LH secretion and response to GnRH during seasonal anoestrus of the Père David's deer hind (*Elaphurus davidianus*)

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Summary. The pattern of LH secretion and response to exogenous GnRH was determined on 5 occasions during seasonal anoestrus of the Père David's deer hind. LH pulse frequency was low (3.3 ± 0.6 pulses/18 h) in early anoestrus (February), increased significantly in mid-anoestrus (April; 8.4 ± 1.4 pulses/18 h) and thereafter declined slightly in late anoestrus (June; 6.3 ± 0.25 pulses/18 h). Mean LH concentrations also showed significant changes during anoestrus with higher levels in mid-anoestrus (April; 0.85 ± 0.12 ng/ml) when compared with other times (0.53 ± 0.05 , 0.60 ± 0.10 , 0.68 ± 0.06 and 0.71 ± 0.05 ng/ml for February, March, May and June, respectively). LH pulse amplitude showed no significant changes during the study. The LH response to intravenous injections of $2 \mu\text{g}$ GnRH was lowest in early anoestrus (February), increased significantly in mid-anoestrus (April) and remained high through late anoestrus. The response during the luteal phase was similar to that seen during late anoestrus. These results indicate that seasonal anoestrus in the Père David's deer hind is not a uniform state but is characterized by an early period of 'deep' anoestrus.

Keywords: LH; deer; GnRH; seasonal anoestrus; breeding season

Introduction

Luteinizing hormone (LH) is secreted by the pituitary in discrete pulses (Katongole *et al.*, 1971) which are a direct consequence of episodic release of gonadotrophin-releasing hormone (GnRH) from the hypothalamus (Clarke & Cummins, 1982). During seasonal anoestrus in the ewe, LH pulse frequency is reduced (Scaramuzzi & Baird, 1977; Yuthasastrakosol *et al.*, 1977), presumably due to a reduction in GnRH pulse frequency. A change in pituitary responsiveness to GnRH during anoestrus has not been reported in sheep. In the absence of adequate gonadotrophic stimulation, ovarian follicles do not undergo the final stages of development which are required for ovulation to occur (McNatty *et al.*, 1984). Replacement with either GnRH or LH during seasonal anoestrus in the ewe results in preovulatory follicle development and ovulation (McNatty *et al.*, 1981; McLeod *et al.*, 1982; McNeilly *et al.*, 1982).

LH pulse frequency is low and does not change during seasonal anoestrus in the ewe. Further, sensitivity to oestradiol negative feedback on LH secretion undergoes relatively abrupt changes, decreasing and increasing acutely at the onset and termination of the breeding season respectively (Legan *et al.*, 1977). Taken together, these data suggest that seasonal anoestrus in the ewe is a

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uniform physiological state in which there is high sensitivity to oestradiol negative feedback resulting in low LH pulse frequency (Karsch *et al.*, 1985). Therefore it would be predicted that in the period leading to the onset of the breeding season there would be an increase in LH pulse frequency which would account for increased follicle development and the subsequent events which lead to ovulation. This has proved difficult to demonstrate (Walton *et al.*, 1980; Poulton *et al.*, 1987) except when ewes have been sampled very close to the onset of the breeding season (I'Anson & Legan, 1988).

Genetic selection and the associated domestication of sheep has resulted in breeds with various degrees of seasonality. For example non-pregnant Welsh mountain, Suffolk and Dorset horn ewes show repeated oestrous cycles for periods of 182, 191 and 265 days per year (Legan & Karsch, 1979; Webster & Haresign, 1983). In contrast wild ungulates such as the red and Père David's deer hinds show a more restricted breeding season with reproductive cycles in non-pregnant animals occurring for 160 days per year (Curlewis *et al.*, 1988a; Loudon *et al.*, 1989). The present study was undertaken to determine whether the model for LH secretion during anoestrus in the ewe can also be applied to a more seasonal species such as the Père David's deer (*Elaphurus davidianus*). We wished to determine (a) whether there is a change in the pattern of LH secretion from early to late anoestrus and (b) whether there is a change in the response to administered GnRH over the same period. The response to GnRH was compared with that recorded during the luteal phase of a reproductive cycle.

Materials and Methods

Animals. Five tame adult Père David's deer hinds (live weight 173 ± 5.2 kg, s.e.m.) were used in the study. Between experiments they were maintained on pasture at Whipsnade Park, Bedfordshire (latitude $50^{\circ}30'N$). At least 2 days before each period of intensive sampling was to begin, hinds were moved indoors and housed individually in pens (4×3 m) and on deep litter and under natural photoperiod and environmental temperature. Dim red artificial light was used to assist collection of blood samples during periods of darkness.

Collection of blood samples and experimental design. The day before each of 5 periods of intensive blood sampling, hinds were sedated with xylazine (40 mg, s.c.; Bayer Pharmaceuticals, Leverkusen, West Germany) and a jugular cannula was inserted. The sedative effects of xylazine lasted 2–4 h with hinds apparently normal on the following day. On subsequent days it was possible to collect blood from the jugular cannula without need for physical restraint of the deer.

Profiles of plasma LH concentrations were determined on 5 occasions at 4- or 5-week intervals during seasonal anoestrus (11 February, 17 March, 23 April, 27 May and 24 June). On each of these occasions, blood samples (4 ml) were collected at 15-min intervals for 18 h. One hind from which the cannula was lost was omitted from blood sampling on 24 June.

On the day after each of the LH profile bleeds, the animals were subjected to a GnRH challenge. Each hind was given 4 intravenous injections (via the jugular vein cannula) of 2.0 µg GnRH (Lutal; Fabwerke Hoechst A.G., Frankfurt, West Germany) in 2.0 ml sterile saline (0.9% NaCl), administered at 2-h intervals. Preliminary studies in anoestrous hinds showed that this was the lowest dose of GnRH which consistently increased plasma LH in all hinds. Blood samples were taken at 15-min intervals for 7.5 h. The first GnRH injection was given immediately after the second blood sample. One hind was omitted from the GnRH challenges on 26 May (B74) and 24 June (Y12) due to lost cannulae.

The response to repeated injections of 2 µg GnRH was also determined on Day 8 of the luteal phase after oestrus had been synchronized with progesterone-impregnated silicone-elastomer implants (Sil-Estrus, Ceva Ltd, Sydney, Australia) and injection of prostaglandin (Estrumate; Coopers Animal Health, Crewe, Cheshire, UK) as previously described (Loudon *et al.*, 1990).

In all periods of intensive blood sampling, blood was collected into heparinized tubes, centrifuged immediately and the plasma stored at $-20^{\circ}C$ until assayed.

Duration of seasonal anoestrus. The duration of seasonal anoestrus was determined by measuring progesterone concentration in plasma samples which were collected twice weekly by jugular venepuncture between 1 December 1986 and 18 August 1987. The onset of seasonal anoestrus was defined as the date when plasma progesterone concentrations fell below 0.3 ng/ml following the last luteal phase of the breeding season. The onset of the breeding season was defined as the date of the first increase in progesterone concentrations above 1 ng/ml for more than two consecutive samples.

Radioimmunoassays. Plasma LH concentrations were measured using an ovine LH radioimmunoassay (McLeod *et al.*, 1982) which has been validated for deer LH (Loudon *et al.*, 1990). The limit of sensitivity of the assay was 0.2 ng/ml and the intra- and inter-assay coefficients of variation were 8.6 and 9.8% respectively. Progesterone concentrations were measured as previously described (Curlewis *et al.*, 1988a). The limit of sensitivity of the assay was 0.08 ng/ml and the intra- and inter-assay coefficients of variation were 3.8 and 10.5% respectively.

Analysis of data. An LH episode was defined by the criteria of Loudon *et al.* (1990); i.e. (i) an increase in plasma LH concentrations of at least 4 times the intra-assay coefficient of variation, (ii) no more than one sample point between the preceding trough and peak value, and (iii) there was at least 1 sample point between the peak and succeeding trough. LH pulse frequency (pulses/18 h) and mean LH were determined. Pulse amplitude was calculated by subtracting the concentration at the preceding trough from the peak value.

The response to GnRH was calculated as the difference in plasma LH concentrations between the sample taken immediately before injection and the peak response occurring within 30 min of injection. In 81% of cases, the maximum LH concentration was recorded in the sample taken 15 min after injection of GnRH.

Statistical analyses were performed using within subject, repeated measure analysis of variance. When significant treatment effects were identified, group means were compared by least significant difference. Data from the GnRH challenge were not normally distributed and were therefore log transformed before analysis. Analyses of pulsatile LH characteristics during anoestrus were on non-transformed data.

Results

Duration of seasonal anoestrus

Mean date for the onset of anoestrus was 2 January (± 4.7 days; s.e.m.). Plasma progesterone concentrations then remained low (< 0.3 ng/ml) in all animals until the period between 21 and 31 July when transient increases in progesterone concentration to 0.3–1.0 ng/ml occurred in all hinds. The breeding season began on 4 August in all hinds.

Plasma LH profiles during anoestrus

Mean LH concentrations, LH pulse frequency and pulse amplitude recorded in the 18-h sampling periods throughout anoestrus are summarized in Fig. 1. There was a significant ($P < 0.01$, ANOVA) change in pulse frequency over the period of seasonal anoestrus. In early anoestrus mean LH pulse frequency was low (3.3 ± 0.6 and 3.7 ± 0.62 pulses/18 h in February and March respectively), increased significantly ($P < 0.01$) in mid-anoestrus (April, 8.4 ± 1.40 pulses/18 h), and remained high in late anoestrus (7.0 ± 1.41 and 6.3 ± 0.25 pulses/18 h in May and June respectively).

Mean LH concentrations were significantly greater ($P < 0.05$; see Fig. 1) in mid-anoestrus (0.85 ± 0.12 ng/ml, April) than in early (0.53 ± 0.05 and 0.60 ± 0.10 ng/ml in February and March respectively) and late anoestrus (0.68 ± 0.06 and 0.71 ± 0.05 ng/ml in May and June respectively).

Although changes in LH pulse amplitude followed a trend similar to those of pulse frequency and mean LH (i.e. highest in mid-anoestrus), these were not significant ($P > 0.1$).

Response to GnRH during anoestrus and the breeding season

Mean LH concentrations during the GnRH challenges and mean LH response to GnRH during anoestrus and the luteal phase of the oestrous cycle are summarized in Figs 2 and 3 respectively. The mean LH response changed significantly ($P < 0.01$) during the anoestrous period. Response was lowest in early anoestrus (February, 1.2 ± 0.12 ng/ml), increased significantly ($P < 0.01$) in mid-anoestrus (April, 2.3 ± 0.60 ng/ml) and remained increased until late anoestrus (June, 2.3 ± 0.49 ng/ml). The response to GnRH during the luteal phase (3.0 ± 0.8 ng/ml) was similar to that seen during mid- to late anoestrus but was significantly ($P < 0.01$) greater than that recorded in early anoestrus (February and March). When all animals and all stages of the season were combined, there was also a significant effect ($P < 0.01$) of the order of injection of GnRH. The

Fig. 1. Summary of LH pulse characteristics during seasonal anoestrus of the Père David's deer hind ($N = 5$). ** $P < 0.01$, * $P < 0.05$ compared with February values.

response to the third injection was significantly greater ($P < 0.01$) than the response to the first and fourth injections. There was no interaction between time of year and response to repeated injection ($P > 0.1$).

Discussion

This study of endogenous and GnRH-induced patterns of LH secretion in the Père David's deer hind demonstrates that changes in LH release occur over the period of seasonal anoestrus. In untreated hinds, mean LH concentrations and LH pulse frequencies were significantly higher in mid-anoestrus than in early anoestrus. In addition, the magnitude of the LH response to administration of GnRH was significantly greater in mid- and late anoestrus, than in early anoestrus. Such variation in gonadotrophin secretion during the period of acyclicity has not previously been reported for a seasonally breeding species. The lower LH pulse frequency and LH response to GnRH in early anoestrus suggests that suppression of the reproductive axis is greatest at this time, supporting the novel concept that these animals are more 'deeply' anoestrus immediately after the end of the breeding season.

It is well established that, in the ewe, mean LH concentrations are higher during the breeding season than during seasonal anoestrus. In addition, studies involving ovariectomized, oestradiol-treated ewes have shown that mean LH concentrations fall abruptly at the onset of seasonal anoestrus and remain at a low level throughout the anoestrous period (Legan & Karsch, 1979; Webster & Haresign, 1983). There is little information available regarding episodic patterns of LH secretion at different times during the anoestrous period in sheep. Studies of ovariectomized,

Fig. 2. Mean $\pm$ s.e.m. plasma LH concentrations in Père David's deer hinds treated every 2 h with 2.0 μ g GnRH (arrow) on 5 occasions during seasonal anoestrus and during the luteal phase.

oestrogen-implanted ewes indicated no changes in the frequency of LH episodes until as little as 10–20 days (Walton *et al.*, 1980) before the first ovulation of the breeding season, while in the ovary-intact ewe an increase in LH pulse frequency was only seen 7–28 days (I'Anson & Legan, 1988) before the first ovulation of the breeding season. The results from the present study do not preclude the occurrence of a further increase in LH pulse frequency in the hind immediately before

Fig. 3. LH response to injection of 2 µg GnRH on 5 occasions during seasonal anoestrus and at Day 8 of the luteal phase of the Père David's deer hind ($N = 5$). On each occasion 4 injections were given at 2-h intervals.

the onset of the breeding season, as the last profile of LH secretion undertaken (24 June) was almost 6 weeks before the first full-length luteal phase of the breeding season.

In the ewe there is an inverse relationship between LH pulse amplitude and frequency which is evident both during anoestrus and the breeding season (Clarke & Cummins, 1985). This relationship was not found in the present study of the hind. On the contrary pulse amplitude showed an increase, albeit non-significant, when pulse frequency increased. Similarly, it has been reported that the large-amplitude pulses that occur every 8–12 h during the luteal phase in the ewe were not evident in the hind (Loudon *et al.*, 1990).

The pulse frequency recorded in mid-anoestrus (April) approached that seen during the follicular phase of the oestrous cycle (0.59 pulses/h or 10.6 pulses/18 h; Loudon *et al.*, 1990). Presumably in mid-anoestrus, this pulse frequency is not sufficient to promote fully preovulatory follicle development. Alternatively, FSH concentrations may have been inadequate. A further possibility is that plasma prolactin concentrations may be important in the control of follicle development or ovulation in this species. Although prolactin does not appear to be important in controlling seasonal reproduction in the ewe (Niswender, 1974; Land *et al.*, 1980; Rodway *et al.*, 1983), in the red deer hind, suppression of prolactin with bromocriptine is associated with a delay in the onset of seasonal anoestrus (Curlewis *et al.*, 1988b) and prolactin concentrations are increased during anoestrus in the Père David's deer hind (Loudon *et al.*, 1989). Further studies are required to determine whether the seasonal changes in prolactin are causally linked with seasonal reproduction in this species.

We are not aware of published information on follicular dynamics or oestradiol secretion during seasonal anoestrus in the hind. It is therefore not possible to relate the patterns of LH secretion reported here to functional changes in the ovary. However, in experiments in which pulsed or continuous administration of GnRH was used to induce ovulation during anoestrus, clear differences were observed between animals treated in early (February and March) and late (May and June) anoestrus (McLeod *et al.*, 1991). Significantly fewer hinds ovulated in response to this treatment in early than late anoestrus (23% compared with 75%, respectively). It is possible

that the poorer response to GnRH treatment in early anoestrus reflects differences in follicle populations or responsiveness to GnRH at the time of treatment.

There have been few studies of gonadotrophin secretion in response to exogenous GnRH given at different stages of seasonal anoestrus in the female. In the ovariectomized ewe, the LH response to GnRH is diminished during anoestrus (Land *et al.*, 1979; Bittman *et al.*, 1985) while in the intact ewe the response to a supra-physiological dose of GnRH does not change across anoestrus (Reeves *et al.*, 1974). In the present study, the LH response to GnRH in the hind was poor in early anoestrus and showed a significant increase from early to late anoestrus. Indeed the response in late anoestrus (June) was not significantly different from that seen in the luteal phase of the oestrous cycle. Furthermore we have again shown in a separate study that there is a significant increase in pituitary responsiveness to GnRH between early and late anoestrus (McLeod *et al.*, 1991). Although an increase in pituitary responsiveness from early to late anoestrus may be due to the priming effect of an increasing frequency of endogenous GnRH pulses, this cannot account for the response recorded in the luteal phase. At this time LH pulse frequency is similar to that in early anoestrus and yet the pituitary response to GnRH is significantly greater. Bittman *et al.* (1985) have shown that a physiological dose of GnRH releases LH in the anoestrous ewe, concluding that low LH pulse frequency during anoestrus is unlikely to be due to the failure of the pituitary to respond to endogenous GnRH. However, in view of the large change in pituitary responsiveness to GnRH during anoestrus in the hind, it remains possible that a failure of the pituitary to respond to GnRH may contribute to the low LH pulse frequency characteristic of early anoestrus.

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