Release of Luteinizing Hormone by Synthetic LH-Releasing Hormone in the Ewe and Ram[1] (36251)

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Recently we established the chemical structure of porcine LH-releasing hormone (LH-RH). It proved to be a decapeptide having the following amino acid sequence: (pyro)Glu – His – Trp – Ser – Tyr – Gly – Leu – Arg – Pro – Gly – NH_2 (1, 2). The synthetic decapeptide corresponding to this structure stimulated the release of LH in rats (3), hamsters (4), and human beings (5). The synthetic material also augmented the release of FSH and LH from rat pituitaries *in vitro* (6).

In a previous study (7), we demonstrated that intracarotid administration of natural LH-RH preparation in the ewes, wethers, and rams resulted in a significant increase in serum LH at all dosages used ranging from 1 to 27 μg. The maximum responses in the ewes and rams appeared to be obtained with 3 μg of the LH-RH preparation; larger doses showed no greater effect. The maximum levels of serum LH detected after administration of LH-RH in the diestrus ewes were well below reported preovulatory levels of LH.

It appears then, that the pituitary of the diestrous ewe is relatively refractory to LH-RH, although the pituitary responsiveness increases at estrus (8). However, the possibility that very much greater doses of LH-RH might induce a different pattern of LH-release in the diestrous ewe could not be excluded. The scarcity of natural LH-RH prevented us from using very high doses of this material. However, the availability of synthetic LH-RH made it possible to investigate this problem.

Materials and Methods. Preparation of synthetic LH-RH. Synthetic LH-RH was prepared by the solid phase method (9), according to the established amino acid sequence of porcine LH-RH (1, 2), and then purified by countercurrent distribution on the basis of tests in ovariectomized estrogen–progesterone-pretreated rats (10). This synthetic LH-RH preparation possessed only 25% of the LH-RH activity of the pure natural LH-RH (AVS 77-33, No. 215-269), or of pure synthetic LH-RH, but was four times more potent than the preparation AVS 77-3, No. 320-339, which was used in our previous studies in the sheep (8, 11).

In the present studies, various amounts of this synthetic LH-RH dissolved in 2.5 ml of 0.9% NaCl were injected into the carotoid artery over a 2 min period.

Assay of serum LH. A double antibody radioimmunoassay, as described in detail by Reeves *et al.* (11), was used for determining LH levels in the serum of the experimental animals. Each serum was assayed in duplicate; and the mean value was obtained. LH levels were expressed in terms of NIH-LH-S-17. The absence of cross reactivity of purified LH-RH with the antiserum in this assay was established previously (11).

Experimental animals. Three normal, white faced ewes and one vasectomized ram were used in this study. The sheep were maintained at near-constant temperature (78 ± 1°F) and fed alfalfa pellets and given water *ad libitum*. Anestrus was induced by daily exposure to artificial light for 16 hr, alternating with 8 hr of darkness. The stage of anestrus was also confirmed by the use of the vasectomized ram. Each animal was surgically prepared with a carotid loop as previously described by Bone *et al.* (12). The carotid loop facilitates intracarotid injection

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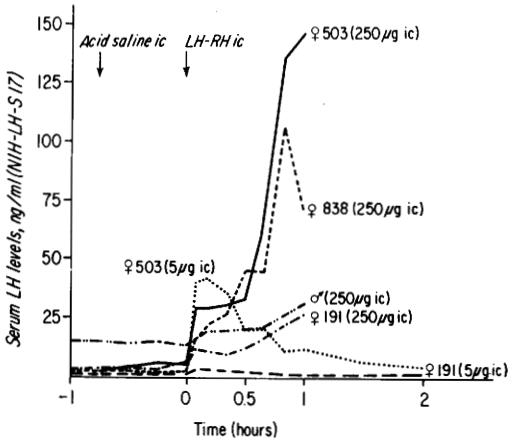

FIG. 1. Serum LH levels after ic injection of synthetic LH-RH in sheep.

of the conscious sheep. Prior to the experiments, an indwelling catheter (Ga 14) was placed in the jugular vein.

Results. In Expt. 1 (Fig. 1), saline as well as small or large doses of LH-RH were injected into the carotoid artery of the sheep. Intracarotid (ic) injection of acidified saline did not change serum LH levels of the sheep. Administration of 5 μg of LH-RH induced a considerable rise of serum LH in one ewe (No. 503) and a slight, but significant rise, in another ewe (No. 191). In both animals, the peak of serum LH levels was reached at 10 min after injection. This was followed by a gradual fall and serum LH levels returned to preinjection levels within 2 hr after injection. In the same animals, ic injection of 250 μg of LH-RH resulted in a prompt rise in serum LH 5 min after the injection, but the serum LH remained at the same level for 25 min, after which time, the LH levels started rising again. During the first 30 min, the increases in serum LH after injection of small and large doses of LH-RH were similar to one another. In one ewe (No. 191), the LH levels after 250 μg of LH-RH were approximately 3 times greater than those after injection of 5 μg of LH-RH. In another ewe (No. 503), the levels after injection of the greater dose of LH-RH were somewhat smaller than those obtained after smaller dose of LH-RH. However, 1 hr after injection of large doses of LH-RH, considerable rise of LH was observed; 26 ng/ml in ewe No. 191 and 145 ng/ml in ewe No. 503. In another ewe, No. 838, serum LH levels increased gradually for the first 40 min and then rose sharply. In the vasectomized ram, the resting levels of LH were moderately elevated compared to those in anestrous female sheep. After injection of 250 μg of LH-RH, serum LH levels rose in a fashion similar to that seen in the ewe, although the magnitude of the rise was smaller in the ram than that seen in the ewes.

In Expt. 2 (Fig. 2), two anestrous ewes, one sensitive (No. 503) and one rather insensitive (No. 191) to LH-RH, were injected intramuscularly (im) with 250 μg of LH-RH. The peak in LH-RH was reached 1.5 hr after the injection. In both ewes, serum LH

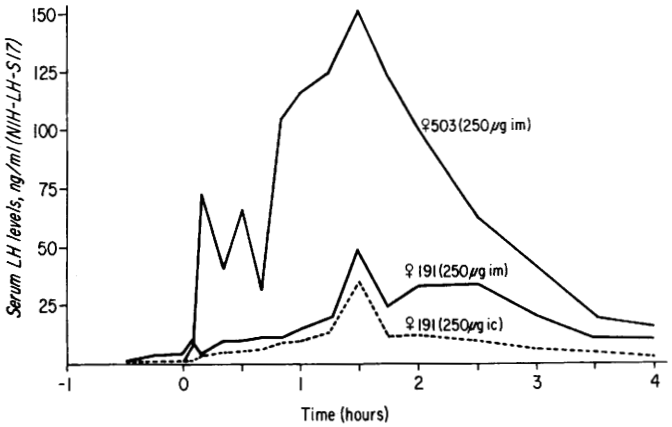

FIG. 2. Serum LH levels after im and ic injection of synthetic LH-RH in sheep.

levels rose within 5–10 min after the injection and remained at similar levels for about 40 min, and then started rising again. The peak was reached at 1.5 hr after injection in both ewes. This was followed by a gradual decrease in plasma LH. At 4 hr after injection, LH had still not returned to the preinjection levels. The pattern of serum LH levels after im injection of 250 μg of LH-RH in ewe No. 191 was similar to the pattern following ic injection.

Discussion. These results clearly demonstrate that the patterns of change in serum LH levels after ic injection of small and large doses of LH-RH were different in the anestrous ewes and the vasectomized ram used. With a relatively small dose, the responses were monophasic, the peak being reached at 5–10 min after injection. With a larger dose of LH-RH they were biphasic. It is interesting that during the first phase of elevation there was no clear-cut dose–response relationship. This is in agreement with our previous findings in diestrous ewes (7).

Although the mechanism of the biphasic response which was observed in the sheep injected with a large dose of LH-RH is unknown, the following possibilities can be considered. Firstly, the pituitary LH cells may be sensitized during the first phase of stimulation. When the dose of LH is large, LH-RH remains in circulation in a sufficient concentration during the time when the pituitary sensitivity keeps increasing. Subsequently, increase of LH release takes place. Sensitization of LH cells could be the result of direct action of LH-RH or represent an indirect effect through stimulation of estrogen secretion from the ovaries (13).

In our previous studies on diestrous ewes, the ceiling of response in LH was readily reached after a relatively small dose of LH-RH. A greater dose of LH-RH failed to induce greater response in LH (7). A limited amount of natural LH-RH did not permit us to test the effect of an enormous dose of LH-RH at that time. The present studies with considerably larger doses of synthetic LH-RH showed that greater responses could be induced in anestrous sheep. Although the maximum responses appeared to be different from one ewe to another, the peak levels of serum LH were of the same order of magnitude as those observed at the time of preovulatory surge of LH (11).

Summary. Large or small doses of synthetic LH-RH were injected into the carotid artery or intramuscularly in three anestrous ewes and one vasectomized ram. Intracarotid injection of 5 μg of synthetic LH-RH elicited monophasic responses in LH-release; the highest levels of serum LH were reached within 5–10 min after injection of LH-RH, followed by a gradual decrease. Injection of 250 μg of LH-RH resulted in a rise of serum LH within 5–10 min which was indistinguishable in magnitude from that after 5 μg of LH-RH. These levels remained steady or kept rising very gradually for 25 min or longer, then again started rising sharply. The peak levels were observed at 1.5 hr after injection, followed by a gradual decrease. Four hours after injection of LH-RH, serum LH levels were still higher than during the preinjection levels. Intramuscular injection of a large dose of LH-RH elicited patterns similar to those observed following intracarotid injection of LH-RH. The magnitude of response after intramuscular injection was the same or greater than that induced by the intracarotid injection of the same dose of LH-RH.

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