Luteinizing Hormone Response to Pulsatile Luteinizing Hormone-Releasing Hormone in Prepubertal Heifers

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ABSTRACT

The effects of 12 hourly 5-μg injections of luteinizing hormone-releasing hormone on luteinizing hormone release, were examined in 18 prepubertal Holstein heifers at 4, 7, or 10 mo of age. During a 6-h pretreatment period, mean serum luteinizing hormone concentrations and mean number of endogenous luteinizing hormone episodes per hour were not influenced by age. The 12-h treatment regimen induced a pulsatile release of luteinizing hormone in all heifers. The magnitude, pattern, and total amount of luteinizing hormone released were not influenced by age. However, in the 4 and 10-mo-old age groups, magnitude of luteinizing hormone response to the 3rd hourly injection of luteinizing hormone-releasing hormone was greater than the response to the second injection. Magnitudes of luteinizing hormone responses decreased with time after the 4th hourly injection through the 12th injection and patterns of decline appeared similar among the three age groups.

The pituitary of the prepubertal dairy heifer is able to respond to an hourly pulsatile administration of luteinizing hormone-releasing hormone and this treatment regimen appears to produce a self-priming effect on luteinizing hormone release.

INTRODUCTION

The concepts of the development of sexual maturity of mammals are incompletely defined. In particular, details for the heifer are lacking. Exact knowledge of the mechanisms of sexual development in the heifer could contribute to better understanding of the problem of delayed puberty in certain types of cattle. Also, exact knowledge of sexual development may be useful to devise successful superovulation schemes in prepubertal dairy heifers, methods that could increase speed and accuracy of identification of genetically superior individuals.

The female lamb appears to conform to the “gonadostat” hypothesis of sexual maturation by exhibiting a change in frequency (increase) of pulsatile luteinizing hormone (LH) secretion in response to rising estradiol concentrations during development (7, 16). In the heifer, it is not known whether frequency of pulsatile LH secretion increases in response to rising serum estradiol concentrations. Prepubertal heifers show no differences in LH response to a single intravenous injection of luteinizing hormone-releasing hormone (LHRH) at 3, 6, or 9 mo of age (1). Prepubertal bulls display no dose-response differences in LH response to a single intramuscular injection of LHRH throughout the prepubertal period (14).

If the heifer conforms to the gonadostat hypothesis, then one possible consequence of an increase in the frequency of endogenous LHRH release could be a progressive increase in pituitary responsiveness predicated on a self-priming effect. Such a self-priming effect could be a result of up-regulation of pituitary LHRH receptors and concomitant increases in synthesis and transfer of LH from a storage to a releasable pool. Possibly, an up-regulation of the pituitary occurs during the prepubertal period of the heifer.

This study was to determine the pituitary LH response to a pulsatile administration of LHRH in Holstein heifers at 4, 7, and 10 mo of age.

MATERIALS AND METHODS

Eighteen prepubertal Holstein heifers from the University of Georgia dairy herd and from a cooperator herd were used. Calves were separated into three age groups: 4, 7, and 10 mo of age.
age with six animals in each group. Initial serum progesterone concentrations were determined.

All animals were fitted with an indwelling jugular catheter 1 d before the trial. The catheter (i.d/o.d: 1.3/2.3 mm; TYGON Microbore Tubing, Fisher Scientific, Pittsburgh, PA) was introduced approximately 15 to 20 cm into a jugular vein via an 11-ga thin-wall trocar cannulation needle; the catheter was secured to the neck of the animal by suture. Approximately 30 cm of catheter tubing remained outside the skin of the animal to facilitate blood sampling. The catheter was covered by a patch of elastic bandage (ELASTOPLAST, Beiersdorf, Inc., Norwalk, CT), which then was wrapped twice around the neck of the animal. Calves were tied in head stanchions throughout sampling and given Coastal bermuda grass hay and water ad libitum.

On the day of the trial, catheters were exposed by removal from the patch and 5-ml blood samples were drawn from each animal at 15-min intervals for 6 h beginning at 0730 h. Concentrations of LH in these samples were used to determine mean serum pre-treatment LH concentrations and mean number of endogenous LH pulses (to be considered a pulse required two standard deviations above the previous sample). At the end of the 6-h pretreatment, each heifer was given 12 successive i.v. pulses of 5 μg LHRH (CEVA Laboratories, North Chicago, IL) in 1-ml sterile saline (35 g NaCl/L) at 1-h intervals. Blood sampling at 15-min intervals was continued throughout LHRH treatment and for 12-h posttreatment. During the 12-h treatment, blood samples at the time of the LHRH infusion were collected immediately before the infusion. Blood samples were stored overnight at 4°C and then centrifuged for 15 min at 2000 × g until they were assayed for LH and progesterone. Concentrations of LH were determined for all samples, whereas concentrations of progesterone were determined only in the first sample drawn at the beginning of the pretreatment period for all animals.

Serum LH concentrations were quantified by a double antibody radioimmunoassay (4). Rabbit antiserum was raised against bovine LH (NIH-LH-B9) by the multiple site injection technique (19). Purified bovine LH was used for iodination (LER-1072-2) and for standards (NIH-LH-B10). Aliquots of 2.5 μg LH in 10.0 μl of sodium bicarbonate were iodinated by the chloramine-T method (9) with modifications.

The assay protocol included the simultaneous addition of 250 μl of 0.01 M phosphate buffer (pH 7.5) containing .1% gelatin (PBS-G); 250 μl of serum sample or standard in PBS-G; 200 μl of rabbit antiovine LH serum; and 100 μl of PBS-G containing 125I-LH (10,000 cpm) into a 12 × 75-mm test tube. The antiovine LH serum was diluted at 1:280,000 with PBS-G containing 1:400 normal rabbit serum. Non-specific binding tubes contained 200 μl of 1:400 normal rabbit serum in place of the anti-bovine LH antibody. All tubes were vortexed and incubated for 24 h at 4°C. On d 2, 200 μl of sheep anti-rabbit serum diluted 1:25 in PBS-G plus 500 μl of .05 M phosphate buffer (pH 7.5) containing 6% polyethylene glycol (60 g/L) were added simultaneously to all tubes. Tubes were then vortexed and allowed to incubate for 15 min at 4°C. After incubation, tubes were centrifuged for 30 min at 2200 × g to separate the antibody-bound [125I]LH (precipitate) from the free [125I]LH (supernate). The supernatant fluid was decanted and the radioactivity remaining in the pellet was counted in a scintillation counter (model 1285 Tracor Analytic Inc., Elk Grove Village, IL). Dilution of the first antibody produced binding of 18 to 23% of the [125I]LH. Standard curves consisted of tubes containing .156, .3125, .625, 1.25, 2.5, 5.0, and 10.0 ng LH.

Initial validation of the assay indicated that a dose response curve for a pool of bovine sera was parallel to the standard curve for NIH-LH-B10 (P>.5). When 1.0, 2.0, 4.0, or 6.0 ng LH were added to sera, .90 ± .04, 1.59 ± .02, 2.99 ± .05, and 4.45 ± .28 ng, respectively, were quantitatively recovered (79 ± 7%, n = 8). Sensitivity of the assay was estimated to be .6 ng per tube. Intraassay and interassay coefficients of variation were 8.1 and 23.1%, respectively.

The progesterone assay was performed as previously reported (5). The intraassay and interassay coefficients of variation were 3.5 and 6.7%, respectively.

Data were analyzed using the general linear models procedure of the statistical analysis system (2). Analysis included a repeated measures analysis of variance of LH with time.
TABLE 1. Mean serum luteinizing hormone concentrations during pretreatment, luteinizing hormone-releasing hormone treatment, and posttreatment in 4, 7, and 10-mo-old Holstein heifers.

<table>
<thead>
<tr>
<th>Age group (mo)</th>
<th>Pretreatment</th>
<th>Treatment</th>
<th>Posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>SE</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>10</td>
<td>.96</td>
<td>.01</td>
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\(^1 n = 6.\)

as the continuous variable and age of the animal as the classification variable.

RESULTS

All heifers displayed mean serum progesterone concentrations less than 1.0 ng/ml (.35 ± .14; mean ± SE; n = 18). Mean serum LH concentrations during the three sampling periods for the three age groups are shown in Table 1. There were no differences (P > .05) for serum LH concentrations due to age within the three sampling periods. Mean serum LH concentration during the 6-h pretreatment for all individuals was 1.01 ± .04 ng/ml (mean ± SE; n = 18). The LHRH treatment raised mean serum LH concentrations to 7.22 ± .72 ng/ml (mean ± SE; NE = 18) during the 12-h treatment.

The mean number of endogenous LH pulses/h for the 6-h pretreatment and 12-h posttreatment samplings are shown in Table 2. There were no differences (P > .05) for mean number of endogenous LH pulses per hour due to age within the pretreatment or posttreatment periods.

Individual serum LH profiles during the entire 30-h sampling period are shown in Figures 1 through 3. Pulsatile LH activity during the 6-h pretreatment period was best exemplified by a 4 and 10 mo heifer (Figures 1c, 3e, respectively). Four of 18 individuals (22%) representing all age groups showed no evidence of pulsatile LH activity during pretreatment and posttreatment sampling periods (Figures 1e; 2c; 3c, d). All individuals responded to the LHRH treatment regimen with pulsatile release of LH. However, 6 of 18 heifers (33%) did not respond to every LHRH treatment with a release of LH (Figures 1e, f; 2a, d, e; 3d), and 4 of 18 heifers (22%) exhibited more than one LH pulse in response to a given LHRH treatment regimen (Figures 1f; 2c; 3d).

No differences were (P > .05) due to age for the magnitude of LH release in response to LHRH, as shown in Figure 5. Regression analysis of LH release over the 12-h treatment showed that the pattern of LH release was not influenced (P > .05) by age of the animal. Analysis of LH release during the 6 h pretreat-

TABLE 2. Mean number endogenous luteinizing hormone pulses pretreatment and posttreatment in 4, 7, and 10-mo-old Holstein heifers.

<table>
<thead>
<tr>
<th>Age group (mo)</th>
<th>Pretreatment</th>
<th>Posttreatment</th>
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<tbody>
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<td></td>
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<td>SE</td>
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<tr>
<td>10</td>
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<td>.051</td>
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\(^1 n = 6.\)
Figure 1. Luteinizing hormone (LH) profiles for individual 4-mo-old heifers over 30-h sampling period. Samples taken at 15-min intervals. Hour 1 to 6 pretreatment; h 7 to 18 treatment, animals received hourly pulses of luteinizing hormone-releasing hormone; h 19 to 30 posttreatment.
Figure 2. Luteinizing hormone (LH) profiles for individual 7-mo-old heifers over 30-h sampling period. Samples taken at 15-min intervals. Hour 1 to 6 pretreatment; h 7 to 18 treatment, animals received hourly pulses of luteinizing hormone-releasing hormone; h 19 to 30 posttreatment.
Figure 3. Luteinizing hormone (LH) profiles for individual 10-mo-old heifers over 30-h sampling period. Samples taken at 15-min intervals. Hour 1 to 6 pretreatment; h 7 to 18 treatment, animals received hourly pulses of luteinizing hormone-releasing hormone; h 19 to 30 posttreatment.
Figure 4. Mean luteinizing hormone (LH) concentrations of 4, 7, and 10-mo-old heifers during 6-h pretreatment sampling.

Figure 5. Mean luteinizing hormone (LH) concentrations of 4, 7, and 10-mo-old heifers during 12-h treatment. Each animal received 5 μg luteinizing hormone-releasing hormone at hourly intervals, on the hour. (Note difference in scale of LH concentrations from Figures 4 and 6.)
that heifers used in this trial were prepubertal at the time of the study.

Mean serum LH concentrations observed pretreatment are comparable with those previously reported for prepubertal beef and dairy heifers (8, 10, 18). From the present data there were no differences for the frequency of endogenous LH pulses due to age during the 6 h pretreatment; this observation agrees with findings by McLeod et al. (12) for the prepubertal beef heifer but contrasts with the report by Schams et al. (17) for the prepubertal dairy heifer. Noted differences could be attributed to varying experimental procedures.

Pituitary release of LH into serum was evident within 15 min for the first LHRH treatment in 17 of 18 individuals. This finding agrees with that of Barnes et al. (1) for the response of prepubertal dairy heifers to a single 200 μg LHRH injection. It is noted that the individual not responding to the first LHRH treatment (Figure 2e) did not respond until the third LHRH treatment; there is no explanation for such a delayed response in this individual.

The LH release in response to the first 3 hourly LHRH treatments in this study is similar to that observed by Schams et al. (17), who administered 1 μg LHRH/kg body weight to prepubertal dairy heifers monthly until puberty. The LHRH treatment regimen in the present study was approximately .02 μg LHRH/kg body weight for each of the 12 injections. McLeod et al. (12) administered 9 consecutive 5-μg injections of LHRH at 2-h intervals to prepubertal Hereford × Friesian crossbred heifers at 4 and 10 mo of age. They used 2 animals in each age group and observed no differences due to age for LH response to the LHRH treatments.

In the present study, we observed no significant differences (P>.05) due to age for LH response to 12 hourly 5-μg injections of LHRH. However, LH response to the third LHRH treatment was greater (P<.05) than the response to the first LHRH treatment of the 4 and 10-mo-old heifers but not for the 7-mo-old group. These increases in LH responses to subsequent LHRH treatments may be due to self-priming effects of LHRH on LH release as reported both in prepubertal rats and in dispersed bovine pituitary cell cultures (13, 15).

However, the apparent self-priming effect observed in the present study simply may reflect a cumulative increase in serum LH concentrations, because LH failed to return to pretreatment baseline values between the first four LHRH treatments in 15 of 18 heifers (83%). Heifers that did not display any apparent self-priming effect were those 4 that displayed lower than average LH release in response to LHRH treatment. McLeod et al. (12) did not observe any increase in LH response to repeated LHRH injections at 2-h intervals, and LH returned to baseline between LHRH treatments. These various observations cumulatively indicate that there may be a critical frequency of LHRH stimulation that may prime pituitary gonadotrophs in the prepubertal heifer.

Magnitudes of the LH responses to the LHRH decreased progressively after the fourth LHRH treatment in the present study, although all individuals continued to respond to succes-

**Figure 6. Mean luteinizing hormone (LH) concentrations of 4, 7, and 10-mo-old heifers during 12-h posttreatment.**

**Table 3.** Area under the luteinizing hormone curve during the 12-hr treatment of study in 4, 7, and 10-mo-old Holstein heifers.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Area</th>
<th>SE</th>
</tr>
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<tbody>
<tr>
<td>(mo)</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>6845.6</td>
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<tr>
<td>10</td>
<td>5485.3</td>
<td>1259.7</td>
</tr>
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1n = 6.
sive LHRH treatments throughout the 12 injections. This pattern of decreasing response contrasts with observations by McLeod et al. (12) who found no decrease in magnitude of the LH response to 9 consecutive 5-μg LHRH injections every 2 h. Possible explanations for the decreasing LH response observed in the present study using an hourly injection scheme include: 1) a decrease of a readily releasable pool of LH at a rate that exceeded synthesis and transfer of LH from a storage to a releasable pool (6); 2) down-regulation of LHRH receptors on pituitary gonadotrophs (11); or 3) steroidal feedback modulation of pituitary responsiveness to LHRH as a consequence of possible induction of ovarian steroidogenesis by LHRH treatment.

Analysis of the area under the LH curve indicated that the total LH released by LHRH was not influenced by age. These observations contradict those of Schams et al. (17) who observed for prepubertal dairy heifers that magnitude of LH response (measured as area under the dose-response curve) to a single monthly injection of 1 μg of LHRH/kg body weight increased throughout the prepubertal period.

Reports (12, 17) indicate subtle differences in endocrine responses between dairy and beef females. The observations of this study for dairy heifers show no increase in LH pulse frequency with increasing age during prepuberty. This observation agrees with that of McLeod et al. (12) for prepubertal Hereford x Friesian heifers but conflicts with that of Schams et al. (17) for prepubertal dairy heifers. Again, noted differences may result from differing experimental procedures.

No differences for pituitary sensitivity to a constant dose of LHRH due to age were observed in the present study. McLeod et al. (12) did not observe any dose-response differences for LH responses to three doses of LHRH (0.5, 2.0, or 5.0 μg) between beef x dairy heifers at 4 or 10 mo of age. However, results of the present study indicate evidence of a self-priming effect of LHRH on LH release in the prepubertal heifer. To our knowledge this is the first report of such an effect in immature heifers.

ACKNOWLEDGMENTS

The authors express appreciation to Terry Kiser, Department of Animal and Dairy Science, University of Georgia, for help with the research and radioimmunoassays, and Leo E. Reichert, Jr., of Albany Medical College, Albany, NY, for the iodination hormone. The authors also thank Myron Brown of Ceva Laboratories, Inc., Overland Park, KS, for the gift of the synthetic luteinizing hormone-releasing hormone. Appreciation is also extended to Maribeth Johnson for statistical analysis of the data and to Camie Thomas for preparation of the manuscript.

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