CHANGES IN THE CONCENTRATION OF LUTEINIZING HORMONE IN PLASMA OF RAMS FOLLOWING ADMINISTRATION OF OESTRADIOL, PROGESTERONE OR TESTOSTERONE

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Fluctuations in the basal level of luteinizing hormone (LH) in the plasma of ewes have been measured by the radioimmunoassay technique (Niswender, Roche, Foster & Midgley, 1968; Goding, Catt, Brown, Kaltenbach, Cumming & Mole, 1969). Goding *et al.* (1969) and Bolt, Kelley & Hawk (1970) reported that the injection of oestradiol into the ewe caused LH release. In rams, however, the basal level of plasma LH is not well defined and any effect exerted by exogenous steroids on plasma LH is not known. This study was designed to measure the basal level of LH in the plasma of adult male sheep and to determine whether exogenous oestradiol, progesterone or testosterone would alter the basal LH level.

Sixteen sexually active Rambouillet rams, weighing about 100 kg and ranging in age from 2 to 5 years, were divided into four equal groups. One-half of the animals in each group had been vasectomized for at least 1 year. All rams were penned together and were not exposed to ewes throughout the test period.

The following agents were administered in 5 ml of corn oil by a single intramuscular injection at 08.00 hours: (1) oestradiol-17 β , 5 mg; (2) testosterone, 5 mg; (3) progesterone, 100 mg; (4) corn oil control. Jugular blood was collected at the initiation of treatment and on eleven subsequent occasions over the 53-hr test period as indicated in Text-figs. 1 and 2. Plasma was separated by centrifugation and stored at -15°C until the time of assay.

Determinations of plasma LH were made with the double-antibody radioimmunoassay procedures described and validated by Niswender, Reichert, Midgley & Nalbandov (1969) with few modifications. LER 1056-C2 ^{131}I -LH was prepared by the method of Greenwood, Hunter & Glover (1963). The anti-LH serum (GDN No. 15) was used at a dilution of 1 : 64,000. The sheep anti-rabbit γ -globulin (DJB, 5XI) was used at a dilution of 1 : 80. NIH-LH-s12 was the reference standard. Sixteen concentrations of standard from 25 pg to 25 ng/tube and seventy-seven unknown samples were used in each of three assays. A laboratory standard plasma sample, S141, was also included with each assay. Each plasma sample was assayed at three levels (0.075, 0.1 and 0.15 ml/assay

tube) and the mean used in calculations. Samples from each ram were assayed in the order collected, but the order of rams was selected at random.

The specificity of the assay in the author's hands was tested with prolactin, NIH-s8; growth hormone, NIH-s8; follicle stimulating hormone, NIH-s5 and

TEXT-FIG. 1. Fluctuations in plasma LH following a single injection of corn oil in two fertile (—, — · — · —) and two vasectomized (----, - - - -) rams. The line either above or below each point indicates assay variation for each determination (standard error of the 0.075, 0.100 and 0.150 ml determination on each plasma sample). nd = non-detectable (i.e. <0.21 ng LH/ml of plasma). Time of injection of corn oil is indicated (↓).

TEXT-FIG. 2. Fluctuations in plasma LH following a single injection of either oestradiol (----), testosterone (—) or progesterone (- - - -) with four rams per group. Plasma LH over the same time span in four control rams is shown Text-fig. 1. Standard errors about each point indicate variation among rams within a treatment. nd = non-detectable (i.e. <0.21 ng LH/ml of plasma). Time of the respective steroid injection is indicated (↓).

adrenocorticotrophin (Sigma). An 'LH value' was obtained for each preparation, but in no case was the 'LH value' greater than 0.2% of the weight of the preparation tested.

The results from each assay were computed separately. The minimum

detectable value was the amount of LH standard which yielded 95% of the counts/min in the buffer control tubes; this value was 0.21 ng/ml of plasma. The lower limit of the 95% confidence interval for the buffer controls was 96.3% of the average counts/min obtained in buffer control tubes. The minimum detectable value was used for samples with a non-detectable LH level.

The mean concentration of LH in forty-eight samples obtained over the 53-hr test period from four control rams was 2.3 ± 0.35 ng/ml of plasma (Mean $\pm$ S.E.). There were no apparent differences between fertile and vasectomized rams. Text-figure 1 illustrates the unstable nature of plasma LH concentration in these rams. In one ram, values ranged from 12.8 ng/ml to a non-detectable value within 5 hr. These fluctuations do not appear to be due to assay variation, as indicated by the small standard error about the mean of the three determinations per plasma sample (Text-fig. 1). The plot of the LH pattern for individual rams shown in Text-fig. 1 suggests that rams may release LH in brief surges rather than at a continuous rate. The timing of these surges is yet to be defined. There did not appear to be any diurnal pattern in these surges but a longer collection period will be required before the possibility of diurnal variation can be eliminated. Peterson, Midgley & Jaffe (1968) also failed to observe diurnal variation in the concentration of LH in plasma from men.

In comparison with the mean plasma LH concentration of 2.3 ng/ml for control rams, the basal level of plasma LH obtained from fourteen cycling ewes in a separate experiment was 0.84 ± 0.10 ng/ml of plasma. The mean was obtained from 168 samples collected at 2-hr intervals from 06.00 to 16.00 hours on Days 3, 5, 7, 9, 11, 13 or 15 of the cycle. Regarding the limitations of comparing results from two experiments, samples from the ewes were assayed with procedures identical to those used for the rams. In addition, the LH concentration obtained on the same laboratory standard (S141) was 19.0 ± 0.8 ng/ml for the nine assays with cycling ewes compared with 18.5 ± 2.6 ng/ml for the three assays with rams.

In rams, a single injection of either oestradiol, testosterone or progesterone resulted in a marked depression in the plasma LH concentration (Text-fig. 2). These steroids also markedly reduced the fluctuations in the levels of plasma LH when compared to the levels in the control rams (Text-fig. 1 versus Text-fig. 2). The mean LH concentration in samples obtained from 3 to 27 hr after either oestradiol, testosterone or progesterone injection was 0.51 ± 0.09 , 0.78 ± 0.31 and 0.60 ± 0.09 ng/ml of plasma, respectively, compared to 2.3 ± 0.35 ng/ml in samples from control rams. The reduction in both the concentration of LH and the fluctuation in the concentration of LH was less apparent between 27 and 53 hr after the respective steroid injection. Both the timing and the magnitude of these changes may be dose dependent. There were no apparent differences between vasectomized and fertile rams within any of the three treatment groups. Peterson *et al.* (1968) and Odell, Ross & Rayford (1967) reported that testosterone propionate or ethinyloestradiol both depress plasma LH in men.

An injection of oestradiol in the anoestrous ewe (Goding *et al.*, 1969) or the cycling ewe (Bolt *et al.*, 1970) causes a brief but sizable increase in plasma LH. However, oestradiol did not produce this effect in rams.

The results of this experiment suggest that rams normally release LH in surges

rather than at a continuous rate and that these surges are either decreased in amplitude or eliminated following the injection of sufficient exogenous oestradiol, testosterone or progesterone.

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