Effect of time after castration on secretion of LHRH and LH in the ram

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Summary. Hypophysial portal blood and peripheral blood were obtained from conscious, unrestrained rams to measure simultaneously the secretion of LHRH and LH in entire rams and rams which had been castrated for 2–15 days (short-term castration) and for 1–6 months (long-term castration). The apparatus for portal blood collection was surgically implanted using a transnasal trans-sphenoidal approach and, 4–5 days later, portal blood and peripheral blood were collected simultaneously at 10-min intervals for 8–9 h from 15 sheep. LHRH was clearly secreted in pulses in all three physiological conditions, but there were marked differences in pulse frequencies, which averaged 1 pulse/2–4 h in entire rams, 1 pulse/70 min in short-term castrated rams and 1 pulse/36 min in long-term castrated rams. In entire and short-term castrated animals, LH profiles were also clearly pulsatile and each LHRH pulse in hypophysial portal blood was associated with an LH pulse in the peripheral blood. In long-term castrated animals, LH pulses were not as well defined, because of the high basal levels and small pulse amplitudes, and the temporal relationship between LHRH and LH pulses was not always clear. These results demonstrate the pulsatile nature of LHRH secretion under the three physiological conditions and suggest that the irregular LH profiles characteristic of long-term castrates are due to an inability of the pituitary gland to transduce accurately the hypothalamic signal. The very high frequency of the LHRH pulses may be one of the major reasons for this, and is probably also responsible for the high rate of LH secretion in the long-term castrated animal.

Keywords: LHRH; LH; rams; castration; pulsatility

Introduction

In the intact ram, blood concentrations of luteinizing hormone (LH) are characterized by pulses of low amplitude and low frequency (Bolt, 1971; Katongole *et al.*, 1974; Sanford *et al.*, 1974; D'Occhio *et al.*, 1982) and there is general agreement that the frequency of these pulses increases as animals pass from the non-breeding season to the breeding season (Sanford *et al.*, 1974; Lincoln, 1976; Schanbacher & Ford, 1976; D'Occhio *et al.*, 1984). LH secretion by the pituitary gland is controlled by an LHRH pulse generator in the hypothalamus (review: Lincoln *et al.*, 1985). This concept of an LHRH pulse generator was the result of many indirect and some direct demonstrations of the relationship between LHRH and LH secretion. Antibodies to LHRH will block LH pulses in the ram and ewe (Lincoln & Fraser, 1979; Caraty *et al.*, 1984) and the pulsatile pattern of LH secretion can be restored by pulsatile delivery of an LHRH agonist (Caraty *et al.*, 1984; Adams & Adams, 1986). Most conclusive is the demonstration in the hypophysial portal blood of ewes that LHRH is secreted in a pulsatile manner (Caraty *et al.*, 1982; Clarke & Cummins, 1982). Castrated rams have appreciably higher mean LH concentrations (Pelletier, 1968) and show more frequent pulses of LH than do intact rams (Riggs & Malven, 1974; Schanbacher & D'Occhio, 1984) due to the withdrawal of steroid feedback. It seems likely that similar increases in the secretion of LHRH underlie this effect, but the LH profiles of castrated animals are often irregular and the pulses are

difficult to discern (Montgomery *et al.*, 1985). It is possible, therefore, that LHRH is secreted in a non-pulsatile mode under these conditions. In the present report a modified version of the surgical technique developed by Clarke & Cummins (1982, 1985) was used to collect hypophysial portal blood in conscious rams and study the patterns of LHRH secretion in intact, short-term and long-term castrated animals, in an attempt to elucidate the effect of castration on the temporal relationship between LHRH and LH secretion.

Materials and Methods

Animals and surgery. Romanov rams were used for this study. The normal breeding season for this breed extends from September to February at latitude 47°N. The time of the year at which the 22 rams were sampled is shown in Table 1. For surgery anaesthesia was induced with pentothal (10 mg/kg body weight) and maintained with halothane (4–5% in oxygen) at 700 ml/min. Castrations were performed via the scrotal route. Implantation of the apparatus for portal blood collection was performed using the transnasal approach of Clarke & Cummins (1982, 1985). The apparatus was constructed from a 12-gauge needle (the upper cannula) and a 15-gauge needle (the lower cannula) joined with dental acrylic. The tip of the upper 'portal' cannula was situated 2 mm ahead of the tip of the lower 'portal' cannula and the two tips were surrounded by a polyethylene cone (8 mm diameter). The apparatus was introduced into the tunnel in the sphenoid bone and the tip of the upper cannula was pushed directly towards the portal vessels until the polyethylene cone reached the anterior surface of the pituitary gland. The space in the sphenoid bone was then filled with dental acrylic. The bone plate was replaced, skin sutured and the needles were filled with heparinized saline (100 i.u. heparin/ml 0.15 M-NaCl) and capped.

Experimental procedure. The animals were left 4–5 days for recovery after anaesthesia and healing of the tissue damaged during the surgery. The 'portal' cannulas were flushed daily with heparinized saline. Two catheters were then inserted into both jugulars of 2 animals, which were placed side by side in two small pens on the floor, without any other form of restraint and left for 24 h before blood collection. Food and water were given *ad libitum* and the animals could see each other. One of the catheters was used for injections of heparin, and the other was connected to a peristaltic pump and was used for collection of peripheral blood. After an initial dose of heparin (25 000 i.u.), peripheral blood samples were obtained every 10 min. Additional doses of heparin (5000 i.u.) were given every 30 min. After about 2–3 h, the lower 'portal' cannula was connected to a peristaltic pump and continual suction was applied. A needle with a sharp, flattened end was introduced into the upper 'portal' cannula and 4–5 small lesions (2–3 mm deep and distributed in a circle) were made in the hypophysial portal network. Blood from these lesions was collected every 10 min in glass tubes containing $50 \mu\text{l } 10^{-3}$ M-bacitracin (Sigma) and maintained in an ice bath (temperature 0–2°C) until centrifuged. At the end of each collection period a sample of jugular blood was taken. The samples were centrifuged at 0°C and 3000 g for 20 min and the supernatants were frozen (–15°C) until assayed. Care was taken to maintain portal blood samples at about 0°C during all manipulations. The day after blood collection, animals were killed by decapitation, the pituitary gland was removed and the traces of the lesions were examined.

Hormone assays. LHRH, LH and prolactin were measured in duplicate aliquants of plasma by specific radioimmunoassays described previously (Caraty *et al.*, 1980; Pelletier *et al.*, 1982; Kann, 1971). The sensitivities of the assays were 4 pg synthetic LHRH/ml (UCB-Bioproducts, Brussels, Belgium), 0.5 ng LH/ml (CNRS-LH-M3) and 0.3 ng prolactin/ml (NIH-PS6). The intra-assay and inter-assay coefficients of variation were 8.0 and 12.6%, 10 and 12% and 4 and 6% for LHRH, LH and prolactin, respectively. Determinations of LHRH concentrations in 0.5 ml samples of portal plasma were made after extraction with 4 ml methanol. After shaking, the plasma and methanol were left for 12 h at –15°C and centrifuged at 3000 g. The supernatants were then dried using a Speed Vac concentrator (Savant Instrument, NY, U.S.A.) and re-dissolved in 0.25 ml buffer. Known quantities of synthetic LHRH were added to peripheral plasma samples and submitted to similar extraction procedures to allow calculation of recovery (usually 70–80%). The plasma concentrations were corrected for these losses.

Analysis of the data. LHRH pulses were defined when the value of a given sample exceeded that of the previous sample by at least 3 standard deviations. When the LHRH baseline was below the detection limit of the assay, pulses were defined as having occurred when assay values were greater than 3 times the assay sensitivities. LH pulses were defined as described by Goodman & Karsch (1980). Estimation of 95% confidence limits of samples were calculated using the NIH Radioimmunoassay computer program of D. Rodbard (NICHD, NIH, Bethesda, MD, U.S.A.).

Results

Portal blood sampling

Out of 22 sheep used for the experiment, portal blood was successfully collected from 15. For 2 sheep, the surgery was not successful and for the others portal blood flow was erratic and blood

Fig. 1. LHRH and LH profiles from (a) 3 entire rams, (b) 3 short-term castrated rams and (c) 3 long-term castrated rams. Pulses of LHRH or LH are indicated by black arrows. White triangles indicate the time when the portal blood network was lesioned.

Table 1. LHRH interpulse interval and pulse amplitudes in the hypophysial portal blood of entire, short-term castrated and long-term castrated rams

Group	Sheep no.	Time of year	Delay after castration	LHRH interpulse intervals (min)*	Mean $\pm$ s.e.m. LHRH pulse amplitude (pg/ml)†
Entire rams	12	Aug.	—	160 (1)	32.5 (2)
	13	Aug.	—	100 (1)	23.5 (2)
	14	Aug.	—	—‡	60 (1)
	15	July	—	—‡	35 (1)
Short-term castrated rams	1	June	2 days	80 (3)	24.2 $\pm$ 2.8 (4)
	2	Feb.	4 days	80 (3)	86.2 $\pm$ 10.9 (4)
	3	Jan.	4 days	50 (2)	34.3 (3)
	4	Sept.	4 days	71 (3)	34.0 $\pm$ 2.5 (4)
	5	June	15 days	70 (4)	26.4 $\pm$ 3.5 (5)
Long-term castrated rams	6	Aug.	1 month	37 (4)	21.4 $\pm$ 1.4 (5)
	7	May	3 months	35 (8)	32.8 $\pm$ 1.9 (9)
	8	Jan.	4 months	33 (7)	35.7 $\pm$ 4.9 (8)
	9	Aug.	5 months	40 (6)	27.1 $\pm$ 2.2 (7)
	10	March	5 months	38 (10)	16.2 $\pm$ 1.9 (11)
	11	March	6 months	33 (8)	17.2 $\pm$ 1.8 (9)

*No. of intervals observed.

†No. of LHRH pulses observed.

‡1 pulse only observed.

collection was abandoned. The place and shape of the lesions differed between animals due to variations in the shape of the pituitary gland. When the lesions were higher in the body of the pituitary gland, large portal vessels (100–150 μ m diameter) were cut and when the lesions were lower, smaller vessels were cut (50 μ m diameter). As the blood from the lesions was a mixture of portal blood and blood from the pituitary, the proportion of portal blood was not the same between animals and LHRH pulse amplitude could not be compared between sheep.

Entire rams

Figure 1 shows LHRH and LH secretion in 3 rams during the time of the year when there is a seasonal increase in LH pulse frequency (before the breeding season). The frequency ranged from one pulse/2 h (for Ram 13) to one pulse/4 h (for Ram 14) and was similar to the frequency measured 1 week previously, before surgery when the animals were in their usual environment (data not shown). During the period of portal blood collection, only 6 LHRH pulses were detected in the 4 animals and each of these pulses was accompanied by an LH pulse. No LH pulse was observed without a coincident LHRH pulse. The LHRH pulse amplitudes for these animals ranged from 20 to 60 pg/ml (Table 1). Plasma prolactin concentrations (mean $\pm$ s.e.m.) were 343 $\pm$ 72 ng/ml for the 4 animals sampled in their usual environment 1 week before surgery and 383 $\pm$ 38 ng/ml when they were sampled during portal blood collection.

Short-term castration

Clear patterns of LH secretion were observed for all short-term castrated rams and profiles from 3 of them are shown in Fig. 1(b). The frequency of LH pulses varied from about 1 pulse/60 min to 1 pulse/90 min and pulse amplitudes ranged from 5 to 10 ng/ml. LHRH interpulse intervals were similar for all animals in this group with a mean of 70.2 min. Mean LHRH pulse amplitudes were between 20 and 35 pg/ml, except for Ram 2 (86.2 pg/ml) in which the lesions in

the portal vessels were near the median eminence. Again, for all animals, LH pulses were always synchronized with an LHRH pulse and each LHRH pulse was accompanied by an LH pulse.

Long-term castration

Long-term castrated rams were characterized by a very high LHRH pulse frequency (mean pulse interval 36.0 min) and amplitude (15–35 pg/ml). LH secretion in these animals was characterized by a higher basal level and smaller pulse amplitudes compared with the intact or short-term castrated rams. The data in Fig. 1(c) from Rams 8 and 9 show that the LH values were always higher than 5 ng/ml and always less than 11 ng/ml. In Ram 7 LH pulse amplitude was greatly reduced after the portal vessels were lesioned. For all animals each LH pulse was synchronous with an LHRH pulse, but LHRH pulses were not always associated with LH pulses.

Discussion

The present report is the first demonstration of LHRH release in the hypophysial portal blood of conscious, unrestrained rams. Portal blood was collected successfully over many hours and in 70% of the animals used. The absence of variations of plasma prolactin concentrations and the normal profiles of LH observed may indicate that the conditions of blood sampling are suitable for these investigations.

The results indicate, for all three physiological conditions studied, the pulsatile nature of the LHRH secretion in rams, as shown previously in ewes (Caraty *et al.*, 1982; Clarke & Cummins, 1982). This has also been observed in other species, including rats (Sarkar & Fink, 1980) and monkeys (Carmel *et al.*, 1976). LHRH pulses were always clearly greater than assay sensitivity, with peak values ranging from 10 to 100 pg/ml, but they were often defined by only one high value between two undetectable values. This indicates that LHRH pulses are very brief secretory events, and that future studies should use more frequent sampling.

LHRH profiles in hypophysial portal blood showed a strong temporal correlation with the LH profile in peripheral blood in entire and short-term castrated animals, suggesting a simple, direct cause–effect relationship. In long-term castrated animals LHRH secretion was also pulsatile but the frequency was higher than in intact or short-term castrated animals. In addition, the LH pulses were not clearly defined in these animals, due to high basal levels and small pulse amplitudes, probably because of a slower rate of disappearance from the circulation (Montgomery *et al.*, 1984). In these rams, individual LHRH pulses were not always associated with a ‘significant’ increase in LH concentration peripheral blood. This lack of correlation in long-term castrated animals may be partly explained by the effect of high LHRH pulse frequencies, coupled with an increase in the half-life of LH, leading to an increase in basal level. This high basal level effectively reduces the LH pulse amplitude and does not allow observation of clear, easily defined pulses. It could be argued that the interruption of part of the portal circulation in the pituitary gland would decrease the strength of the hypothalamic signal, thereby reducing LH pulse amplitude, but, as shown by Ram 8, LH secretion was not clearly pulsatile before the portal network was lesioned.

The range of LHRH peak heights varied between 10 and 100 pg/ml. This agreed with the indirect estimates of LHRH concentrations in portal blood of 60–200 pg/ml (Lincoln, 1976) needed to induce a normal LH pulse. These peak heights are much lower than the range of 2–1500 pg/ml reported by Clarke & Cummins (1985) for ovariectomized ewes. This difference is probably due more to the site of the lesions than to any other factor. The total hypothalamic content of LHRH varies from 40 to 100 ng for ovariectomized and oestrous ewes (Wheaton, 1979) and from 20 to 100 ng for castrated and entire rams (Caraty, 1983), and so it appears likely that values of 1000 pg/ml in portal blood could only be obtained by lesions placed in the median eminence. Moreover, from a technical viewpoint, this difference in positioning of the lesions may explain why, under the conditions of our experiment, there was no leakage of cerebrospinal fluid and the portal

blood volume remained constant in 10-min fractions throughout the period of sampling. On the other hand, there is difficulty in standardizing the site and magnitude of the lesions in the hypophysial portal network, and this leads to variation between animals in the number and size of portal vessels lesioned, and in LHRH pulse amplitudes.

Nevertheless, in spite of the limitation of this technique, especially the inability to compare LHRH pulse amplitudes between sheep, the narrow range of LHRH peak values measured in this experiment suggest that the major control of the hypothalamus on the pituitary is due to the variation of the LHRH pulse frequency. The high frequency of LHRH pulses observed in long-term castrated animals may be partly responsible for the increase of basal concentrations of LH associated with a decrease of the pulse amplitude.

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