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Concentrations of Luteinizing Hormone in Ovariectomized Ewes and the Effect of Disturbance, Starvation and Vascular Cannulation

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Abstract

Neither routine experimental procedures, including vascular cannulation and venipuncture, nor starvation or disturbance caused significant fluctuations in luteinizing hormone (LH) concentrations in ovariectomized ewes. Samples obtained at 5-min intervals revealed episodic LH releases occurring every 10–35 min. Hourly sampling over 3 days revealed large LH variations but no distinct diurnal variation.

Introduction

In two reports (Roche *et al.* 1970; Reeves *et al.* 1972) no consistent pattern could be found in luteinizing hormone (LH) concentrations in ovariectomized ewes when daily blood samples were taken. More frequent sampling on only one day revealed no apparent diurnal variation in LH concentration but Reeves *et al.* (1972) described rhythmic oscillations in LH with a periodicity of $52 \pm 10 \cdot 5$ min. Butler *et al.* (1972) took blood samples from ovariectomized ewes every 15 min for 3–4 h on several days, and found clearly defined rhythmic pulsatile releases of LH every 45–60 min. It appears that when ovariectomized ewes are sampled more frequently, there is variation in LH concentration. Ewes were therefore sampled more frequently and over an extended period of time in order to provide a more detailed analysis of rhythmic oscillations and diurnal variation in LH concentrations.

Since stress has been found to cause release of certain pituitary hormones (Meyer and Knobil 1967) and ovulation in anoestrous ewes (Braden and Moule 1964), and since experimental ewes are frequently subjected to many apparently stressing routine procedures, it was considered important to firstly evaluate the effect of the following procedures on plasma LH concentration of ovariectomized ewes: vascular cannulation for blood collection, venipuncture with restraint, starvation, and disturbance and yarding of animals.

Materials and Methods

General

All ewes involved in these experiments had been bilaterally ovariectomized for 4 months. All blood samples were centrifuged immediately following collection and plasma was stored at $-20°C$ until assayed for LH by radioimmunoassay (Goding *et al.* 1969). Between assay variation was $\pm 20\%$. All samples for each ewe in any experiment were analysed within the one assay. Between assay variation was accounted for by the use of correction factors calculated from values obtained for internal standards analysed in each assay for at least 200 assays.

Experiment 1

An indwelling catheter was inserted into the jugular vein of each of 25 ovariectomized ewes. The ewes were then penned in a shed for 3 days prior to the initiation of a 20-h blood sampling schedule. This 3-day period is referred to as the pretreatment period. The first 8 h of the 20-h sampling schedule are referred to as the treatment period and the remaining 12 h as the post-treatment period. Blood samples were obtained (per indwelling catheter unless indicated otherwise) from every ewe once each hour during the period 1800–2300 h of the first day of the pretreatment period and throughout the treatment and post-treatment periods. Five ewes were randomly allocated to each of five groups as follows:

Group 1. *Control* animals—no further treatment.

Group 2. *Venipuncture*—ewes were restrained and blood samples were obtained from the jugular vein by aspiration through a 19-gauge needle during the treatment period. In the post-treatment period, samples were taken from the indwelling catheter.

Group 3. *Vascular cannulation*—in each ewe the other jugular vein was cannulated just prior to the commencement of the treatment period. Blood samples were obtained from the catheters inserted 3 days previously.

Group 4. *Starvation*—feed was withheld for the last 2 days of the pretreatment period and during the treatment period.

Group 5. *Disturbance*—the ewes were driven around a yard for 5 min prior to each blood sample being taken in the treatment period, and were penned during the post-treatment period.

All LH results were subjected to an analysis of variance.

Experiment 2

Jugular vein blood samples were taken (using indwelling catheters) from two ovariectomized ewes every 5 min for 3 h and from five ovariectomized ewes every hour for 72 h.

LH results obtained each day from the five ewes were arbitrarily grouped into four periods:

Period 1. 0901–1500 h

Period 2. 1501–2100 h

Period 3. 2101–0300 h

Period 4. 0301–0900 h

Mean plasma LH concentrations were calculated for each period and the data subjected to analysis of variance, treating each blood sample as a replicate. Results were analysed for rhythmic oscillations and presence of diurnal variations.

Results and Discussion

Experiment 1

There was considerable variation in the plasma LH concentrations observed in ovariectomized ewes following no treatment, or routine experimental procedures (disturbance, starvation, venipuncture or vascular cannulation). The statistical analysis indicated that there was significant variation in LH concentrations between periods and ewes within each treatment and between ewes within treatments. Fluctuations in LH concentrations were different for ewes within each treatment, and within each period. The actual treatments *per se* did not have a significant effect on fluctuations in LH concentrations. The LH data from the four experimental treatments were pooled, and are presented with the data from the control group in Fig. 1. Even though the ewes exhibited outward signs of stress when they were subjected to cannulation, venipuncture and disturbance, fluctuations in LH concentrations were not affected. The variation in LH levels that did occur was due to inherent animal variation.

Experiment 2

Since the method of collection of blood samples did not affect LH concentrations *per se*, ewes in this experiment were sampled using indwelling catheters. The LH results obtained when ewes were sampled at 5-min intervals are presented in Fig. 2. There was significant variation in LH concentration within and between the animals during the period studied. The LH values ranged between 7 and 24 ng/ml but there were no clear-cut patterns of periodicity. However, the intervals between releases of LH (increases of greater than 3 ng/ml in 5 min) ranged in duration from 10 to 35 min with a mean (±s.e.) of 19·2±1·5 min. Episodic releases of LH were observed in our ewes two to three times more frequently than in ewes studied by Butler *et al.* (1972) and Reeves *et al.* (1972). However, these two groups of workers sampled their ewes only every 15 min so may have missed some of the episodic releases.

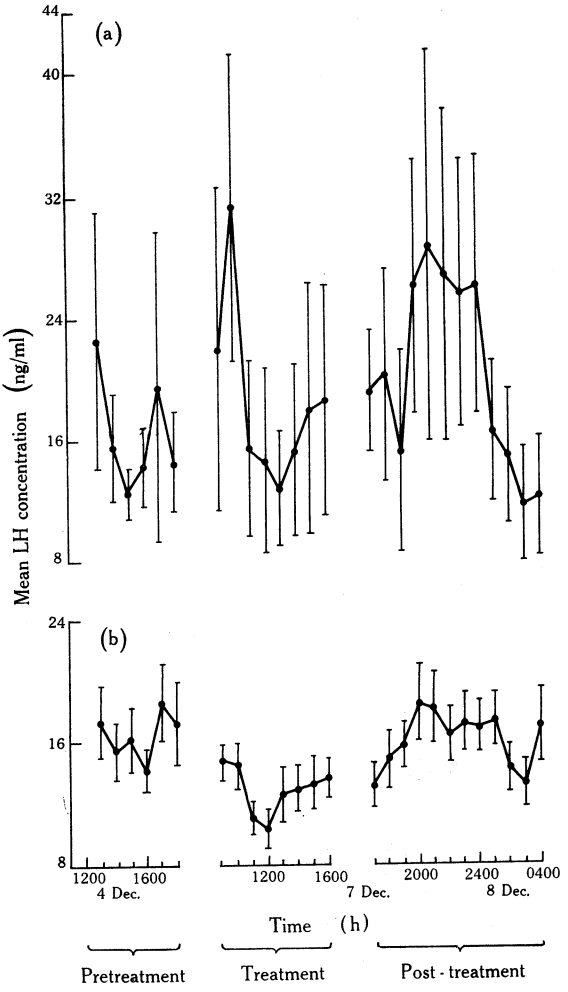

Fig. 1. Effect of routine experimental procedures on plasma LH concentrations in ovariectomized ewes. (*a*) Data from the control group (group 1), $n = 5$. (*b*) Data pooled from the experimental groups (groups 2–5), $n = 20$. Standard errors are shown by vertical bars.

When ewes were bled hourly for 72 h, there was significant variation in LH concentrations between ewes ($P<0·01$), and between ewes within days ($P<0·01$), but not between the selected periods of the day. The results are summarized in

Table 1. The lack of diurnal variation in plasma LH concentration, which was also reported by Roche *et al.* (1970) who bled ewes every 6 h for 1 day only, is at variance with the results of Butler *et al.* (1972). Reasons for these differing results are not clear. Clearly, however, even more detailed LH profiles on a larger number of ewes appear necessary to establish the pattern of LH release in the ovariectomized ewe. Our results suggest that individual ewe variation is so large that it overrides other influencing factors. This emphasizes the need for adequate replicates in endocrinological studies.

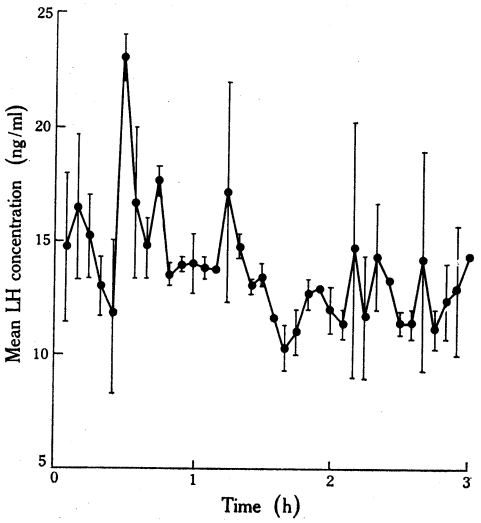

Fig. 2. Mean plasma LH concentration for two ovariectomized ewes sampled at 5-min intervals. Standard errors are shown by vertical bars.

Table 1. Summary of mean plasma LH concentrations from ovariectomized ewes, sampled hourly for 3 days

Each value is the mean (±s.e.) of six hourly blood samples taken from each of five ewes (n = 30). Values are expressed in ng/ml

Day	Time (h) of the day			
	0901–1500	1501–2100	2101–0300	0301–0900
1	18·5±3·0	19·5±2·7	23·0±3·4	16·7±2·0
2	13·5±1·5	13·3±1·6	13·4±1·7	16·2±2·0
3	13·5±1·8	17·9±1·9	19·0±2·0	16·2±1·5

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