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Effect of Testosterone and Estradiol-17β Alone and in Combination on LH and FSH Concentrations in Blood Serum and Pituitary of Geldings and in Serum after Administration of GnRH[1]

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ABSTRACT

Sixteen stallions were castrated and 30 days later assigned to 1 of 4 treatments: testosterone propionate (T, 175 μg/kg BW), estradiol-17β-3-benzoate (E, 44 μg/kg BW), the combination of both steroids (TE) or control (safflower oil). These dosages were administered every other day for 18 days (low dose) and then were doubled (high dose). Treatment at the high dose was continued for 27 days and the geldings were sacrificed. Treatment at the low dose resulted in concentrations of testosterone and estradiol in serum similar to those in intact stallions during the breeding season. Concentrations of LH and FSH in peripheral serum increased by 4–10-fold after castration and stabilized in ∿20 days. Concentrations of LH in serum were suppressed by T and even more rapidly by TE. Estradiol alone increased concentrations of LH in serum at the low dose. Compared with levels in control geldings, concentrations of LH in the pituitary glands were suppressed by ∿50% in T or TE treated geldings, whereas they were increased by 10-fold in E treated geldings. Concentrations of FSH in serum were rapidly suppressed by E or TE. Testosterone alone suppressed concentrations of FSH in serum at the high dose. Compared with control geldings, all treatments resulted in elevated concentrations of FSH in the pituitary gland; the effect of T was ∿3-fold greater than for E. The magnitude of increase in concentrations of LH and FSH in serum after an injection of GnRH generally reflected the concentrations of these hormones in the pituitary. However, E in combination with T decreased release of LH in response to GnRH, even though pituitary concentrations of LH were similar in T and TE treated geldings. Estradiol also decreased the release of FSH in response to GnRH in E treated geldings, compared with controls, even though pituitary concentrations of FSH were ∿2-fold greater.

INTRODUCTION

In the male, the effects of androgens and estrogens on gonadotropin synthesis and secretion differ among species. Testosterone and other androgens generally suppress the synthesis and release of luteinizing hormone (LH; Gay and Bogdanove, 1969; Steinberger and Smith, 1977; Drouin et al., 1978). There are reports where testosterone alone did not inhibit secretion of LH in castrates (Gay and Dever, 1971; Resko et al., 1977). Estrogens often stimulate synthesis and secretion of LH in castrate males (Kulin and Reiter, 1976; Hodges and Hearn, 1978). In males of some species, estrogen suppresses secretion of LH (Karsch and Foster, 1975; Resko et al., 1977; Buhl., 1978).

The effects of testosterone on synthesis and secretion of follicle-stimulating hormone (FSH) in males also varies among species. Testosterone may be inhibitory (Crim and Geschwind, 1972; Steinberger and Smith, 1977) or have no effect (Gay and Bogdanove, 1969; Resko et al., 1977) on secretion of FSH. Often testosterone causes an accumulation of FSH in the pituitary gland (Greep and Jones, 1950; Gay and Bogdanove, 1969; Steinberger and Chowdhury, 1977). In contrast, estrogens generally suppress secretion of FSH in males (Steinberger and Chowdhury, 1977; Resko et al., 1977; Schanbacher and Ford, 1977; Sawin et al., 1978).

Stallions produce large amounts of estradiol and estrone compared with the males of most mammalian species (Beall, 1940; Goldzieher and Roberts, 1952; Nyman et al., 1959; Bedrak

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and Samuels, 1969). The purpose of this experiment was to study the role of estradiol and testosterone in the regulation of secretion of LH and FSH in male horses. The effects of these steroids on serum concentrations of LH and FSH after an injection of gonadotropin releasing hormone (GnRH) were also studied. The response to GnRH was compared with the concentrations of LH and FSH in the pituitary glands.

MATERIALS AND METHODS

Sixteen stallions of light-horse breeds, between the ages of 3–17 years, were castrated (4/day) on November 5, 8, December 1 and 4. Testosterone propionate (T) and estradiol-17β-3-benzoate (E) were obtained from Sigma Chemical Co. A 16 mg/ml solution of T, a 4 mg/ml solution of E and a solution of both steroids at these concentrations were made with safflower oil. Beginning on Day 30 after castration, each gelding received 1 of 4 treatments: 1) 175 μg T/kg BW, 2) 44 μg E/kg BW, 3) both steroids at these dosages (TE), or 4) oil only. Steroids were administered s.c. every other day (low dose). On Day 48 after castration, the dosages were doubled (high dose) and injections were continued every other day until sacrifice (∿Day 75 after castration).

On Day 15 after initiation of treatment at each dose level (Day 45 and 63 after castration, respectively), each gelding was given an i.v. injection of GnRH (LHRH, US Biochemical Corp.) at 1.0 μg/kg BW at ∿1000 h.

On the day of castration blood samples (30 ml) were drawn at −2 h, −1 h and immediately prior to removal of the testes and then at 1, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 60 h after castration. On Days 5, 10, 15, 20, 25, 29 and every 3rd day thereafter to Day 68 after castration, samples were drawn at 0800 and 1600 h. When GnRH was injected, 10 ml samples were drawn −2 h, −1 h and immediately prior to injection and then at 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 300 and 360 min after injection of GnRH. For frequent bleedings on a given day, an indwelling catheter (10.2 cm long, 18 gauge, Becton-Dickinson and Co.) was positioned in the jugular vein and sutured in place. Blood samples were allowed to clot at ∿20°C for 30–60 min and then stored overnight at 5°C. Serum was harvested by centrifugation and stored at −15°C.

At sacrifice of the animals, the pituitary glands were removed, weighed with the capsule intact and then frozen in liquid nitrogen. Upon thawing, a portion of the adenohypophysis was homogenized in 0.01 M phosphate buffered saline (PBS, 0.15 M NaCl, pH 7.4) at a concentration of 3.25 mg wet tissue/ml. The homogenates were centrifuged at 10,000 × g for 20 min and the supernatants were diluted 1:100 in PBS containing 0.1% gelatin for assay of LH and FSH.

Testosterone was quantitated in extracts of 0.5 or 1.0 ml aliquots of serum by radioimmunoassay as described by Berndtson et al. (1974). Estradiol was quantitated in extracts of 1 ml aliquots of serum as described by Thompson et al. (1978). Luteinizing hormone was quantitated in serum and pituitary homogenates as described by Nett et al. (1975). Samples of serum were assayed in duplicate at 50 or 200 μl and the diluted pituitary homogenates were assayed at 4, 20, 100 or 500 μl. Follicle stimulating hormone was quantitated as described by Nett et al. (1978). Sample sizes were similar to those for the LH radioimmunoassay. The sensitivities of the LH and FSH radioimmunoassays were 0.4 and 3.4 ng/tube, respectively. For each hormone, all samples for a given animal were processed in one assay.

Data were analyzed by analyses of variance in a split-plot design (Gill and Hafs, 1971) when treatment groups were considered individually. For data immediately after castration, one-way analyses of variance were performed. Statistical significance was determined by Duncan's multiple range test (Steel and Torrie, 1960). When variances within groups differed due to treatment, separate analyses were performed for groups with like variances in addition to the overall analysis. Regression analysis was used to compare rates of change in hormonal concentrations over time (Draper and Smith, 1966).

RESULTS

After castration, concentrations of testosterone and estradiol decreased rapidly and stabilized within ∿6 h. Injections of T and E resulted in slow, gradual increases in serum testosterone and estradiol concentrations which were maintained for at least 48 h. The concentration of testosterone in peripheral serum of T, E, TE and control geldings averaged 1.44, 0.05, 1.34 and 0.05 ng/ml during treatment at the low dose level; at the high dose level, the corresponding concentrations were 2.70, 0.06, 2.51 and 0.05 ng/ml. Estradiol concentrations in peripheral serum of T, E, TE and control geldings averaged 2, 81, 98 and 2 pg/ml during treatment at the low dose level; at the high dose level, the corresponding concentrations were 10, 163, 210 and 2 pg/ml.

Concentrations of LH in peripheral serum began to increase ∿4–6 h after castration (Fig. 1). The rate of increase in concentrations of LH was greater ($P<0.05$) from 4–12 h after castration then during the later periods (24–60 h).

The long term effects of castration and steroid treatment on concentrations of LH are presented in Fig. 2. The serum concentrations of LH after castration were extremely variable and did not correlate with precastration values. Averaged over all groups, concentrations of LH increased ($P<0.05$) ∿14-fold from castration to Day 29. During treatment at the low dose, concentrations of LH in T and TE treated geldings decreased ($P<0.05$); the rate of decrease in LH concentrations was greater ($P<0.05$) in TE treated than in T treated geldings. Concentrations of LH in E treated

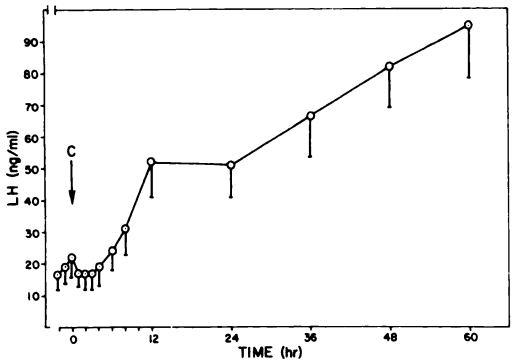

FIG. 1. Effect of castration on concentrations of LH in peripheral serum (n = 16). SEM denoted by vertical lines.

geldings were higher ($P<0.05$) than in control geldings during treatment at the low dose level. At the higher dose level, concentrations of LH in T and TE treated geldings stabilized at 2–6 ng/ml, which were lower ($P<0.05$) than precastration concentrations. By Day 56 postcastration, the higher dose of E decreased the elevated concentrations of LH in E treated geldings to values similar to those in control geldings.

Concentrations of FSH in peripheral serum increased ($P<0.05$) in all geldings after castration (Fig. 3) and stabilized by Days 20–25 postcastration. Concentrations of FSH in serum after castration were variable and were not correlated to precastration concentrations. Concentrations of FSH in serum of stallions prior to castration ranged from 46–262 ng/ml and averaged 105 ± 63 (SD) ng/ml. Concentrations of FSH in serum decreased ($P<0.05$) in E and TE treated geldings and were not different from precastration values by the end of treatment at the low dose level. Treatment at the higher dosage of E resulted in no further suppression of concentrations of FSH.

Treatment with T suppressed serum concentrations of FSH by ∿40% at the low dose level (Fig. 3). Mean concentrations of FSH remained higher ($P<0.05$) than precastration values until ∿Day 56. Even though mean concentrations of FSH in T treated geldings were eventually suppressed to values similar to those in E and TE treated geldings, there were large fluctuations in concentrations from day to day which were not evident in the latter 2 groups.

Since the concentrations of LH and FSH in serum differed ($P<0.05$) among groups prior to injection of GnRH, the initial changes in concentrations of LH or FSH (maximum concentration in the first h minus preinjection concentration) were used to compare responses among groups. During treatment at the low dose level (Fig. 4A), the initial change in concentrations of LH in TE treated geldings (69 ng/ml) was less ($P<0.05$) than observed in T treated (247 ng/ml), E treated (335 ng/ml) or control geldings (327 ng/ml). The concentrations of LH in E treated geldings remained elevated ($P<0.05$) during the first 6 h after treatment with GnRH.

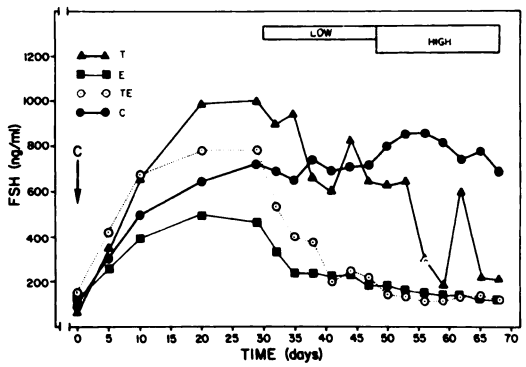

FIG. 2. Effects of castration and steroid treatment on concentrations of LH in peripheral serum. Castration is denoted by the arrow and the periods of treatment are indicated by the bars. The pooled SEMs were: 1) 42.5 ng/ml for all groups prior to treatment; 2) 39.7 ng/ml for E treated and control geldings during treatment; and 3) 17.8 ng/ml for T and TE treated geldings during treatment.

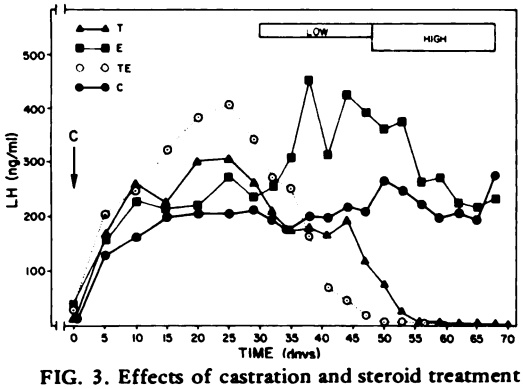

FIG. 3. Effects of castration and steroid treatment on concentrations of FSH in peripheral serum. Castration is denoted by the arrow and the periods of treatment are indicated by the bars. The pooled SEMs were: 1) 82.1 ng/ml for all groups prior to treatment; 2) 28.9 ng/ml for E and TE treated geldings during treatment; and 3) 99.0 ng/ml for T treated and control geldings during treatment.

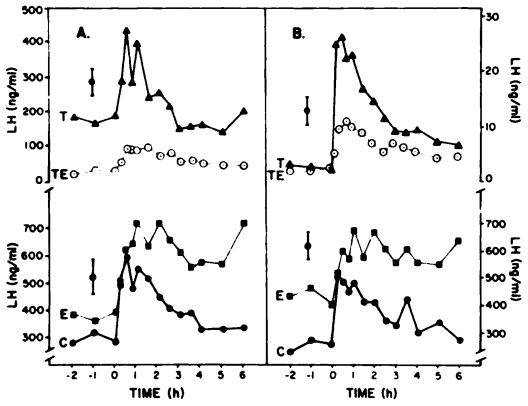

FIG. 4. Concentrations of LH in peripheral serum after an injection of GnRH after 15 days of treatment at A) the low dose of steroid; B) the higher dose level. GnRH was administered (1 μg/kg BW) immediately after the blood sample was drawn at 0 h. The vertical lines denote ± the pooled SEM for each pair of treatment groups.

Prior to injection of GnRH at the higher dose of steroid, concentrations of LH in E treated and control geldings were 100-fold greater ($P<0.05$) than in T and TE treated geldings (Fig. 4B). The initial increase in concentrations of LH after GnRH was greater ($P<0.05$) in T treated than in TE treated geldings (25 vs 8 ng/ml), but the responses in both groups were lower ($P<0.05$) than in E treated or control geldings (285 and 235 ng/ml, respectively). The initial increases in concentrations of LH in E treated and control geldings were similar, whereas the concentrations of LH remained elevated in E treated geldings during the 6 h sampling period.

After injection of GnRH during treatment at the low dose of steroid, the initial increases in serum concentrations of FSH were similar in T and TE treated geldings (881 and 850 ng/ml, respectively), but were higher ($P<0.05$) than in E treated or control geldings (396 and 528 ng/ml, respectively; Fig. 5A). During treatment at the higher dose level, the initial increase in concentrations of FSH in T treated geldings (1510 ng/ml) was higher ($P<0.05$) than in E, TE treated or control geldings (227, 574 and 438 ng/ml, respectively; Fig. 5B). The response in E treated geldings was also lower ($P<0.05$) than in TE treated or control geldings.

Pituitary concentrations of LH and FSH are presented in Table 1. Compared with control values, the concentrations of LH in the pituitaries of T or TE treated geldings were reduced ($P<0.05$) by ∿50%. The concentrations of LH in pituitaries of E treated geldings were increased ∿11-fold. Pituitary concentrations of FSH were higher ($P<0.05$) in T and TE treated geldings than in E treated or control geldings.

DISCUSSION

The rate of increase in serum concentrations of LH after castration was biphasic, increasing more rapidly from 6–12 h than from 24–60 h. A similar pattern in the rate of increase in concentrations of LH after castration was reported by Gay and Midgley (1969) in the rat and may reflect two distinct phases of LH secretion: 1) an increase in the secretion of stored hormone due to release caused by negative feedback after castration, and 2) an increase in synthesis and release of new hormone. The transition period, seen as a plateau in Fig. 1, was probably due to the depletion of stored hormone and a lag in release until synthesis was stimulated maximally.

Peripheral concentrations of hormones in these geldings were proportional to secretion rates if clearance rates of LH and FSH were not affected by steroid treatment. This assumption is probably valid, since clearance rates of gonadotropins in other species were not affected by castration, pregnancy, anestrus or stage of the estrous cycle (Kohler et al., 1968; (Coble et al., 1969; Gay and Midgley, 1969; Akbar et al., 1974).

The concentrations of LH in serum of T and TE treated geldings were suppressed after

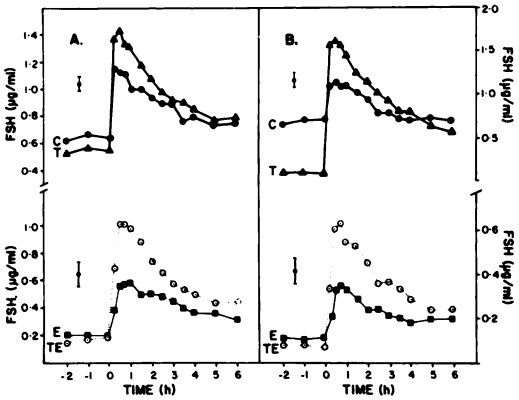

FIG. 5. Concentrations of FSH in peripheral serum after an injection of GnRH after 15 days of treatment at A) the low dose of steroid; B) the higher dose level. GnRH was administered (1 μg/kg BW) immediately after the blood sample was drawn at 0 h. The vertical lines denote ± the pooled SEM for each pair of treatment groups.

TABLE 1. Mean concentration of LH and FSH in pituitary glands of steroid treated and control geldings.

Hormone (mean ± SEM)	Group[a]			
	T	TE	E	C
LH (μg/mg)[b]	1.88 ± 0.14[ce]	2.05 ± 0.61[ce]	43.23 ± 4.57[d]	3.84 ± 0.44
FSH (μg/mg)[b]	104.8 ± 40.3[d]	72.0 ± 16.3[cf]	23.6 ± 8.4[g]	12.2 ± 0.6

[a]Each group consisted of 4 geldings which received either testosterone propionate (T, 175 μg/kg BW), estradiol benzoate (E, 44 μg/kg BW), the combination of both steroids at these dosages (TE) or oil only (C) every other day beginning on Day 30 after castration and twice these dosages from Day 48 until sacrifice on ∿Day 75.

[b]Micrograms hormone/mg wet tissue.

[c]$P<0.05$ from controls.

[d]$P<0.01$ from controls.

[e]$P<0.01$ from E treated geldings.

[f]$P<0.05$ from E treated geldings.

[g]$P<0.08$ from controls.

initiation of treatment. Thus, testosterone is a potent inhibitor of LH secretion in the gelding as it is in the stallion (Hoyer, 1978). Estradiol in combination with T caused a significantly greater rate of decrease in LH secretion than T alone. A synergistic effect of E with T on LH has been reported for male rats (Ewing et al., 1977) and rhesus monkeys (Resko et al., 1977), whereas T alone did not suppress secretion of LH in male rhesus monkeys.

The stimulatory effect of E alone on secretion of LH during treatment at the low dose level was similar to that reported for ovariectomized pony mares (Garcia and Ginther, 1978). There was no large surge in concentrations of LH in serum due to injection of E as reported for the monkey (Hodges and Hearn, 1978; Steiner et al., 1978). The higher dose of E caused a gradual return of the elevated LH concentrations in E treated geldings to values similar to those in control geldings. The concentrations of estradiol maintained by E treatment at the higher dose level were ∿50% higher than those reported for intact stallions during the breeding season (Thompson et al., 1978). It is likely that larger doses of E would have suppressed secretion of LH even further.

Estradiol was a potent inhibitor of FSH secretion and there was no additional effect due to the presence of testosterone. Estradiol treatment did not suppress FSH secretion to the extent that T treatment suppressed LH secretion. After 18 days of treatment at the high dose level, serum concentrations of FSH in geldings receiving E were not different from the precastration values. However, mean concentrations of LH in serum of geldings receiving T were suppressed to between 30–60% of precastration values by the high dose of steroid. Thus, it is possible that some other testicular factor (inhibin?) interacts with E in the intact stallion to regulate the secretion of FSH.

Testosterone at the low dose level had little inhibitory effect on secretion of FSH. There was significant aromatization of testosterone to estradiol in T treated geldings at the higher dose level, resulting in peripheral concentrations of estradiol of 10 pg/ml. The inhibitory effects of T on FSH release may have been due only to the estradiol produced.

The inhibitory effect of T on secretion of LH was also evident in pituitary concentrations of LH. Thus, it appears that T suppresses synthesis as well as secretion of LH. Estradiol alone resulted in a large accumulation of LH in the pituitary gland. Since peripheral concentrations of LH in E treated geldings at the end of treatment at the high dose level were similar to those in control geldings, the accumulation of LH in the pituitary gland was probably due to a greater suppression of release than of synthesis.

The long term suppression of FSH secretion in E and TE treated geldings was not associated with reduced pituitary concentrations of FSH. If E suppressed FSH production to the extent that T suppressed LH production, then a reduced pituitary concentration of FSH would be expected. Thus, high doses of E apparently

did not totally suppress FSH production in these geldings.

Testosterone treatment resulted in a large accumulation of FSH in the pituitary gland, even in the presence of estradiol. This phenomenon has been reported for male rats (Greep and Jones, 1950; Gay and Bogdanove, 1969; Steinberger and Chowdhury, 1977), but does not appear to be due to increased synthesis of FSH (Steinberger and Chowdhury, 1977). This accumulation may represent a physiologic shift of FSH into a storage form of the hormone, rather than simply a buildup due to suppressed secretion (Steinberger and Duckett, 1968).

The secretion of LH and FSH in these geldings in response to administration of GnRH generally reflected the pituitary concentrations of these hormones. The synergistic inhibition of E and T on secretion of LH was also evident after injection of GnRH. Since pituitary concentrations of LH in T and TE treated geldings were similar at sacrifice, the diminished response to GnRH in TE treated geldings was apparently due to a diminished responsiveness of the gonadotropes to GnRH.

The patterns of change in serum concentrations of LH and FSH after administration of GnRH were generally characteristic of a rapid, transient release of hormone into the circulation. The maintenance of elevated concentrations of LH in E treated geldings was probably due to a high rate of synthesis already in progress which was readily stimulated by GnRH. The initial increase in concentrations of LH in E treated geldings was not different from that in control geldings. This may have been due to similar pools of releasable hormone in the 2 groups, even though pituitary concentrations differed by 10-fold.

Despite the large differences in pituitary concentrations of FSH among groups, there was no indication of sustained secretion of FSH after injection of GnRH. During treatment at the low dose level, when concentrations of FSH in peripheral serum of E and TE treated geldings were suppressed equally, the increase in FSH concentrations in response to administration of GnRH was 2-fold greater in TE treated geldings. Thus, the difference in pituitary concentration of FSH between E and TE treated geldings observed at sacrifice was probably established during treatment at the low dose level.

During treatment at the higher dose level, the initial increase in concentrations of FSH after administration of GnRH in E treated geldings was lower than in control geldings, even though pituitary concentrations were ∿2-fold greater. Thus, E appears to inhibit directly the response of the gonadotropes to GnRH.

The large accumulation of FSH in the pituitary glands of T treated geldings resulted in a dramatic initial increase in concentrations of FSH in serum after administration of GnRH. This is in contrast to the sustained release of LH seen in E treated geldings. If this sustained release of LH was due to a high rate of synthesis in E treated geldings, then this was not the case for FSH in T treated geldings, although readily releasable stores of FSH were high.

Since secretion of both LH and FSH was stimulated by GnRH, these data are consistent with the hypothesis that there is one hypothalamic releasing hormone for both gonadotropins. Because of the large differences in secretion and in pituitary concentrations of LH and FSH due to treatment, the simplest model would involve two separate gonadotropes for the production of LH and FSH (Steinberger et al., 1973). If there is only one hypothalamic releasing hormone for both LH and FSH in the horse, then at least some of the effects due to steroids observed in this experiment were at the level of the pituitary gland.

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