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Hypothalamic control of the post-castration rise in serum LH concentration in rams

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Summary. Sexually mature rams were left intact, castrated (wethers), castrated and implanted with testosterone, or castrated, implanted with testosterone and pulse-infused every hour with LHRH. Serum concentrations of LH increased rapidly during the first week after castration and at 14 days had reached values of 13·1 ± 2·2 ng/ml (mean ± s.e.m.) and were characterized by a rhythmic, pulsatile pattern of secretion (1·6 ± 0·1 pulses/h). Testosterone prevented the post-castration rise in serum LH in wethers (1·0 ± 0·5 ng/ml; 0 pulses/h), but a castrate-type secretory pattern of LH was obtained when LHRH and testosterone were administered concurrently (10·7 ± 0·8 ng/ml; 1·0 pulse/h). We conclude that the hypothalamus (rather than the pituitary) is a principal site for the negative feedback of androgen in rams and that an increased frequency of LHRH discharge into the hypothalamo–hypophysial portal system contributes significantly to the post-castration rise in serum LH.

Introduction

The modulating influence of gonadal steroids on gonadotrophin secretion in rams is revealed by the dramatic increases in serum LH concentration which follow castration (Riggs & Malven, 1974; D'Occhio, Schanbacher & Kinder, 1982). The rapidity of the post-castration rise in serum LH also indicates that testosterone, alone or in combination with other testicular steroids, imposes continuous negative feedback on gonadotrophin secretion (Schanbacher & Ford, 1977; D'Occhio *et al.*, 1983a). A question yet to be resolved, however, is whether steroid feedback in rams occurs primarily at the level of the hypothalamus or anterior pituitary gland.

Two aspects of the LH secretory profile which distinguish between the intact and castrated ram, and which provide information regarding the site of negative feedback, are LH pulse frequency and pulse amplitude (Schanbacher, 1980a; D'Occhio *et al.*, 1983b). Changes in LH pulse frequency, for example, reflect activity of the LH-releasing hormone (LHRH) pulse generator thought to reside within the hypothalamus, while changes in LH pulse amplitude may be interpreted either as changes in LHRH pulse amplitude or direct actions of testicular steroids on the pituitary. Frequency of LHRH input from the hypothalamus can also influence LH secretion (Wildt *et al.*, 1981). To determine the contribution of increased frequency of hypothalamic LHRH release to the post-castration rise in serum LH we examined the nature of LH secretion in testosterone-implanted castrated rams given pulsatile infusions of LHRH.

Materials and Methods

Animals and experimental procedures. In early December, 16 mature crossbred rams (85·2 ± 1·9 (s.e.m.) kg body weight) were brought into an environmentally-controlled facility providing 16-h

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daylengths and randomly assigned to one of four treatments. Long days were imposed to increase the sensitivity of implanted animals to testosterone negative feedback (Pelletier & Ortavant, 1975a, b; Schanbacher, 1980a). Four rams were left intact (Group 1) while the 12 remaining rams were surgically castrated under xylazine (Rompun; Haverlockhart, Shawnee, Kansas) analgesia. Four wethers served as unimplanted castrate controls (Group 2), whereas whethers in Groups 3 and 4 were, at the time of castration, implanted with four Silastic capsules (Dow Corning, Midland, MI, U.S.A.) filled with crystalline testosterone (Schanbacher, 1980b; D'Occhio *et al.*, 1982, 1983a, b). Wethers in Group 4 were also pulse infused every 60 min via Harvard infusion pumps with a 30-sec intravenous dose of LHRH (5 ng/kg body wt in saline) starting immediately after castration and testosterone replacement (Day 0). At Day-2, all rams were equipped with a chronic jugular cannula (Intramedic polyethylene tubing; 1·19 mm i.d., 1·7 mm o.d.; Clay Adams, Parsippany, NJ, U.S.A.) and placed in metabolism crates. On Days −1, 7 and 14, jugular blood samples were collected every 10 min for 12·5 h starting at 08:00 h. All animals were challenged with an intravenous dose of LHRH (5 ng/kg) at 20:00 h. Additional blood samples were collected from the jugular cannula every 10 min for a 4-h period (08:00–12:00 h) on Days 1, 2 and 4.

Hormone assays. Serum concentrations of LH and testosterone were determined by the double-antibody radioimmunoassay procedures described by Schanbacher & Ford (1976) and Schanbacher & D'Occhio (1982), respectively. Testosterone was assayed in all samples collected from Group 1 rams but only in hourly samples for animals in Groups 2, 3 and 4. Sensitivities of these assays were 0·5 ng LH/ml and 0·2 ng testosterone/ml. The intra- and interassay coefficients of variation were both $<8\%$ for LH and $<10\%$ for testosterone, based on assay duplicates.

Statistical analyses. Mean concentrations were calculated for LH and testosterone from the values determined on Days −1, 1, 2, 4, 7 and 14 of the study. Secretory episodes of LH were recorded as peaks if (1) they were preceded by at least two successive values that showed a progressive decline or represented basal levels and (2) the increases were at least twice the particular intra-assay coefficient of variation (D'Occhio *et al.*, 1982). These episodes were used to calculate LH pulse frequency. Magnitude of the LH response to exogenous LHRH represented pulse amplitude and was derived by subtracting the LH concentration at the time of injection from the peak LH concentration (D'Occhio *et al.*, 1983b). Differences between treatments were assessed by analysis of variance and the least significant difference test.

Results

Serum testosterone

Before castration, all 16 rams had appreciable levels of serum testosterone which were either basal (1·5–3·0 ng/ml) or variable depending on the frequency of LH secretory episodes. Mean serum testosterone concentrations for the rams within each of the four treatment groups on Day −1 are shown in Table 1. Intact rams (Group 1) had similar testosterone levels throughout the course of the study (Day −1 to Day 14), but castration resulted in a precipitous fall in serum testosterone within 24 h (Day 1). Testosterone was undetectable in all Group-2 wethers from Day 2 onwards but remained at levels typical of intact rams in all wethers treated with testosterone (Groups 3 and 4) (Table 1). The pulsatile nature of testosterone secretion in rams and the stability of this steroid in serum of implanted wethers are demonstrated in Text-fig. 1.

Serum LH

LH profiles for representative animals within each of the four treatment groups are shown in Text-fig. 1. Characteristics of these profiles are summarized by treatment in Table 1. The most obvious differences between groups are in the frequency of LH secretory episodes and the overall

Table 1. Mean serum concentrations of testosterone and luteinizing hormone (LH), the frequency of endogenous LH pulses, and the amplitude of LHRH-induced LH release (Δ) in rams (Group 1), wethers (Group 2), wethers implanted with testosterone (Group 3) and wethers implanted with testosterone and infused hourly with LHRH (Group 4)

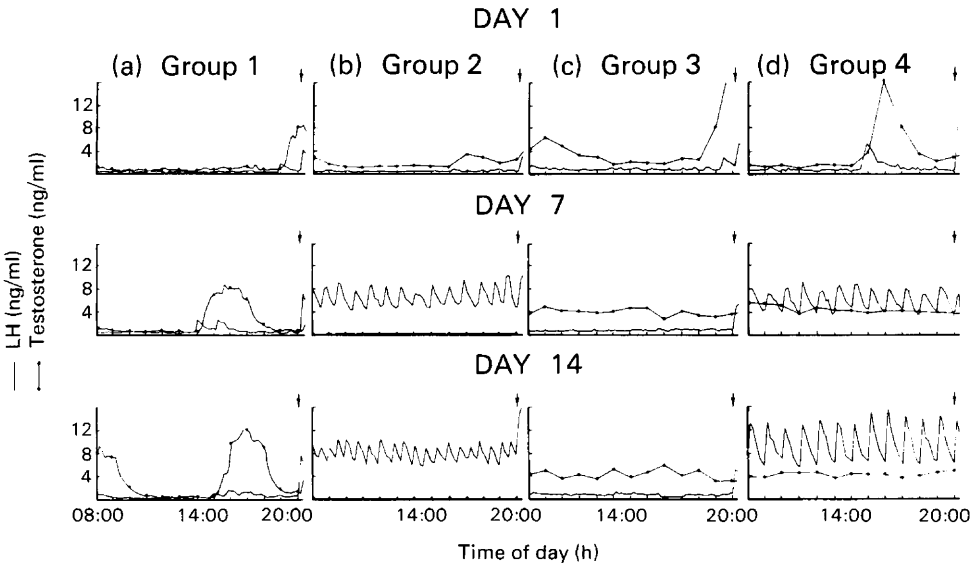
	Group 1	Group 2	Group 3†	Group 4‡
Day −1				
Testosterone (ng/ml)	7·4 ± 3·9	5·7 ± 1·8	4·4 ± 1·3	5·4 ± 1·8
LH				
Conc. (ng/ml)	0·6 ± 0·1	0·8 ± 0·1	0·7 ± 0·2	1·0 ± 0·2
Frequency (pulses/h)	0·2 ± 0·1	0·1 ± 0·05	0·1 ± 0·04	0·1 ± 0·02
Δ (ng/ml)	2·7 ± 0·6	4·7 ± 1·3	2·8 ± 0·5	4·7 ± 1·4
Day 1				
Testosterone (ng/ml)	4·9 ± 2·2	<0·22**	4·6 ± 0·5	5·7 ± 0·7
LH (ng/ml)	1·1 ± 0·2*	2·7 ± 0·9	0·9 ± 0·2*	7·2 ± 1·4*
Day 2				
Testosterone (ng/ml)	5·0 ± 2·4	<0·20**	4·0 ± 0·4	5·3 ± 0·7
LH (ng/ml)	1·1 ± 0·01*	6·6 ± 2·5	0·7 ± 0·2*	6·6 ± 1·3
Day 4				
Testosterone (ng/ml)	5·1 ± 2·5	<0·20**	4·1 ± 0·4	5·7 ± 1·1
LH (ng/ml)	1·1 ± 0·04*	11·9 ± 3·3	1·3 ± 0·3*	6·8 ± 1·8*
Day 7				
Testosterone (ng/ml)	5·2 ± 2·3	<0·20**	4·1 ± 0·1	5·8 ± 1·7
LH				
Conc. (ng/ml)	1·0 ± 0·06*	12·5 ± 2·6	0·6 ± 0·1*	6·5 ± 0·6*
Frequency (pulses/h)	0·2 ± 0·07*	1·3 ± 0·1	0·02 ± 0·02*	1·0 ± 0·0*
Δ (ng/ml)	4·7 ± 1·3*	7·3 ± 1·1	2·8 ± 0·7*	6·1 ± 0·4
Day 14				
Testosterone (ng/ml)	4·5 ± 1·1	<0·20**	3·3 ± 0·5	5·2 ± 1·2
LH				
Conc. (ng/ml)	0·7 ± 0·04*	13·2 ± 2·2	1·1 ± 0·5	10·7 ± 0·8
Frequency (pulses/h)	0·1 ± 0·06*	1·6 ± 0·1	0·0*	1·0 ± 0·0*
Δ (ng/ml)	4·2 ± 1·2	10·1 ± 1·1	2·8 ± 0·7*	7·6 ± 1·6

Values are mean ± s.e.m. (N = 4).
† Four testosterone capsules at the time of castration (Day 0).
‡ Four testosterone capsules and pulse-infused with LHRH (5 ng/kg once every hour) starting on Day 0.
* $P < 0·01$ compared with Group 2.
** $P < 0·01$ compared with Group 1.

mean concentrations of this hormone. Intact rams showed only an occasional pulse of LH as did castrates implanted with testosterone. In the absence of testosterone, LH pulse frequency increased dramatically, and by Day 14 after castration, LH pulses occurred about every 37 min (i.e. 1·6 pulses/h) in Group-2 wethers. The amplitude of these pulses generally increased after castration but considerable variation was observed between animals. The combination of increased frequency and amplitude of the LH pulses resulted in elevated mean serum LH concentration which had plateaued by Day 4.

Testosterone prevented the post-castration rise in LH when provided as replacement therapy commensurate with castration (Group 3), but did not prevent LHRH-induced hypersecretion of LH (Group 4). In Group-4 wethers there was a 7-fold increase in serum LH concentration within 24 h and values approached those of Group-2 wethers by Day 14, in spite of serum testosterone concentrations comparable to those of intact rams (Table 1).

Responsiveness of the pituitary to exogenous LHRH was assessed in all animals on Days 7 and 14 of the study and the results are summarized in Table 1. Wethers (Group 2) were more responsive than rams (Group 1) to a single injection of LHRH, while testosterone-implanted wethers (Group 3), which exhibited few if any endogenous LH pulses, gave the smallest LH release of all groups. In contrast, wethers in Group 4 (LHRH + testosterone) released an amount of LH that, by Day 14, was not significantly different from that of Group-2 untreated wethers.



Text-fig. 1. Secretory profiles of LH and testosterone from a representative ram in Group 1 (a), wether in Group 2 (b), testosterone-implanted wether in Group 3 (c) and testosterone-implanted, LHRH-infused wether in Group 4 (d) on Day −1, Day 7 and Day 14 after castration. The arrows denote the time (20:00 h) at which the LHRH challenge dose (5 ng/kg) was given.

Discussion

The results of the present study indicate that the rhythmic pulse pattern in LH secretion observed in wethers develops quickly after castration of mature rams and that the pituitary had an inherent capacity to secrete considerable LH. Since it is now confirmed that pulses of LH in sheep are synchronized to episodes of LHRH release into the median eminence (Levine, Pau, Ramirez & Jackson, 1982) and hypothalamo–hypophysial portal blood (Clarke & Cummins, 1982), it can be concluded that the castrate-type pattern of LH results from an increased frequency of LHRH input to the pituitary. Testosterone would therefore appear to prevent the post-castration rise in LH (D'Occhio *et al.*, 1983a, b) by suppressing activity of the presumptive LHRH pulse generator (Knobil, 1981). This may be the principal mode of action of testosterone since a 'castrate-like' pattern of LH secretion was observed in wethers treated with both testosterone and LHRH. The dose–response relationship between serum testosterone concentration and frequency of LH pulses in male sheep provided further evidence that this steroid can modulate the LHRH pulse generator (D'Occhio *et al.*, 1982). Androgen binding sites have been observed in hypothalamic tissue of intact rams (Pelletier & Caraty, 1981).

Patterns of LH secretion are also a function of dosage and frequency of LHRH administration (Santen & Ruby, 1979; Wildt *et al.*, 1981). An intravenous dosage of 5 ng LHRH per kg body weight produces LH pulses in testosterone-implanted wethers which are nearly identical to those that occur naturally in intact rams (D'Occhio *et al.*, 1982). However, in rams (Lincoln, 1978) and steroid-implanted wethers (Schanbacher, 1980a), LH pulse amplitudes vary with dosage of LHRH administered. A single injection of LHRH (5 ng/kg) into the testosterone-implanted wethers of Group 3 resulted in a relatively small amount of LH being released (i.e. the pulse amplitude was approximately 2·8 ng/ml on Days 7 and 14), whereas when this same dose was repeatedly given at 60-min intervals to the testosterone-implanted wethers of Group 4, LH release was increased 2- to

3-fold (i.e. the pulse amplitude was between 6·1 ng/ml (Day 7) and 7·6 ng/ml (Day 14)). In the light of these findings, it would be particularly interesting to monitor LH secretion in testosterone-implanted wethers that were infused with LHRH at a pulse rate which more closely mimicked the pulse-frequency observed in unimplanted castrates.

Although our results suggest that the hypothalamus may be the principal site where testosterone acts to regulate LH secretion, presumably to prevent the occurrence of LHRH pulses, direct modulating effects on the pituitary cannot be excluded. Not only have androgen receptors been identified in anterior pituitary cytosol (Thieulant & Pelletier, 1979; Clarke, Mitchelhill, Zachariah, Findlay & Funder, 1982; Schanbacher, Winters, Rehm & D'Occhio, 1984), but pulse-infusion of intact males with LHRH (Lincoln, 1979; Schanbacher, 1984) leads to dampened LH release when compared to that in castrates or castrates implanted with testosterone. This most probably results from gonadotrophin-induced androgen excess in the intact male. In conclusion, increased activity of the LHRH pulse generator is important for manifestation of the post-castration LH rise in rams; however, the importance of a change in androgen feedback on the pituitary gonadotroph remains uncertain.

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