LUTEINIZING HORMONE SECRETION DURING THE OESTROUS CYCLE OF THE EWE AS DETERMINED BY RADIO-IMMUNOASSAY

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This note presents preliminary results on the use of a radio-immunoassay method involving the use of ^{125}I LH prepared by the method of Greenwood, Hunter & Glover (1963) for the determination of LH in the blood of ewes throughout the oestrous cycle. Particular attention was paid to the period around oestrus. In the assay, 0.3 ml plasma is incubated for 72 hr at 4°C with 100 pg ^{125}I LH ($\sim 100\ \mu\text{c}/\mu\text{g}$, vol 0.1 ml in phosphate buffer, 0.05 M, pH 7.4, containing 0.25% bovine serum albumin, Fraction V) and with 1.0 ml of 1:250,000 LH antiserum, also diluted in phosphate buffer. After incubation, during which the ^{125}I LH and LH present in plasma compete for binding to the antiserum, 150 mg talc is added to adsorb the hormone not bound by the antiserum (Rosselin, Assan, Yalow & Berson, 1966). The talc is then separated by centrifugation, washed with 1.5 ml phosphate buffer and its radio-activity counted in a γ -spectrometer. The radio-activity of ^{125}I LH adsorbed to talc in the absence of antiserum is used as a measure of the labelled hormone available for binding to antiserum. From this measure, the ratio of antiserum-bound radio-activity (B) to radio-activity present on talc (F) is determined. This ratio is related to a curve obtained from similarly treated standard LH solutions to which 0.3 ml sheep plasma is added, immediately before the talc, to allow for non-specific binding of hormone to plasma constituents. ^{125}I LH and standard LH solutions were prepared from NIH-LH-s1.

The antiserum used was prepared in a mare against bovine LH, and examination of its specificity (Text-fig. 1) showed that, as expected, it bound bovine LH even more strongly than ovine LH. In addition, the antiserum bound both ovine and bovine thyrotrophic hormone (TSH) almost as well as ovine LH, and there was a small reaction against ovine follicle stimulating hormone (FSH); there was no reaction against ovine growth hormone (GH), adrenocorticotrophic hormone (ACTH), ovine prolactin, pregnant mare serum (PMSG) or human chorionic gonadotrophin (HCG). Serial dilution of a sheep plasma, estimated to have a high LH concentration, gave a curve essentially parallel to that of NIH-LH-s1. The completeness of competition between TSH and LH has negated attempts to adsorb anti-TSH activity from the antiserum. In the present study, the problem of relative contributions of TSH and LH was overcome

by comparing the levels of LH in normal ewes with those in ewes treated with thyroxine to inhibit TSH release.

Assays were performed on plasma obtained by jugular venipuncture in twenty trained Merino and Border Leicester $\times$ Merino ewes throughout the oestrous cycle. Blood was collected once in 24 hr, except at the expected time of oestrus when samples were taken for several days at intervals which varied from

TEXT-FIG. 1. Cross-reaction of hormonal preparations with bovine LH antiserum. $\times$, NIH-LH-B5; $\bullet$, NIH-LH-S1; $\circ$, sheep plasma serially diluted; Δ , NIH-TSH-S2; $\triangle$, NIH-TSH-B3; $\square$, NIH-FSH-S1. ---, No cross-reaction with ovine GH, up to 256 ng/ml (potency 2 i.u./mg); ACTH (Acton, Andrews Laboratories) up to 20 m-units/ml; prolactin (NIH-P-36) up to 256 ng/ml; PMSG (Burroughs Wellcome), up to 50 i.u./ml; HCG (Schering), up to 20 i.u./ml.

4 hr to 30 min. The onset and cessation of oestrus were determined by testing ewes individually with vasectomized rams at 2 or 4 hr intervals, commencing 1 to 2 days before the expected onset of oestrus. Thyroxine-treated ewes were injected subcutaneously with 1 mg L-thyroxine/day commencing 3 to 4 days before expected oestrus, and were given a further injection of 1 or 2 mg L-thyroxine intravenously at the start of oestrus.

The changes in LH concentration of jugular plasma throughout the oestrous cycle in two representative ewes are shown in Text-fig. 2; this pattern of response was common to all animals. The concentration of LH in plasma was low during most of the oestrous cycle, ranging from 5 to 15 ng/ml. There

was a steady decline in LH concentration from the end of oestrus to Day 14 or 15 of the cycle. Thereafter, there was an increase which, however, was small compared with the dramatic rise in jugular LH concentration which occurred shortly after the start of oestrus. From observations on forty oestrous periods, this rise began 4.6 hr (S.D. ± 4.3) after the start of oestrus and reached a peak

TEXT-FIG. 2. Changes in LH concentration of jugular plasma throughout the oestrous cycle of two Border Leicester $\times$ Merino ewes. Horizontal black bars represent period of oestrus. The day on which oestrus started is indicated as Day 0.

by 12.0 hr (S.D. ± 4.2). Peak values ranging from 46 to 460 ng/ml (mean 172) were recorded at this time and by 20.7 hr after onset of oestrus (S.D. ± 5.3) values had decreased almost to pre-oestrous levels. There was no detectable difference between thyroxine-treated and untreated ewes in the magnitude or timing of the peak during oestrus, indicating that the peak represents secretion of LH at this time, and is unlikely to be due to TSH secretion. The peak occurred 19.9 hr (S.D. ± 4.6) before the end of oestrus, and 25.7 hr before ovulation as

determined by laparotomy in five ewes. There was no apparent difference between Merino and Border Leicester $\times$ Merino ewes.

These results are consistent with the recent observations of Pelletier, Kann, Dolais & Rosselin (1968) and Geschwind & Dewey (1968), who also used radio-immunoassay to determine LH in the plasma of ewes throughout the oestrous cycle. Further, the results provide accurate information on the time relationships between the limits of the oestrous period and the oestrous LH peak. The rise in LH during oestrus occurred apparently several hours later than the time at which pharmacological agents will uniformly block ovulation in the ewe (Robertson & Rakha, 1965; Radford, 1966). It seems likely, then, that the neural stimulus for LH release and LH release itself are not synchronous in this species, a suggestion made first by Robertson & Rakha (1965) on the basis of their data on pharmacological blockade of ovulation, together with earlier studies on the pituitary content of gonadotrophin (Robertson & Hutchinson, 1962).

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