Effect of prolonged infusion of small doses of LH-RH on the release of LH and ovulation in ewes during mid-anoestrus

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Domański & Kochman (1968) showed that sheep were more responsive to infusion of purified hypothalamic extract into the adenohypophysis in late anoestrus than in mid-anoestrus. Natural (Schally, Redding, Matsuo & Arimura, 1972) and synthetic (Matsuo, Arimura, Nair & Schally, 1971) LH-RH have been used to induce ovulation in many species, including sheep (Reeves *et al.*, 1972). Administration of a single intravenous injection of 150 µg synthetic LH-RH to seasonally anoestrous ewes induces LH release in all injected animals, but the LH peak is much smaller than that observed at oestrus and luteal function, assessed by peripheral progesterone levels, is absent in most animals (Crichton, Foster, Haresign & Scott, 1975). When the LH-RH was given as five injections the LH peak resembled that at natural ovulation but plasma progesterone levels were still low. Shareha, Ward & Birchall (1976) gave much smaller doses of LH-RH (25 µg/day in continuous infusions for 2 days) to anoestrous ewes but the results were again unsatisfactory.

Jeffcoate, Greenwood & Holland (1974) showed that the rate of disappearance of LH-RH from the circulation in normal human subjects is represented as a double exponential with half-lives of the two components being 5.3 and 27.4 min. Kochman, Kerdelhué, Zor & Jutisz (1975) reported that degradation of the hormone, caused by polypeptidases in the hypothalamic and pituitary tissues, occurs within 30 min. We therefore investigated the effects of prolonged infusions of small doses of LH-RH into anoestrous ewes.

Methods

Merino sheep, 15–18 months old, in mid-anoestrus were given an infusion of LH-RH into the internal maxillary artery for one 6-h period a day for 6 consecutive days. The daily infusion dose was 3.0 µg synthetic LH-RH. Before infusion each ewe was laparotomized; their ovaries were small, pale and contained small Graafian follicles which were only 2–3 mm in diameter.

Blood samples were withdrawn through an indwelling jugular vein cannula at 4-h intervals throughout the 6-day period of infusions. Plasma samples were stored at –20°C until assay for LH concentration by a double-antibody radioimmunoassay (Stupnicki & Madej, 1976). The sensitivity of the assay was 0.03 ng/ml and the within-assay error was 10% at 1 ng/ml and 4% at 5 ng/ml. All the samples were measured in duplicate.

Blood samples for progesterone determinations were taken from 4 treated ewes at 4, 7 and 9 days after the induced ovulation and the results were compared with the plasma progesterone concentration during the luteal phase of 5 normally cyclic sheep. Progesterone was measured by the competitive protein-binding technique described by Holtan, Nett & Estergreen (1975). The sensitivity of the assay (in our tests) was 0.1 ng/ml; the coefficient of variation within an assay was 9–15% and between assays was 10.5–17.2%. Laparotomy was undertaken 2 days after the end of infusion and the ovaries and uteri of all ewes were examined.

Results and discussion

Inspection of the ovaries and the occurrence of plasma LH elevations indicated that 8 out of 11 of the treated ewes had ovulated on the 4th, 5th or 6th day of LH-RH infusion (Text-figs 1 and 2).

Text-fig. 1. Peripheral plasma LH concentration in 5 infused ewes, 4 of which (×—× No. 1658; ---- No. 257; No. 896; — No. 884) ovulated and showed very well developed corpora lutea, while for one (— No. 399) there was no observed reaction of the ovaries.

Text-fig. 2. Plasma LH concentrations in (a) Ewe 343, (b) Ewe 481, (c) Ewe 439 and (d) Ewe 285. All the ewes ovulated and showed well developed CL: the progesterone concentrations (ng/ml) for each animal on Days 4, 7 and 9 after ovulation were: (a) 0.61, 1.05, 1.12; (b) 0.35, 1.51, 1.63; (c) 0.53, 1.58, 1.20; (d) 0.37, 1.45, 1.50.

Two ewes did not respond, and one (No. 399) had only a small elevation of LH and no ovarian response. The ovaries of the ovulating animals were 2–3 times larger than before treatment and the corpora lutea seemed as large as those known to be functional. The uteri were also enlarged and appeared similar to the uteri of animals after ovulation at a normal oestrus.

The basal plasma LH concentrations during the first 3 days of infusion were lower (0.92 ± 0.032 (S.E.M.) ng/ml) than those during the next 3 days (2.47 ± 0.059 ng/ml, $P < 0.05$; days on which

Table 1. The mean $\pm$ S.E.M. concentrations of progesterone in the peripheral plasma of 5 normally cyclic ewes (Day 0 = day of ovulation))

Day	2	3	4	5	6	7	8
Progesterone conc. (ng/ml)	0.26 ± 0.035	0.41 ± 0.02	0.65 ± 0.07	0.81 ± 0.045	0.89 ± 0.05	1.28 ± 0.10	1.20 ± 0.12
Day	9	10	11	12	13	14	
Progesterone conc. (ng/ml)	1.21 ± 0.15	1.37 ± 0.25	1.20 ± 0.21	1.20 ± 0.25	1.15 ± 0.26	0.41 ± 0.03	

preovulatory peaks were seen were excluded from statistical analysis). The preovulatory peaks of LH in the 8 ovulating animals occurred on the 4th, 5th or 6th day of infusion; their values (34.72 ± 4.38 ng/ml) were much smaller than those observed at pro-oestrus in cyclic ewes (97.92 ± 11.4 ng/ml, $P < 0.01$).

The plasma progesterone levels after the induced ovulation (see Text-fig. 2) were very similar to those found in cyclic ewes in this study (Table 1) and by Thorburn, Bassett & Smith (1969) who used a similar method.

These results indicate that ovulation can be induced in sheep without the use of a very high dose of neurohormone. This is in accordance with low concentrations of LH-RH which have been found in hypophysial portal blood of rats during the oestrous cycle, i.e. < 12 pg/ml during pro-oestrus and 58 ± 7.3 pg/ml during oestrus (Eskay, Oliver, Ben-Jonathan & Porter, 1975).

It has been suggested that LH-RH has a priming effect on the responsiveness of the anterior pituitary gland to subsequent infusions of the neurohormone (Fink, Aiyer, Jamieson & Chiappa, 1975). Such an effect could be involved in anoestrous sheep, because in our experiments the pre-ovulatory surge of LH did not occur until the 4th day of LH-RH infusion. It is also possible that the higher basal plasma concentrations of LH during the last 3 days of infusion stimulate the gonadal hormone secretion which in turn may modulate the responsiveness of the pituitary gland and the ovaries themselves.

The ability of the sheep ovary to respond and ovulate in late anoestrus or during the breeding season is probably due to the release of hypothalamic neuro- and gonadotrophic hormones at a higher tonic rate than during mid-anoestrus. Administration of LH-RH for 9.5 h, as in the experiments of Crighton *et al.* (1975), or during 2 days as in the experiments of Shareha *et al.* (1976) was probably for too short a time to render the ovaries ready for ovulation. It is also possible that during prolonged infusion of LH-RH the release of FSH occurs before that of LH, as it has been shown at natural pro-oestrus in the rat (Wakamatsu, Sekigushi & Inoué, 1975). FSH itself also possesses the capacity to stimulate follicular adenylate cyclase, steroidogenesis and ovum maturation (Tsafiriri *et al.*, 1976).

The present experiments show, however, that anoestrous sheep will respond by ovulation to prolonged infusion of low doses of LH-RH.

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