GONADOTROPIN RELEASING HORMONE-INDUCED CHANGE IN SERUM LUTEINIZING HORMONE, TESTOSTERONE AND ANDROSTENEDIONE IN BULLS, STEERS AND STEERS GIVEN TESTOSTERONE

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SUMMARY

Two experiments were conducted to determine the effect of gonadal hormones on LH release by successive injections of Gonadotropin Releasing Hormone (GnRH). In the first experiment 1 month-old Holstein bulls were used. Bulls were assigned to be: 1) left intact (n=5), 2) castrated (n=6), or 3) castrated and given 10 mg testosterone thrice daily for 15 days (n=4). All animals received three intramuscular injections of 20 µg GnRH administered at 12-hr intervals beginning 14 days post-castration. Average magnitude of LH release by GnRH, measured as area under the response curve was greatest (P<.01) in steers and least (P<.01) in steers given testosterone. Magnitude of LH release in response to the first GnRH challenge was greater (P<.01) than that of the second or third. Serum androstenedione was increased (P<.05) after GnRH treatment. These data indicate that testicular hormones affect the ability of the pituitary of bulls to respond to GnRH as early as 1 month of age.

A second experiment utilized pubertal bulls and was conducted in two parts. In the first part, four bulls were left intact, four were castrated and four were castrated then given testosterone (20 mg) thrice daily from the day of castration (day 0) through day twenty-one. On day 21, each animal was given an intramuscular injection of 40 µg GnRH at 0900, 1300 and 1700 hour. Magnitude of increase in serum LH concentration averaged overall injections was less (P<.05) in steers given testosterone than in untreated steers or bulls. Similar to results with prepubertal bulls, the increase in serum LH that occurred after the first GnRH injection was greater (P<.001) than that after the second or third GnRH injection. These same animals were used in the second part of this experiment. Treatment of the two groups of steers was reversed from days 22 to 28. On day 28 each animal was given an intramuscular injection of 40 µg GnRH at 0900, 1300 and 1700 hour. Magnitude of increase in serum LH was greater (P<.01) in steers given testosterone from days 21 to 28 than in intact bulls and least (P<.01) in steers that had been given testosterone for 21 days post-castration. Following the first GnRH injection, magnitude of LH release was greater (P<.01) than the comparable average after the second or third injections. In pubertal bulls, serum testosterone and androstenedione was increased (P<.01) after each GnRH injection and the increases were quantitatively similar on day 21 and 28. These results suggest that a series of GnRH injections may be more useful than single injection in evaluating the capabilities of the pituitary to release luteinizing hormone. In addition, the degree to which the pituitary recovers its ability to respond to a second GnRH injection may be related to rate of LH biosynthesis.

(Key Words: Luteinizing Hormone, Testosterone, Androstenedione, Gonadotropin Releasing Hormone, Castration, Puberty.)

INTRODUCTION

Orchidectomy causes increased basal concentrations of serum luteinizing hormone (LH) and a marked increase in magnitude of LH release

1080 JOURNAL OF ANIMAL SCIENCE, Vol. 44, No. 6 (1977)
by gonadotropin releasing hormone (GnRH) in pubertal bulls (Mongkonpunya et al., 1974a), sexually mature rams (Galloway and Pelletier, 1975) and rats (Debeljuk et al., 1973). Testosterone given to steers (Mongkonpunya et al., 1974a) or wethers (Galloway and Pelletier, 1975) reduced serum LH concentrations to those of intact controls, but magnitude of LH release by GnRH was unaffected by testosterone. However, in those experiments, steers had been castrated for 3 weeks and wethers for more than 6 months when GnRH was given. Testosterone replacement begun immediately post-castration may prevent the increase in pituitary sensitivity to GnRH. In addition, it is not known whether castration increases the magnitude of LH release by GnRH in sexually immature bulls. Therefore, objectives of experiments reported here were to determine: 1) whether basal serum LH concentration and magnitude of LH release by GnRH are increased after castration in 1-month-old bulls, and 2) if exogenous testosterone could prevent or reverse the post-castration increase in magnitude of LH release by GnRH in immature and pubertal bulls.

A secondary objective of these experiments was to determine whether castration affects magnitude of LH release in response to a series of GnRH injections. Chakraborty et al. (1973), Rippel et al. (1974) and Mongkonpunya et al. (1975) observed that the degree of increase in serum LH concentrations in response to a series of GnRH injections was greatest after the first injection. In addition, the difference between magnitude of LH release by the first and subsequent injections of GnRH in ewes was reduced as the injection interval was increased (Rippel et al., 1974). To the extent that this rate of recovery might be related to rate of synthesis of LH, it was of interest to determine how conditions thought to alter rate of LH synthesis might affect change in magnitude of LH release by consecutive injections of GnRH.

MATERIALS AND METHODS

General. Bulls were assigned to be: 1) left intact; 2) castrated, or 3) castrated and given exogenous testosterone. Castrations were done on the first day of an experiment which was designated day zero. Testosterone was injected intramuscularly in 1 ml safflower oil thrice daily beginning immediately after castration.

The ability of a series of GnRH3 injections to cause LH release was determined on specific days indicated for each experiment. Each GnRH test series was given as a sequence of three intramuscular injections of GnRH in 1 ml .85% NaCl. Jugular blood was collected via cannulae at intervals before and after each GnRH injection as indicated in the figures for each experiment. An index of total LH response to GnRH was derived by measuring the area under the curve formed by plotting serum LH (ng/ml) against time after GnRH injection excluding basal LH concentrations, i.e., average of -20 and 0 samples. Area was measured using a polar planimeter. Serum LH (Convey et al., 1976), testosterone and androstenedione (Mongkonpunya et al., 1974b) concentrations were measured by radioimmunoassays previously described. Variance in hormone concentrations after GnRH were analyzed by methods described by Gill and Hafs (1971) for repeat measurements on individual animals. Selected comparisons were made using Orthogonal contrasts (Sokal and Rohlf, 1969).

Experiment One. Fifteen bulls were approximately 1 month old and averaged 71 ± 3 kg body weight when used in this experiment. Five bulls were left intact, six were castrated and four were castrated and given testosterone (10 mg) at 0700, 1500 and 2300 hr daily for 15 days. On day 13, jugular blood was collected at hourly intervals for 6 consecutive hr beginning at 0900. This blood was used to estimate serum testosterone and androstenedione concentrations. Gonadotropin releasing hormone (20 µg) was given at 0900 and 2100 hr on day 15.

Experiment Two. Twelve bulls, approximately 9 months old and averaging 165 ± 7 kg body weight, were used. This experiment consisted of two parts. In the first part, four bulls were left intact, four were castrated and four were castrated and given testosterone (10 mg) at 0700, 1500 and 2300 hr daily for 21 days. On day 21, each animal was given GnRH (40 µg) at 0900, 1300 and 1700 hour. Jugular blood was collected prior to and following GnRH and assayed for LH, testosterone and androstenedione concentrations.

The same animals were used in the second part of this experiment. Treatment of the two groups of steers were reversed from days 22 to

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3Synthetic GnRH was generously supplied by Dr. R. Rippel, Abbott Laboratories, North Chicago, IL.
Thus, steers given no testosterone from days 0 to 21 were given testosterone (20 mg) thrice daily through day 28 and steers given testosterone from days 0 to 21 were given no steroid from day 21 to 28. On day 28, all bulls and steers were given three injections of GnRH (40 μg) according to the schedule described for day 21 above. Luteinizing hormone data from parts one and two of this experiment were analyzed separately. Serum testosterone and androstenedione concentrations following injection of testosterone were similar on day 21 and 28. Therefore, data for each hormone were pooled and changes in serum testosterone and androstenedione concentration during an 8-hr period following testosterone injections was analyzed by multiple regression analysis (Steel and Torrie, 1960).

RESULTS

Experiment One. Serum testosterone concentrations during 6 hr on day 13 (the day prior to GnRH injection) of 1-month-old bulls, steers and steers given testosterone are shown in figure 1. In bulls, serum testosterone averaged 1.1 ± .06 ng/ml (X ± SE) over all sampling times and this average was not different (P>.05) from that for steers given no testosterone (.89 ± .04 ng/ml). In steers given testosterone, serum concentrations averaged 10.3 ± 2.4 ng/ml at 2 hr after injection (0900) and decreased (P<.01) to 1.7 ± .1 ng/ml immediately before the next testosterone injection was to be given; i.e., 1500 hour. Assuming that testosterone concentrations shown in figure 1 represent those after each testosterone injection, then testosterone averaged ≈10 ng/ml at the time of the first and third GnRH injection (0900 hr, day 15) and 4 ng/ml at the time of the second GnRH injection (2100 hr). The latter represents a time 6 hr after a testosterone injection at which time serum testosterone would presumably be equivalent to that at 1300 hr (figure 1).

Changes in serum LH concentration with time after GnRH (20 μg) in 1-month-old bulls, steers and steers given testosterone (10 mg 3 x daily).
BOVINE SERUM HORMONES AFTER GNRH

(P<.01) effect of treatment, injection (1, 2 or 3) and treatment by injection interaction. Magnitude of LH release (ng ml⁻¹ min x 10²) averaged over all injections was greater (P<.01) in steers (11.3 ± 1.1) than in bulls (7.9 ± 1.1) and least (P<.01) in steers given testosterone (4.4 ± 1). In addition, magnitude of LH release (ng ml⁻¹ min x 10²) averaged over all treatments was 10.2 ± .9 after the first GnRH injection; greater than the corresponding average resulting from the second (7.3 ± .5) or third (7.5 ± 1.2) injections of GnRH.

A significant interaction of the main effects resulted from a greater (P<.01) increase in LH release after the first GnRH injection than after the second or third injections in bulls and in steers given testosterone but not in untreated steers. The increase in serum LH concentration after the first GnRH injection was similar among treatment groups and significant effects due to treatment were revealed only after the second and third GnRH injections when the order of response was castrate > intact > castrate given testosterone (P<.01).

In immature bulls, changes in serum androstenedione and testosterone concentration with time were similar (P>.05) after each GnRH injection. Therefore, results were averaged over all injections and these data are given in figure 3. Serum androstenedione increased (P<.05) from .25 ± .04 ng/ml at time zero, to a peak of .47 ± .1 ng/ml by 2 hr after GnRH. Serum testosterone increased slightly from .47 ± .06 ng/ml at 0 hr to .79 ± .13 ng/ml at 3 hr, however, this increase was not significant (P>.05).

**Experiment Two—Serum Androgens.** Serum testosterone and androstenedione concentrations following testosterone or GnRH injections were not different (P>.05) between days 21 and 28. Therefore, data for each hormone were pooled for analysis and the pooled data are presented in figure 4. Inasmuch as serum testosterone and androstenedione decreased rapidly from peaks which occurred immediately following injection, serum testosterone and androstenedione concentrations at the time of each GnRH injection varied markedly. Thus, testosterone (ng/ml) and androstenedione (pg/ml) averaged 20 and 1,100 at the time of the first and third GnRH injections, and was 5 and 200 at the time the second GnRH injection was given (figure 4).

Serum testosterone concentration in intact bulls increased (P<.001) with time after GnRH injection (figure 4). Thus, serum testosterone concentration averaged over treatment days and time after injection was 4.5 ± .3 ng/ml after the first GnRH injection which was greater (P>.01) than the comparable average after the second (3 ± .9) or third (2.7 ± .3) GnRH injections. Serum androstenedione concentration increased after GnRH and magnitude of increase was similar after each injection. On the average, serum androstenedione (pg/ml) prior to each GnRH injection was 316 ± 38 and increased (P<.05) to 502 ± 50 at 1 hr after GnRH then returned to baseline (366 ± 20) by 3 hr after GnRH. Serum testosterone and androstene-
dione concentrations were unchanged in untreated steers after GnRH and averaged .24 ± .02 ng/ml and 150 ± 12 pg/ml, respectively.

**Luteinizing Hormone, Day 21.** Serum LH concentrations prior to and after GnRH on day 21 are given in figure 5. Average serum LH concentration at 20 min prior to the first GnRH injection on day 21 was 6.5 ± .9 ng/ml in steers which was greater (P<.05) than the comparable concentration for bulls (2.4 ± .38) or steers given testosterone (1.8 ± .3). Analysis of variance of these data revealed a significant effect of treatment (P<.05) and injection (P<.001). Thus, the magnitude of increase in serum LH concentration (ng ml⁻¹ min × 10²) averaged over all injections was less (P<.05) in steers given testosterone (17.8 ± 4.1) than in untreated steers (27.7 ± 7.9) or bulls (30.8 ± 5.8). The increase in serum LH concentration that occurred after the first GnRH injection when averaged over treatment groups was 47.7 ± 7.4 ng ml⁻¹ min × 10² and was greater (P<.001) than the corresponding concentrations after the second (17.8 ± 2.2) and third (13.6 ± 3.2) GnRH injections.

**Luteinizing Hormone, Day 28.** Serum LH concentration prior to and after GnRH on day 28 are given in figure 6. On day 28, serum LH concentrations at 20 min prior to the first GnRH injection averaged 1.3 ± .1 ng/ml in steers given testosterone from day 21 to 28 post-castration and this level was not different from that of intact bulls (1.1 ± .1 ng/ml). In contrast, LH in serum of steers in which testosterone injections were discontinued from days 21 to 28 post-castration, averaged 6.1 ± .8 ng/ml which was greater than that of intact bulls and steers receiving testosterone.

Magnitude of LH release by GnRH given on day 28 was significantly (P<.01) influenced by treatment and injection and the interaction of the main effects was significant (P<.01). Thus, magnitude of LH release (ng ml⁻¹ min × 10²) averaged over all injections was greater (P<.01) in steers given testosterone from 21 to 28 days post-castration (105.1 ± 25.2) than in intact bulls (35.3 ± 4) and least (P<.01) in steers (12.9 ± 2.8) that had been given testosterone for 21 days post-castration (figure 6). Following the first GnRH injection, magnitude of LH release averaged over all treatments was 94.2 ± 24.1 ng ml⁻¹ min × 10² which was greater (P<.01) than comparable values after the second (32.8 ± 5.6) and third (26.4 ± 5.3) injection. The magnitude of LH release in steers given testosterone for 21 days post-castration was similar (P>.05) after each GnRH injection whereas magnitude of LH release in steers given testosterone from days 21 to 28 and bulls decreased from the first to second injection but not from the second to the third injection (figure 6).
**Discussion**

The increase in basal serum LH concentration and magnitude of LH release induced by GnRH that occurred following castration in these prepubertal bulls indicates that testicular secretions normally exert a negative feedback on LH release in bulls as early as 1 month of age. That exogenous testosterone inhibits the post-castration increase in basal and GnRH-induced LH release in immature bulls demonstrates that the mechanism by which testosterone feeds back on the hypothalamo-hypophysial axis to suppress LH release has also matured in bulls by 1 month of age. This experiment does not, however, provide insight as to what testicular secretion is involved in negative feedback control of LH release in prepubertal bulls.

Exogenous testosterone was effective in preventing a post-castration increase in basal and GnRH-induced LH release. However, the amount of testosterone present in serum of these immature bulls was very low, i.e., not different from that of the untreated steers of similar age. On this basis, it seems unlikely that testosterone is the hormone which normally maintains serum LH at relatively low concentrations prior to puberty. A similar argument can be made against androstenedione as the hormone responsible for negative feedback control of LH secretion before puberty in bulls. Unquestionably, castration removes some testicular secretion which allows an increase in basal LH secretion and enhanced LH release by GnRH. However, the nature of this substance is not currently known.

These results also suggest that a series of GnRH injections may be more useful in evaluating the capabilities of the pituitary to release LH than a single injection. Apparently the pituitary will completely recover its ability to release LH if the time allowed for recovery is sufficiently long. Recovery of the pituitary's ability to respond to a second injection of GnRH may be related to its ability to regenerate releasable stores of luteinizing hormone. This may involve de novo synthesis of LH or perhaps an intercompartmental shift of this hormone to a readily releasable pool. Wakabayashi and Tamoaki (1967) measured rate of LH biosynthesis in male rats and observed an increase in LH synthesis in pituitaries of castrates relative to intact controls. This increase in LH biosynthesis was inhibited by daily testosterone treatment begun at the time of
castration. Experimental treatments of the bulls used in the present experiments were similar to treatments used by the latter investigators. Therefore, it seems a reasonable assumption that biosynthesis of LH was probably increased in steers and that this increase was suppressed by thrice daily testosterone injections. The results observed in these bovine males at 1 month suggest that under conditions that enhance LH synthesis (castration), the pituitary is capable of completely restoring its ability to release LH during a 12-hr injection interval. Under conditions where pituitary LH secretion was presumably normal (intact bull), recovery of the pituitary’s ability to release LH in response to GnRH was complete after 12 hr but was still greater than that of steers given testosterone. The shorter injection interval (4 hr) used for treatment of pubertal males apparently did not allow sufficient time for complete recovery of the pituitary to release LH. However, the effect of treatment on degree of recovery of the pituitary’s ability to release LH followed the same trend as for month-old bulls. These results suggest, but do not prove, that the capacity of the pituitary to restore its ability to respond to GnRH following exposure to a previous GnRH injection is related to rate of LH synthesis.

Apparently, exogenous testosterone can prevent the increase in magnitude of LH release by GnRH which occurs following castration. However, 7 days of testosterone treatment cannot reverse the castration effect when replacement therapy is delayed for 3 weeks. In contrast, the effect of testosterone on basal LH release occurs relatively quickly. Thus, exogenous testosterone decreased basal serum LH concentrations in sexually mature wethers (Galloway and Pelletier, 1975) and bulls castrated at puberty (Mongkonpunya et al., 1975) but did not decrease magnitude of LH release by GnRH to levels characteristic of intact males. In the aforementioned experiments, rams were castrated 6 months and steers at 3 weeks before testosterone was given.

Mongkonpunya et al. (1975) previously reported that testosterone given to steers from days 21 to 28 post-castration decreased basal LH concentrations but did not decrease magnitude of GnRH-induced LH release on day 28 to levels observed prior to castration. Results reported herein are consistent with our previous report inasmuch as testosterone from days 21 to 28 post-castration decreased basal LH in steers and did not reduce magnitude of LH release by GnRH to values characteristic of intact control bulls. In fact, in these experiments, testosterone for 7 days actually enhanced the increase in serum LH concentration that occurred after each GnRH injection. Interestingly, the converse result occurred with steers given testosterone for 21 days post-castration then given no testosterone from days 21 to 28; i.e., basal LH increased and GnRH-induced LH release remained less than that of intact control bulls. From these, and previous studies (Mongkonpunya et al., 1975; McCarthy and Swanson, 1976) it appears that the negative feedback effect of testosterone on LH release occurs relatively quickly, whereas shifts in rate of LH biosynthesis and/or rate of entry into a releasable pool after castration occurs only after a period of weeks. Although GnRH-induced LH release was similar in intact pubertal bulls and steers castrated for 21 days, basal serum LH concentrations were greater in the latter, suggesting that both LH synthesis and release were increased. Results on day 28 may be explained as follows: Giving previously untreated steers testosterone for 7 days resulted in inhibition of LH release but inhibition of LH synthesis probably occurred at a much slower rate or not at all. Thus, on day 28 releaseable pools of LH were increased due to inhibition of LH release and a continued relatively high rate of LH synthesis. Thus, magnitude of LH release was enhanced relative to that of intact bulls or that of these same steers on day 21. The converse argument applies to results observed with those steers given testosterone for 21 days then no testosterone to day 28.

Changes in serum testosterone and androstenedione observed after GnRH in these prepubertal and pubertal bulls are consistent with previous reports from this laboratory. Thus, testosterone concentration is increased by GnRH in serum of mature (Zolman and Convey, 1975) and pubertal bulls but not in sexually immature bulls (4 months or less) Mongkonpunya et al., 1975). However, serum androstenedione concentration was increased after GnRH at each of these developmental stages. Hooker (1970) reported that differentiation of mesenchymal cells of the testicular interstitium in bull calves began at about 3.5 months of age and developed into competent secretory cells during the next 3.5 months. Although serum testosterone concentrations in testosterone injected steers decreased markedly
during the injection interval, the absolute testosterone concentration at the time GnRH was given did not noticeably influence magnitude of LH release by GnRH. The degree to which serum testosterone increased in intact bulls after each GnRH injection was directly related to the magnitude of LH release. Whether the decrease in testosterone release into the blood that occurred from the first to the second and third GnRH injection resulted from the associated decrease in LH release by GnRH or refractoriness of the testis to LH is equivocal.

LITERATURE CITED


