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Negative Feedback Regulation of Pulsatile LH Secretion During Treatment with an LHRH Antagonist in Rams

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Suppression of LH and testosterone secretion in sexually active rams by the short-term administration of an LHRH antagonist results in a compensatory increase in the release of LHRH from the hypothalamus. This is inferred from the observed increase in the frequency of LH pulses in peripheral blood during the period of recovery when the pituitary regains its responsiveness to LHRH. To investigate the nature of the inhibitory feedback signal which triggers this compensatory response, a single intravenous injection of 1 mg of an LHRH antagonist (28 μg/kg; N-Ac-D-pCl-Phe 1, D-pCl-Phe 2, D-Trp 3, D-hArg (Et 2) 6, D-Ala 10, LHRH) was given to groups of intact, testosterone-implanted castrated and castrated rams housed under stimulatory short days. Pulsatile LH secretion was monitored in blood samples collected every 10 min for 34 h. The treatment caused an immediate blockade of LH pulses in all three groups of rams followed by a progressive recovery of LH secretion from 12–30 h. Compared to the pretreatment period, intact rams showed a significant increase in frequency of LH pulses during the recovery period. Castrated rams did not show this increase, with or without supplementary testosterone. Since the circulating testosterone concentration decreased after the blockage of LH secretion in the intact rams, but not in the castrated or testosterone-implanted castrated rams, we conclude that it is the reduction in the steroid negative feedback signal which leads to a compensatory increase in the activity of the LH pulse generator.

Key words: LHRH antagonists, steriod feedback, hypothalamus, pituitary gland, testis, testosterone, pulsatile LH secretion.

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In a recent study, we provided evidence that the short-term inhibition of LH and testosterone secretion in sexually active rams by the IV injection of a potent LH releasing hormone (LHRH) antagonist leads to an increase in the frequency of LH pulses, and, by inference, an increase in the secretion of LHRH from the hypothalamus (Lincoln and Fraser, 1987). This was evident during the recovery period from the effect of the antagonist when the pituitary gland first regained its ability to respond to the pulsatile signals emanating from the hypothalamus, assuming a 1:1 relationship between LHRH and LH, as is well established in the ram (Caraty and Locatelli, 1988). The increase in LHRH

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secretion appears as a compensatory response triggered by the decline in the circulating concentrations of gonadal steriods, or a change in some other feedback signal normally involved in the autoregulation of LHRH (Bedran de Castro et al, 1985, De Paolo et al, 1987). There is good experimental evidence for the role of gonadal steriods in the negative feedback control of LHRH/LH in the ram (Bolt, 1971; Schanbacher and Ford, 1977; Parrott and Davies, 1979; D'Occhio et al, 1983; Lincoln 1984), but the possible involvement of the "short loop" signals has not been studied.

The aim of this project was to establish whether the increase in LH pulse frequency following treatment with LHRH antagonist is caused by the decline of testosterone secretion or results from some other change in the mechanism of homeostasis. To assess this, pulsatile LH secretion was measured in groups of intact, testosterone-implanted castrated, and castrated rams following the injection of LHRH antagonist. The prediction was that if the change in testosterone is a prerequisite for an increase in frequency of LH pulses, then there will be no response in castrated rams or castrated rams with an exogenous source of testosterone. However, if the inhibitory feedback involves other mechanisms, all groups may respond.

Materials and Methods

Animals

Sixteen adult Soay rams aged 3–6 yr and mean live weight of 36.0 ± 1.5 kg were kept in light-controlled rooms at the AFRC Dryden field station near Edinburgh. They were exposed to an artificial regimen of alternating 16 wk periods of long days (16 h light: 8 h darkness, 16L:8D) and short days (8L:16D) to induce a cycle in reproduction every 32 wks (Lincoln and Short, 1980). To permit the collection of sequential blood samples, a cannula was inserted into the jugular vein on the day before the experiment and kept patent with heparinised saline. All blood samples were heparinised and centrifuged within 30 min. The plasma was stored at −20°C until required for the hormone assays.

Treatments

Five of the rams were castrated and immediately implanted with Silastic envelopes containing testosterone capable of maintaining constant peripheral blood concentrations of testosterone in the low physiological range for the breeding season (Lincoln, 1984). Five rams were castrated 2 wks before the study and received no supplementary testosterone. The remaining six rams were intact controls.

The LHRH antagonist (N-Ac-D-pCl-Phe 1, D-pCl-Phe 2, D-Trp 3, D-hArg (Et 2) 6, D-Ala 10, LHRH) was kindly donated by Syntex (Palo Alto, CA, product code RS-18286; Nestor et al, 1983). It was dissolved in saline (9 g NaCl/l) and 40% propylene glycol. After 12 wks exposure to short days, when the intact rams had fully enlarged testes, all animals were given a single IV injection of 1 mg LHRH antagonist (28 μg/kg) capable of supressing LH secretion for about 12h (Lincoln and Fraser, 1987). Blood samples were collected at 10 min intervals for 6 h before and continued for 32 h after the treatment, with a 6 h gap, 12–18 h after the injection. The blood sampling started at 8 AM at the beginning of the light phase and dim red illumination was used to aid the sampling during the dark phase.

Radioimmunoassays

The plasma concentrations of LH were measured by RIA using the method of McNeilly et al (1986). The sensitivity of the assay (90% B/Bo) was 0.2–0.4 μg/l NIH-LH-S18 (NIDDK, Bethesda, MD) and the mean intra- and interassay coefficients of variation (cv) were 8.3 and 13.7% respectively, based on high, medium, and low quality control plasma samples in a total of 14 assays. The plasma levels of testosterone were measured by RIA using the extraction method of Corker and Davidson (1978) and an iodinated tracer (Sharpe and Bartlett, 1985). The intraassay cv was < 12% and the sensitivity 0.4 nmol/l. All plasma samples for each animal were measured in duplicate within the same assay.

Analysis

The LH profiles, based on the 10 min blood samples, were analysed for significant peaks using the criteria of two consecutive high values, at least one of which exceeded the mean of the preceeding basal values by twice the intraassay cv (Fraser and Lincoln, 1980). The effects of the treatment with the LHRH antagonist were assessed using a split-plot analysis of variance (ANOVA). The sampling periods were subdivided into pretreatment and posttreatment 6 h blocks, and mean values were determined for each animal for comparison between the pretreatment period using ANOVA followed by the Newman Keuls test. The division into 6 h blocks was preselected since this is the minimum time period to adequately assess the frequency of LH pulses in sexually active rams (Lincoln and Short, 1980). The values shown in Table 1 are group mean hormone concentrations and pulse frequencies for each time block. The inter-pulse interval for LH values during the recovery period was based on the first five LH peaks after the period of suppression.

Results

The effects of the single IV injection of 1 mg LHRH antagonist (28μg/kg) on the blood plasma concentrations of LH and testosterone are illustrated for representative intact, castrated, and testosterone-implanted castrated rams in Figs. 1–3. The results are summarised in Table 1.

The treatment resulted in an immediate blockage

TABLE 1. Summary of changes in LH and testosterone concentrations in blood plasma from intact (I, n = 6), testosterone-implanted castrate (C + T, n = 5) and castrate (C, n = 5) Soay rams housed under stimulatory short days (8L:16D), before (pretreatment) and after (posttreatment 1–4) an IV injection of LHRH antagonist (RS 18286, 28 μg/kg) based on blood samples collected every 10 min for periods of 6 h

		Pretreatment	*Posttreatment*			
			1	2	3	4
		(0–6 h)	(6–12 h)	(18–24 h)	(24–30 h)	(30–34 h)
LH mean (μg/l)	I	2.72	1.49†	1.87	2.24	2.77
		±0.35	±0.13	±0.23	±0.27	±0.46
	C + T	22.84	9.27†	10.89*	15.39	20.68
		±1.37	±0.95	±2.06	±1.45	±2.37
	C	22.46	7.57†	12.50*	21.94	31.44
		±1.78	±0.39	±1.25	±2.59	±4.60
LH pulse frequency (peaks/6 h)	I	3.67	0.66†	5.17*	5.17*	4.44
		±0.33	±0.33	±0.39	±0.30	±0.37
	C + T	4.20	0.80†	2.40†	4.25	3.85
		±0.36	±0.37	±0.50	±0.41	±0.73
	C	6.50	0†	5.50	5.75	5.92
		±0.87		±0.43	±0.48	±1.01
Testosterone mean (nmol/l)	I	47.71	17.94†	27.24*	33.42	40.39
		±2.95	±1.56	±2.12	±4.00	±2.95
	C + T	12.94	12.32	12.25	12.08	11.52
		±2.32	±1.53	±1.18	±1.46	±0.56
	C	< 1.20	< 1.20	< 1.20	< 1.20	< 1.20

Statistical comparison: ANOVA with Newman Keuls test; significantly different from corresponding pretreatment value.
*$p < 0.05$, †$p < 0.01$.

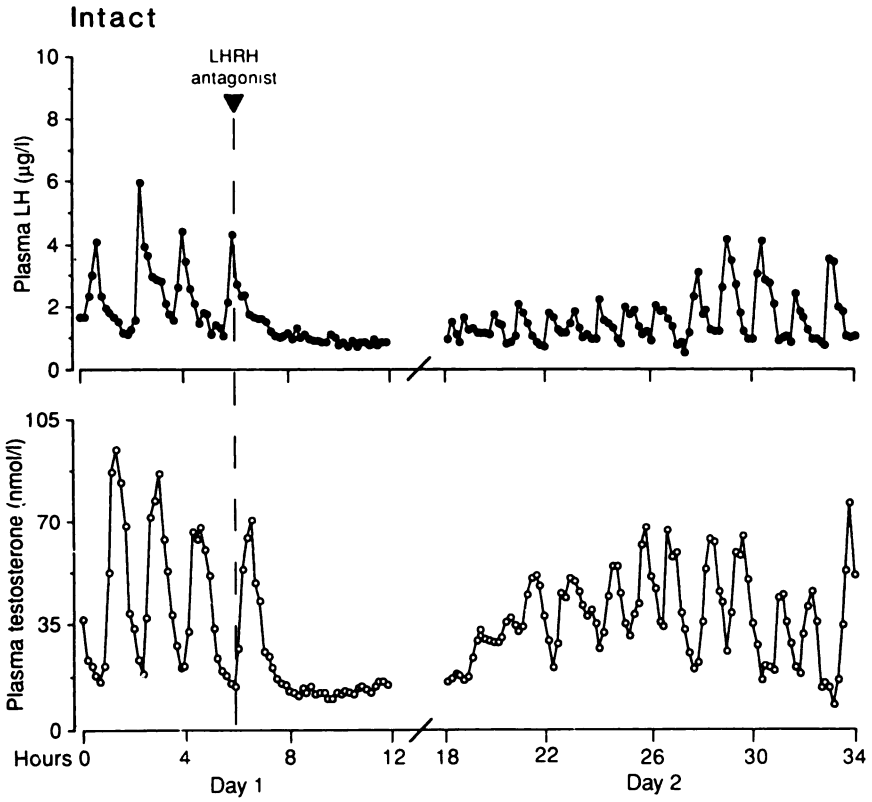

Fig. 1. Blood plasma concentrations of LH and testosterone in a representative intact adult Soay ram before and after the single IV injection of 1 mg LHRH antagonist (28 μg/kg) based on blood samples collected at 10 min intervals during a period of 34 h. Note the immediate blockade of LH pulses following the LHRH antagonist and the progressive recovery of pituitary responsiveness 12–20 h after the treatment.

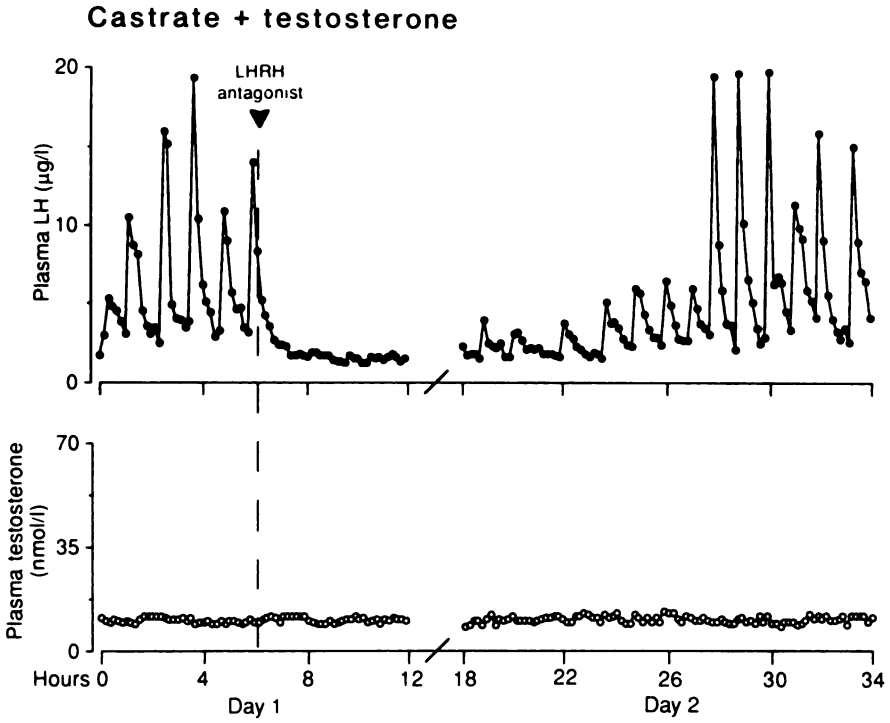

Fig. 2. Blood plasma concentrations of LH and testosterone in a representative testosterone implant castrated adult Soay ram before and after the single IV injection of 1 mg LHRH antagonist (28 µg/kg based on blood samples collected at 10 min intervals during a period of 34 h. Note the immediate blockage of LH pulses following the LHRH antagonist and the progressive recovery of pituitary responsiveness 12–20 h after the treatment.

of the pulsatile secretion of LH in all three groups and the inhibitory effect persisted for 12–18 h. In the intact rams, there was a corresponding decline in the plasma levels of testosterone, while in the implanted castrates the circulating levels of testosterone remained relatively constant due to the exogenous source of hormone. In the castrates receiving no supplementation, the plasma testosterone levels were persistently low at the detection limit of the assay (Table 1).

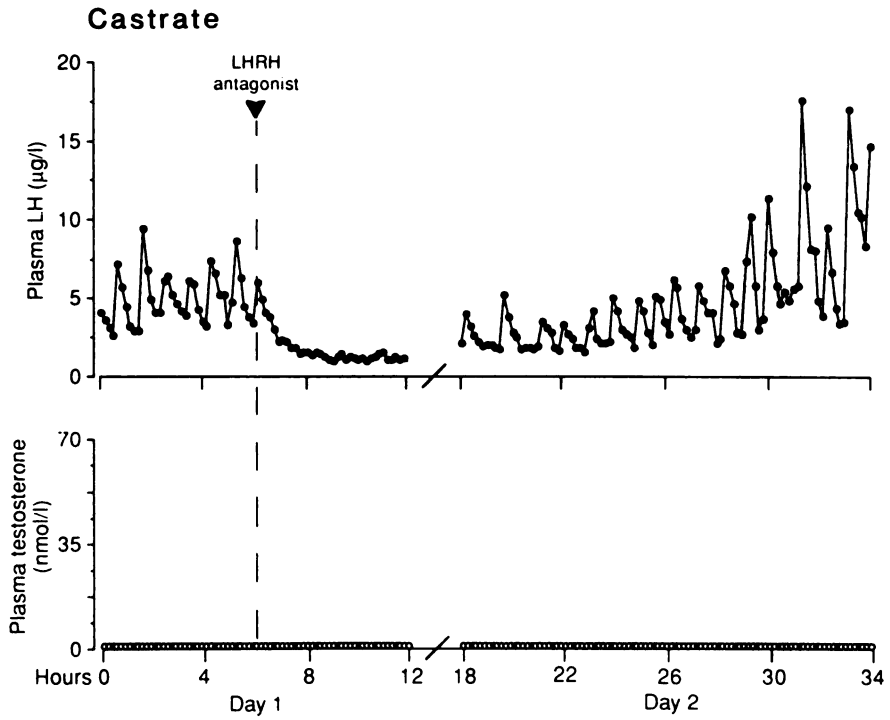

Fig. 3. Blood plasma concentrations of LH and testosterone in a representative castrated adult Soay ram before and after the single IV injection of 1 mg LHRH antagonist (28 µg/kg based on blood samples collected at 10 min intervals during a period of 34 h. Note the immediate blockage of LH pulses following the LHRH antagonist and the progressive recovery of pituitary responsiveness 12–20 h after the treatment.

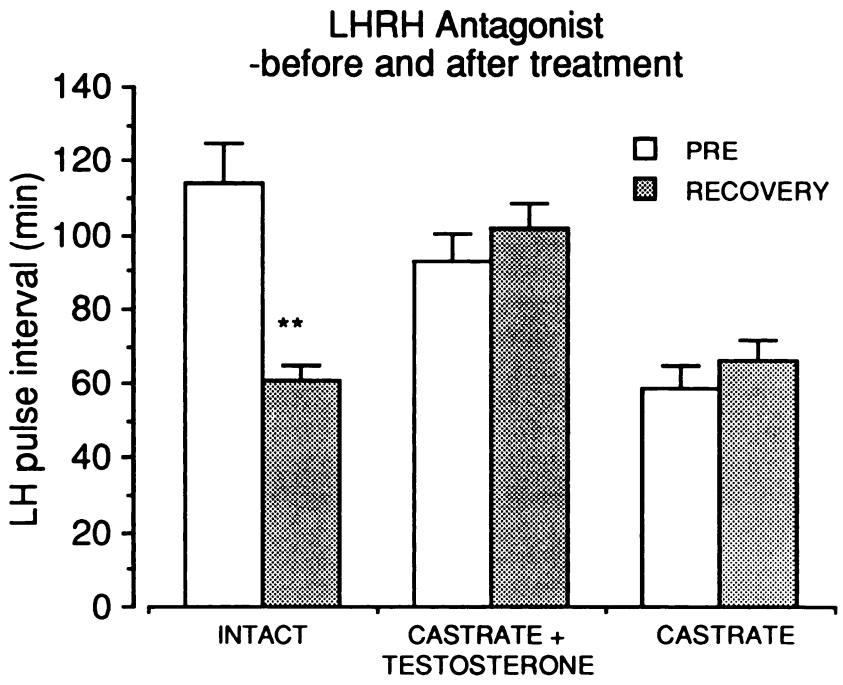

Fig. 4. LH inter-pulse interval (mean ± SEM, n = 5–6) in intact, testosterone-implanted castrated, and castrated Soay rams before and after the single IV injection of 1 mg LHRH antagonist (28 μg/kg) based on blood samples collected at 10 min intervals as in Figs. 1–3. The inter-pulse interval after the treatment was assessed for the first 5 LH pulses during the period of recovery.

During the recovery from the effects of the LHRH antagonist, LH pulses were again evident in all groups; the pulses progressively increased in amplitude until reaching the pretreatment values after 30 h (Figs 1–3). At the onset of pulsatile LH secretion following the period of suppression, the frequency of LH pulses was increased compared to pretreatment values in the intact rams, but not in the castrated rams with or without supplementary testosterone (Table 1). Since the rams in each group were not exactly synchronized in the time of recovery, this effect was best illustrated by assessing the inter-pulse interval for the first 5 LH pulses to reappear in each animal as summarized in Fig. 4. The interval was significantly reduced ($P < 0.01$) compared to the pretreatment value in the intact rams, but not in the other two groups.

Discussion

The peripheral administration of LHRH antagonist was very effective at blocking the pulsatile secretion of LH in the rams, acting within 10 min to fully supress LH pulses for up to 12 h. This effect was equally apparent in the castrated animals, which were initially showing a high frequency of LH pulses, and in the testosterone-implanted castrated and intact animals which were releasing LH at a lower pulse frequency. This is consistent with the action of the antagonist on the gonadotrophs, blocking the stimulatory influence of pulses of LHRH arising from the neurosecretory cells in the hypothalamus. The progressive increase in amplitude of the LH pulses at 12–18 h after the antagonist, presumably reflects the gradual recovery of pituitary responsiveness to endogenous LHRH as the antagonist is metabolized and LHRH receptors become available on the gonadotrophs.

The return of pituitary responsiveness makes it possible to infer the pattern of LHRH secretion from the hypothalamus following the treatment with LHRH antagonist. In the intact rams, there was an increase in LH pulses at this time, indicating an increase in hypothalamic drive to the pituitary gland as first described for rams treated with low, medium, and high doses of the same antagonist (Lincoln and Fraser, 1987). However, there was no similar increase in the frequency of LH pulses following the LHRH antagonist in the castrated rams with or without supplementary testosterone. The principle difference between these groups was in the changes in the circulating concentrations of testosterone, declining only in the intact rams due to the blockade of LH secretion. This strongly indicates that the increase in LH pulses evident in the intact rams during the recovery from the treatment with LHRH antagonist reflects the

compensatory increase in pulsatile LHRH secretion from the hypothalamus, triggered by the decrease in the negative feedback effect of gonadal steriods.

In the testosterone-implanted castrates, the frequency of LH pulses was less than in the castrates, but greater than in the intact rams. This indicates that the negative feedback influence of exogenous testosterone on the LH pulse generator in the implanted group was less than the feedback normally operating in the intact ram to regulate LH secretion. The circulating concentration of testosterone in the treated castrate was relatively low and much more constant than the levels in intact rams, which may account for the differences in the feedback signal. However, it has been shown that removal of the testosterone implant from a castrated ram exposed to short days leads to a progressive increase in the frequency of LH pulses beginning within 6 h (G.A. Lincoln, unpublished observation). Thus, the implanted castrate provides a sensitive model to observe changes in the negative feedback effects of gonadal steroids. In the current study, the treatment with the LHRH antagonist caused no change in the frequency of LH pulses in the testosterone implanted castrates, therefore, it is evident that the antagonist failed to modify the negative feedback control of LH.

These experiments provide no support for the involvement of "short-loop" feedback mechanisms whereby LH inhibits the release of LHRH, or LHRH inhibits its own release. This could have resulted in an increase in LHRH/LH secretion in either group of castrated rams due to the blockade of LH secretion, or due to the blockade of putative autoregulatory LHRH receptors in the hypothalamus. The simplest conclusion is that treatment with LHRH antagonist results in an increase in the secretion of LHRH from the hypothalamus only if there is a corresponding decrease in gonadal steriods as occurs in the normal intact male. It should be possible to measure this change directly by collecting blood samples from the pituitary portal system in conscious intact animals. In the current experimental protocol, it was only possible to deduce the changes as an increase in the frequency of LH pulses as the pituitary gland regained its responsiveness after treatment with the LHRH antagonist.

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