Modification of Acute Testosterone Responsiveness to Luteinizing Hormone by Follicle-Stimulating Hormone and Luteinizing Hormone in the Domestic Cockerel

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ABSTRACT

Pretreatment of newly hatched cockerels with low doses of highly purified ostrich or ovine follicle-stimulating hormone (FSH) (1 µg/injection × 5 or 7 injections over 2.5 or 3.5 days) enhanced subsequent acute in vivo testosterone responses to a range of doses of NIH ovine luteinizing hormone (LH). Similar pretreatment with corresponding LH preparations or with much higher doses of NIH-LH (25 µg/injection) enhanced responses to moderate and high doses of NIH-LH, but had no effect on responses to low doses. When combined with FSH in pretreatment, the high doses of NIH-LH suppressed the stimulatory effect of FSH on these latter responses. Other results indicated that the effects of FSH preparations on responsiveness to LH were not due to LH contamination: the activity of the ovine FSH was not reduced by immunoneutralization of its LH contamination, and very low doses of ovine LH, equivalent to amounts that might have contaminated the effective doses of the FSH preparation, had no effect on responsiveness to LH.

In slightly older cockerels, pretreatment with highly purified ovine FSH (1 µg/injection × 14 injection over 7 days, from Days 4–10 after hatching) had effects similar to those in the younger cockerels. Similar pretreatment with NIH-LH (25 µg/injection) suppressed the acute testosterone response to a low dose of NIH-LH, but seemed to enhance that to a higher dose (though this latter effect was not statistically significant). Thus, in the cockerel, FSH can enhance responsiveness of the testis to acute LH stimulation of testosterone production and LH can also modulate testicular responsiveness to itself.

INTRODUCTION

Current evidence indicates that acute testicular androgen secretion in birds is regulated principally by luteinizing hormone (LH); follicle-stimulating hormone (FSH) is essentially inactive both in stimulating this process by itself and in increasing the acute androgen response when given concurrently with or shortly before LH (Jenkins et al., 1978; Maung and Follett, 1978; Chase, 1982). Such pronounced specificity for LH has also been established in mammals (reviewed by Bartke et al., 1978). In reptiles, however, testicular androgen secretion is acutely responsive to both gonadotropins, and indeed FSH preparations are often more potent than corresponding LH preparations (reviewed by Licht et al., 1977; Callard et al., 1978). These comparative data raise the possibility that FSH may have retained some action on testicular androgen secretion in birds and mammals that is not apparent in studies of only acute stimulation.

From the effects of mammalian gonadotropin preparations on interstitial cell cytology and comb development in hypophysectomized domestic cockerels, Nalbandov et al. (1946) concluded that during prolonged treatment FSH can “synergize” with LH in stimulating testicular androgen secretion. Similar conclusions were drawn in several early studies of the effects of gonadotropin preparations on interstitial cells and sex accessories in immature rats (Greep et al., 1936; Evans et al., 1937; Parlow and Reichert, 1963). More recent studies of immature rats, involving both direct and indirect measurements of testicular androgen secretion, have indicated that several days of FSH treatment can enhance subsequent androgen responsiveness to acute LH stimulation (Odell and Swerdloff, 1976; Chen et al., 1976, 1977; van Beurden et al., 1976; Selin and Moger, 1977; Moger and Murphy,
1982). The investigations reported here were designed principally to determine whether a similar effect of FSH could be demonstrated in domestic cockerels. Studies of rats have also shown that LH can have multiple effects on testicular responsiveness to itself (reviewed by Purvis and Hansson, 1978; Payne et al., 1982), and the experiments described here included tests to determine whether LH can have similar effects in cockerels.

MATERIALS AND METHODS

Birds

Domestic cockerels (single-comb white leghorn) were obtained from H and N Inc., Petaula, CA, on the day of hatching and were used during the next 10 days. The ages of the birds and the conditions under which they were maintained are given with the experimental results.

Hormones

Hormone preparations used in these experiments were: ovine LH, NIH-S19 from the National Institutes of Health (Bethesda, MD); ovine FSH, G4-211B, and ovine LH, G3-256DA, from H. Papkoff (Hormone Research Laboratory and Reproductive Endocrinology Center, University of California, San Francisco); and ostrich FSH, G36B, and ostrich LH, G36BRB, from H. Papkoff and P. Licht (Dept. of Zoology, University of California, Berkeley). The ovine LH and FSH preparations from Papkoff are about 2–3 times and 30–50 times, respectively, as potent as corresponding NIH-S1 standards in all bioassays, radioimmunoassays (RIA), and radioreceptor assays in which they have been tested, and cross-contamination between them is <1%. The ostrich gonadotropin preparations are also highly purified and relatively free of cross-contamination (Papkoff et al., 1982; Bona Gallo et al., 1983).

Pretreatment and Acute LH Stimulation

All injections, for pretreatment and for acute stimulation, were given subcutaneously (in the back of the neck) in 0.2 ml of 0.01 M phosphate-buffered physiological saline, pH 7.2–7.4, containing 0.1% gelatin (PBSg). The basic protocol for all experiments consisted of pretreatment with FSH and/or LH (or saline) for several days (generally 2 injections/day) prior to tests of acute responses to LH (or saline) the next day (about 16 h after the last pretreatment injection). Hormone preparations and doses used in pretreatment and duration of pretreatment varied among experiments; details are given with experimental results. For tests of acute responses to LH, birds were injected with saline or one of several doses of NIH-LH and bled 2 h later from the jugular vein into heparinized glass tubes. Plasma was obtained by centrifugation (1800 g X 10 min at 4–6°C) and assayed for testosterone by RIA.

Immunneutralization of LH

After a series of experiments had demonstrated that pretreatment with the ostrich and ovine FSH preparations could enhance subsequent acute testosterone responses to LH, an experiment (Experiment IV) was designed to determine whether these effects could be due to LH contamination rather than intrinsic FSH activity. A rabbit antiserum generated (by H. Papkoff and S. W. Farmer) against the β-subunit of ovine LH (LH-A/S) was used to “neutralize” LH activity in the highly purified ovine FSH and LH preparations (G4-211B and G3-256DA). Solutions containing 50 μg of FSH or 1, 5, or 10 μg of LH in 250 μl of PBSg were incubated with 10 μl of LH-A/S or normal rabbit serum (NRS) for 48 h at 6°C. Hormone-antibody complexes were precipitated with 100 μl of a goat anti-rabbit γ-globulin serum. FSH was precipitated at 6°C and separated from unbound hormone by centrifugation (1800 g X 10 min at 4–6°C). Radioreceptor assays of the supernatants containing unbound hormones, using homogenates of porcine ovary and 125I-labeled human FSH and ovine LH as radioligands (after the methods of Licht and Midgley, 1976), indicated that 10 μl of the LH-A/S had no effect on the FSH binding activity of 50 μg of FSH but could “neutralize” the LH binding activity of as much as 8 μg of LH. It was concluded, therefore, that this volume of the LH-A/S could “neutralize” far more LH than the 0.5 μg (or less) that could be expected to contaminate 50 μg of the FSH preparation. Cockerels were pretreated with amounts of the supernatants from the NRS- and LH-A/S-treated FSH preparations equivalent to the doses of the original FSH preparation used in previous experiments or with doses of the untreated LH preparation equivalent to amounts that might reasonably be expected to contaminate those doses of the original FSH preparation.

Testosterone Radioimmunoassay

Plasma testosterone concentrations were determined by RIA as described previously (Chase, 1982). Although this assay is not entirely specific for testosterone (having about 38% cross-reactivity with 5α-dihydrotestosterone), results are expressed as "testosterone" throughout this paper.

Testis Size

Effects of hormone treatments on testis size were also determined in these experiments. Analysis of data from several early experiments indicated that testis weight followed the same pattern and differed by the same magnitude among groups as did gonadosomatic index, and that testis weight did not differ among groups given different acute LH treatments after the same pretreatment. Therefore, data concerning testis size are expressed as weights of paired testes and, in most cases, represent values from only one acute LH treatment group from each pretreatment group or pooled values from several such groups.

Statistical Analysis

Statistical analyses of all data were done with Duncan’s multiple range test (α=0.05) after logarithmic transformation to make variances homogeneous according to Cochran’s C test or Bartlett’s Box-F test (α=0.05).
RESULTS

Experiment I (Fig. 1 and Table 1)

Cockerels were held from the day of hatching on long photoperiods (16L:8D), given food and water ad lib, and pretreated for 7 days, from Days 4–10 after hatching, with 2 daily injections of saline, 1 μg of highly purified ovine FSH (G4-211B), or 25 μg of ovine LH (NIH-S19) (given 4 h after the beginning and 4 h before the end of the light phase). The following morning, about 16 h after the last pretreatment injections, birds were injected with saline or with 5 or 25 μg of NIH-LH and bled 2 h later. Regardless of pretreatment, plasma testosterone concentrations were very low (<25 pg/ml) in all birds acutely injected with saline. In saline-pretreated controls, plasma testosterone concentrations were increased in relation to the dose of LH injected acutely. Pretreatment with FSH significantly enhanced the acute response to the low dose of LH, whereas pretreatment with LH significantly suppressed this response. Both pretreatments seemed to enhance slightly the acute response to the high dose of LH, but these effects were not statistically significant. Both gonadotropin pretreatments increased testis weight significantly over the value for saline-pretreated controls, and the effect of the highly purified FSH preparation (total dose=14 μg) was about twice that of the NIH-LH preparation (total dose=350 μg).

Subsequent experiments were done with newly hatched cockerels that were held on short photoperiods (12L:12D) and fasted but allowed access to water. Birds were pretreated for only 3 or 4 days with saline or gonadotropin(s) given as 1 injection on Day 1 and 2 daily injections thereafter (2 h after the beginning and 2 h before the end of the light phase). Effects of pretreatments were assessed as in the previous experiment with older cockerels. As in that experiment, none of the gonadotropin pretreatments significantly altered plasma testosterone concentrations following acute injection of saline compared to values for saline-pretreated controls (<25 pg/ml). Therefore, in Figs. 2 and 3 data for groups of birds acutely injected with saline have been omitted.

### TABLE 1. Effects of FSH and LH pretreatments on testis weights in young domestic cockerels.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>Testis weight (mg/pair)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment I</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>22.25 ± 0.85 (15)a</td>
</tr>
<tr>
<td>FSH</td>
<td>31.58 ± 1.50 (14)c</td>
</tr>
<tr>
<td>LH</td>
<td>26.95 ± 1.58 (15)b</td>
</tr>
<tr>
<td><strong>Experiment II</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>5.75 ± 0.40 (15)a</td>
</tr>
<tr>
<td>FSH</td>
<td>13.36 ± 0.98 (10)b</td>
</tr>
<tr>
<td>LH</td>
<td>15.87 ± 1.11 (9)b</td>
</tr>
<tr>
<td>FSH + LH</td>
<td>20.09 ± 1.10 (9)c</td>
</tr>
<tr>
<td><strong>Experiment III</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>5.68 ± 0.32 (9)a</td>
</tr>
<tr>
<td>FSH</td>
<td>16.20 ± 1.34 (10)c</td>
</tr>
<tr>
<td>LH</td>
<td>10.72 ± 0.29 (10)b</td>
</tr>
<tr>
<td>FSH + LH</td>
<td>21.46 ± 2.01 (10)d</td>
</tr>
<tr>
<td><strong>Experiment IV</strong></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>4.00 ± 0.25 (19)a</td>
</tr>
<tr>
<td>FSH + NRS</td>
<td>10.78 ± 1.39 (6)b</td>
</tr>
<tr>
<td>FSH + LH-A/S</td>
<td>13.10 ± 1.51 (6)b</td>
</tr>
<tr>
<td>LH (10 ng)</td>
<td>4.94 ± 0.24 (10)a</td>
</tr>
<tr>
<td>LH (1 ng)</td>
<td>4.29 ± 0.25 (10)a</td>
</tr>
</tbody>
</table>

*Details concerning ages of birds, conditions under which they were maintained, and hormone preparations, dosages, and durations used in pretreatments are included with descriptions of experimental results. Testis weights are given as means ± standard errors (n). Superscript letters denote groupings determined by Duncan's multiple test (α=0.05); values with the same superscripts are not significantly different.
and data are illustrated only for groups acutely injected with doses of LH that elevated plasma testosterone concentrations significantly compared to values for groups acutely injected with saline.

**Experiment II (Fig. 2 and Table 1)**

Newly hatched cockerels were pretreated for 4 days with saline, the same doses of the same ovine hormone preparations used in Experiment I (1 µg of FSH, G4-211B, or 25 µg of LH, NIH-S19, per injection), or a combination of these. In saline-pretreated birds, acute testosterone responses to LH were minimal; responses to 27 µg and 81 µg of LH were no greater than those illustrated for 3 µg and 9 µg. FSH pretreatment significantly enhanced the acute responses to all doses of LH with which it was tested. Pretreatment with LH alone had no effect on the acute response to the low dose of LH, but, when combined with FSH in pretreatment, LH significantly suppressed the stimulatory effect of FSH on this response. Pretreatment with LH alone or with FSH and LH in combination significantly enhanced the acute responses to higher doses of LH. All gonadotropin pretreatments increased testis weight significantly over the value for saline-pretreated controls, and the effect of both gonadotropins in combination was significantly greater than the effect of either gonadotropin alone (total doses=7 µg of FSH and 175 µg of LH).

The dose-dependencies of the effects observed in the previous experiments were examined in an experiment of essentially identical design using the same ovine FSH preparation (G4-211B) but a more highly purified ovine LH preparation (G3-256DA), both at doses of 0.25 and 1 µg per injection (results not illustrated). The effects of the higher pretreatment dose of gonadotropins were the same as in Experiment II: FSH pretreatment enhanced the acute response to a low dose of LH (1 µg), whereas LH pretreatment had no effect on the acute response to that dose of LH but enhanced the response to a higher dose (5 µg); both pretreatments increased testis weight, FSH significantly more so than LH in this experiment. At the lower pretreatment dose, neither gonadotropin preparation had a significant effect on acute responses to LH, and only FSH increased testis weight significantly.

**Experiment III (Fig. 3 and Table 1)**

The effects of gonadotropin pretreatments on responsiveness to acute LH stimulation were confirmed (in part) using the same protocol but with ostrich FSH and LH, at doses of 1 µg per injection, alone and in combination. The results were essentially the same as in Experiment II, except that, at the doses used, when combined with ostrich FSH in pretreatment, ostrich LH
did not significantly suppress the effect of FSH on the acute response to the low dose of LH (1 \( \mu \)g) but did significantly enhance the effect of FSH on the acute response to the higher dose of LH (10 \( \mu \)g). The pretreatment effects on testis weight were also essentially the same as in Experiment II, except that the effect of FSH was significantly greater than that of LH.

The fact that the effects of ostrich and ovine gonadotropins were essentially the same was further confirmed in an experiment in which newly hatched cockerels were pretreated for only 3 days with saline or with ostrich FSH or LH or ovine FSH (G4-211B) or LH (G3-256DA) at doses of 1 \( \mu \)g per injection (results not illustrated). All gonadotropin pretreatments significantly enhanced subsequent acute responses to 5 \( \mu \)g of NIH-LH and significantly increased testis weights, and there were no significant differences among the effects of the various gonadotropin preparations.

Experiment IV (Fig. 4 and Table 1)

In order to confirm that the effects of FSH preparations were due to intrinsic FSH activity, rather than LH contamination, newly hatched cockerels were pretreated for 4 days with saline, the equivalent of 1 \( \mu \)g per injection of ovine FSH (G4-211B) that had been treated with either normal rabbit serum (NRS) or rabbit anti-ovine LH\( \beta \) serum (LH-A/S), or 10 ng or 1 ng per injection of highly purified ovine LH (G3-256DA), doses equivalent to the amounts that would be present in 1 \( \mu \)g of the FSH preparation if it were 1% or 0.1% contaminated with LH. The effects of these pretreatments on testis weight and on the acute testosterone response to 5 \( \mu \)g of NIH-LH were then determined. Pretreatment with either FSH preparation significantly increased testis weight and enhanced the acute testosterone response to LH. The LH-A/S-treated FSH preparation was as effective in both regards as the NRS-treated FSH preparation, and the effects of these serum-treated FSH preparations were essentially equivalent to those of untreated FSH preparations in previous experiments. Pretreatment with the very low doses of LH had no significant effect on either testis weight or the acute testosterone response to LH.

**DISCUSSION**

The results of these investigations indicate that in very young domestic cockerels, FSH, given at low doses in several treatment schedules, can enhance acute testosterone responses to subsequent LH stimulation, even though, at the same doses, FSH cannot acutely stimulate testosterone secretion by itself or increase acute testosterone responses when given concurrently with LH (Chase, 1982). This action of FSH was observed with highly purified preparations of the hormone from both the ostrich and the sheep, and there were no striking differences between the effects of the two species of FSH. Although FSH clearly increased the magnitudes of acute responses to a wide range of doses of LH, whether it also affected sensitivity to LH cannot be determined from these experiments.

In these studies, all FSH-induced increases in testicular responsiveness to LH were accompanied by increases in testicular weight, presumably resulting principally from effects of the hormone on the seminiferous tubules. It is possible that the effective FSH treatments also caused increases in the numbers of Leydig cells in the testes, but several observations argue against this being the only (or even the principal) means by which FSH increased testicular responsiveness to LH: in cockerels of both ages, FSH treatments that increased testicular weight and responsiveness to LH did not elevate basal plasma testosterone concentrations, and, in the
older cockerels, an FSH treatment that increased testicular weight and the response to a low dose of LH did not significantly increase the response to a high dose of LH. The effects of FSH on testicular responsiveness to LH thus seem to include an effect on Leydig cells beyond just stimulating an increase in their number. Whatever their specific mechanisms, these actions of FSH may be mediated by cells in the seminiferous tubules.

Similar effects of FSH on responsiveness to LH have been reported in the immature rat (Odell and Swerdloff, 1976; Chen et al., 1976, 1977; van Beurden et al., 1976; Selin and Moger, 1977; Purvis et al., 1979; Moger and Murphy, 1982). Such enhancement by FSH of responsiveness to LH may be important in the process of sexual maturation. Indeed, the greater responsiveness to acute LH stimulation in the older, fed, and photostimulated cockerels (compared to that of the younger, fasted, and photoinhibited cockerels) may have been due, at least in part, to endogenous FSH having an action similar to that demonstrated with exogenous FSH. This action of FSH may also be important during the onset of reproductive activity in seasonally breeding birds and mammals.

In the present studies, the ability of exogenous FSH preparations to enhance responsiveness to LH seems to represent intrinsic activity of FSH and not merely the activity of LH contamination. Not only were low doses of highly purified ostrich and ovine FSH preparations active in enhancing acute responses to LH, but the activity of the ovine FSH preparation in this regard was not reduced by pretreatment with a volume of anti-LH serum sufficient to "neutralize" far more LH than could reasonably be expected to contaminate it. Furthermore, very low doses of highly purified ovine LH, equivalent to the amounts that might reasonably be expected to contaminate an effective dose of this FSH preparation, had no effect on the acute testosterone response to LH stimulation. However, these experiments done with intact cockerels do not rule out the possibility that the enhancement by FSH of responsiveness to LH requires the presence of at least small amounts of LH (endogenous in this case) to be apparent. Whether the effect of FSH on responsiveness to LH is due to intrinsic activity of FSH has also been examined, with conflicting results, in hypophysectomized immature rats. In one such study, pretreatment with anti-LH serum greatly reduced or abolished the ability of a fairly crude ovine FSH preparation to enhance responsiveness to LH, and the effects of this FSH preparation were duplicated with low doses of LH (approximating the amounts that might have contaminated the effective doses of FSH) but not with a more highly purified ovine FSH preparation (at doses equivalent in FSH activity to those of the crude preparation) (Purvis et al., 1979). In other studies, however, the effects of FSH were not duplicated with such low doses of LH (Chen et al., 1976; van Beurden et al., 1976). Moreover, in a more recent study, a fairly crude ovine FSH preparation and more highly purified ovine and bovine FSH preparations, at doses equivalent in FSH activity, all enhanced responsiveness to LH, and pretreatment with neuraminidase, shown to destroy in vivo activity of FSH but not of LH, greatly reduced or abolished the effects of the highly purified FSH preparations on responsiveness (Moger and Murphy, 1982). Although apparently at odds with each other, all of these various results are compatible with the idea suggested above that the effect of FSH on responsiveness to LH requires the presence of some small amount of LH to be apparent.

The present studies also indicate that, in addition to acutely stimulating testicular androgen secretion, LH can modulate acute responsiveness to itself in very young domestic cockerels. At the doses tested, LH preparations from both the ostrich and the sheep consistently enhanced acute testosterone responses to "moderate" and "high" doses of LH, at least in the younger cockerels, and there were no striking differences between the effects of equivalent doses of the two species of LH. The effects of LH on these responses were neither additive nor synergistic with those of FSH. The effects of LH on acute responses to "low" doses of LH were somewhat more complicated. In the younger cockerels, neither low doses of highly purified ostrich or ovine LH nor much higher doses of NIH-LH (given alone in pretreatment) affected the minimal acute response to a "low" dose of LH; low doses of ostrich LH did not significantly suppress the stimulatory effect of FSH on this response, but much higher doses of NIH-LH did. In the older cockerels, in which acute responses to LH were much greater, high doses of NIH-LH suppressed almost completely the acute response to a "low" dose of LH. This latter effect may have
been due to LH suppressing the action of endogenous FSH in the same way it suppressed the action of exogenous FSH in the younger cockerels.

LH has also been shown to have multiple effects on responsiveness to itself or human chorionic gonadotropin (hCG) in the rat. Treatment with low to moderate doses of LH increases maximum responses but reduces sensitivity of rat Leydig cells to hCG, and treatment with higher doses of LH further reduces sensitivity and also reduces maximum responses (reviewed by Purvis and Hansson, 1978; Payne et al., 1982). As in the present experiments with cockerels, no additive or synergistic effects of LH on the FSH-induced increases in responsiveness to LH have been observed in experiments with rats in which they might have been apparent (van Beurden et al., 1976; Selin and Moger, 1977).

There is evidence that the actions of FSH and LH on testicular responsiveness to LH (or hCG) in the rat are mediated by effects on LH receptors, the adenylly cyclase-cyclic AMP phosphodiesterase system, and various steroidogenic enzymes (Odell and Swerdloff, 1976; Chen et al., 1976, 1977; Murono and Payne, 1979; Purvis and Hansson, 1978; Payne et al., 1982). These possibilities provide a framework for investigating the mechanisms mediating the actions of gonadotropins demonstrated by the present investigations in the domestic cockerel. In both species, the physiological roles of these gonadotropin actions in the regulation of testicular androgen secretion, as well as their biochemical mechanisms, remain to be clarified.

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