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Luteinizing Hormone Release by Gonadotropin Releasing Hormone Before and After Castration in Bulls[1] (38459)

K. MONGKONPUNYA,[2] H. D. HAFS, E. M. CONVEY, W. D. OXENDER, AND T. M. LOUIS

Animal Reproduction Laboratory, Department of Dairy Science, Michigan State University, East Lansing, Michigan 48824

The interaction of gonadal steroids and gonadotropin releasing hormone (GnRH) in causing pituitary LH release is imperfectly understood, especially in males. Magnitude of LH release by exogenous GnRH is increased in castrate male rats (1, 2), pigs (3), and sheep (4) compared with intact males. These results suggest a role for testicular hormones in modification of releasing hormone stimulation of LH release. Yet, we found no reports describing attempts to reduce the exaggerated LH response to GnRH in castrate males with exogenous androgens. Therefore, our primary objective in the present investigation was to compare magnitude of GnRH induced LH release prior to castration, following castration and during testosterone replacement.

We have previously reported that LH is released episodically in mature intact bulls (5) and that increases in serum LH were accompanied, or closely followed, by elevations in serum testosterone. Whether these acute elevations in serum testosterone play a role in regulating LH release is not clear. Therefore our secondary objective was to determine effects of castration and testosterone replacement on patterns of episodic changes in serum LH.

Materials and Methods. Six Holstein bulls were 9 mo old and averaged 173 kg when used in these experiments. The experimental protocol is shown in Table I. Briefly, blood samples were collected at hourly intervals for 24 hr at 2 days prior to castration, at 21 days postcastration, and at 23 days postcastration, at which time testosterone treatment (20 mg, im, thrice daily) had been underway for 24 hr. Each bull was given GnRH[3] (40 μg, iv) at 24 hr before castration, at 7 and 14 days postcastration, and at 28 days postcastration, which followed 6 days of testosterone treatment. Blood was collected via jugular cannulae at frequent intervals before and after each treatment. Details of blood sampling intervals are shown with results. Blood was allowed to clot at room temperature for 2–3 hr, held at 5° for approximately 15 hr, centrifuged at 2500*g* for 15 min, and sera stored at −20° until assayed for LH and testosterone as previously described (6, 7). Bovine LH[4] (NIH B-8) was used as standard.

Analysis of variance for a split plot design combined with Scheffe's interval was used to determine significance of differences in magnitude of LH released in response to the standard dose of GnRH. Significance of differences in number or magnitude of episodic increases in LH or testosterone that occurred prior to and after castration and after testosterone replacement treatment were tested by Tukey's honestly significant difference procedure (8).

Results. Serum LH and testosterone after GnRH. Changes in serum LH concentration in response to a standard GnRH challenge administered prior to and after castration and after testosterone replacement are listed in Table II and Fig. 1. Before GnRH, LH (ng/ml) was low in serum collected prior to castration (1.0 ± 0.4) relative to comparable means for Days 7 (3.4 ± 0.4) and 14 (7.1 ± 0.6) postcastration. At 28 days after castration, after testosterone injections for 6 days, serum LH concentration (ng/ml) averaged

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[2] Present address: Kasetsart University, Bangkok 9, Thailand.

[3] GnRH was generously supplied by Dr. R. Rippel, Abbott Laboratories, North Chicago, IL.

[4] Bovine LH (NIH B-8) was generously provided by The Endocrinology Study Section, NIH.

TABLE I. Experimental Protocol.

Experimental event	Time relative to castration	Time of day
	(days)	(hr)
Jugular cannulation	−3	1600–1800
Hourly blood samples	−2	900 Day −2 to 800 Day −1
40 μg GnRH[a]	−1	1000
Castration	0	1000
40 μg GnRH[a]	7	1000
40 μg GnRH[a]	14	1000
Hourly blood samples	21	900 Day 21 to 800 Day 22
3 × daily testosterone (20 mg[b])	22-28	800,1600 and 2400
Hourly blood samples	23	1000 Day 23 to 900 Day 24
40 μg GnRH[a]	28	1000

[a] Administered intravenously.
[b] Administered intramuscularly.

1.9 ± 0.7, lower ($P < 0.05$) than the comparable value for Day 14 after castration. Peak serum LH concentration (ng/ml) and area under the LH response curve (ng ml^{-1} min × 10^2) after 40 μg GnRH was 21 and 16.6 prior to castration. Comparable means at Day 7 postcastration were not significantly changed relative to precastration values, but on Day 14 after castration the GnRH induced LH peak was significantly increased ($P < 0.05$). At Day 28, after 6 days of testosterone treatment, the LH response to GnRH remained greater than precastration values and area under the LH response curve had increased relative to the comparable value at 7 days postcastration.

Testosterone was quantified in sera from intact bulls after GnRH (Fig. 2). Prior to GnRH administration, testosterone averaged 4.0 ± 1.5 ng/ml, then increased ($P < 0.05$) to 8.9 ± 1.4 ng/ml at 0.75 hr after GnRH, peaked (9.9 ± 1.3 ng/ml) at 1.5 hr, then progressively declined to pretreatment levels within 4 hr after treatment.

Episodic changes in serum LH and testosterone. The number of episodic increases in serum LH concentration (> 0.5 ng/ml) in samples collected at 1-hr intervals during 24 hr, averaged 3.7 prior to castration and was increased ($P < 0.05$) to 6.5 on Day 21 postcastration. But, the magnitude (Δ) of increase in LH concentration in these episodic events was not affected by castration, averaging 2.7 ± 0.6 and 3.2 ± 0.4 ng/ml, respectively (Table III). At 24 hr following initiation of thrice daily testosterone injections, the number (6.0 ± 0.6) and magnitude (3.6 ± 0.5 ng/ml) of episodic releases of LH were unchanged ($P > 0.05$) relative to the comparable means for 21 days postcastration. Prior to castration, each change in serum LH coincided with or preceded by 1 hr an increase in serum testosterone which averaged 12.0 ± 0.7 ng/ml.

Exogenous testosterone. Following injection of exogenous testosterone on Day 2 of testosterone treatment, serum testosterone increased

TABLE II. Serum LH Response to Gonadotropin Releasing Hormone Administered Prior to and after Castration and Following Testosterone Replacement.[a]

Criterion	Day relative to castration			
	−1	7	14	28
				(Testosterone for 6 days)
Pretreatment LH (ng/ml)	1.0 ± 0.4[b]	3.4 ± 0.4[b,c]	7.1 ± o.6[d]	1.9 ± 0.7[b,c]
Peak LH (ng/ml)	21.0 ± 2.6[b]	38.0 ± 2.6[b,c]	52.6 ± 3.1[c]	57.6 ± 13.5[c]
Area under LH response curve[a]	16.6 ± 3.1[b]	19.7 ± 3.3[b]	27.0 ± 2.7[b,c]	36.3 ± 8.9[c]

[a] Values are means ± standard errors for six animals; values having the same superscripts are not different.

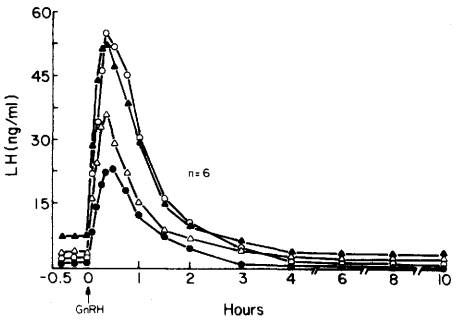

FIG. 1. Serum LH response to 40 μg GnRH (iv) at 1 day before castration (● —— ●), 7 (Δ —— Δ) and 14 days (▲ —— ▲) after castration, or at 28 days postcastration, after testosterone treatment for 6 days (○——○).

from an average of 3.8 ng/ml at time zero to 12.2 ng/ml at 1 hr postinjection then decreased linearly ($P < 0.01$) over the remainder of the interinjection period (Fig. 3). Average serum LH concentration did not change significantly during the 8-hr period following exogenous testosterone.

Discussion. Enhanced release of pituitary LH by GnRH after castration in these bulls suggests that gonadal factors normally decrease pituitary responsiveness to GnRH. Failure to reduce the LH response to GnRH by administering exogenous testosterone at a dose sufficient to reduce base-line LH concentrations, suggests that the gonadal factor(s) is not testosterone. Similarly, testosterone propionate did not effect magnitude of LH released by GnRH when administered to intact male rats (9). Alternatively, the time required to reverse castration effects on basal LH release may be much less than that required to reverse castration effects on pituitary sensitivity to GnRH, but, because GnRH was administered repeatedly to the same bulls in this experiment, we cannot rule out the possibility of carry-over as well as time effects in these studies. For example, the magnitude of LH release in response to GnRH in rats increases with time after castration (1). Similarly, in our bulls the response to GnRH appeared to be greater at 14 than at 7 days postcastration although this increase was not significant. However, the possibility that the LH response would have been increased further on Day 28 if testosterone treatments had not been administered for 7 days cannot be determined from this experimental design. Similarly, steroid treatment may have different effects depending on time of administration after castration. Estradiol injections decreased the magnitude of LH release after GnRH when given at 1 day postcastration but the same dose of estradiol at 7, 30, or 60 days postcastration augmented the LH response (1).

Precastration serum LH and testosterone for the six bulls in this experiment are similar to values for mature dairy bulls previously reported from this laboratory (5). Our results indicate that LH was not released acutely in response to withdrawal of testosterone following castration, but basal LH increased in serum collected at 7 days after castration. Odell and co-workers (10) reported that serum LH concentration first increased in serum of male and female calves at 2–4 days following gonadectomy.

Episodic release of LH has been observed in intact bulls (5, 11) and rams (12), and results of the present investigation confirm those observations. In addition, we observed continuation of the episodic releases of LH after gonadectomy, similar to results recently reported for castrate rats (13). The greater frequency of bursts of LH release after castration probably results from removal of negative feedback of the gonadal steroids. However, testosterone replacement after castration did not significantly reduce the frequency of LH release. These results suggest that the frequency of the episodic release of LH in bulls is controlled by gonadal factors other than testosterone. It is unlikely that the dose of testosterone administered was subminimal, since serum concentrations resulting from this dose fell within the normal range for this species (1). But since hourly samples were collected only on the second day of testosterone replacement we cannot rule out the possibility that more time is required to reestablish steroid control over LH release.

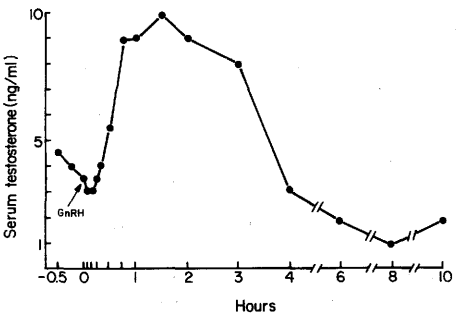

FIG. 2. Average serum testosterone after 40 μg GnRH (iv) in six intact bulls.

TABLE III. Number and Magnitude (Δ) of Episodic Increases[a] in Serum Luteinizing Hormone During 24-hr Periods Before and after Castration and after Testosterone Replacement in Bulls.

	Days relative to castration					
	2 days before		21 days after		23 days, testosterone for 24 hr[b]	
Bull	No.	Δ	No.	Δ	No.	Δ
1	4	2.1	6	4.9	7	4.8
2	5	1.0	6	3.4	7	3.4
3	3	3.7	7	4.0	6	3.6
4	4	4.4	6	2.4	7	4.9
5	3	3.4	7	2.0	6	2.0
6	3	1.4	7	2.8	3	2.7
Av	3.7 ± 0.3[c]	2.7 ± 0.6	6.5 ± 0.2	3.2 ± 0.4	6.0 ± 0.6	3.6 ± 0.5

[a] To be counted as an increase, the change in serum concentration had to exceed 0.5 ng/ml.

[b] Testosterone (20 mg) administered im three times daily.

[c] Significantly less ($P < 0.05$) than the comparable averages at 21 and 28 days after castration.

Although testosterone replacement in castrate bulls did not restore the frequency of episodic releases of LH to the low levels characteristic of these bulls when intact, it did reduce average baseline LH concentrations in the serum. Similarly, estradiol injections suppressed the increase in baseline LH that normally occur after gonadectomy in male rats (9).

Summary. Six 9-mo-old Holstein bulls were used to compare the magnitude of LH release in response to gonadotropin releasing hormone administered prior to and after castration and following testosterone replacement. In addition, effects of castration and testosterone replacement on patterns of episodic LH release were investigated. GnRH caused release of LH before and after castration, but the magnitude of LH increase in response to GnRH increased 2.5-fold at 14 days postcastration relative to precastration changes. Testosterone replacement did not reduce the magnitude of LH response to GnRH to precastration levels. LH concentration in serum collected at hourly intervals prior to castration averaged 1.1 ng/ml, only 17% of the comparable value at 21 days after castration (6.7 ng/ml). The number of episodic increases in serum LH concentration during a 24-hr period prior to castration averaged 3.7 and increased to 6.5 at 21 days postcastration. Testosterone replacement restored neither the average number nor the magnitude of LH increase to levels characteristic of the precastration period. We conclude that LH release in response to GnRH is increased after castration and the increase is not reversed by testosterone. In addition, LH is released episodically in bulls, and peaks of LH normally are closely followed by increased testosterone in serum. Castration increases the frequency but not the magnitude of LH increase and testosterone does not restore frequency to precastration levels.

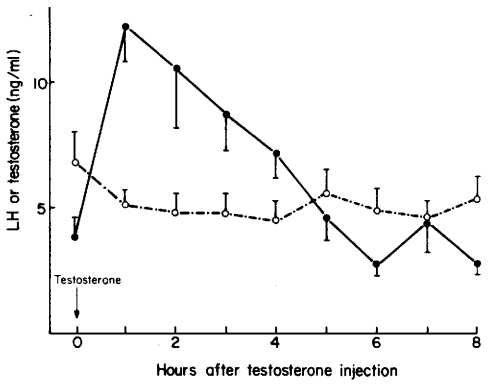

FIG. 3. Average serum LH (O—·—·—O) and testosterone (● —— ●) concentration during exogenous testosterone (20 mg, im) administration for six steers. Each point represents the mean and standard error of two observations in each steer.

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