EFFECTS OF DIHYDROTESTOSTERONE BENZOATE ADMINISTRATION ON GONADOTROPIN SECRETION IN OVARIECTOMIZED PONY MARES

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ABSTRACT

Eight long-term ovariectomized pony mares were treated with either dihydrotestosterone (DHT) benzoate (400 µg/kg body weight) in safflower oil or an equivalent amount of oil every other day for 21 d to determine the effects of DHT on follicle stimulating hormone (FSH) and luteinizing hormone (LH) concentrations in blood samples drawn once daily and after administration of three successive injections of gonadotropin releasing hormone (GnRH). The GnRH injections were given at 4-h intervals on the day following the last DHT or oil injection. Treatment with DHT benzoate did not alter (P>.10) concentrations of FSH or LH in daily blood samples relative to controls. The FSH and LH response, assessed by areas under the GnRH curves, decreased (P<.05) from the first to third injection of GnRH when averaged over both groups of mares. There was no effect of DHT treatment on FSH response to GnRH. There was an interaction (P<.05) between treatment and GnRH injection for LH areas; areas decreased (P<.05) for DHT-treated mares from the first to third GnRH injection but were unchanged for control mares. It seems that DHT alone cannot mimic the stimulatory effects of testosterone on FSH production and secretion as observed in previous experiments with ovariectomized and intact mares. Moreover, because intact mares have been shown previously to respond to DHT treatment with an increase in GnRH-induced FSH secretion, it appears that some mechanism is lost in long-term ovariectomized mares, making them unresponsive to DHT treatment.

(Key Words: Testosterone, Follicle Stimulating Hormone, Luteinizing Hormone, Gonadotropin Releasing Hormone, Mares.)

Introduction

Testosterone propionate (TP) treatment of ovariectomized and intact mares causes an increase in the follicle stimulating hormone (FSH) response to administration of gonadotropin releasing hormone (GnRH; Thompson et al., 1983c, 1984). This same stimulatory effect of TP treatment on FSH secretion has been reported for geldings (Thompson et al., 1979). Reville-Moroz et al. (1984) showed that the increased FSH response in pony mares after TP treatment was partially due to an increase in de novo production of FSH. Moreover, Thompson et al. (1983c) reported that the common androgenic metabolite of testosterone, dihydrotestosterone (DHT), also caused an increase in the FSH response after administration of GnRH in intact cyclic mares. Also in that study, treatment with either TP or estradiol benzoate suppressed FSH concentrations in daily blood samples, whereas treatment with DHT did not (Thompson et al., 1983c). Thus, TP administration resulted in both androgenic and estrogenic effects in intact mares.

Because 1) the effects of TP treatment on FSH secretion were similar in intact and ovariectomized mares and 2) DHT treatment increased the FSH response to GnRH in intact mares without suppressing daily FSH secretion, we hypothesized that DHT treatment of ovariectomized mares would increase the FSH response to GnRH without affecting daily hormonal secretion. Thus, the present experiment was designed to determine the effects of DHT treatment on concentrations of FSH and luteinizing hormone (LH) in daily blood samples and on the response of these gonadotropins to administration of GnRH in long-term ovariectomized pony mares.

Materials and Methods

Eight long-term (> 6 mo) ovariectomized pony mares between the ages of 3 and 20 yr were used. All mares were kept on pasture and were supplemented with grass hay as needed to maintain good body condition. Beginning on...
May 21 (d 1), blood samples (15 ml) were drawn from each mare by jugular venipuncture at approximately 0800 h; daily blood samples were drawn in this manner through d 26. On d 6, each mare received a subcutaneous injection of either DHT benzoate (400 µg/kg body weight) in safflower oil (DHT-treated mares) or an equivalent amount of safflower oil (control mares). Injections of DHT benzoate and oil were repeated every other day through d 26. On d 27, an indwelling catheter (14 gauge) was inserted into one jugular vein of each mare and five samples of blood were drawn at 15-min intervals. Immediately after the fifth blood sample was drawn, each mare was injected with GnRH (1 µg/kg body weight) through the jugular catheter; blood samples were then collected at 15, 30, 45, 60, 90, 120, 180 and 240 min after injection of GnRH. Immediately after the 240-min sample was drawn, a second injection of GnRH was administered and post-GnRH blood samples were again drawn as described above. A third injection of GnRH was administered immediately following the 240-min sample after the second GnRH injection and the above post-GnRH sampling schedule was again repeated. Blood samples were placed at 5°C until centrifugation; plasma was harvested and stored at -15°C.

Concentrations of FSH and LH in plasma were determined by radioimmunoassay as described previously (Thompson et al., 1983a,b). Partially purified equine FSH (LER-1686-2)\(^3\) and LH (LER-958-1)\(^3\) were used as standards. Hormonal concentrations were adjusted before analysis for pretreatment differences between groups by subtracting each animal’s pretreatment average from all its subsequent values and then adding back the grand mean of all animals as described by Thompson et al. (1977). Adjusted data for hormonal concentrations over time were analyzed by analysis of variance in a split-plot design (Gill and Hafs, 1971). Areas under the three GnRH-response curves (table 1) indicated that the FSH response decreased (P<.05) from the first to third injection of GnRH for both groups of mares; however, there was no effect of DHT treatment nor any interaction between treatment and GnRH injection.

There was an interaction (P<.05) between DHT treatment and GnRH injection for area sample for each GnRH injection). Areas were analyzed by analysis of variance in a split-plot design with successive GnRH injections as subplots. Duncan’s multiple range test was used to determine significant differences between means (Steel and Torrie, 1960).

**Results**

Concentrations of FSH and LH in daily blood samples for DHT-treated and control mares are presented in figure 1. There was no significant effect of DHT treatment on concentrations of either hormone during the experimental period.

Concentrations of FSH and LH in response to the three successive GnRH injections are presented in figure 2. There was an effect (P<.01) of time in the analyses of variance for both gonadotropins, whereas there was no significant effect of DHT treatment nor any treatment × time interaction. Analysis of the areas under the GnRH-response curves (table 1) indicated that the FSH response decreased (P<.05) from the first to third injection of GnRH for both groups of mares; however, there was no effect of DHT treatment nor any interaction between treatment and GnRH injection.

There was an interaction (P<.05) between DHT treatment and GnRH injection for area

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Figure 1. Concentrations of FSH and LH in daily blood samples of control (C) and DHT-treated (T) mares. Treatment was initiated on d 6 (arrows). Pooled standard errors calculated from the analyses of variance were 26.4 ng/ml for FSH and 13.7 ng/ml for LH.
Figure 2. Concentrations of FSH and LH in plasma of control (C) and DHT-treated (T) mares administered GnRH at 0, 4 and 8 h on the day following the last DHT or oil injection. Pooled standard errors calculated from the analyses of variance were 23.4 ng/ml for FSH and 10.5 ng/ml for LH.

under the GnRH-response curve for LH (table 1). That is, area under the curve decreased from the first to second and third injections of GnRH for the DHT-treated mares but not for the control mares.

Discussion

Previous experiments have shown that treatment of ovariectomized mares, intact mares and geldings with TP consistently results in an approximately threefold increase in the FSH response to administration of GnRH (Thompson et al., 1979; Wallace, 1980; Thompson et al., 1983c, 1984; Reville-Moroz et al., 1984). All of those experiments were conducted in a manner similar to the present one with regard to numbers of animals per group, mode of steroid treatment and dosage of GnRH. The lack of effect of DHT treatment in the present study was consistent among mares, as was the stimulatory effect of TP in the previous experiments. Thus, we are confident that the results reported herein adequately characterize a true lack of effect of DHT at this dosage on LH and FSH secretion in the long-term ovariectomized mare. Moreover, because this dosage of DHT benzoate resulted in a significant stimulation of FSH secretion after GnRH in intact cyclic mares (Thompson et al., 1983c), it is unlikely that dosage of steroid was responsible for the lack of effect in these ovariectomized pony mares.

The TP-induced stimulation of FSH secretion after administration of GnRH results partly from an increase in de novo production of FSH (Reville-Moroz et al., 1984) and suppression of daily secretion of FSH (Wallace, 1980; Thompson et al., 1983c, 1984). Thompson et al. (1983c) showed that the increase in FSH response to administration of GnRH in intact mares was a classical androgenic effect because it could be produced by treatment with either TP or DHT benzoate. In that same experiment, it was concluded that the suppression of daily secretion of FSH was an estrogenic

TABLE 1. MEAN AREAS UNDER THE THREE SUCCESSIVE GN RH-RESPONSE CURVES FOR FOLLICLE STIMULATING HORMONE (FSH) AND LUTEINIZING HORMONE (LH) FOR OVARIECTOMIZED PONY MARES TREATED WITH DIHYDROTESTOSTERONE (TREATED) OR OIL (CONTROLS) a

<table>
<thead>
<tr>
<th>Hormone</th>
<th>GnRH</th>
<th>Group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Treated</td>
</tr>
<tr>
<td>FSH</td>
<td>1</td>
<td>267 b</td>
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<tr>
<td></td>
<td>2</td>
<td>184 bc</td>
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<td></td>
<td>3</td>
<td>69 c</td>
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<tr>
<td>LH</td>
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<td>136 b</td>
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<tr>
<td></td>
<td>2</td>
<td>66 c</td>
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<tr>
<td></td>
<td>3</td>
<td>51 c</td>
</tr>
</tbody>
</table>

aMares were treated every other day for 21 d and then administered GnRH on the following day.

b, cMeans within a column for each hormone with no like superscript differ (P<.05). Residual mean squares from the analyses of variance were 7,892 for FSH and 1,337 for LH.
effect because it could be produced by treatment with either TP or estradiol benzoate (Thompson et al., 1983c) but not with DHT benzoate. In the present experiment, treatment of long-term ovariectomized mares with DHT benzoate did not alter the FSH response to GnRH administration nor the concentrations of FSH in daily blood samples. Thus, it seems that the ability of the intact mare to respond to DHT is lost in the long-term ovariectomized mare.

Although the stimulatory effect of DHT on FSH secretion observed in intact mares (Thompson et al., 1983c) was not observed for the long-term ovariectomized mares in the present experiment, the FSH response to TP treatment is the same for both types of mares (Wallace, 1980; Thompson et al., 1983c, 1984; Reville-Moroz et al., 1984). This may mean that other metabolites of testosterone, such as estrogens or hydroxylated forms of testosterone, or testosterone itself, may be necessary for the androgenic stimulation of FSH production and secretion in the mare. In rats, DHT acts synergistically with estrogen to increase aggressiveness in males (Finney and Erpino, 1976) and stimulates masculine behavior in females (Baum et al., 1974). In both of those studies, the combined androgen and estrogen treatment appeared to affect the central nervous system rather than peripheral reproductive structures (Baum et al., 1974; Finney and Erpino, 1976). Thus, further research is needed with horses to determine whether estrogen in combination with DHT treatment will mimic the stimulatory effects of TP treatment on FSH secretion.

The decrease in FSH response from the first to third injection of GnRH (table 1) indicates that partial depletion of releasable reserves occurred. The average FSH responses (based on areas) to the second and third GnRH injections, respectively, were 79 and 42% of the first response. In ovariectomized ponies treated with TP, Reville-Moroz et al. (1984) found that a single injection of GnRH released 59% of the FSH calculated to be in the pituitary before GnRH injection; the release was 45% in control mares in that experiment. If the first GnRH injection in the present experiment also released approximately 45% of the total FSH in the pituitary, then the amount secreted for the second and third GnRH injections would be approximately 35 and 19%, respectively, based on their areas relative to the first GnRH.

There was a significant decrease in LH response from the first to second and third GnRH injections for DHT-treated mares, whereas there was no significant change in response for control mares (table 1). However, for each GnRH injection, there was no difference between DHT-treated and control mares. Because there was also no significant effect of DHT treatment on LH concentrations in daily blood samples, it is unlikely that this difference in response to successive GnRH injections was due to DHT treatment. Even in TP-treated mares, in which FSH response to GnRH was increased threefold (Thompson et al., 1984), LH response was reduced by only 25%. Moreover, Reville-Moroz et al. (1984) reported that TP treatment for 11 d did not significantly alter de novo production of LH in pituitaries of pony mares.

In conclusion, treatment of long-term ovariectomized pony mares with DHT benzoate at a dosage equivalent to that used for TP in previous experiments (Thompson et al., 1979; Wallace, 1980; Thompson et al., 1983c, 1984; Reville-Moroz et al., 1984) did not significantly alter daily LH or FSH secretion nor the response of these gonadotropins to three successive injections of GnRH. Because intact mares do respond to DHT and TP treatment in a similar manner (Thompson et al., 1983c), it appears that some important factor is lost in mares ovariectomized for at least 6 mo. Moreover, because TP treatment causes a response in long-term ovariectomized mares whereas DHT does not, it appears that some other moiety (or metabolite) of TP is required (other than DHT) to produce the previously observed androgen response.

Literature Cited


