Effect of pregnancy and exogenous interferon on synchronous pulsatile release of oxytocin and luteolytic prostaglandin $F_{2\alpha}$ in red deer (*Cervus elaphus*)

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Three experiments were carried out to investigate the secretion of luteolytic hormones in red deer hinds during the oestrous cycle, early pregnancy and after administration of interferon, the putative pregnancy recognition signal. Three groups of hinds ($n = 8-9$ per group) were treated with progesterone-impregnated intravaginal controlled internal drug releasing (CIDR) devices for 13 days (device withdrawal = day 0). Group 1 ($n = 9$) served as controls; Group 2 ($n = 8$) received injections of 4 mg recombinant bovine interferon- α_1 twice a day on days 13–18; Group 3 ($n = 9$) were run with a fertile stag on days 0–3. Plasma samples collected each day on days 16–23 were analysed for progesterone. Plasma samples collected each hour for 16 h on days 4, 10, 16, 18 were analysed for oxytocin and the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) metabolite (PGFM). Plasma progesterone concentrations declined to $< 1 \text{ ng ml}^{-1}$ between days 18 and 25 in control hinds indicating that luteolysis had occurred, whereas there was no endocrine evidence of luteolysis in interferon-treated or pregnant hinds. Control hinds (6/9) exhibited synchronous pulses of oxytocin and $PGF_{2\alpha}$ secretion on day 18, a greater proportion than on any other day in these hinds or on any day in the interferon-treated or mated hinds ($P < 0.05$). In a second experiment, close synchrony in secretion of oxytocin and $PGF_{2\alpha}$ pulses was evident in an unmated hind when samples were collected every 12 min on day 18. In a third experiment, oxytocin-induced $PGF_{2\alpha}$ secretion was potentiated by oxytocin administration at an interval of 1 h and inhibited by administration at a 6 h interval ($P < 0.05$). These results suggest that synchronous pulsatile secretion of oxytocin and $PGF_{2\alpha}$ induces luteolysis and is suppressed by pregnancy or administration of interferon. Oxytocin-induced alterations in uterine oxytocin sensitivity may underlie the pulsatile nature of luteolytic hormone secretion in red deer.

Introduction

Survival of over a third of extant deer species is threatened by the activities of man (Goombridge, 1994) and consequently there is considerable interest in the application of reproductive technology to the conservation of endangered cervid species (Asher *et al.*, 1988a; Waldhalm *et al.*, 1989; Jabbour and Asher, 1991; Fennessy *et al.*, 1994; Jabbour *et al.*, 1994a). The techniques of artificial insemination, superovulation–embryo transfer and *in vitro* fertilization offer considerable advantages over natural mating for propagation of small populations. However, application of these techniques is often associated with lower conception rates than are observed in naturally mated animals. A possible contributing factor to such conception loss is failure of maternal recognition of pregnancy, the

conceptus signal that alters maternal physiology to avert luteolysis and allow pregnancy to proceed (Short, 1969; Bainbridge *et al.*, 1996a).

The duration of the red deer oestrous cycle is approximately 18–19 days (Guinness *et al.*, 1971; Krzywinski and Jaczewski, 1978). Oestrus may be artificially synchronized with intravaginal progesterone-releasing devices and approximately 18 days after device withdrawal plasma concentrations of progesterone decline rapidly due to luteolysis in unmated hinds. Luteolysis does not occur in pregnant hinds, although the mechanism of suppression of luteolysis during pregnancy has not been investigated (Bainbridge *et al.*, 1996a).

It has been proposed that the embryonic pregnancy recognition signal in red deer is an interferon since administration of interferon- α delays luteolysis in red deer hinds by suppressing uterine oxytocin sensitivity (Bainbridge *et al.*, 1996a) and interferon is present in uterine flushings from pregnant red deer (Flint, 1995). Despite this, there have been no published reports of the effects of exogenous interferons on endogenous secretion of luteolytic hormones.

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The potent luteolytic action of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) analogues provides evidence that endometrial $PGF_{2\alpha}$ is the primary luteolysin in red deer (Asher *et al.*, 1995; Jabbour *et al.*, 1994b) and its secretion may result from a positive feedback interaction with luteal oxytocin secretion (Flint and Sheldrick, 1983; Flint *et al.*, 1991, 1994a) regulated by ovarian steroids (Bainbridge *et al.*, 1996a, b). While episodic secretion of oxytocin and $PGF_{2\alpha}$ has been detected around the time of luteolysis in fallow deer (*Dama dama*) (Asher *et al.*, 1988b), no temporal association between release of the two hormones has been established in any species of deer.

The present study was designed to determine the pattern of secretion of oxytocin and $PGF_{2\alpha}$ during the luteal phase of non-pregnant red deer hinds and the effects of pregnancy and exogenous interferon administration on this pattern. In addition, steroid-treated ovariectomized hinds were used to investigate the effects of repeated oxytocin stimuli on uterine oxytocin sensitivity.

Materials and Methods

Source and standardization of interferon

Recombinant bovine interferon- α_1 1 was generously donated by R. Steiger at CIBA-Geigy Ltd, Basle, Switzerland. The antiviral activity of the preparation was measured by the Madin-Darby bovine kidney cell–Semliki Forest virus cytopathic effect inhibition assay described by Abayasekara *et al.* (1995) and was found to be 1.32×10^8 units mg^{-1} protein.

Animals

The study was conducted at the ADAS Rosemaund Research Centre, Hereford ($52^\circ 10'N$) in autumn 1994 and used 29 mature intact red deer hinds with a mean ($\pm$ SEM) liveweight of 103.8 ± 6.3 kg and 11 mature ovariectomized hinds with a mean liveweight of 110.1 ± 13.2 kg. The animals were contained in a fenced grass paddock. The animals were held in groups, in covered, strawed pens (40 m^2) during the periods of intensive blood sampling.

Experiment 1: secretion of luteolytic hormone in pregnant, non-pregnant and interferon-treated hinds

A total of 26 hinds were allocated to one of three treatment groups ($n = 8$ –9 per group) adjusted for liveweight. Each hind was treated with two progesterone-releasing intravaginal Controlled Internal Drug Releasing devices (CIDR type G; 0.3 g progesterone per device; InterAg (NZ) Ltd, Hamilton). The first CIDR device was inserted for 8 days and was replaced with a second device that was left in place for 5 days. The time of removal of the second CIDR device was taken as day 0.

Group 1 ($n = 9$) received no further treatment and served as unmated controls. Group 2 ($n = 8$) were unmated and received i.m. injections of 4 mg recombinant bovine interferon- α_1 1 in 1.6 ml sterile water twice a day from days 13 to 18. Hinds

in Group 3 ($n = 9$) were run with a fertile stag from days 0 to 3, and all were subsequently diagnosed pregnant by ultrasonography at day 55.

Blood samples were collected each day by jugular venepuncture on days 16–23. Hinds were restrained manually in a drop-floor crush for withdrawal of 5 ml of blood by jugular venepuncture into heparinized tubes. These samples were centrifuged (500 g, for 10 min) immediately at 4°C and the plasma was stored at -20°C before determination of progesterone. Further blood samples (10 ml) were collected at intervals of 1 h for 16 h on days 4 and 10 (Group 1) and days 16 and 18 (Groups 1, 2 and 3). These samples were divided into two aliquots before storage at -20°C . One aliquot was used to determine oxytocin concentrations and the other aliquot to determine concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM), the pulmonary metabolite of $PGF_{2\alpha}$.

Experiment 2: temporal association of oxytocin and prostaglandin $F_{2\alpha}$ secretion

Three intact hinds were treated with two progesterone-releasing CIDR devices. The first CIDR device was inserted for 8 days and was replaced with a second device that was left in place for 5 days. The time of removal of the second CIDR device was taken as day 0. Blood samples (10 ml) were collected via indwelling jugular catheters from the three hinds at intervals of 12 min for a period of 16 h on day 18. The plasma samples were divided into two aliquots, stored at -20°C before assay and used to determine concentrations of oxytocin and PGFM.

Experiment 3: effect of repeated stimulation by oxytocin on uterine $PGF_{2\alpha}$ secretion

Eleven ovariectomized hinds were allocated to one of two treatment groups adjusted for liveweight. Each hind was treated with two progesterone-releasing CIDR devices. The first CIDR device was inserted for 8 days and was replaced with a second device that was left in place for 5 days. The time of removal of the second CIDR device was taken as 0 h. All animals received i.m. injections of 100 μg oestradiol benzoate at 0, 12, 24 and 36 h.

At 48 h, all hinds received the first of two oxytocin challenges (Bainbridge *et al.*, 1996b). A second oxytocin challenge was administered after an interval of either 1 h (Group 1, $n = 6$) or 6 h (Group 2, $n = 5$). For each challenge 4 μg synthetic oxytocin (Sigma Chemical Company, St Louis, MO) in sterile water was injected into the jugular vein and blood samples (5 ml) were collected at -30 , -20 , -10 , 2, 5, 10, 20, 30 and 40 min from this injection. Plasma was subsequently analysed for concentrations of PGFM.

Radioimmunoassays

Plasma concentrations of progesterone were measured by a radioimmunoassay method validated for red deer (Argo and Loudon, 1992). The interassay coefficients of variation for control samples with mean concentrations of 3.57, 0.42 and

Fig. 1. Plasma progesterone concentrations of (a) control, (b) interferon-treated and (c) pregnant hinds. Each graph is a superimposition of the individual profiles of all hinds in the treatment group. Profiles of animals that exhibited coincident pulses of secretion of oxytocin and prostaglandin $F_{2\alpha}$ on day 18 are denoted by thick lines. CIDR device: controlled internal drug release device impregnated with progesterone. Day 0 is day of removal of the device.

0.18 ng ml⁻¹ were 9.4, 8.1 and 24.7%, respectively, and the intra-assay coefficients of variation were 3.9%, 6.0% and 9.4%, respectively. Assay sensitivity was 35 pg ml⁻¹ (defined as sample concentration corresponding to the mean minus twice the standard deviation of the total binding). Samples with concentrations exceeding the maximum detectable on the standard curve were repeated after fivefold dilution in charcoal-treated plasma from red deer stags.

Plasma concentrations of oxytocin were measured by a radioimmunoassay method previously described by Stevenson *et al.* (1991) with the following modifications: 2 ml plasma samples were extracted on C₁₈ cartridges (Waters Sep-Pak; Millipore Corporation, Bedford, MA) and assay separation was achieved using 50 µl antibody-coated cellulose suspension per tube (Sac-Cel; IDS, Boldon). Extraction and assay of serial dilutions of oxytocin in charcoal-treated plasma from ovariectomized hinds produced a dilution curve parallel to a standard curve diluted in assay buffer and indicated an extraction recovery of 75%. The interassay coefficients of variation for controls with mean concentrations of 33.2, 6.94 and 2.43 pg ml⁻¹ were 7.6%, 13.3% and 13.1%, respectively, and the intra-assay coefficients of variation were 5.2%, 8.2% and 7.9%, respectively. Assay sensitivity (defined above) was 0.4 pg ml⁻¹.

Plasma concentrations of PGFM were measured in duplicate by a radioimmunoassay method validated for red deer (Bainbridge *et al.*, 1996b). The interassay coefficients of variation for controls with mean concentrations of 340.0 and 60.2 pg ml⁻¹ were 12.8% and 12.3%, respectively, and the intra-assay coefficients of variation were 10.3% and 8.1% respectively. Assay sensitivity (defined above) was 30 pg ml⁻¹.

Statistical analyses

In Expt 1, a pulse of hormone secretion was defined as a period when the plasma hormone concentration exceeded the mean + 2 × SD for that hind. Basal plasma hormone concentration was defined as the mean plasma concentration in one hind on one day excluding pulse values. Data were analysed by χ^2 test (incidence of episodes of hormone secretion) and

analysis of variance with least-squares difference *post hoc* comparisons (basal plasma hormone concentrations).

In Expt 2, the onset of a pulse of hormone secretion was defined as the first timepoint at which the plasma concentration first exceeded the mean + 2 × SD of previous baseline values. The end of a pulse of hormone secretion was defined as the first timepoint at which the plasma concentration declined below this threshold.

In Expt 3, the magnitude of the PGFM response after an oxytocin challenge was defined as the highest PGFM concentration after the challenge minus the mean of the three data points immediately before the challenge. The data were analysed by *t* test (magnitude of first PGFM response) and paired *t* test (alteration in PGFM response after the second challenge).

Results

Experiment 1: secretion of luteolytic hormone in pregnant, non-pregnant and interferon-treated hinds

Individual progesterone profiles of the hinds used in Expt 1 are shown (Fig. 1). Plasma progesterone concentrations in unmated hinds declined to < 1 ng ml⁻¹ between day 18 and days 21–23, indicating that this was the period of luteolysis. There was no endocrine evidence of luteolysis in the interferon-treated or pregnant hinds during this period.

Individual oxytocin and PGFM profiles of the hinds in Groups 1–3 are shown (Figs 2–4). The proportion of hinds in these treatment groups exhibiting at least one synchronous pulse of PGFM and oxytocin is shown (Table 1). A greater proportion of hinds in the control group exhibited at least one such synchronous pulse on day 18 than on days 4, 10 or 16 ($P < 0.05$) and this proportion also exceeded that in the interferon-treated and pregnant groups on day 18 ($P < 0.05$).

Intra-group comparisons of basal plasma concentrations of PGFM are made (Fig. 5a). Inter-group comparisons demonstrated that basal plasma concentrations of PGFM were greater in control and interferon-treated hinds than in pregnant hinds on day 16 ($P < 0.05$) and this relationship was reversed on day 18 ($P < 0.05$).

Fig. 2. Plasma concentrations on day 18 of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM, ●) and oxytocin (○) in individual hinds treated with progesterone only (Group 1, $n = 9$). Day 0 is the day the progesterone-releasing device was removed. Vertical arrows denote coincident episodes of secretion when the plasma concentration of each hormone was greater than the mean $+ 2 \times \text{SD}$ for that hind.

Intra-group comparisons of basal plasma concentrations of oxytocin are made (Fig. 5b). Inter-group comparisons were as follows: on day 18, basal plasma concentrations of oxytocin in control hinds were greater than those in pregnant hinds which were in turn greater than in interferon-treated hinds ($P < 0.05$, $P < 0.05$, respectively).

Experiment 2: temporal association of oxytocin and prostaglandin $F_{2\alpha}$ secretion

One hind exhibited single pulses of both PGFM and oxytocin (Fig. 6). The maximum concentrations of both hormones occurred at the same timepoint. The onset of the PGFM pulse preceded that of the pulse of oxytocin secretion by 48 min. The duration of the PGFM and oxytocin pulses were 3.6 h and 3.2 h, respectively. No increase in secretion of either hormone was observed in the other two hinds.

Experiment 3: effect of repeated stimulation by oxytocin on uterine prostaglandin $F_{2\alpha}$ secretion

The magnitude of the PGFM response to the initial oxytocin challenge (Fig. 7) did not differ between Groups 1 and 2 (288.1 ± 99.1 versus 429.8 ± 240.8 pg ml^{-1} , $P > 0.2$). The PGFM response to the second oxytocin challenge was greater than that to the first challenge in Group 1 (644.8 ± 186.9 versus 288.1 ± 99.1 pg ml^{-1} , $P < 0.05$), but was less than that to the first challenge in Group 2 (103.2 ± 47.2 versus 429.8 ± 240.8 pg ml^{-1} , $P < 0.1$).

Discussion

The suppression of luteolysis in red deer induced by interferon treatment and pregnancy is similar to that previously reported by Bainbridge *et al.* (1996a). In that study, an identical protocol of interferon treatment to that used in the present study

Fig. 3. Plasma concentrations on day 18 of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM, ●) and oxytocin (○) of individual hinds pretreated with progesterone and then treated with 4 mg recombinant bovine interferon- α_1 on days 13–18 (Group 2, $n = 8$). Day 0 is the day the progesterone-releasing device was removed.

delayed luteolysis by approximately 10 days by suppressing oxytocin-induced $PGF_{2\alpha}$ secretion. From these data, it is proposed that an increase in uterine sensitivity to oxytocin precipitates luteolysis in non-pregnant hinds, but that this increase is suppressed by an antiluteolytic interferon produced by the embryo in pregnant hinds.

Luteolysis was associated with synchronous pulses of oxytocin and $PGF_{2\alpha}$ secretion in unmated control hinds, and this is similar to observations in sheep and cattle (Flint and Sheldrick, 1983; Hooper *et al.*, 1986; Moore *et al.*, 1986; Vighio and Liptrap, 1986). While the marked recrudescence of uterine sensitivity to oxytocin that occurs in red deer hinds immediately before luteolysis (Bainbridge *et al.*, 1996a) is thought to be a major initiating factor of luteolysis, it is probable that subsequent synchronous episodic release of oxytocin and $PGF_{2\alpha}$ is the actual mechanism that causes the corpus luteum to regress. The frequency of the pulses cannot be determined from the present data, since the inter-pulse interval appears to be similar to or greater than the period (16 h) over which plasma samples were collected. The hormonal pulses are notably less

frequent than those in cattle and sheep. This quantitative difference suggests that fewer $PGF_{2\alpha}$ pulses may be required to induce luteolysis, since there is no evidence that pulsatile secretion continues for a longer period before luteolysis than in sheep and cattle. The three unmated, control hinds exhibited no detectable synchronous pulses on day 18, although the progesterone data indicate that the onset of luteolysis may have occurred later in these hinds.

The synchrony of oxytocin and $PGF_{2\alpha}$ secretion detected in plasma samples collected each hour supports the utero-ovarian positive-feedback model of luteolysis (Flint and Sheldrick, 1983; Flint *et al.*, 1990) in red deer. Other evidence for this model is that administration of $PGF_{2\alpha}$ analogues induces secretion of oxytocin, a hormone present in high concentrations in the corpus luteum of red deer (Flint *et al.*, 1991), and that exogenous oxytocin causes the release of uterine $PGF_{2\alpha}$ (Flint *et al.*, 1994a; Bainbridge *et al.*, 1996a, b), the primary luteolysin in red deer (Jabbour *et al.*, 1994b; Asher *et al.*, 1995; Bainbridge *et al.*, 1995). More frequent plasma sampling, every 12 min, confirms the close synchrony between secretion of the

Fig. 4. Plasma concentrations on day 18 of 13,14-dihydro-15-keto prostaglandin F_{2α} (PGFM, ●) and oxytocin (○) of pregnant hinds pretreated with progesterone (Group 3, n = 9). Day 0 is the day the progesterone-releasing device was removed.

Table 1. The proportion of hinds in each treatment group exhibiting coincident pulses^a of secretion of both oxytocin and prostaglandin F_{2α}

Treatment group	Day of experiment			
	4	10	16	18
Control	0/9	0/9	1/9	6/9
Interferon-treated			0/8	0/8
Pregnant			1/9	0/9

^aA hormone pulse was defined as a period when the plasma hormone concentration was greater than the mean + 2 × SD for that hind.

two hormones. At the beginning of the pulses, the concentration of PGFM increased significantly above the baseline 48 min before the oxytocin concentrations increased. Although this result is similar to an effect reported in sheep (Moore *et al.*,

1986) and may be interpreted as evidence for uterine PGF_{2α} being the initiating stimulus of the pulse, it may simply reflect differences between the variability of basal secretion of the two hormones, or differing sensitivity of the two hormone assays. Both pregnancy and the administration of exogenous interferon suppressed the synchronous episodic release of PGF_{2α} and oxytocin observed at the end of the luteal phase in control unmated hinds. The effect of pregnancy is similar to that observed in bovids (Homeida and Cooke, 1983; Hooper *et al.*, 1986) and provides further evidence that control of episodic hormone release is of importance in maternal recognition of pregnancy in red deer. The observation that exogenous interferons also suppress episodic release of PGF_{2α} and oxytocin supports the hypothesis that the embryonic pregnancy-recognition signal is an interferon. This hypothesis is in agreement with the findings of other studies demonstrating that trophoblast interferon genes are present in another cervid, the white-tailed deer (*Odocoileus virginianus*) (Leaman and Roberts, 1992), that uterine flushings from pregnant red deer hinds exhibit antiviral activity (Flint, 1995) and that

Fig. 5. Mean ($\pm$ SEM) basal (that is, excluding pulse values) plasma concentrations on days 4, 10, 16 and 18 of (a) 13,14-dihydro-15-keto prostaglandin F_{2u} (PGFM) and (b) oxytocin in control, interferon-treated and pregnant hinds pretreated with progesterone. Day 0 is the day the progesterone-releasing device was removed. Columns with different superscripts differ significantly ($P < 0.05$) within treatment groups.

administration of exogenous interferon extends the luteal phase in red deer by suppressing uterine oxytocin sensitivity (Bainbridge *et al.*, 1996a).

Basal plasma concentrations of oxytocin in control hinds increased in the first half of the luteal phase, presumably as a result of the formation of luteal tissue following ovulation, as occurs in sheep (Flint and Sheldrick, 1983). This observation is further evidence of secretion of oxytocin by the cervid corpus luteum into the systemic circulation (Flint *et al.*, 1991, 1994b). The increase was not associated with an increase in basal or pulsatile PGFM release by day 10, and we suggest that this is because uterine oxytocin sensitivity is at a minimum during the mid-luteal phase in red deer (Bainbridge *et al.*, 1996a). The decline in basal oxytocin concentrations in mated and interferon-treated hinds between days 16 and 18 is notable since, unlike the control hinds, this is a period over which the corpus luteum is maintained because of suppression of uterine oxytocin sensitivity (Bainbridge *et al.*, 1996a). However, there are few data from any ruminant species relating to the control of basal luteal oxytocin secretion, although it is not thought that interferon exerts a direct inhibitory effect on luteal

oxytocin secretion in red deer (D. R. J. Bainbridge and H. N. Jabbour, unpublished data).

Basal plasma concentrations of PGFM were not increased at the time of luteolysis and indeed were higher in pregnant than in non-pregnant hinds on day 18. Administration of interferon may have induced increases in basal prostaglandin secretion by activating phospholipase A_2 , as is known to occur in ruminant cells (Yedgar *et al.*, 1990). These results suggest that it is unlikely that the luteolytic effect of PGF_{2u} is dependent upon its basal secretion, since this parameter has little effect on the occurrence or otherwise of luteal regression. We propose that, as in sheep (Flint and Sheldrick, 1983; Hooper *et al.*, 1986; Moore *et al.*, 1986; Flint *et al.*, 1990), increased episodic rather than basal secretion of PGFM is required to precipitate luteolysis. It is known that the luteolytic potency of exogenous prostaglandin is increased after intermittent, rather than continuous, administration in ewes (Schramm *et al.*, 1983).

Administration of an oxytocin challenge to steroid-treated ovariectomized hinds induced an enhanced PGFM response to a second challenge administered 1 h later and a reduced

Fig. 6. Plasma concentrations on day 18 of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM, ●) and oxytocin (○) in a hind treated with progesterone only. Day 0 is the day the progesterone-releasing device was removed. Squares indicate the timepoint for each hormone when the plasma concentration first exceeded the mean + 2 × SD of all preceding basal values.

Fig. 7. Plasma concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) after two oxytocin challenges at intervals of (a) 1 h ($n = 6$) or (b) 6 h ($n = 5$). The animals had been ovariectomized before the experiment and pretreated with progesterone-releasing controlled internal drug releasing (CIDR) devices and i.m. injections of oestradiol benzoate. The first challenge was administered 48 h after removal of the CIDR device. Vertical arrows denote times of administration of oxytocin.

response to a second challenge administered 6 h later. This transient potentiation followed by refractoriness after oxytocin administration is in contrast to findings in sheep, in which refractoriness is induced within 1 h which wanes over a period greater than 6 h (Sheldrick and Flint, 1986). This discrepancy may result from a qualitative difference between the species, or

from a quantitative difference in the timescale of the response which may have led to the potentiation phase being undetected in ewes. While the cellular mechanisms underlying such alterations in uterine oxytocin sensitivity have not been investigated in red deer, they may act to enhance the episodic nature of hormone release at luteolysis.

The present study has provided data regarding several aspects of luteolysis and the maternal recognition of pregnancy in red deer. Luteal regression is probably induced by synchronous episodic release of uterine PGF_{2α} and luteal/pituitary oxytocin resulting from increased uterine oxytocin sensitivity (Bainbridge *et al.*, 1996a). The physiological relevance of these phenomena is supported by their suppression during pregnancy and after administration of interferon, a putative pregnancy-recognition signal from the embryo. The attack phase of each pulse probably results mainly from the development of a uterine-ovarian PGF_{2α}-oxytocin positive-feedback loop or transient potentiation of uterine sensitivity to oxytocin. Similarly, the decay phase of the pulses and the interpulse interval may be regulated by the development of uterine oxytocin refractoriness, although other factors such as luteal refractoriness to PGF_{2α} cannot be discounted.

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