion. Plasma LH concentrations on the first 4 days of continuous infusion of GnRH are shown in Fig. 2. Within 1 h of insertion of the osmotic mini-pumps, LH concentrations rose from mean pretreatment levels of 1.3 ± 0.20 ng/ml to reach eventually a mean maximum concentration of 17.1 ± 3.09 ng/ml. In 5 cows, the maximum LH concentration was attained by a mean time of 4.1 ± 0.5 h (range 3.0–5.8 h) after the start of infusion, whereas in the remaining animal (Cow 1, Fig. 1) LH concentrations continued to increase throughout the period of blood sampling on the day of mini-pump insertion. Mean LH concentrations returned to pretreatment levels by Day 2 (Cows 2, 3 & 6) or Day 3 (Cows 1, 4 & 5), and thereafter remained low for the duration of continuous administration of GnRH. In all cows there was a significant increase in plasma LH concentrations in response to each of the i.v. injections of GnRH. However, the magnitude of this response was significantly ($P < 0.01$) less to the second GnRH injection than to the first (see Fig. 3 and Table 1). A summary of these LH responses to GnRH is presented in Table 1.

Mean LH concentrations 2 days before GnRH infusion began (1.1 ± 0.08 ng/ml) did not differ significantly from those after 7 (1.1 ± 0.08 ng/ml) or 13 (0.9 ± 0.06 ng/ml) days of continuous infusion of GnRH (Fig. 4). In addition, there were no significant differences in the frequency of LH

Fig. 2. Mean ($\pm$ s.e.m.) plasma LH concentrations in post-partum beef cows before and in response to continuous infusion of GnRH (20 μ g/h) delivered s.c. by osmotic minipump. The time of minipump insertion is shown by the arrow. Blood samples were taken at 10-min intervals.

Fig. 3. Mean ($\pm$ s.e.m.) plasma LH concentrations in post-partum beef cows before and in response to i.v. injections of 10 μ g GnRH given the day before ($\bullet$ — $\bullet$) and on the 14th day ($\circ$ — $\circ$) of a period of continuous infusion of 20 μ g GnRH/h.

episodes recorded on these days (3.3, 4.3 and 3.2 episodes/6 h on Days -2, 7 and 13 respectively). However, the mean amplitude of the LH episodes monitored decreased over the period of GnRH infusion (1.8 ± 0.16 , 1.5 ± 0.07 and 1.3 ± 0.10 ng/ml on Days -2, 7 and 13) and was significantly ($P < 0.05$) lower on Day 13 than on Day -2 (Fig. 4).

Progesterone concentrations

In Cows 2, 3 and 6, plasma progesterone concentrations were low (mean $\pm$ s.e.m. 0.4 ± 0.1 ng/ml) before the administration of GnRH. Values remained low throughout the treatment period in Cow 2,

Table 1. Liveweight, progesterone (P₄) status* at the time of treatment and characteristics of LH secretion in post-partum beef cows in response to continuous s.c. infusion (20 µg/h) and i.v. injections (10 µg/injection) of GnRH

Cow	Continuous GnRH infusion					First GnRH injection					Second GnRH injection			
	Liveweight (kg)	P ₄ status	Pretreatment LH conc. (ng/ml)	Maximum LH conc. (ng/ml)	Time to max. LH conc. (h)	P ₄ status	Pretreatment LH conc. (ng/ml)	Maximum LH conc. (ng/ml)	Area of LH response†	P ₄ status	Pretreatment LH conc. (ng/ml)	Maximum LH conc. (ng/ml)	Area of LH response†	
1	485	+	1.0	5.9	8.7	+	0.9	4.6	217	+	0.8	2.5	107	
2	544	—	1.7	23.7	5.8	—	1.3	9.3	609	—	1.0	2.1	63	
3	486	—	2.1	18.9	3.3	—	0.6	4.5	471	+	0.8	1.5	57	
4	528	+	0.9	23.4	3.8	+	0.9	5.6	193	—	0.9	2.5	66	
5	481	+	1.0	21.0	3.0	+	1.0	7.5	246	+	0.8	2.8	68	
6	488	—	1.2	9.4	4.7	—	1.4	7.0	354	+	1.2	2.2	34	
Mean														
± s.e.m.	502 ± 11.0		1.3 ± 0.20	17.1 ± 3.09	4.9 ± 0.86		1.0 ± 0.12	6.4 ± 0.76	384 ± 67.3		0.9 ± 0.07	2.3 ± 0.19	66 ± 9.1	

* + Indicates luteal-phase concentrations; - indicates follicular-phase concentrations.
†Arbitrary units.

Fig. 4. LH profiles of individual post-partum beef cows 2 days before the start and on the 7th and 13th days of a 14-day period of continuous infusion of 20 μ g GnRH/h. Increases in plasma LH concentrations that are deemed to be episodes are indicated ($\blacktriangledown$). The progesterone status of the animals at the time of sampling are indicated as high (P+, luteal-phase concentrations) or low (P-, follicular-phase concentrations). Blood samples were taken at 10-min intervals.

but in Cows 3 and 6 they rose by the 4th day of GnRH infusion (indicative of luteal activity in response to GnRH treatment) and were maintained at luteal-phase concentrations (5–10 ng/ml) thereafter. In contrast, in the remaining 3 animals progesterone concentrations were elevated at the start of GnRH treatment, and either remained high throughout the treatment period (Cow 1) or returned to basal levels by the 10th (Cow 4) or 14th (Cow 5) day of infusion, indicative of luteolysis.

Discussion

When post-partum cows were infused s.c. with GnRH at the rate of 20 μ g/h for 14 days, plasma LH concentrations rose immediately after the start of treatment, but returned to pretreatment levels within 48 h, with a pattern of secretion similar to that reported earlier in response to continuous administration of GnRH (Jagger *et al.*, 1987). Previously, refractoriness of the pituitary to prolonged GnRH infusion has been attributed to 'down regulation', a term used to describe the loss of

receptors in a target organ following excessive exposure to the stimulatory hormone (Belchetz *et al.*, 1978; Knobil, 1980). Indeed, it has been demonstrated, both *in vivo* and *in vitro*, that continuous infusion of GnRH reduces pituitary receptor numbers in sheep (Nett *et al.*, 1981; Loumaye & Catt, 1982; Khalid *et al.*, 1987). Chakraborty *et al.* (1974) suggested as an alternative explanation that depletion of pituitary LH reserves may account for the cessation in LH release. However, i.v. injection of a large bolus of GnRH will evoke further LH release, at least early in the refractory period (Schuiling *et al.*, 1976; Nett *et al.*, 1981). A third possible explanation is that the short-lived elevation in plasma LH concentrations after the start of GnRH infusion, may be due to transient 'up regulation' of GnRH receptor populations resulting from an increase in plasma oestradiol concentrations. For example, rat pituitary gonadotrophs can be sensitized to GnRH by pretreatment with oestrogen, and desensitized by androgen pretreatment (Labrie *et al.*, 1979). Furthermore, in the cow, plasma oestrogen concentrations increase markedly within 30 min of GnRH injection (Peters *et al.*, 1985).

It is unlikely in this experiment that the transient nature of the elevation in LH concentrations was due to failure of the osmotic minipumps; the precision and reliability characteristics of these devices have been well-established (Theeuwes & Yum, 1976). In addition, although the *in-vitro* incubation of the minipumps was carried out after their specified effective life-time (14 days), the rate of GnRH delivery, as determined by RIA of the infusate, was still comparable to the nominal delivery rate, at least for those from 2 animals (20.5 µg/h, Cow 1, and 16.2 µg, Cow 4; see Fig. 1). The pumps from the remaining 4 animals showed a lower level of release, but indicated that the devices had been delivering GnRH over the specified period. The GnRH content of the stock solution was unaffected by incubation (5.07 and 5.03 µg/ml for incubated and non-incubated solutions respectively).

The pattern with which LH concentrations increased at the start of continuous administration of GnRH is similar to that seen at the onset of the preovulatory LH surge in the naturally-cycling animal (Walters & Schallenger, 1984), and is comparable to that observed in response to high-frequency (10-min intervals) GnRH pulses in oestrogen-treated ovariectomized ewes (Clarke & Cummins, 1985). Clarke & Cummins (1985) also refer to an increased flow rate in the pituitary portal blood vessels that is concomitant with the increased frequency of GnRH pulses. The pattern of GnRH that induces preovulatory surge gonadotrophin release in the naturally cyclic animal may therefore be effectively the same as that which occurs with continuous low-level GnRH administration (present study).

Within this study differences between animals in the magnitude of the initial LH response to GnRH infusion may reflect differences in steroid hormone concentrations or in endogenous GnRH release. In Cow 1, in which the pattern of LH release that followed insertion of the osmotic minipump differed from that of the other 5 animals (see Table 1), the progesterone profile suggests that an endogenous preovulatory LH surge may have occurred 48–72 h earlier. After a naturally-occurring or an induced preovulatory surge, the pituitary is insensitive to further stimulation for 72–96 h (Foster & Crighton, 1976; Crighton & Foster, 1977).

The magnitude of the LH response to the first bolus i.v. injection of GnRH varied widely between cows (maximum LH concentration range, 4.48–9.28 ng/ml). In addition, similar variation occurred in the LH response to the second i.v. GnRH injection given after 14 days of continuous infusion (range 1.45–2.80 ng/ml). The magnitude of the response to the first and second bolus GnRH injections was not related to progesterone status or to the responses to previous GnRH treatment. However, the ability of this dose of GnRH to release LH was significantly reduced (maximum LH concentration was 64.6% less and the area of the LH response was 84.8% less) by the period of GnRH infusion. This reduction in sensitivity to GnRH injections has also been demonstrated in sheep pituitary cells subjected to continuous administration of GnRH *in vitro* (McIntosh & McIntosh, 1985). The fact that the pituitary did respond at all after 14 days of continuous infusion could be explained simply by differences in the amount of GnRH to which the pituitary gonadotrophs were exposed. The infusion dose of 20 µg/h represents only 0.33 µg

delivered subcutaneously in 1 min, compared to the 10 µg bolus injection which was delivered intravenously in 3–5 sec. In heifers the pituitary response to subcutaneous administration of GnRH is only 50–60% that which follows intravenous administration (J. P. Jagger, B. J. McLeod, A. R. Peters & G. E. Lamming, unpublished data).

When the initial, short-lived stimulatory phase of the response to continuous infusion of GnRH ended, mean plasma LH concentrations returned to pretreatment levels. Indeed, the pattern of LH secretion evident throughout the remainder of the period of continuous infusion was similar to that recorded over the pretreatment period (see Figs 1 & 3). Mean LH concentrations (including LH episodes) and mean LH episode frequency after 7 and 13 days of GnRH infusion were not significantly different from those recorded before treatment (Table 2). However, the mean amplitude of episodes recorded on Day 13 was significantly lower ($P < 0.05$) than that recorded on Day -2. Nevertheless, LH episode amplitude decreased by only 27% over the GnRH infusion period, compared with a reduction of 65% in the amplitude of LH release in response to the bolus injections of exogenous GnRH.

Changing progesterone status did not appear to be responsible for changes in response to exogenous GnRH. For example, over the stimulatory phase of the GnRH infusion period plasma progesterone concentrations were high (6.7 ± 1.8 ng/ml) in 3 cows and low (0.4 ± 0.1 ng/ml) in the remaining 3 animals. In addition, progesterone levels were high in 3/6 cows at the time of the first bolus injection of GnRH, and in 4/6 cows at the time of the second. None of the LH responses to GnRH was related to plasma progesterone concentrations. Furthermore, it is unlikely that changes in the amplitude of endogenous LH episodes were due to changes in progesterone levels. Rahe *et al.* (1980) reported that LH episode amplitude is increased during the luteal phase of the oestrous cycle, and progesterone concentrations were elevated in 3/5, 5/6 and 4/6 of our cows on Days -2, 7 and 13, respectively.

This experiment, along with earlier work in the sheep (McLeod *et al.*, 1983; Wright *et al.*, 1983b) and the cow (Jagger *et al.*, 1987), demonstrates that continuous infusion of low doses of GnRH can stimulate gonadotrophin release without incurring long-term pituitary down-regulation. However, this conclusion is in direct conflict with that derived from experiments with primates, in which continuous administration of GnRH induced a state of total quiescence in the pituitary and in gonadotrophin secretion (Belchetz *et al.*, 1978; Knobil *et al.*, 1980; Crowley & McArthur, 1980; Leyendecker & Wildt, 1983). Our results are more in accord with those of Levine *et al.* (1985) who emphasized the importance of synergism between oestrogens and GnRH in inducing a maximum pituitary response in terms of LH secretion. This is especially relevant in the cow, since we have previously shown that a rise in plasma oestradiol concentrations occurs within 30 min of administration of low levels of GnRH (Peters *et al.*, 1985). These widely different views on the effects of continuous administration of GnRH could be related to species. For example, data drawn from the arcuate-lesioned monkey may bear no relation to the gonadotrophin response to GnRH in the intact sheep and cow. Similarly, the ovariectomized, oestrogen-treated sheep model used by Clarke & Cummins (1985), or the ovariectomized monkey model of Levine *et al.* (1985), does not truly duplicate the intact animal because oestradiol replacement therapy does not mimic the varying ovarian steroid environment that is characteristic of the intact animal. It is also possible that these different responses could be due to differences in the natural hormone patterns which control reproductive cycles in domestic animals and primates. The relatively short follicular phase of the oestrous cycle in the sheep and the cow, compared with that of the menstrual cycle in primates, indicates that only a short period (48–72 h) of GnRH stimulation is required for the development of preovulatory follicles and the induction of ovulation.

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