SERUM LH SUPPRESSION BY ESTRADIOL BUT NOT BY TESTOSTERONE OR PROGESTERONE IN WETHERS

Lee A. Edgerton and Clifton A. Baile

Applebrook Research Center, SmithKline Corporation, West Chester, PA 19380

SUMMARY

The objective of this experiment was to determine the effect of steroids, previously shown to depress serum luteinizing hormone (LH) in rams, upon serum LH in orchiectomized sheep. Accordingly serum LH concentration was monitored during the intravenous injection of testosterone, progesterone or estradiol. Neither testosterone at rates of 12.5, 125 or 1,250 µg/hr nor progesterone at 125, 1,250 or 12,500 µg/hr significantly depressed LH during an 8-hr injection period. Estradiol, however, injected for 12 hr at only 1 µg/hr, depressed LH from 22 ng/ml to 11 ng/ml within 4 hours. Although the temporal response to this low dose of estradiol may have been slower, the depression in serum LH concentration appeared to be as great with 1 µg/hr as with 12 µg/hr indicating an all or none response. Two of the three steroids reported to depress serum LH in rams failed to do so in wethers. Thus, the steroid signals available for acute control of LH in this animal model are more limited.

(Key Words: LH Control, Wethers, Steroid Feedback.)

INTRODUCTION

If one chooses to study the control of luteinizing hormone (LH), then wethers provide a potential advantage over intact animals since normal LH concentrations in rams fluctuate from only a few nanograms per milliliter of serum to nondetectable levels (Bolt, 1971). Since published reports of steroid effects on LH in the ram indicate only suppression, the investigator is at a disadvantage if he starts with low levels. Therefore, it was decided to investigate the effects of testosterone, progesterone and estradiol upon LH concentration in wethers which had elevated basal LH concentrations. These three steroids had been shown by Bolt (1971) to suppress LH within 8 hr in the intact male.

MATERIALS AND METHODS

Twenty-seven mature western wethers (about 60 kg) were housed indoors and fed a 60% concentrate pelleted ration ad libitum. The animals, which were obtained from a commercial supplier, were 10 to 14 months of age and presumably had been castrated prior to 1 month of age. The animal room was maintained at 21 ± 2 C (range) and illuminated at all times.

In experiment I, seven wethers were injected continuously for 8 hr via a silastic jugular cannula with vehicle (Ethanol: .9% NaCl, 50:50 by volume) or testosterone at 12.5, 125 or 1,250 µg/hour. Syringe pumps were used to inject 2.5 ml/hr in each case. Each animal received each treatment in random sequence with a 48-hr interval between the start of each injection. Blood samples were taken immediately prior to the start of each injection (0 time) and at 15 min, 30 min and 1, 2, 3, 4, 6 and 8 hours.

In experiment II, eight wethers received in random sequence each of the following treatments: vehicle, .125, 1.25 or 12.5 mg progesterone/hour. As in experiment I, each injection was continuous for 8 hr with a minimum 48-hr interval between the start of each injection. Blood samples were taken immediately prior to the start of each injection (0 time) and at 15 min, 30 min and 1, 2, 3, 4, 6 and 8 hours.
Experiment III was conducted similarly with vehicle and estradiol-17β at doses of 3, 6 and 12 μg/hr, except that four wethers were used and each injection was continuous for 12 hours. Blood samples were taken at 0 time and hourly from 4 to 16 hr after the start of injection. Thus, in contrast to the other experiments, blood sampling continued 4 hr beyond the end of the injection period.

In experiment IV, each of eight wethers received in random sequence vehicle or estradiol at doses of 1, 3 or 6 μg/hr for a 4-hr period. Blood samples were taken at 0 time, 15 and 30 min and at 1, 2, 3 and 4 hours.

In all experiments blood samples were collected via jugular venipuncture. Samples remained at room temperature for at least 1 hr, and then were held overnight at 4 C prior to centrifugation after which serum was decanted and frozen until assay of LH by radioimmunoassay (RIA).

Data were analyzed for each experiment as described for randomized complete-block designs (Steel and Torrie, 1960). In addition, in experiment IV, orthogonal comparisons were made between each of the latter 6 time periods and all previous periods (Steel and Torrie, 1960).

**Radioimmunoassay of LH.** Development of the assay was patterned after Oxender et al. (1972) with the following exceptions. Rabbit antisera to either bovine or ovine LH were produced following a single injection of .1, .5, or 1.0 mg of NIH-LH (B8 or S18) according to the procedure of Vaitukaitis et al. (1971). Pertussis vaccine 5, fluid, bulk, 4XDO6-D and pertussis vaccine, crude, Lot BP0695 were used alternately in this procedure. Antisera to bovine LH from rabbit 397 was selected for use in the assay at a dilution of 1:100,000 (final concentration in assay tube was 1:500,000). The second antibody was produced as described (Oxender et al., 1972) except that the goats were immunized against both rabbit 6 and guinea pig 7 gamma globulins. Normal rabbit serum was diluted 1:200 in .05 M EDTA-phosphate buffered saline. The 1% egg white albumin in .01 M phosphate buffered saline (PBS) was replaced by .1% Knox gelatin in PBS and 3 ml of .2% gelatin:PBS was used in place of PBS just prior to centrifugation. Figure 1 illustrates the standard curve obtained when inhibition of 125I bound (logit scale) was made a function of the logarithm of standards ranging from .05 to 12.8 ng NIH-LH-S18. Parallel inhibition with serial dilutions of serum and pituitary extracts was also observed (figure 1). When standard ovine LH (1.6, 3.2 and 6.4 ng) was added to serum, assay recovery values ranged from 75 to 116% of the theoretical value. Specificity of the assay was proven by the negligible inhibition of binding produced by other pituitary hormones (table 1). The correlation between biological and radioimmunological values for pituitary preparations shown in table 1, including thyroid stimulating hormone (TSH) preparations, was .92. The extensive cross reaction with both ovine and bovine TSH (NIH preparations) may be due to retention of immunological activity of LH even though biological activity of LH was destroyed by oxidation with hydrogen peroxide as described (Reichert, 1961).

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5 Pertussis preparations were kindly supplied by Dr. R. Hosley of Eli Lilly Corporation.
6 Nutritional Biochemicals Corporation, Cleveland, OH 44128.
7 Miles Laboratories, Inc., Kankakee, IL 60901.
TABLE 1. HORMONE CONTENT OF VARIOUS PITUITARY PREPARATIONS

<table>
<thead>
<tr>
<th>Hormone</th>
<th>NIH#</th>
<th>TSHa</th>
<th>OAADb</th>
<th>RIAc</th>
<th>Bio/RIA</th>
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<tr>
<td>OLH</td>
<td>LER-1056-C2</td>
<td>1.73</td>
<td>3.4</td>
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</tr>
<tr>
<td>BLH</td>
<td>LER-1072-2</td>
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<td>3.4</td>
<td>.5</td>
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</tr>
<tr>
<td>OLH</td>
<td>S18</td>
<td>1.03</td>
<td>1.03</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>BLH</td>
<td>B8</td>
<td>1.03</td>
<td>1.03</td>
<td>1.0</td>
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</tr>
<tr>
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<td>S7</td>
<td>3.87</td>
<td>.01</td>
<td>.8</td>
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<tr>
<td>BTHS</td>
<td>B6</td>
<td>2.54</td>
<td>.02</td>
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</tr>
<tr>
<td>BTS (Pierce)</td>
<td>30</td>
<td>.005</td>
<td>.0003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPRL</td>
<td>B3</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
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<tr>
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<td>B1</td>
<td>.01</td>
<td>.002</td>
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</tr>
</tbody>
</table>

aUSP units/mg.
bOAAD (LH bioassay) values (units/mg) are those provided by NIH, using NIH-LH-S1 as a reference. Since LH contamination of other hormone preparations is listed as less than the value shown we have not calculated the Bio/RIA index for those preparations.
cValues are relative to OLH-S18 which was arbitrarily designated 1.03 for comparison with bioassay values.

dence supporting lack of inhibition by TSH per se was obtained by demonstrating (1) reduced inhibition with a more potent TSH preparation (table 1) and (2) the lack of increased inhibition in blood serum following injection of up to 100 µg thyrotropin releasing hormone (TRH), although as little as 5 µg TRH tripled immunassayable TSH (L. A. Edgerton, unpublished data). Sensitivity of the assay is .5 ng LH/ml.

The cumulative within assay coefficient of variation (calculated from four standard serums by the method of Rodbard, 1971) was 5.5%. This value is applicable for assay variation within experiments. The cumulative between assay coefficient of variation, however, was 32% and accounts for much of the variation in baseline concentrations of serum LH between experiments.

RESULTS

In experiment I (figure 2) the mean concentrations of serum LH for each testosterone dose preceding injection (0 time) ranged from 15 to 25 ng/ml and were only slightly lower (12 to 21 ng/ml) at 8 hours. Analysis of variance indicated that neither testosterone treatment nor time after the start of treatment accounted for significant amounts of variation (P>.1). Similarly, progesterone treatment in experiment II did not account for a significant amount of variation in LH concentration (P>.1). Mean concentrations of LH ranged from 24 to 29 ng/ml for the three progesterone doses prior to injection and from 18 to 29 ng/ml at 8 hr (figure 3).

In experiment III, estradiol suppressed serum LH concentration maximally by 4 hr and the response was not dose dependent in that 3 µg/hr was as effective as 12 µg/hr (figure 4). No significant increase in LH was observed during the 4 hr (hr 13 to 16) after administration of exogenous hormone was halted although a few samples with high LH (20 to 46 ng/ml) implied that suppression may have ended soon. The higher mean serum LH concentration for the

Figure 2. Effects of testosterone injected IV upon serum levels of LH in mature wethers. Each bar represents the mean of six or seven samples (± SEM). Solid bar = control (Ethanol:saline, 50:50), stippled bar = 12.5 µg/hr, open bar = 1.25 µg/hr, hatched bar = 1,250 µg/hr. Fifteen- and 30-min values follow time 0 and the bars between 2 and 4 hr represent values 3 hr after the start of injection.
control group at time 0 is due to one animal whose concentration consistently approached or exceeded 100 ng/ml on that day. In this case the control day followed the 12 μg/hr treatment with estradiol-17β, thus allowing speculation about a rebound effect.

The final experiment (IV) confirmed that estradiol treatment significantly depresses serum LH concentration within 4 hours (figure 5). In addition to the effects of treatment (P<.01) and time (P<.005) the interaction of treatment and time (P<.005) implied that serum LH concentration may have been suppressed more rapidly by the larger doses of estradiol. To further clarify the time and dose effects a separate analysis was performed excluding the control treatment. In this analysis, orthogonal comparisons of time showed that the first significant depression of LH concentration occurred at 2 hours. The treatment by time interaction (P<.1) suggested that this suppression may have resulted from the 3 and 6 μg/hr doses of estradiol, whereas 1 μg/hr may have been ineffective until later. By 4 hr, serum LH was suppressed by all estradiol doses to about 10 ng/ml, less than one-half the preinjection concentration, and equivalent to the level of suppression observed over 12 hr in experiment III.

Discussion

Results of this study should be evaluated keeping in mind the precautionary note of Schwartz and McCormack (1972) that “there is no reason to believe that a normally responding hypothalamic-pituitary axis still exists after long term castration”. Nevertheless, the suppression of LH in these mature wethers by estradiol indicates that some portion of the steroid feedback system is still functional and thus justifies a comparison of these results to those with intact animals and to the results of similar tests in other species.

Sampling interval and the large variation in LH concentration observed in these wethers may have prevented detection of subtle effects of testosterone upon LH secretion. However, the failure of testosterone to suppress serum levels of LH in these wethers is in distinct contrast to the effect of this steroid in rams as reported by Bolt (1971). Random surges of LH release were markedly suppressed in the ram
within 3 hr following a single intramuscular (IM) injection of testosterone. In the present study total steroid received over the 8-hr period ranged from 2 to 200% of the IM dose of testosterone administered by Bolt (1971). Thus the observed difference cannot be easily rationalized on the basis of route of administration or dose level. Rather, it appears that long term castration results either in an altered feedback mechanism or that the mechanism is dependent upon some additional steroids provided by the testis. Additional evidence for this has been presented by Galloway and Pelletier (1975) who suppressed serum LH in wethers castrated for 6 months by large (200 mg) doses of testosterone propionate every 48 hr for 2 weeks. However, even though large doses of this propionate derivative suppressed serum LH to levels typical of intact rams, the quantitative LH response to luteinizing hormone releasing hormone remained in the high range characteristic of wethers.

The failure of testosterone to suppress serum LH in these mature wethers appears to be in contrast not only to the results of similar treatment of rams (Bolt, 1971) but also to the effect of androgens in castrate male and female rats (Gay and Dever, 1971; Beyer et al., 1972). These latter studies may, however, only reflect differences in dose and length of treatment rather than a species difference. Indirect support of the idea that castration alters testosterone feedback is present in the report of Radford and Wallace (1971). These investigators induced follicular and ovulatory activity in ovaries of anestrous ewes with administration of testosterone propionate. This suggests an influence on gonadotropins in the intact animal but they were unable to influence serum LH concentration with a similar treatment in ovariectomized ewes.

Like treatment with exogenous testosterone, progesterone also failed to significantly suppress serum LH concentration in the present study. This is again in contrast to the effect of exogenous progesterone in the intact ram (Bolt, 1971). Progesterone doses in the present study ranged from 1 to 100% of the IM dose used for rams (Bolt, 1971). Since neither serum steroid concentrations nor clearance rates were determined in the present study or in Bolt's (1971) study, it is impossible to determine directly whether the intravenous administration in the present study produced circulating levels of hormone equivalent to those from IM injections. However, the following indirect evidence suggests that circulating levels of steroid in the present study would have been higher unless significantly greater clearance rates occurred in these wethers. Yuthasastroskosol et al. (1974) found a peak serum concentration of 3 ng/ml 4 hr after intramuscular administration of 30 mg of progesterone to ovariectomized ewes. This serum concentration would result from a theoretical absorption rate of 14.7 μg progesterone/min based upon a metabolic clearance rate of 3.675 liters/min and a head extraction of 25% (Bedford et al., 1972) for non-pregnant sheep. The injection rate for 30 mg given intravenously over an 8-hr period would be 62.5 μg progesterone/min, more than fourfold the rate apparently achieved by IM injection. Thus, neither dose nor route of administration appear to be plausible explanations for the contrasting results between wethers and rams.

These results are, however, consistent with the failure of progesterone to depress serum LH in castrate male rats (Kingsley and Bogdanove, 1973). They are also consistent with lack of serum LH suppression by progesterone in ovariectomized female monkeys, rats and ewes (Yamaji et al., 1972; Schwartz and McCormack, 1972; Scaramuzzi et al., 1971). This latter observation, however, lends little support for the hypothesis of change in feedback following castration since progesterone is generally considered to act in the intact female only by blocking estrogen induced surges of serum LH. Since blockade of estrogen induced LH surges can be accomplished by progesterone in ovariectomized ewes (Scaramuzzi et al., 1971) the alteration in progesterone feedback after castration in sheep may be limited to the male.

Of the three steroids tested by Bolt (1971) in rams, estradiol alone appears to retain its relative effect on LH secretion in the castrate. Here there is a clear resemblance to the situation in females where estradiol continues to manifest an effect on LH release in the ovariectomized ewe (Scaramuzzi et al., 1971). All doses tested in our experiments produced a depression in serum LH concentration within 4 hours. The response appeared to be an all or none effect, 1 μg/hr suppressing LH as much as 12 μg/hr, although the 1 μg/hr dose may have produced the response more slowly than the higher doses. Although estradiol has the same relative response in wethers as in rams (Bolt, 1971) it does not depress serum LH to the low
levels typical of rams. Rather, LH remains at a level about three-fold higher than that observed in rams (L. A. Edgerton, *unpublished data*; Bolt, 1971).

It is possible that higher doses of either testosterone or progesterone may have suppressed serum LH concentration. The suppression of serum LH in wethers with the more potent propionate derivative of testosterone given IM at doses 20 to 40 times greater than in the present study indicate this is true for testosterone (Pelletier, 1970; Galloway and Pelletier, 1975).

It is also possible that longer exposure to either testosterone or progesterone may have suppressed serum LH concentration and that estradiol might with time suppress LH to concentrations typical of the intact male. However, results from successive test days gave no hint of such an effect and the report of Riggs and Malven (1974) suggests that estradiol may not suppress LH to concentrations typical of the intact ram even after 3 weeks exposure to estradiol. Thus, although our results can only apply to wethers which have been castrated for a long period, they indicate an apparent quantitative change in effect of estrogen upon LH release and an apparent loss of feedback sensitivity of testosterone and progesterone in the mature castrate male.

These observations indicate that the castrate male sheep provides a model with greater potential range in LH concentration and thus may be superior to the intact animal for many studies. However, the steroid options for control of LH release in this model are reduced from those in the intact male.

**LITERATURE CITED**


