Pituitary responsiveness to synthetic LH-RH and pituitary LH content at various reproductive stages in the sheep

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Summary. In sheep the basal concentration of LH in jugular vein plasma was significantly higher during the first 50 days of gestation than in late pregnancy or at parturition. The pituitary response to a single i.v. injection of 200 µg synthetic LH-RH was determined at different stages of gestation and compared with that of anoestrous and cyclic sheep. Pituitary response to LH-RH decreased progressively with advancing gestation: by 56 days after mating the response had declined to 35% and by parturition to 14% of the value in anoestrous sheep. The pituitary response to LH-RH increased after parturition and the pattern of recovery differed in non-lactating and lactating sheep. By 63 days *post partum* the response to LH-RH in non-lactating and lactating animals had returned to values similar to those in sheep during anoestrus and sheep during the luteal phase of the oestrous cycle.

A decrease in pituitary responsiveness during pregnancy was associated with a decrease in pituitary content of LH. The quantity of LH released in response to a standard injection of LH-RH was linearly related to pituitary LH content.

Introduction

In sheep the preovulatory surge of LH is stimulated by a positive feedback effect of oestrogen. The infusion or the injection of oestradiol-17β into anoestrous or ovariectomized animals causes a sharp rise in plasma LH after a latent period of about 12 h (Goding *et al.*, 1969; Pelletier & Signoret, 1969; Radford, Wheatley & Wallace, 1969; Scaramuzzi, Tillson, Thorneycroft & Caldwell, 1971). Pituitary responsiveness, however, is influenced by the levels of ovarian hormones. Treatment with oestradiol-17β or LH-RH has a greater effect on LH release at the onset of behavioural oestrus than during the luteal phase (Bolt, Kelly & Hawk, 1971; Cumming, Brown, Blockey & Goding, 1971; Foster & Crighton, 1976). In the present study we have looked for possible changes in pituitary responsiveness to LH-RH during the reproductive cycle and investigated the relationship between responsiveness and the concentration of pituitary LH. A further aim was to examine the influence of lactation on pituitary sensitivity to LH-RH. Preliminary reports of this work have been published previously (Jenkin & Heap, 1974; Jenkin, 1975).

Materials and Methods

Animals

Multiparous Clun Forest or Finnish Landrace ewes were used in all experiments except where stated. The day of oestrus (Day 0) and the time of gestation were determined from daily records of marking by a vasectomized or intact ram fitted with a 'Sire-Sine' harness and crayon. Sheep were brought in from a paddock adjacent to the animal house and placed in individual pens at least 24 h before an experiment. They were allowed free access to food and water throughout.

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Experiments with LH-RH were repeated on the same animal at intervals of not less than 16 days. Details of the various groups are given in the Tables. Sheep which were not mated during the normal breeding season and were studied during seasonal anoestrus are described as anoestrous. Animals that became pregnant during the normal breeding season and were used for experiments after parturition and during seasonal anoestrus are described as *post partum* (lactating or non-lactating). Lambs were kept with lactating ewes and not separated from them during the experimental period. Pregnant animals were brought into the animal house at least 4 days before the expected date of parturition and remained indoors until 2 weeks *post partum*.

Plasma LH

The concentration of LH was measured in peripheral plasma to determine the basal value at various reproductive stages to compare results in anoestrous and cyclic ewes with those in pregnant, parturient and post-partum animals. Particular attention was given to animals near term to determine the effect of a sharp rise in plasma unconjugated oestrogen level which occurs during the last 48 h of gestation. The post-partum ewes were allocated to groups according to whether they were lactating (with lambs) or not lactating (lambs removed at birth). Blood samples were taken by jugular venepuncture. All samples were centrifuged within 15 min of collection and plasma was stored at -15°C .

Treatment with LH-RH and blood sampling

Sheep were given 200 μg of the synthetic decapeptide, LH-RH (Hoechst Pharmaceuticals), through a polyvinyl catheter (Portex NT3, SH90, 1.4 mm i.d., 2.0 mm o.d.) inserted on the day before an experiment 10 cm into a jugular vein by the method of Seldinger (1953). In preliminary experiments a dose of 200 μg LH-RH was found to produce a maximum plasma LH concentration in anoestrous ewes similar to that found at oestrus. Blood samples (2.5 ml) were taken from the same catheter into heparinized plastic syringes every 10–15 min for 30 min before and 120 min after injection, and every 20–30 min for a further 200 min.

Pituitary responsiveness to LH-RH

The responsiveness of the pituitary to LH-RH was assessed by measuring the maximum plasma concentration of LH after LH-RH injection (maximum peak height), the time taken to reach this value (time to maximum peak height), and the area under the curve of LH release from 0–320 min (area under curve) calculated by the trapezoidal rule (Abramowitz & Stegun, 1968). Plasma LH values returned to preinjection levels within 320 min after injection so that the area, expressed in (ng/ml) τ , was proportional to the total amount of LH released (τ represents the period of the experiment from 0–320 min). The injection of 250 μg TRH in 2 ml saline or of 2 ml saline alone as control procedures did not increase LH concentration in peripheral plasma.

Pituitary content of LH

Sheep were anaesthetized with halothane (Fluothane: I.C.I. Pharmaceuticals) and exsanguinated. The anterior pituitary was removed, weighed and immediately frozen in liquid nitrogen and stored at -15°C . Before assay, the pituitary was thawed at 4°C , homogenized in 8 ml cold 0.1 M-ammonium sulphate, pH 5.5 (Dick & Gay, 1969), using a polytetrafluoroethylene mortar, and centrifuged at 4°C for 30 min at 17,000 g. The supernatant was removed and assayed for LH and results obtained from at least two dilutions were used in subsequent calculations. The results were linear over the range of dilutions used (1 in 25 to 1 in 1000, $y = 13.74 - 0.02x$, $r = 0.66$, $P < 0.05$, $n = 12$). Storage of supernatants for up to 1 year at -15°C did not affect the quantity of immunoreactive LH present.

Radioimmunoassay of LH

The radioimmunoassay was similar to the solid-phase method described by Goding *et al.* (1969).

The antiserum (LH 3/5) was raised in adult New Zealand White rabbits injected with 2 mg NIH-LH-S16 emulsified in Freund's complete adjuvant at 0 and 21 days (i.m.) and 77 days (s.c.) and was obtained 79 days after the first injection. It was stored at -15°C as a 1 in 100 solution in 0.05 M-carbonate buffer, pH 9.2, and further diluted to a titre of 1 in 10,000 before use.

Purified ovine LH (LER-1056-C2) was iodinated by the chloramine-T method of Greenwood, Hunter & Glover (1963) using a remotely operated system (Henville & Jenkin, 1973). Iodinated LH was separated from free iodine on a column of Sephadex G25 fine, 5×1 cm. The ^{125}I -labelled LH fraction was added to a column of Whatman CF11 cellulose, 2×0.5 cm, washed with 20 ml 0.05 M-phosphate buffer, pH 7.5, to remove unreacted ^{125}I and damaged or fragmented ^{125}I -labelled LH (Hunter, Willoughby & Strong, 1968), and undamaged LH was eluted with 0.05 M-phosphate buffer containing 5% (w/v) bovine serum albumin. ^{125}I -labelled LH was stored at 4°C for up to 3 weeks without loss of immunoreactivity. In each assay ^{125}I -labelled LH was diluted to 6.0×10^4 d/min/0.1 ml with 0.01 M-phosphate buffer containing 0.15% NaCl, 0.1% bovine serum albumin and 0.01% merthiolate, pH 7.4 (Buffer A). NIH-LH-S17 was dissolved in Buffer A to provide assay standards over the range 0.097–50 ng/0.8 ml. Results were expressed in terms of NIH-LH-S17 which has a biological potency of 1.01 NIH-LH-S1.

Assays were carried out in polystyrene tubes (Luckham LP3) prepared by filling with 1 ml LH antiserum at 1 in 10,000 dilution. After standing overnight in a saturated atmosphere at room temperature, the antiserum was removed by aspiration and stored at 4°C for further use. Tubes were washed twice with 0.9% NaCl and once with Buffer A. Coated tubes could be stored at 4°C for several weeks before assay.

Plasma samples (0.2 ml and 0.8 ml Buffer A) and standards (0.8 ml and 0.2 ml plasma from a hypophysectomized goat) were added to assay tubes and, after mixing, were incubated for 8 h at room temperature in a humid atmosphere. Pituitary extracts were diluted and assayed in Buffer A alone. Approximately 6.0×10^4 d/min ^{125}I -labelled LH was added in 0.1 ml Buffer A and the tubes incubated for a further 18–24 h at 4°C . The contents were then aspirated, the tubes washed twice with tap water, and the bound radioactivity measured. The calibration curve was fitted by computer to an asymptotic decay curve (Walters, 1974) and the sample concentration calculated as the mean of duplicates or triplicates (ng/ml) by interpolation (Broad, Challis, Heap, Jenkin & Walters, 1972).

The antiserum showed no significant cross-reaction with NIH-FSH-S8, NIH-GH-S10 or NIH-P-S7 over the range of 0.1 to 250 ng/ml. TSH showed an apparent cross-reaction of 10% at 50% binding of ^{125}I -labelled LH which was due to contamination of the TSH preparation with immunoreactive LH (Jenkin, 1975). The measurement of known amounts of NIH-LH-S17 added to Buffer A or to plasma from a hypophysectomized goat plotted against the amount added gave regression lines that were highly significant ($y = 1.20x$, $r = 0.998$, $P < 0.001$, $n = 39$; and $y = 1.01x$, $r = 0.957$, $P < 0.001$, $n = 46$, respectively). The lowest concentration of LH distinguishable from zero was 0.75 ± 0.20 (S.E.M.) ng/ml. The determination of a standard amount of LH in buffer (7.1 ng/ml) or plasma (5.1 ng/ml) gave a standard deviation of ± 0.8 ng/ml (5 assays) and ± 1.5 ng/ml (31 assays) respectively.

Results

Plasma LH concentrations

These are shown in Table 1 for sheep at various stages of the reproductive cycle. The concentrations during seasonal anoestrus and in the luteal phase of the oestrous cycle were low. During gestation the plasma LH concentration was highest in the first 7 weeks, and was significantly lower between 16 and 21 weeks, and was even lower by the time of parturition. Within 7 weeks after parturition plasma LH values were significantly higher than those at term and were similar to those in early pregnancy. There was no significant difference in lactating or non-lactating anoestrous sheep at different times after parturition.

Table 1. Mean $\pm$ S.E.M. plasma LH concentrations in the jugular vein of sheep

Reproductive state of ewe	Group	Time of sampling (weeks <i>post coitum</i>)	No. of samples	No. of animals	LH conc. (ng/ml)	Statistical significance†
Anoestrous	A	Anoestrous period (April–August)	27	11	1.8 $\pm$ 0.4	>F*** >B, >E*
Cyclic	B	Days 5–13 of oestrous cycle	46	15	1.0 $\pm$ 0.2	<A, <C*
Pregnant	C & D	2–7	23	4	1.7 $\pm$ 0.4	>F*** >B, >E*
	E	10–16	23	4	1.2 $\pm$ 0.3	N.S.
	F	16–20	99	15	1.0 $\pm$ 0.1	<A** <C*
Parturient	G	20–21 (near term)‡	62	15	0.7 $\pm$ 0.1	<A, <C, <I*** <J**
<i>Post partum</i> (lactating)	H	1–2	21	15	0.9 $\pm$ 0.3	N.S.
	I	3–5	8	6	0.9 $\pm$ 0.4	N.S.
	J	5–7	6	6	1.7 $\pm$ 0.8	>F***
	K	8–10	8	6	1.5 $\pm$ 0.4	>F**
<i>Post partum</i> (not lactating)	L	1–2	6	3	0.4 $\pm$ 0.2	Not significantly different from Groups H, I, J or K
	M	3–5	11	6	0.6 $\pm$ 0.1	
	N	5–7	11	6	1.6 $\pm$ 0.4	
	O	8–10	17	7	1.7 $\pm$ 0.5	

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, N.S. = no significant difference ($P > 0.05$).

† Measured by multifactorial analysis of variance.

‡ 3 days before to 3 days after parturition.

Table 2. The release of LH (mean $\pm$ S.E.M.) after the injection of LH-RH in sheep

Reproductive state	Group	Time of LH-RH injections (weeks <i>post coitum</i>)	No. of animals	Maximum LH conc. (ng/ml)	Area under the curve of LH release (ng/ml)r	Total peak area as % of anoestrous value
Anoestrous	A	Anoestrous period (April–August)	11	63.2 $\pm$ 7.7	8030 $\pm$ 897	100
Cyclic	B	Days 5–13 of oestrous cycle	15	63.8 $\pm$ 12.0	6885 $\pm$ 1232	85.7
Pregnant	C	2–4	4	49.6 $\pm$ 14.6	5764 $\pm$ 1919	71.8
	D	6–9	4	19.0 $\pm$ 4.2	2832 $\pm$ 796	35.3
	E	10–13	6	14.1 $\pm$ 2.4	1836 $\pm$ 411	22.9
	F	15–20	9	14.0 $\pm$ 2.9	2131 $\pm$ 508	26.5
Parturient*	G	20–21	6	8.8 $\pm$ 1.6	1159 $\pm$ 131	14.4
<i>Post partum</i> (lactating)	H	1–2	7	29.6 $\pm$ 7.5	3959 $\pm$ 965	49.3
	I	3–5	7	30.6 $\pm$ 5.2	3997 $\pm$ 714	49.8
	J	5–7	6	38.4 $\pm$ 9.4	5212 $\pm$ 1130	64.9
	K	8–10	6	60.3 $\pm$ 18.3	7488 $\pm$ 1436	93.3
<i>Post partum</i> (not lactating)	L	1–2	3	30.2 $\pm$ 10.3	4212 $\pm$ 1488	52.5
	M	3–5	6	76.8 $\pm$ 21.2	8947 $\pm$ 1087	111.4
	N	5–7	7	53.7 $\pm$ 4.2	7939 $\pm$ 559	98.9
	O	8–10	7	61.1 $\pm$ 14.1	7383 $\pm$ 1480	91.9

* Days 0–5 after parturition.

Pituitary responsiveness to LH-RH

The responsiveness of the pituitary to LH-RH was determined in 102 experiments on 37 sheep at various times of the reproductive cycle (Table 2). The time from LH-RH injection to the maximum LH value found in plasma (time to maximum peak height) did not differ significantly at any time during the reproductive cycle, the mean value for all groups being 100 ± 2 (S.E.M.) min. The magnitude of the response, however, showed a marked change as determined by the highest concentration of LH observed (ng/ml plasma) or by the area under the curve of LH release. Both estimations were closely correlated ($r = 0.96$, $P < 0.001$, $y = 0.0088x - 4.43$, $n = 102$) and pituitary responsiveness is subsequently described in terms of the latter estimation.

The area under the curve of LH release after LH-RH treatment was high in sheep during anoestrus, in the luteal phase of the oestrous cycle, and during the first 4 weeks of gestation. As pregnancy progressed the pituitary responsiveness to LH-RH declined to 23% of that in anoestrous animals, whereas at parturition it was only 14.4% of the value at anoestrus.

In contrast to the gradual decrease in pituitary responsiveness during pregnancy, recovery after parturition was rapid. By 14 days *post partum* pituitary LH release in response to LH-RH in lactating and non-lactating sheep was about half that found in anoestrous animals. Thereafter the pattern of recovery in lactating sheep was different from that in non-lactating animals. By 3–5 weeks after parturition LH release was 50% and 111% in lactating and non-lactating animals, respectively, of the mean value found in anoestrous animals. By 8–10 weeks *post partum* LH release was similar to anoestrous values in all post-parturient sheep.

Table 3. Mean $\pm$ S.E.M. pituitary LH concentration and content in sheep at various reproductive stages

Reproductive state	Group	Time of collection (weeks <i>post coitum</i>)	No. of animals	Pituitary conc. (μ g/g tissue)	Pituitary content (μ g)	Statistical significance of pituitary conc.
Anoestrous	A	Anoestrous period (April–August)	7	1135.0 ± 302.3	754.5 ± 150.2	>F*, <C**
Cyclic	B	Days 5–13 of oestrous cycle	5	1354.5 ± 207.5	874.0 ± 121.8	<C**, >F**, >H* >E**, >G**
Pregnant	C	2–4	3†	$2890.4 \pm 282.1†$	735 ± 45.5	>A**, >E***, >H***, >K*** >B**, >F***, >I*** >D***, >G***, >J***
	D	6–9	7	872.9 ± 175.7	535.0 ± 116.6	<C***, >G* >F*
	E	10–16	8	505.7 ± 107.5	304.0 ± 64.9	<B**, <J* <C***
	F	16–20	6	174.8 ± 125.7	183.1 ± 64.9	<A*, <D*, <J** <B**, <I*, <K* <C***
	G	20–21	3	120.8 ± 56.0	111.8 ± 17.7	<B**, <D*, <J* <C***, <I**, <K**
<i>Post partum</i> (lactating)	H	1–2	4	511.5 ± 145.4	441.1 ± 187.5	<B* <C***
	I	3–5	4	819.2 ± 117.1	713.6 ± 147.7	<C***, >G** >F*
	J	5–7	4	1127.0 ± 217.7	814.9 ± 147.0	<C***, >F** >E*, >G*
	K	8–10	3	846.1 ± 89.4	1017.2 ± 53.5	N.S.
<i>Post partum</i> (not lactating)	M	3–5	4	1082.8 ± 57.5	843.0 ± 172.5	} Not significantly different from Groups H, I, J or K
	N	5–7	4	995.0 ± 45.8	987.0 ± 126.1	
	O	8–10	4	1041.0 ± 199.7	909.2 ± 162.6	

Student's *t* test, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; N.S. = no significant difference ($P > 0.05$).

† Pituitaries from sheep in their first pregnancy; all other animals were multiparous.

‡ Day of parturition.

Pituitary concentration and content of LH

The concentration of LH in the anterior pituitary was high in anoestrous, cyclic and early pregnant sheep (Table 3). There was a linear correlation between the mean value of pituitary content of LH and the mean area under the curve of LH in response to LH-RH (Text-fig. 1; $r = 0.898$; $P < 0.001$). The slope of the linear regression was 7.967, and the intercept was not significantly different from zero.

Text-fig. 1. Linear regression of pituitary LH content and LH released (area under the curve) during a 320 min period after a single i.v. injection of 200 μg LH-RH in sheep. The pituitary content of LH was measured at various times during the reproductive cycle and the mean values are given in Table 3; the mean values of pituitary response to LH-RH are given in Table 2.

Discussion

Pituitary responsiveness to a standard intravenous injection of synthetic LH-RH decreased progressively throughout gestation in sheep and by parturition it was only about 14% of that in anoestrous animals. These findings confirm and extend those of other workers (Chamley, Findlay, Cumming, Buckmaster & Goding, 1974a; Chamley, Findlay, Jonas, Cumming & Goding, 1974b). There was a parallel reduction of pituitary LH content which was probably related to the high plasma progesterone concentration (Jenkin & Heap, 1974). The decrease in response to LH-RH observed during pregnancy was not related to daylength since non-pregnant sheep which were in seasonal anoestrus and treated at the same time of year showed a high response to LH-RH (see Table 2). It is also unlikely that the decreased pituitary response observed could be an artefact resulting from an increased systemic clearance of the decapeptide during pregnancy because a similar pattern of decreasing responsiveness was noted when the decapeptide was injected directly into a lateral ventricle of the brain (G. Jenkin & K. Johnson, unpublished observations), or infused into a carotid artery (Chamley *et al.*, 1974a) in conscious animals.

Pituitary responsiveness in anoestrous sheep may be enhanced by LH-RH itself (auto-sensitization) since the amount of LH released after a single injection of the synthetic decapeptide is less than when it is injected repeatedly at 90-min intervals (Crichton, Foster, Haresign & Scott, 1975) or infused continuously for 8 h (Symons, Cunningham & Saba, 1974). In contrast, a single injection of LH-RH results in an acute loss of responsiveness which lasts for 24–28 h (Rippel, Johnson & White, 1974). This acute effect does not result from a depletion of pituitary LH stores which remain high even after repeated LH-RH injections. The chronic decrease in pituitary responsiveness found in gestation seems to represent a different phenomenon in which the mean pituitary response is directly related to the mean pituitary content of LH. If the LH released is derived entirely from pituitary stores, the gradual decrease in response during pregnancy may be due simply to a decreased content. If the LH released after an LH-RH injection were wholly or in part synthesized *de novo*, then the pituitary content would reflect the synthetic capacity at that time.

A question that arises from these findings is whether LH-RH also regulates the synthesis of pituitary LH in sheep, and whether the decline in pituitary LH content in gestation is associated with a chronic decrease in the sensitivity of the pituitary cells to LH-RH or in the production of LH-RH by the hypothalamus. The decrease in pituitary responsiveness we have observed may be due to a depletion of LH-RH receptors in the pituitary. A depletion of receptors may arise from a decrease in the gonadotroph population of the anterior pituitary which is known to occur during pregnancy in some species (Cameron, Foster & Allanson, 1966; Allanson, Cameron & Foster, 1966). In sheep, however, alterations in the gonadotroph population have not so far been conclusively demonstrated (Warbritton & McKenzie, 1937). If chronic desensitization to LH-RH is related to changes in the number of gonadotrophs it is to be noted that their replenishment after parturition is much more rapid than their depletion during gestation.

The sharp rise in oestrogen secretion which occurs during the last 48 h of gestation fails to elicit LH release at parturition in contrast to the positive feedback effect of oestradiol-17 β during the normal oestrous cycle. This lack of LH release presumably derives from the depletion of pituitary LH content and would explain why ovulation rarely, if ever, occurs in the sheep immediately *post partum*. In women, a temporary decrease in pituitary response to LH-RH *post partum* has also been reported (LeMaire, Shapiro, Riggall & Yang, 1974), probably arising from the negative feedback effect of pregnancy hormones, as in sheep.

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