

Release of Bovine Luteinizing Hormone by Purified Porcine and Synthetic Gonadotropin Releasing Hormone¹ (36985)

J. ZOLMAN, E. M. CONVEY, J. H. BRITT, AND H. D. HAFS

Animal Reproduction Laboratory, Department of Dairy Science, Michigan State University, E. Lansing, Michigan 48823

That gonadotropin secretion is controlled by pituitary active neurohumors of hypothalamic origin is now well established. Crude hypothalamic extracts and gonadotropin releasing hormone (GnRH) of natural or synthetic origin increased serum LH in sheep (1-3), rats (1, 4, 5) and humans (6). Similar responses in cattle, not published at the outset of this project, would provide important comparative information and possibly provide a means of treating some types of infertility in the bovine. Accordingly, the present study was undertaken to investigate the effect of synthetic GnRH on release of LH in cows and bulls and to compare the effectiveness of purified porcine GnRH and synthetic GnRH on LH release from steer pituitaries *in vitro*.

Materials and Methods. Synthetic GnRH,² in 10 ml isotonic NaCl, was administered in a single injection via a jugular cannula to each of four yearling Holstein heifers and four mature dairy bulls. Heifers, in the early luteal phase (Days 5-10) of the estrous cycle at treatment, received 5, 20 or 80 μ g and bulls 10, 40 or 160 μ g GnRH on 3 consecutive days in a sequence determined at random for each animal. Sera, prepared from jugular blood collected at intervals before and after treatment, were stored at -20° until assayed for LH as described previously (7).

Whether GnRH would directly effect the bovine pituitary to release LH *in vitro* was determined during constant superfusion incubation of pituitary slices. Detailed methodology of this incubation system has been pub-

lished (8). Briefly, tissue ($\cong 75$ mg) from the peripheral area of the anterior pituitary of individual steers was placed in 9 incubation chambers, then continuously superfused with medium 199 (0.224% sodium bicarbonate) at a rate of 1.0 ml/min for 2 hr, exposed to 0, 1 or 4 ng/ml porcine GnRH² in medium 199 for 20 min, and then superfused in control medium 199 for an additional 40 min. Pituitary tissue from four steers was used and treatments were assigned randomly to tissue explants from each pituitary with three explant replicates per treatment. Effluent media were fraction-collected at 1-min intervals for LH assays. A similar experiment was conducted to compare the efficacy of purified porcine GnRH versus synthetic GnRH in causing LH release *in vitro* using pituitary tissue from a single steer. Peripheral pituitary tissue (three explant replicates per treatment) was exposed to 4 ng/ml synthetic or purified porcine GnRH for 30 min. Medium was fraction-collected and frozen until assayed for LH.

Results. LH release in vivo. Serum LH concentration of bulls prior to GnRH treatment averaged 1.1 ng/ml and increased to peaks of 9.2, 19.3 and 39.1 ng/ml after 10, 40 and 160 μ g GnRH, respectively. The average interval after GnRH to attain these peaks was 20, 53 and 113 min, respectively ($p < .01$). The relationship between dose of GnRH and magnitude of the LH peak is described by the linear function shown in Fig. 1. The interval from GnRH and the magnitude of the LH peak were significantly correlated ($r = .88$; $p < .01$) Neither individual bulls nor days of treatment affected ($p > .05$) the magnitude of the LH peak.

Average serum LH in heifers before GnRH treatment was 0.5 ng/ml; LH increased

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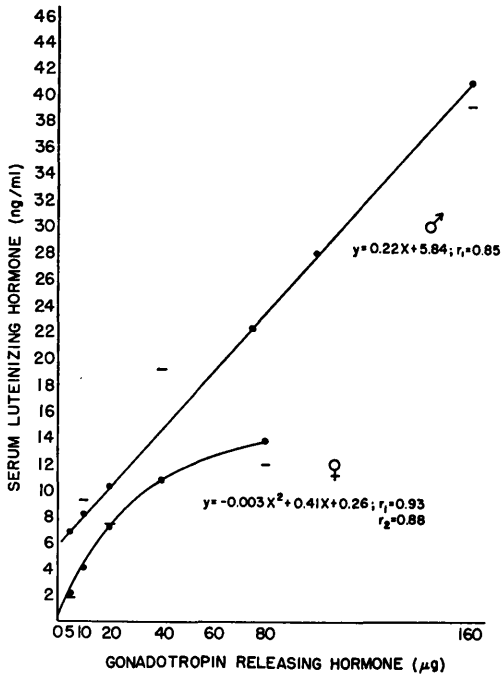


FIG. 1. Time course of the luteinizing hormone response to synthetic gonadotropin releasing hormone for one bull and one heifer.

($p < .001$) to peaks of 1.9, 7.3 and 11.1 ng/ml after 5, 20 and 80 μg GnRH, respectively. In contrast to results for bulls, the relationship between dose of GnRH administered, and LH peak response was best described by a quadratic function (Fig. 1). The LH peaks occurred 13, 16 and 26 min ($p < .01$) after 5, 20 and 80 μg GnRH, respectively, and magnitude of the LH peak and the interval to the peak were significantly correlated ($r = .92$; $p < .01$). Differences in the magnitude of the LH response attributed to day of treatment or to individual heifers were not significant.

The response curves shown in Fig. 2 for an individual bull and an early luteal phase heifer can be considered typical of each group since between animal variation was not significant. These curves show the time course of LH response to GnRH; serum LH in all animals was increased within 5 min after GnRH injection, increased to a peak at a time that was related to the magnitude of the LH peak, and then LH declined pro-

gressively. In addition, the LH response in bulls was characterized by an early rapid increase, followed by a plateau, then a further surge. This response was observed for each bull tested.

LH release in vitro. LH averaged 0.25 ng/ml/min in the effluent media from continually superfused steer pituitary tissue prior to exposure to GnRH; LH increased ($p < .001$) to 0.70 and 1.12 ng/mg/min after 4 min exposure to 1 and 4 ng purified porcine GnRH/ml medium, respectively (Fig. 3). Comparable values were 0.94 and 1.50 ng/mg/min after 20 min. LH in the effluent media did not change significantly without GnRH addition to the medium. Average LH concentration in the effluent media decreased 8 and 33% ($p < .05$) during the 40 min period following exposure to 4 and 1 ng/ml GnRH, respectively, but these LH concentrations were still 340 and 100% greater than control values at the termination of incubation.

LH in the effluent media of steer pituitary exposed to 4 ng synthetic or purified porcine GnRH was 0.46 and 0.52 ng/mg/min immediately prior to exposure and increased linearly ($p < .01$) to 1.72 and 2.48 ng/ml/min, respectively, after 30 min exposure. The increases in media LH concentration with time during exposure to natural or synthetic GnRH were parallel; the difference between response slopes was not significant ($p > .05$). When superfusion with control media was resumed, LH release decreased linearly ($p < .01$) as the incubation period advanced, but was still more than twice the preexposure base line when the incubation was terminated 4 hr after first exposure to GnRH.

Discussion. To our knowledge, this is the first report to demonstrate that synthetic GnRH induces release of LH in heifers and bulls. The magnitude of the LH peak increased linearly with increasing dose up to 160 μg of GnRH in bulls, while the peak LH response in heifers appeared to plateau at 80 μg GnRH, indicating that the male bovine pituitary is more responsive to GnRH than that of females; subject to the restriction that these early luteal phase heifers are characteristic of all ages and all stages of the

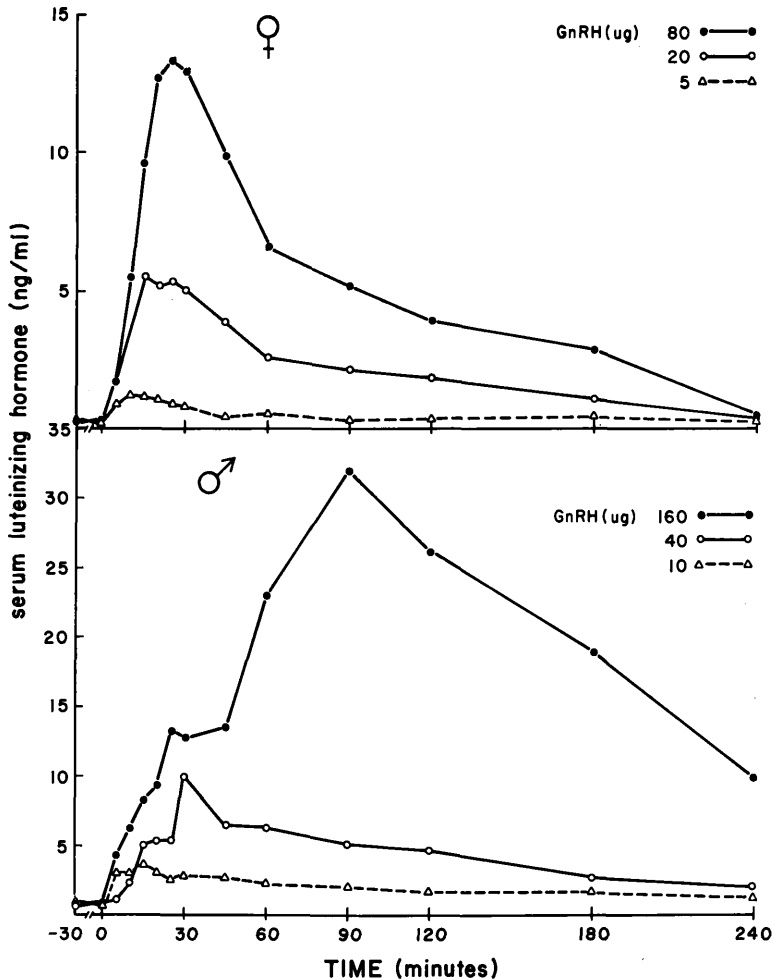


FIG. 2. Peak serum luteinizing hormone response in bulls and heifers after synthetic gonadotropin releasing hormone.

estrous cycle. This is in contrast to reports by others (10, 11) who observed no difference in LH response of diestrus ewes and rams to purified porcine or synthetic GnRH; nor did they observe a dose-response relationship. However, Reeves, Arimura and Schally (10) demonstrated a linear increase in LH response with increasing levels of GnRH in wethers, not unlike the response we observed in bulls. Differences in pituitary responsiveness to GnRH may reflect changes in steroid hormone environment, since increased serum estrogens precede and may trigger the spontaneous LH surge in cattle (9) and pituitary sensitivity to GnRH is maximum during an 8-hr period

around estrus in ewes (10).

The increase in LH which followed the maximum level of GnRH used in heifers in this study was approximately half that which was recently reported from this laboratory (12) to characterize the ovulatory surge of LH (*i.e.*, mean, 25.9; range, 19-35 ng/ml). This comparison suggests that under the conditions of this study, GnRH did not release LH in quantities characteristic of the ovulatory surge. Possibly a single surge of releasing hormone from the hypothalamus is not the exclusive event which mediates the preovulatory surge of LH.

Although the time periods involved are

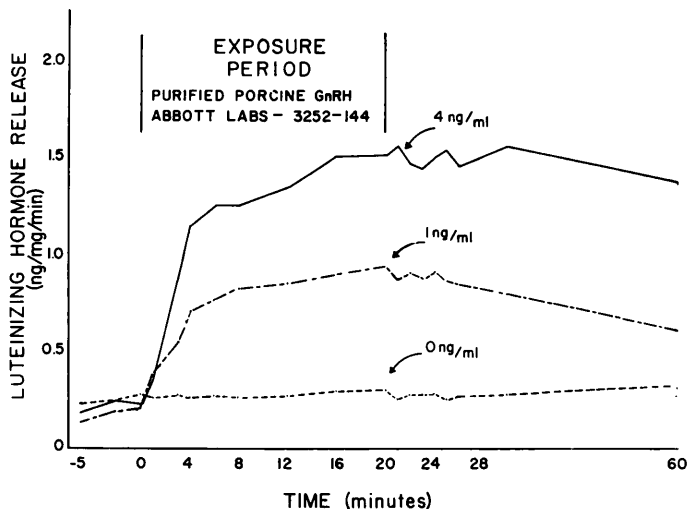


FIG. 3. Luteinizing hormone response of steer pituitary explants to purified porcine releasing hormone in continuous superfusion.

much shorter, the plateau in the LH response that occurred with bulls in this experiment is reminiscent of results reported for anestrous ewes. Thus Arimura *et al.* (11) observed that large doses of GnRH (250 μ g) caused an initial increase in ovine serum LH, followed by a plateau that was sustained for 25 min or longer, then a further LH surge with the second peak occurring at 1.5 hr after GnRH. Perhaps the earlier occurrence and shorter duration of the plateau in serum LH in bulls relative to anestrous ewes (11) is the result of the lower dose of GnRH used; our highest dose of GnRH in bulls (av 783 kg body wt) was only 60% of that used in anestrous ewes when the biphasic response was observed. Failure to detect a similar two-component response in early luteal phase heifers may suggest that this type of response is a condition of the prevailing endocrine environment.

To our knowledge, we are the first to report that the interval to the LH peak after GnRH is directly related to the magnitude of the LH peak. This result suggests that LH in samples taken at a few preset intervals after GnRH (3) may not accurately describe the LH response; it also indicates that the effect of GnRH is prolonged beyond the time when GnRH is presumably removed from the circulation, possibly reflecting binding of GnRH to pituitary cells and continued release for

some time thereafter. This hypothesis is supported by the continued LH release after withdrawal of GnRH *in vitro* (Fig. 3).

Demonstration of a dose-related LH release *in vitro* is evidence that GnRH acts directly on the anterior pituitary. Prolonged release of LH after GnRH was removed from the superfusing medium suggests that only a brief exposure of the pituitary to GnRH is required to induce events which are not immediately reversible. Interpretation of this phenomena will require additional study.

Others have demonstrated large between animal variation in response to synthetic GnRH although it was not verified statistically (3). In contrast, we observed no significant variation among heifers or among bulls, or to day of GnRH treatment. This result suggests that GnRH may be used, at least within a homogeneous population, with high predictability in treatment of cattle.

Summary. LH concentration in serum of heifers averaged 0.5 ng/ml and increased to peaks of 1.9, 7.3 and 11.1 ng/ml after a single iv injection of 5, 20 or 80 μ g GnRH, respectively. Similarly, LH concentration in serum from mature bulls averaged 1.1 ng/ml before GnRH and increased to peaks of 9.2, 19.3 and 39.1 ng/ml after 10, 40 and 160 μ g GnRH, respectively. The interval from GnRH to the LH peak and magnitude of the peak were

positively correlated. Exposure of pituitary explants, in constant superfusion, to 1 or 4 ng GnRH increased LH release from a pre-treatment base line of 0.25 ng/mg/min to 0.94 and 1.50 ng/mg/min after 20 min, respectively. LH release did not return to base line by 40 min after exposure to GnRH. A comparison of purified porcine and synthetic GnRH revealed parallel increases in media LH concentration with time after GnRH.

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