

Inhibition of LH Release as Influenced by LRF Infusion¹ (38652)

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Intra-carotid injections of purified ovine hypothalamic extracts of luteinizing hormone releasing factor (LRF) from mature rams (1) results in peak levels of luteinizing hormone (LH) within 5 min after injection. The castrate is apparently more sensitive to LRF (2) than intact animals. Synthetic LRF given iv to ovariectomized ewes (3) results in a maximal LH response within 15 min. Prepubertal gilts given 16 series of injections of LRF at 5 hr intervals (4) showed higher serum LH concentrations after the first two injections than later injections. Three 100 μg injections of LRF given at 2 hr intervals to cyclic cows (5) tended to depress the LH release by the third injection. Continuous infusion of LRF into anestrus ewes (6) caused an increase in LH secretion which reached a peak at 3 hr and decreased to pretreatment levels by 20 hr. The LRF infusion lowered pituitary LH but did not deplete it. Some preliminary work in this laboratory indicated that infusion of 10 $\mu\text{g/hr}$ of LRF into ovariectomized ewes would produce a rapid rise in plasma LH concentration. After the initial increase plasma LH concentration declined during the next 12 hr. This decrease in LH levels, even though LRF infusion was maintained, suggested that the pituitary was becoming refractory to the LRF. The study reported here was designed to determine the effects of continued infusion of LRF followed by a large single injection of LRF on plasma and pituitary levels of LH.

Materials and Methods. Four sheep which had been ovariectomized 48 days prior to starting the experiment were used in this study. Each jugular vein was cannulated and the cannulas taped in place. One cannula (PE-60) was used for drawing blood samples. The other cannula (PE-50) was at-

tached to an infusion pump. After cannulation, the sheep were put in small pens and blood samples were taken at 30 min intervals for 4 hr. After the initial 4 hr collection period, the ewes were infused with LRF² at a rate of 10 $\mu\text{g/hr}$ for 20 hr. The syringes on the infusion pump delivered the LRF-physiological saline solution at the rate of 1 ml/hr. The syringes containing the LRF solution were maintained in an ice bath throughout the infusion period. After 16 hr of LRF infusion each animal received 1 mg of LRF through the jugular vein cannula. Blood samples were taken at 30 min intervals for the next 4 hr. After completion of the 20 hr of LRF infusion, the ewes were sacrificed by exsanguination; and the pituitary immediately removed. The plasma and pituitary samples were analyzed for LH by a validated RIA procedure (7), utilizing anti-ovine LH No. 573 supplied by G. Niswender and LER-1056-C₂ for iodination.

Results. After 30 min of LRF infusion the mean serum LH levels increased from 6.4 to 54.7 ng/ml (Fig. 1). A peak LH value of 73.2 ng/ml was reached 1.5 hr after beginning infusion. Following the peak value, serum LH concentration declined rapidly to 21 ng within 3 hr. For the next 11.5 hr LH concentration declined slowly to 12 ng/ml. After the 1 mg injection of LRF at 16 hr a nonsignificant increase (8 ng) in LH concentration occurred. After 20 hr of LRF treatment the LH levels (5.8 ng/ml) were similar to the preinfused values (6.4 ng/ml).

Ewe No. 3 had a large increase in serum LH concentration 8.5 hr after the start of LRF infusion (Fig. 2). The same ewe had a more pronounced decline in LH concentration when compared with the other ewes, 1.5 hr after the initial LH surge. These data

¹ This paper is submitted with the approval of the Director of the Arkansas Experiment Station.

² The LRF was supplied by Dr. W. C. Dermody, Parke-Davis, whose help and advice is appreciated.

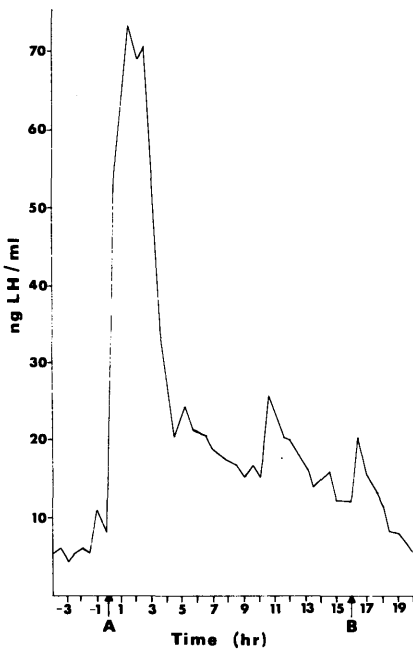


FIG. 1. Effect of LRF infusion (10 $\mu\text{g/hr}$) on mean plasma LH concentration. Point A is the time the 20 hr LRF infusion period is initiated. Point B is the time 1 mg of LRF is given.

suggest that ewe No. 3 may not have been receiving LRF during the 1.5–8.5 hr infusion period. The lack of LRF could have been caused by a temporarily blocked or leaking cannula.

The pituitary LH concentration of the treated animals varied from 369 to 1360 ng/mg of tissue. A direct relationship between the concentration of LH in the pituitary and the amount of LH released when 1 mg of LH was injected was noted (Fig. 3). The plasma LH concentration appears to rise more rapidly as the pituitary LH concentration rises.

Discussion. This experiment verifies previous reports (2 and 3) that LH release occurs after administration of LRF to ovariectomized ewes. Continuous administration of LRF by infusion results in peak levels within 1.5 hr after initiating infusion. With constant infusion of LRF continuing, the plasma concentration of LH drops rapidly after reaching a peak. The drop in plasma LH concentration indicates the pituitary has become partially refractory to

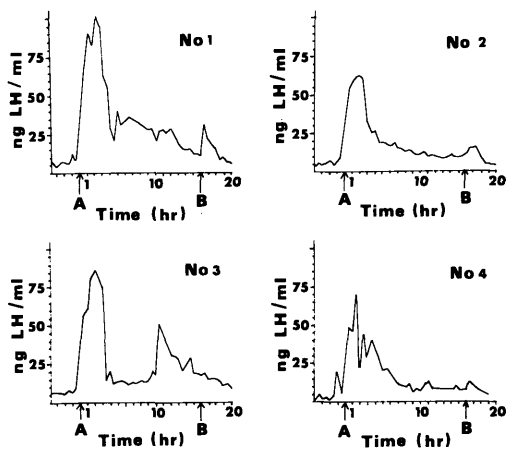


FIG. 2. Effect of LRF infusion on each animal's (No. 1–4) plasma LH concentration. Point A is the time the 20 hr LRF infusion period is initiated. Point B is the time 1 mg of LRF is given.

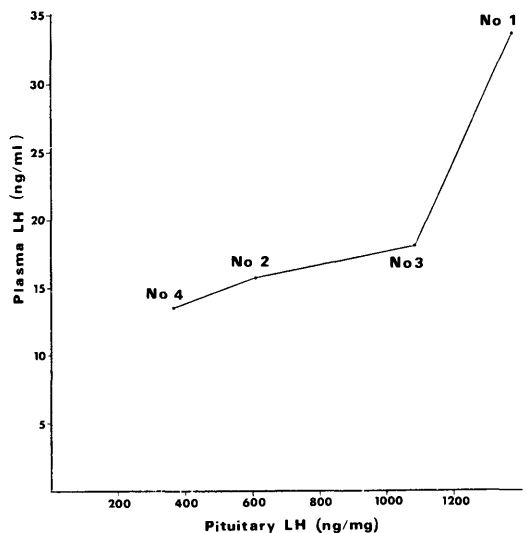


FIG. 3. Relationship between the peak plasma LH concentration after a 1 mg injection of LRF and the pituitary LH concentration after the LRF treatment.

the level of LRF (10 $\mu\text{g/hr}$) being infused. Little effect was noted even when the infused ewes were given 1 mg of LRF. These data indicate that after a period of LRF stimulation, the pituitary is no longer capable of releasing significant quantities of LH even in response to massive doses of LRF. Ewes which had been ovariectomized for 30 days had pituitary LH concentration

of 1105 ng/g (8). Two of the four ewes in the present study had pituitary LH concentrations in this range. Pituitary depletion of LH by LRF does not appear to be the reason for the lack of response to LRF.

Summary. Four ovariectomized ewes were infused with LRF (10 μ g/hr) for 20 hr. Jugular vein blood samples were taken at 30 min intervals. After 16 hr of LRF infusion each animal was given a 1 mg iv injection of LRF. At the end of the 20 hr infusion period, the animals were sacrificed and the pituitary removed. Pituitary and plasma samples were analyzed for LH. Plasma LH concentration reached a peak level of 73 ng/ml, 1.5 hr after starting the LRF infusion. A rapid decline in LH concentration occurred for the next 3 hr. The plasma LH concentration declined slowly to preinfusion levels over the next 15.5 hr. A small insignificant rise in plasma LH concentration occurred when the animals were given 1 mg of LRF after 16 hr of LRF infusion. At the end of the treatment period pituitary LH concentration varied from

369 to 1360 ng/mg. Two of the four ewes had pituitary LH concentrations similar to untreated ovariectomized ewes. The pituitarys' refractoriness to LRF apparently is not due to depletion of pituitary LH.

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Received October 16, 1974. P.S.E.B.M. 1975, Vol. 148.