Plasma LH and FSH responses and ovarian activity in prepubertal heifers treated with repeated injections of low doses of GnRH for 72 h

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Summary. Twelve 5-month-old Hereford × Friesian heifers were injected i.v. with 2.0 µg GnRH at 2-h intervals for 72 h. Blood samples were collected at 15-min intervals from 24 h before the start until 8 h after the end of the GnRH treatment period. Over the 24-h pretreatment period, mean LH concentrations ranged from 0.4 to 2.2 ng/ml and FSH concentrations from 14.1 to 157.4 ng/ml; LH episodes (2–6 episodes/24 h) were evident in all animals. Each injection of GnRH resulted in a distinct episode-like response in LH, but not FSH. Mean LH, but not FSH, concentrations were significantly increased by GnRH treatment. The GnRH-induced LH episodes were of greater magnitude than naturally-occurring episodes (mean maximum concentration 6.7 ± 0.5 and 4.9 ± 0.6 ng/ml respectively). Preovulatory LH surges occurred between 17.0 and 58.8 h after the start of treatment in 9/12 heifers, with a coincident FSH surge in 8 of these animals. This was not followed by normal luteal function. There were no apparent correlations between pretreatment hormone concentrations, and either the pituitary response to GnRH or the occurrence of preovulatory gonadotrophin release.

Introduction

In the naturally-cyclic ewe and cow, the final stages of maturation of preovulatory follicles are associated with a period of high-frequency episodic LH release (Baird, 1978; Rahe, Owens, Fleege, Newton & Harms, 1980). In contrast, LH episodes occur infrequently during periods of ovarian inactivity, for example, seasonal or post-partum anoestrus (Yuthasastrakosol, Palmer & Howland, 1977; Lamming, Wathes & Peters, 1981). Similarly, LH episodes occur infrequently during the prepubertal period in both species, and puberty occurs only after the frequency characteristic of the follicular phase of the oestrous cycle is attained (Ryan & Foster, 1980; Schams, Schallenberger, Gombe & Karg, 1981; Day *et al.*, 1984; McLeod, Haresign, Peters & Lamming, 1984). In contrast, FSH concentrations in the prepubertal heifer, with the exception of the absence of preovulatory surges, do not differ markedly from those recorded during the oestrous cycle of the adult animal (Akbar, Reichert, Dunn, Kaltenbach & Niswender, 1974).

Repeated administration of LH or GnRH to increase LH episode frequency will restore normal ovarian cycles in the post-partum acyclic cow (Riley, Peters & Lamming, 1981; Walters *et al.*, 1982) and the seasonally anoestrous ewe (McLeod, Haresign & Lamming, 1982a, b; McNeilly, O'Connell & Baird, 1982). This treatment stimulates follicle growth and oestradiol production, and eventually results in preovulatory gonadotrophin surges and ovulation. Injections of low doses of GnRH have also been reported to induce episode-like release of LH in prepubertal heifers as young as 4 months of age (McLeod *et al.*, 1984), but in that trial the duration of GnRH treatment was much less than that necessary to induce ovulation in mature cows (Riley *et al.*, 1981). The current experiment was therefore undertaken to determine whether an extended period of treatment with low doses of

GnRH would eventually result in preovulatory surge secretion of gonadotrophins and ovarian activity in young prepubertal heifers.

Materials and Methods

Animals and management

Twelve Hereford × Friesian heifers, aged approximately 5 months (mean ± s.e.m. bodyweight 171 ± 5.3 kg), were housed indoors under conditions of natural daylength and temperature and fed concentrates and hay twice daily. They were run as a single group except during the period of intensive blood sampling, when all animals were restrained individually.

Treatment and blood sampling

After the insertion of a jugular vein catheter 48 h earlier, all animals were given i.v. injections of 2.0 µg GnRH (Lutal: Fabwerke Hoechst A.G., Frankfurt, West Germany) in 2 ml sterile saline (0.9% NaCl, w/v) at 2-h intervals for 72 h. Blood samples (2 ml) for gonadotrophin determination were collected via the jugular vein catheter at 15-min intervals from 24 h before the start, until 8 h after the end of GnRH treatment (a total period of 104 h). Throughout the treatment period the blood samples were taken immediately before administration of GnRH. Blood samples (10 ml) for progesterone determination were collected by jugular venepuncture every 2nd day from 2 days before the start until 24 days after the end of GnRH treatment.

Hormone analyses

LH. Plasma LH concentrations were measured by the specific double-antibody radioimmunoassay method of Webb, Lamming, Haynes, Hafs & Manns (1977). The limit of sensitivity of the assay within this study was 0.4 ng NIH-LH-B9 equiv./ml plasma, and the inter- and intra-assay coefficients of variation were 11.9% ($n = 12$) and 6.3% ($n = 50$) respectively.

FSH. Blood samples were assayed for FSH by the specific heterologous radioimmunoassay of Webb, Lamming, Haynes & Foxcroft (1980). Within this study the limit of sensitivity of the assay was 9.7 ng NIH-FSH-B1 equiv./ml plasma, and the inter- and intra-assay coefficients of variation were 12.8% ($n = 12$) and 8.2% ($n = 50$) respectively.

Progesterone. Plasma progesterone concentrations were determined by the specific radioimmunoassay method previously described (Webb *et al.*, 1977). The limit of sensitivity within this study was 0.2 ng/ml, the inter- and intra-assay coefficients of variation were 12.3% ($n = 8$) and 6.4% ($n = 50$) respectively, and the mean extraction efficiency was 74.8%.

Analysis of data

An LH episode was defined by the criteria previously described by McLeod *et al.* (1982a), i.e. (i) an increase of at least 50% above the preceding baseline value, (ii) at least 2 points between the peak value and the succeeding trough or baseline, and (iii) a rate of decline in concentrations after the peak no greater than that allowed by the known half-life of the hormone. The definition used to characterize a preovulatory surge was (i) that there was a sustained (> 5 h) elevation in LH concentrations above 4.0 ng/ml and (ii) that the peak concentration attained during this time exceeded 10 ng/ml. The onset of this peak was defined as that time when the concentration first reached 4.0 ng/ml.

Normal luteal function was defined as a sustained elevation in plasma progesterone concentrations of > 1.0 ng/ml for at least 10 days, starting within 5 days of the preovulatory LH surge.

Mean LH and FSH concentrations, and mean LH episode frequencies, were determined for individual animals over the pretreatment and treatment periods. Differences between pretreatment and treatment values, and between responsive and non-responsive animals, were assessed by one-way analysis of variance, Students *t* test or χ^2 analysis. In addition, changes in the LH response to GnRH injections were analysed by linear regression and FSH concentrations were subjected to power spectral analysis, a time series technique designed to identify recurring periodicities in the data (McLeod & Craigon, 1985).

All results are expressed as mean $\pm$ s.e.m.

Results

Gonadotrophin concentrations

Individual profiles of plasma LH and FSH concentrations for 3 heifers are shown in Text-fig. 1. These animals are selected to demonstrate both the patterns of gonadotrophin secretion typical of these prepubertal heifers over the pretreatment period and the range of responses observed to GnRH treatment.

During the 24-h pretreatment period, mean plasma LH concentrations in individual heifers ranged from 0.4 to 2.5 ng/ml (Table 1). LH episodes were evident in all animals and occurred with a frequency of 2–6 episodes/24 h. Mean basal LH concentrations (excluding episodic secretion) ranged from 0.4 to 2.3 ng/ml plasma.

In 10/12 heifers, each GnRH injection induced an episode-like release of LH, at least until the onset of a preovulatory LH surge (see Text-fig. 1). In the remaining 2 animals (Heifers 1 and 3), LH episodes occurred in response to most, but not all, injections of GnRH. The mean maximum concentration of GnRH-induced LH episodes (6.7 ± 0.5 ng/ml) was significantly greater ($P < 0.01$) than that of naturally-occurring episodes (4.9 ± 0.6 ng/ml) recorded during the pretreatment period. Furthermore, overall mean LH concentrations over the period extending from the start of GnRH treatment until the onset of the preovulatory LH surge, were significantly greater ($P < 0.001$) than mean pretreatment concentrations (3.0 ± 0.3 ng/ml and 1.4 ± 0.2 ng/ml respectively).

Preovulatory LH surges were recorded in 9/12 heifers and occurred at a mean time of 41.2 ± 4.3

Table 1. Liveweights and characteristics of plasma LH and FSH concentrations over the 24-h pretreatment period in 5-month-old prepubertal heifers

Heifer	Liveweight (kg)	Mean LH conc. (ng/ml)	Basal* LH conc. (ng/ml)	No. of LH episodes/24 h	Mean FSH conc. (ng/ml)
1	158	2.2	1.9	4	29.8
2	162	1.4	1.0	5	38.4
3	176	2.5	2.3	4	63.0
4	148	1.6	1.3	6	18.3
5	156	0.4	0.4	2	62.5
6	174	0.8	0.5	5	19.7
7	188	0.7	0.7	2	28.0
8	174	0.9	0.4	4	134.6
9	200	0.9	0.6	5	49.5
10	164	2.0	1.2	6	14.1
11	156	1.3	0.7	4	157.4
12	206	2.2	1.8	3	153.7
Mean	171 ± 5.3	1.4 ± 0.2	1.1 ± 0.2	4.2 ± 0.4	64.1 ± 15.5

* Excluding endogenous LH episodes.

Text-fig. 1. Plasma LH and FSH concentrations in 3 prepubertal heifers before, and in response to i.v. injections of 2.0 µg GnRH given at 2-h intervals for 72 h. These profiles show (a) Heifer 4 in which the preovulatory LH surge occurred late in the treatment period and was not associated with an FSH surge, (b) Heifer 12 in which there was a coincident surge of LH and FSH early in the treatment period and (c) Heifer 8 in which there was no preovulatory LH/FSH surge. The times of GnRH injections are indicated by the arrows. Blood samples were collected at 15-min intervals.

h (range 17.0 to 58.8 h) after the start of GnRH treatment. Although the time of onset varied widely between animals, the heifers could be classified into 4 groups on the basis of the timing of the preovulatory LH surge (see Table 2), namely, those in which the preovulatory surge occurred early (17.0 h, Heifer 12), intermediate (36.4 ± 1.7 h, Heifers 2, 5, 7 & 9) or late (52.1 ± 3.0 h, Heifers 1, 4, 6 & 10) in the GnRH treatment period, and those in which a surge was not recorded (Heifers 3, 8 & 11). Maximum LH surge concentrations attained ranged from 12.4 to 193.7 ng/ml (see Table 2), and the duration of the preovulatory surge from 5.3 to 10.5 h.

Pretreatment mean FSH concentrations ranged from 14.1 to 157.4 ng/ml plasma in individual animals with a mean value of 64.1 ± 15.5 ng/ml (Table 1). Although FSH levels remained

Table 2. Mean plasma concentrations of LH and FSH, and characteristics of preovulatory gonadotrophin surges in 5-month-old heifers treated with i.v. injections of 2.0 µg GnRH at 2-h intervals for 72 h

Heifer	Mean plasma concentrations*		Preovulatory gonadotrophin surges		
	LH (ng/ml)	FSH (ng/ml)	Time of onset† (h)	Maximum LH conc. (ng/ml)	Maximum FSH conc. (ng/ml)
12	5.7	144.2	17.0	193.7	439.9
7	2.9	31.3	32.3	27.0	61.8
2	3.3	36.9	36.0	48.1	97.8
5	1.4	56.0	36.5	18.0	135.2
9	2.4	29.3	40.8	39.6	115.6
4	3.3	15.9	47.0	12.4	58.6
10	3.4	11.4	47.0	58.8	68.4
1	3.3	36.0	55.7	39.7	70.2
6	3.0	20.5	58.8	27.4	—
3	2.7	37.5	—	—	—
8	1.9	97.4	—	—	—
11	2.2	73.7	—	—	—
Mean	3.0 ± 0.3	49.2 ± 11.2	41.2 ± 4.3	51.5 ± 18.4	130.9 ± 45.2

* Mean concentrations are based on samples collected from the start of GnRH treatment until the onset of the preovulatory surge in those animals exhibiting surges, and throughout the treatment period in those not producing a surge.

† Time from the start of GnRH treatment (see text).

relatively constant in some heifers throughout the 24-h pretreatment period, in others they fell or rose markedly before the start of GnRH treatment (see Text-fig. 1). The mean FSH concentrations (excluding preovulatory surges) of individual animals throughout the period of GnRH treatment ranged from 11.4 to 144.2 ng/ml, giving an overall mean value of 49.2 ± 11.2 ng/ml (see Table 2). These were not significantly different from pretreatment levels. No consistent changes in plasma FSH concentrations were apparent in response to GnRH administration, and few GnRH-induced episodes could be identified by visual appraisal of hormone profiles (see Text-fig. 1). Time series analysis detected a number of recurring periodicities in FSH secretion of apparently random frequency over the pretreatment period. However, during the period of 2 h GnRH injections, a 2-h cycle in FSH secretion was interposed in 8/12 animals. In contrast, such a pattern was apparent in only one heifer over the pretreatment period.

Preovulatory FSH surges (mean maximum concentration of 130.9 ± 45.2 ng/ml) occurred in 8/9 heifers in which preovulatory LH surges were recorded. A further elevation in FSH concentrations was observed 24 h later in those animals in which the sampling period was long enough (see Text-fig. 1b).

There were no significant differences in mean liveweight, pretreatment gonadotrophin concentrations, or in the immediate response to repeated injections of GnRH, between those animals that subsequently produced preovulatory gonadotrophin surges and those that did not.

Progesterone concentrations

Plasma progesterone concentrations were low (mean $\pm$ s.e.m., 0.3 ± 0.1 ng/ml) in all heifers before GnRH treatment. In 3 animals (Heifers 2, 6 & 9) progesterone concentrations rose above 1.0 ng/ml 4 or 5 days after the preovulatory gonadotrophin surge but returned to basal levels 1 or 2 days later, indicative of transient luteal activity. In all other animals progesterone concentrations remained low (0.3 ± 0.1 ng/ml) throughout the sampling period.

Discussion

A characteristic of tonic LH secretion in the prepubertal heifer is the low frequency with which LH episodes occur. The pattern of LH secretion, recorded over the 24 h preceding the GnRH treatment period, was similar to that reported previously for heifers of this age (McLeod *et al.*, 1984). The LH episode frequency (mean rate 4.2 ± 0.4 episodes/24 h) was similar to that reported earlier for the acyclic post-partum cow (Riley *et al.*, 1981), but the mean baseline concentrations of LH (excluding episodes) were markedly higher. Similarly, FSH concentrations (mean 64.1 ± 15.5 ng/ml, range 14.1 to 157.4 ng/ml) in these heifers over the pretreatment period, were in agreement with earlier reports for the prepubertal animal (Gonzalez-Padilla, Wiltbank & Niswender, 1975a; Schams *et al.*, 1981; McLeod *et al.*, 1984).

In this study, a frequency of episodic LH secretion comparable to that occurring during the follicular phase of the oestrous cycle was imposed on 5-month-old heifers by repeated injections of 2 µg GnRH. Previous studies (McLeod *et al.*, 1984) had indicated that this was the minimum dose level required to ensure a consistent LH response to all GnRH injections in heifers of similar ages and weights. Furthermore, work in the seasonally anoestrous ewe has shown that an 8-fold difference in the dose of GnRH has little effect on the size of the induced LH episodes, or on the efficacy of repeated injections of GnRH to induce ovulation (McLeod *et al.*, 1982b). In some animals in this study, the maximum LH concentration recorded decreased with successive injections of GnRH (see Text-fig. 1c). Indeed, when all animals were included in the analysis, there was a significant negative correlation ($P < 0.01$, $r = -0.51$) between maximum LH concentration in response to each GnRH injection, and time from the start of treatment. While this could be interpreted as evidence of pituitary refractoriness, in some animals there were alternating periods of increasing and decreasing sensitivity to GnRH injections (see Text-fig. 1a), which is likely to be the result of steroid modulation of pituitary responsiveness rather than a progressive desensitization. Such fluctuations in pituitary sensitivity to GnRH have also been reported to occur throughout the treatment period when seasonally anoestrous ewes are subjected to continuous infusion of low doses of GnRH (McLeod *et al.*, 1983).

In contrast to the consistent LH episode response to GnRH injections, GnRH-induced FSH episodes were not obvious by simple inspection of hormone profiles. However, time series analysis, which is capable of detecting regularly recurring cycles in secretion even when baseline levels are fluctuating markedly, detected a 2-h cycle in FSH secretion in 8/12 heifers over the GnRH treatment period. Nevertheless, the presence of an FSH response to GnRH injections did not appear to determine whether a preovulatory gonadotrophin surge would occur. The 4 heifers in which time series analysis failed to detect a 2-h pattern of FSH secretion subsequently produced preovulatory LH/FSH surges, while, conversely, a 2-h cycle in FSH concentrations was detected in the 3 animals in which preovulatory surges were not observed.

Staigmiller, Short & Bellows (1979) and Schillo, Dierschke & Hauser (1983) reported that the positive feedback mechanism in heifers becomes functional between 3 and 5 months of age. The occurrence of preovulatory gonadotrophin surges in only a proportion (9/12) of 5-month-old heifers treated with GnRH may therefore reflect differences between individual animals in the maturity of this component of hypothalamo-pituitary activity. These workers also reported that when LH release was induced in 5-month-old heifers by a standard dose of oestradiol, the magnitude of the LH surge varied greatly, indicative of considerable between-animal variation in the sensitivity of the hypothalamo-pituitary axis. Similarly, in the current study the magnitude of the preovulatory LH surges varied widely (maximum concentration 12.4–193.7 ng/ml). There was however, no apparent relationship between the amplitude of the episodic response to GnRH and the timing, magnitude or incidence of preovulatory surges. Such variation, therefore, may merely reflect differences in the ovarian response, and thus in plasma oestradiol concentrations, between animals. Indeed, it is not clear from these results whether the preovulatory LH/FSH surges were produced as a result of the direct effects of GnRH injections, or of the positive feedback effects of

oestradiol from follicles induced to grow by the GnRH-induced increase in LH episode frequency. However, data from seasonally anoestrous ewes subjected to a similar treatment regimen clearly indicate that the preovulatory surge was due to the positive feedback effects of high levels of oestradiol (McLeod & Haresign, 1984). Further work is therefore required to establish the basis of variation in responses observed.

The failure of some animals to produce a preovulatory surge may have been attributable to 'stress' effects incurred by the intensive handling and blood loss (about 20% of total volume over 5 days). It has been demonstrated that hyperadrenal states interfere with GnRH-induced preovulatory LH release (Li & Wagner, 1983).

In all 7 animals in which the blood sampling period continued for long enough, a 2nd rise of FSH was recorded about 24 h after the preovulatory LH/FSH surge, producing a pattern of release similar to that seen in the naturally-cyclic adult animal (Dobson, 1978). This 2nd FSH rise may be due to the escape of the hypothalamo-pituitary axis from the feedback effects of follicular secretions at the time of ovulation.

The absence of normal luteal function in this trial does not necessarily preclude the occurrence of ovulation in these animals, as a proportion of GnRH-induced ovulations in similarly-treated seasonally anoestrous ewes produce abnormal corpora lutea, unless the animals are pretreated with progesterone (McLeod *et al.*, 1982b). In addition, Gonzalez-Padilla, Wiltbank & Niswender (1975b) demonstrated that progesterone pretreatment was required to produce normal corpora lutea in heifers in which ovulation was induced by injection of oestradiol. However, it has yet to be determined whether the GnRH treatment used in the present study induces ovulation in the prepubertal heifer, and, if so, whether progesterone pretreatment influences the functional competence of the resultant corpora lutea.

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