SERUM TESTOSTERONE RESPONSE IN HOLSTEIN BULLS AFTER ADMINISTRATION OF LUTEINIZING HORMONE

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Summary

Seven yearling Holstein bulls were given 0, 20, 40, 60, 80, 100 and 200 μg NIH-LH-B9 during a 7-day complete Latin square design experiment. Treatments were administered via jugular cannulas at 0900 hr, and blood was collected at 60, 30 and 0 min before treatment, at 15-min intervals for 3 hr after treatment and at 30-min intervals from 3 to 7 hr after treatment. Luteinizing hormone (LH) and testosterone concentrations were measured in serum by radioimmunoassay. Area under the LH and testosterone response curves, expressed as nanograms per milliliter x hours, increased (P<.001) with increasing amounts of exogenous LH. Basal LH in serum averaged 1.4 ± .1 ng/ml, and peaks ranged from 3.8 ± .4 to 13.3 ± .4 ng/ml after 40 and 200 μg of exogenous LH, respectively. Basal testosterone in serum of bulls given saline was 1.8 ± .2 ng/ml, and peak responses ranged from 4.1 ± .3 ng/ml to 5.9 ± .5 ng/ml after 40 and 200 μg exogenous LH, respectively. The LH area response to exogenous LH was linear (Y = .4717 + .0352X; P<.001), with testosterone concentration reaching maximum level in response to 100 μg LH (Y = 1.0279 + .1041X + .0001X²; P<.001). On the basis of these data, we suggest that the magnitude of the pulsatile release of LH quantitatively controls the secretion of testosterone from the testes of yearling bulls.

(Key Words: Testosterone, Luteinizing Hormone, Dose-Response, Bulls.)

Introduction

Understanding of the control of testosterone secretion from bovine testes may provide information on variations in reproductive processes that depend on testosterone. Several researchers have reported that elevations in testosterone secretion were preceded by episodic peaks of luteinizing hormone (LH) in the serum (Mongkonpunya et al., 1975; Haynes et al., 1976; Kiser et al., 1978; Welsh et al., 1979a). Similar increases in LH and testosterone followed administration of gonadotropin-releasing hormone (Zolman et al., 1973; Mongkonpunya et al., 1975; Thibier, 1976; Schanbacher and Echternkamp, 1978). Stimulation of testosterone secretion after administration of large amounts (200 μg to 4 mg) of LH has been observed in bulls (Smith et al., 1973; Kiser et al., 1978; Welsh et al., 1979b). However, LH concentrations after these exogenous LH treatments were several fold higher than endogenous LH peaks that normally occur in bulls (Kiser et al., 1978). The ability of the testis to respond to relatively small variations in LH secretion in bulls may be important in view of research indicating that photoperiod (Leining et al., 1978) and stimuli associated with ejaculation (Katangole et al., 1971; Smith et al., 1973) may cause moderate changes in LH secretion in bulls.

The present experiment was designed to determine whether exogenous LH given at amounts estimated to mimic the normal pulsatile release of LH would alter serum testosterone concentrations in bulls. The specific objective was to determine whether testosterone response in bulls was dependent on the dose of LH or whether it was an "all-or-none phenomenon."
Materials and Methods

Seven Holstein bulls, 11 to 12 months of age (210 ± 35 kg), were housed in individual stalls. Jugular cannulas were inserted 2 days before the start of this experiment, which was designed as a complete Latin square. Each bull was given seven treatments of NIH-LH-B9 (0, 20, 40, 60, 80, 100 or 200 µg in saline) administered via the jugular cannula. One treatment was given daily for 7 consecutive days. Beginning at 0800 hr each day, blood was taken at 30-min intervals for 1 hr before saline or LH treatment, then at 15-min intervals for 3 hr and at 30-min intervals for 3 to 7 hr after treatment. Sera were stored at -20°C until assayed for LH and testosterone. Testosterone was measured in serum by radioimmunoassay as described by Kiser et al. (1978), except that the antiserum (MSU #74) was used at a final dilution of 1:12,500 and sheep antirabbit gamma globulin was used to separate free from bound hormone. Percentage of binding of radiolabeled testosterone ranged from 75 to 80%, a value which gave the desired precision and reliability for this assay in our laboratory. The range of the standard curve was 0 to 4 ng, and 20 pg of standard testosterone caused a reduction (P<.05) in binding below the level associated with the zero standard. Dihydrotestosterone crossreacted with the antitestosterone serum (40%), as has been reported by Kiser et al. (1978), but other steroids tested caused a negligible change in displacement of radiolabeled testosterone from the antiserum. Addition of 25, 50, 75 and 100 µl of pooled bull serum resulted in displacement of radiolabeled testosterone from the testosterone antiserum parallel (P>.50) to that caused by the testosterone standards. The intra- and interassay coefficients of variation were 9.2 and 12.9% for a pool of sera from bulls and 12.1 and 14.4% for a pool of sera from steers. LH was quantified in serum by a double antibody radioimmunoassay patterned after that reported by Convey et al. (1976) and validated in our laboratory by Dunlap et al. (1981).

LH and testosterone responses were determined by tracing of the pattern of hormone concentration as a function of time after treatment with a compensating polar planimeter. The resulting area of hormone response (square centimeters) represented the area from base line to base line and back to the origin and was converted to nanograms per milliliter x hours (Thibier, 1976) to allow for a more precise estimate of individual testosterone responses in bulls. Hormone peaks were defined as a concentration that exceeded the average of the previous nadir concentration by 50%. Data were subjected to analysis of variance and regression analysis with the Statistical Analysis System (Barr et al., 1976).

Results and Discussion

Average LH and testosterone concentrations in serum of all bulls after each treatment with LH are presented in figure 1. All bulls exhibited similar LH and testosterone patterns regardless of the order of treatment. In all cases in which an LH peak occurred following treatment, the peak was detected at 15 min after LH. In four of the 49 treatment periods, an endogenous serum LH peak or testosterone surge occurred during or immediately before treatment. Data from these treatment periods were omitted.

Average basal concentrations of LH and testosterone in serum before treatment were 1.4 ± .1 and 1.8 ± .2 ng/ml, respectively. Although the concentrations of both LH and testosterone after 20 µg of exogenous LH were comparable to those after saline treatment, administration of 40 µg of LH caused detectable increases (P<.05) in LH and testosterone. Randomly occurring LH peaks averaged 2.4 ± .9 ng/ml in bulls given saline, while average LH peaks ranged from 3.8 ± .4 to 13.3 ± .4 ng/ml in bulls treated with 40 µg and 200 µg LH, respectively. Peak concentration of LH after 40, 60 and 80 µg of LH were comparable to concentrations after episodic release of LH in bulls given saline treatment and similar to LH peak concentrations reported in bulls by Kiser et al. (1976, 1978) and Mongkonpunya et al. (1975). Peak concentrations of LH in serum after 200 µg LH (13.4 ± 1.1 ng/ml) were three to five times higher than those observed after saline, a finding consistent with the results of Kiser et al. (1978). There was a linear relationship between amount of LH administered and area under the LH response curve (r = .99; P<.001; figure 2). In addition, dose of LH and peak height of LH at 15 min after treatment were correlated (r = .98; P<.001). Presumably, exogenous LH resulted in a linear
range of LH concentration at the level of the testes.

Maximum concentrations of testosterone found during spontaneous surges averaged 4.6 ± .7 ng/ml and maximum concentrations of testosterone surges ranged from 4.1 ± .3 to 5.9 ± .5 ng/ml after 40 and 200 µg LH, respectively. These peak testosterone concentrations are lower in magnitude than those reported by Kiser et al. (1978), Schanbacher (1979) and Welsh et al. (1979b), but similar to that reported by Haynes et al. (1976). The reason for the apparent differences between studies in peak testosterone concentrations observed after endogenous release of LH or after exogenous LH treatment is not clear from the present experiment. However, environmental factors and breed and age of animal may contribute to variation in maximum testosterone response.
Maximum concentrations of testosterone were observed within 1 to 2 hr after LH peaks. The duration of the testosterone response, measured by the length of time that testosterone concentrations in sera were increased above the base line, was 2 to 3 hr and was not affected (P > .05) by dose of LH. Testosterone secretion, as indicated by area under the response curve, was described by a quadratic equation ($r = .99; P < .001$; figure 3). The relationship between testosterone area and dose of LH was linear from 0 to 100 μg; however, the mean testosterone area response to 200 μg LH (11.2 ng/ml × hr) was not different from the mean testosterone response to 100 μg LH (10.6 ng/ml × hr). Results of this experiment indicate that maximum testosterone production occurred after 100 μg LH. Analysis of variance indicated that peak testosterone concentration in serum and testosterone area were affected by dose of LH (P < .001 in both cases). Previously, Kiser et al. (1978) reported that the correlation between area under the response curves for LH and testosterone was greater than .9 after endogenous releases of LH and after PGF$_{2α}$-induced release of LH in bulls. These results, together with those from the present study, would suggest that LH secretion quantitatively controls testosterone secretion in the bull. In contrast, Welsh et al. (1979b) reported that the within-bull correlation for peak LH concentration and the subsequent testosterone concentration ranged from −.06 to .79. This apparent discrepancy may be explained by differences in frequency of blood sampling. Magnitude of LH concentration was determined by 15-min sampling intervals in the present study and by hourly sampling in the study by Welsh et al. (1979b). Frequent sampling probably is necessary to characterize the absolute magnitude of LH concentration.

Previous studies have demonstrated that exogenous LH causes increases in testosterone in bulls (Smith et al., 1973; Kiser et al., 1978). However, the amount of LH administered in those studies (200 μg to 4 mg) raised serum LH concentrations to values several times higher than the endogenous episodic concentrations normally observed (Smith et al., 1973; Kiser et al., 1978; Welsh et al., 1979a). In the present experiment, the amount of LH administered to bulls resulted in serum concentrations of LH more representative of the normal physiological concentrations in bulls. Nonsignificant increases in endogenous serum LH similar to those occasionally found after 20 μg LH in the present study may not always induce testosterone secretion. In other cases, small but detectable increases in endogenous serum LH equivalent to those found after administration of 40 μg LH in this study (which might not be observed by sampling at greater than 15-min intervals) may cause detectable increases in testosterone. Thus, peripheral concentrations of testosterone may increase only marginally after a slight increase.
in serum LH, but increases in testosterone concentrations in the testicular venous discharge may be appreciable (Amann and Ganjam, 1976; Schanbacher and Echternkamp, 1978). Our results support the concept that although increased testosterone secretion may not occur after every release of LH secretion, increases in LH secretion are necessary for increases in testosterone concentrations in the blood. Similarly, in other studies, a 1:1 relationship usually was found between the number of endogenous LH peaks and subsequent testosterone surges (Katangole et al., 1971; Smith et al., 1973; Mongkonpunya et al., 1975; Welsh et al., 1979a).

In addition to the variations in serum LH and testosterone resulting from random episodic secretion, changes in the secretion of these hormones may be caused by some environmental stimuli. Foote et al. (1976) found that testosterone concentrations in sera of bulls were higher in the spring than in the fall, but the authors did not measure LH in the sera. Other data indicate that changes in photoperiod may cause an increase in episodes of LH release by bulls (Leining et al., 1978). More pronounced increases in LH and testosterone in serum were observed after exposure of bulls to stimuli associated with ejaculation (Katangole et al., 1971; Smith et al., 1973). However, these transitory increases in hormone secretion may be non-specific events mediated via the central nervous system. In fact, large increases in growth hormone and prolactin, but not in LH in serum, were observed in bulls between 5 and 30 min after ejaculation (Convey et al., 1971).

More recently, Schanbacher (1979) induced cryptorchidism in bulls and found that the testosterone response to either GnRH-induced LH or purified ovine LH was diminished compared with the testosterone concentration in intact control bulls. Thus, while LH normally controls testosterone secretion, the testis may be modified artificially or perhaps by environmental stimuli, with a resulting variation in testosterone secretion.

In summary, we conclude that testosterone secretion in the bull can be regulated by exogenous LH in a quantitative manner. Therefore, internal or external stimuli that influence the magnitude of LH concentration may also control the total amount of testosterone secreted into the peripheral circulation by the testis of the bull. Delineation of biologically significant events controlled by the absolute concentration of testosterone in the peripheral circulation awaits further investigation.

**Literature Cited**


