Testosterone Pretreatment and the Response of Pituitary LH to Gonadotropin-Releasing Hormone (GnRH) in the Male Dog

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To characterize the effects of testosterone (T) pretreatment on the response of pituitary LH secretion to exogenous gonadotropin-releasing hormone (GnRH), intact male dogs were injected subcutaneously with either oil or 500 μg/kg of T in oil at 1, 3, 6, 12, or 24 hours prior to intravenous GnRH administration (50 ng/kg). The pre-GnRH levels of plasma LH were reduced in all groups of T-treated dogs except in animals given T 1 hour before GnRH. The concentrations of plasma LH during both the peak-response period and the recovery period following GnRH administration in animals injected with T did not differ from those in animals injected with oil. These results indicate that T pretreatment has no effect on the ability of the pituitary to respond to exogenous GnRH at all time periods tested, and imply that direct feedback of T on the pituitary may not be acutely involved in steroid negative feedback in the male dog. Unexpectedly, however, there was some indication that the time of injection of either oil or T could affect the response of the pituitary to GnRH, and this may represent a stress phenomenon.

Key words: GnRH, LH-response, testosterone, dog

The question of direct (pituitary) versus indirect (hypothalamic) regulation of pituitary gonadotropins in the male by androgens and their metabolites is still not fully answered. It is well known that steroids act on the hypothalamus by influencing the secretion of hypothalamic releasing hormones (Davidson, 1969). However, LH and FSH are released in a divergent manner under various physiologic conditions (Bogdanove, 1967), and this may be explained by a differential modulating effect of steroids and other testicular products at the pituitary level. Since specific androgen receptors have been found in the anterior pituitary (Naess et al, 1975), it is possible that androgens have a direct action on LH and FSH release.

In vitro experiments using cultured pituitaries have indicated that testosterone (T) can act in an inhibitory manner on gonadotropin-releasing hormone (GnRH)-induced LH release (Drouin and Labrie, 1976). Others have shown that in the ram (Hopkinson et al, 1974; Galloway and Pelletier, 1975) and rat (Debeljuk et al, 1974), high or massive doses of T-propionate can block the response of LH to GnRH, and these studies lend some support to the possibility of a direct effect of androgens on the pituitary.

Studies in eugonadal human males have indicated that T infusions that raise plasma T concentrations to twice normal values have no effect on the GnRH-induced LH release (Santen, 1975; Winters et al, 1979). However, if these high levels of T are maintained for four weeks, the response of LH to GnRH is eventually reduced (Caminos-Torres et al, 1977). It has been reported that under sedation, T administration 165 minutes prior to GnRH administration had no effect on the response of

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LH in the eugonadal male dog (Jones and Boyns, 1974). In this study, we wish to more thoroughly characterize the effect of T pretreatment on the GnRH-induced LH release in the conscious, eugonadal male dog. The T dose chosen from these studies has been characterized in this laboratory (Falvo et al, 1979) for its effects on plasma LH and T levels in this animal.

Materials and Methods

Eleven adult male mongrel dogs (20.4 to 26.8 kg), fitted with indwelling jugular cannulae, were housed individually in the Southern Illinois University—Carbondale Vivarium under controlled lighting conditions (12:12, lights on 0600 CST). Water and dog chow were available *ad libitum*.

Experiment 1

Six dogs were injected intravenously with saline, 5, 10, 25, 50, 125, or 250 ng/kg of GnRH (Calbiochem-Behring Corp., La Jolla, California). GnRH was administered at four-day intervals at 1000 hours in random fashion. The design was unbalanced, with some animals receiving five or six different treatments and some animals receiving only one or two.

Blood samples were drawn every 20 minutes between 0900 hours and 1000 hours, every 5 minutes between 1005 hours and 1030 hours, and then at 1045, 1100, and every 30 minutes until 1400 hours. Plasma LH levels between 0900 and 1000 hours were termed the baseline, between 1005 and 1200 hours, the response period, between 1005 and 1030 hours, the peak response period, and between 1200 and 1400 hours, the return to baseline period.

Experiment 2

Five dogs were randomly assigned to the partially-balanced block design show in Table 1. For this experiment, dogs received an injection of oil and 50 ng/kg of GnRH during a control trial, and an injection of T (500 μg/kg) plus a similar dose of GnRH during a corresponding treatment trial.

As shown in Table 1, each dog was exposed to two different intervals between the prior injection (oil or T) and administration of GnRH. The design is partially balanced in that each interval between the injections was tested in two separate animals. Each dog was exposed to four experimental interventions: two treatment trials (T) at different preinjection times each preceded by a corresponding control trial (oil). Trials were spaced four days apart.

Blood samples were drawn every 20 minutes, 1 hour prior to GnRH administration (baseline period), at 1010, 1020, 1030 and 1045 hours following GnRH administration (peak response period) and at 1100, 1130 and 1200 hours (the recovery period).

Radioimmunoassay (RIA) of LH

Plasma was collected following centrifugation and stored at −20 C until assayed. Plasma concentrations of plasma LH in all samples were determined in duplicate 200 μl aliquots by a double antibody RIA procedure previously described by DePalatis et al (1978).

Statistical Analysis

Experiment 1 was analyzed using a two-way analysis of variance (Dixon and Massey, 1969) on animal and dosage. Followup evaluation used pairwise t-tests (Dixon and Massey, 1969) on the least squares means, employing the mean square error from the analyses of variance. The dependent variables in the analyses were averages of observations for each animal for the baseline, response, peak response, and recovery time periods.

The Sign test, a nonparametric test (Hollander and Wolfe, 1973), was applied to the data of Experiment 2 and the data were expressed in percentage of LH prior to GnRH administration. The baseline data displayed a distribution amenable to analysis using the Sign test and not to analysis of variance.

Results

Experiment 1

Analysis of the baseline data for LH revealed no significant differences and no further analysis was performed. Analysis of the response period revealed a significant effect for the different doses of GnRH ($F = 4.94$, $df = 6.9$, $P < 0.05$). Table 2 shows the adjusted LH means (means adjusted for individual dog effects) for each GnRH dosage and the *P* value corresponding to the test of equality of dose effects versus the alternative that increased dosage increases the LH response. The LH responses to GnRH doses of 50, 125, and 250 ng/kg

TABLE 1. Design of Experiment 2*

Dog number	1	2	3	4	5
Time (hours) of oil and T	1	1	3	3	6
pretreatment prior to GnRH	12	6	24	12	24

* Time intervals between injections of oil or T (500 μg/kg) and administration of GnRH (50 ng/kg) in five dogs used in this study. For each time interval, the dogs received both oil followed by GnRH and T followed by GnRH. The experiments were performed at least four days apart.

TABLE 2. Doses of GnRH, Adjusted Mean Concentrations of LH and *P* values of the response period (1005–1200 hours) in Experiment 1

GnRH dose (ng/kg)	LH ng/ml Adjusted means	*P* values (vertical versus horizontal)*						
Saline	2.6	Saline	5	10	25	50	125	(GnRH doses)
5	3.7	NS						
10	5.3	NS	NS					
25	7.0	NS	NS	NS				
50	9.3	.05	.05	NS	NS			
125	13.0	.005	.005	.05	.05	NS		
250	15.0	.005	.005	.01	.05	NS	NS	

* *P* values are a comparison of vertical versus the horizontal GnRH doses.

were not significantly different from each other. The two highest doses of GnRH did elicit a significantly greater response in LH when compared to all other doses of GnRH with the exception of the 50 ng/kg dosage. Furthermore, whereas the 50 ng/kg dose of GnRH did cause a significant increase in plasma LH when compared to saline and the 5 ng/kg dose, it did not cause a significant increase when compared to the 25 and 10 ng/kg doses of GnRH. Despite this overlap of statistical significance, the linear trend of the LH response was evident as the dose increased. Analysis of the peak response period also showed a significant effect ($F = 4.93$, $df = 6.9$, $P < 0.05$). Table 3 shows the adjusted means for each GnRH dosage and the corresponding *P* value in a fashion similar to that in Table 2. During the peak response period, a relationship of responses to the doses tested was similar to that observed in the response period. Analysis of data from the return to baseline period revealed no significant difference.

Experiment 2

The results of Experiment 2 are shown in Figs. 1 and 2. Fig. 1 shows levels of plasma LH in one dog following both oil and T pretreatment at 6 and 24 hours prior to GnRH administration. A similar response of LH to GnRH in both oil and T pretreated dogs was observed for all time periods. These results are summarized in Fig. 2. The comparison using the Sign test between plasma LH in the oil and T pretreated dogs during the baseline period indicated that the T-injected dogs had a lower LH level ($r = 8$, $N = 9$, $P < 0.02$) than the oil-injected group, with the exception of the time period when T was injected 1 hour prior to GnRH administration. During the peak response period, little difference between oil and T pretreatment was observed. However, there was an indication of a prior injection effect (note the elevated LH at 1, 6, and 12 hours, and the lower LH at 3 and 24 hours). These differences appeared to be due to individual dog effects ($F = 93.1$, $df = 4.9$, $P < 0.05$). During the after-peak response period, the concentrations of plasma LH in the oil treated dogs were similar to those during the baseline period. Plasma LH levels in the T treated dogs also had returned to values observed in intact untreated dogs.

Discussion

The results of Experiment 1 indicated that in the dog, as in other species examined, GnRH caused a

TABLE 3. Doses of GnRH, Adjusted Mean Concentrations of LH and *P* values of the peak response period (1005–1030 hours) in Experiment 1

GnRH dose (ng/kg)	LH ng/ml Adjusted means	*P* values (vertical versus horizontal)*						
Saline	3.9	Saline	5	10	25	50	125	(GnRH doses)
5	5.3	NS						
10	7.9	NS	NS					
25	9.9	NS	NS	NS				
50	13.3	.05	.05	NS	NS			
125	19.0	.005	.005	.05	.05	NS		
250	21.0	.001	.001	.005	.05	NS	NS	

* P values are a comparison of vertical versus the horizontal GnRH doses.

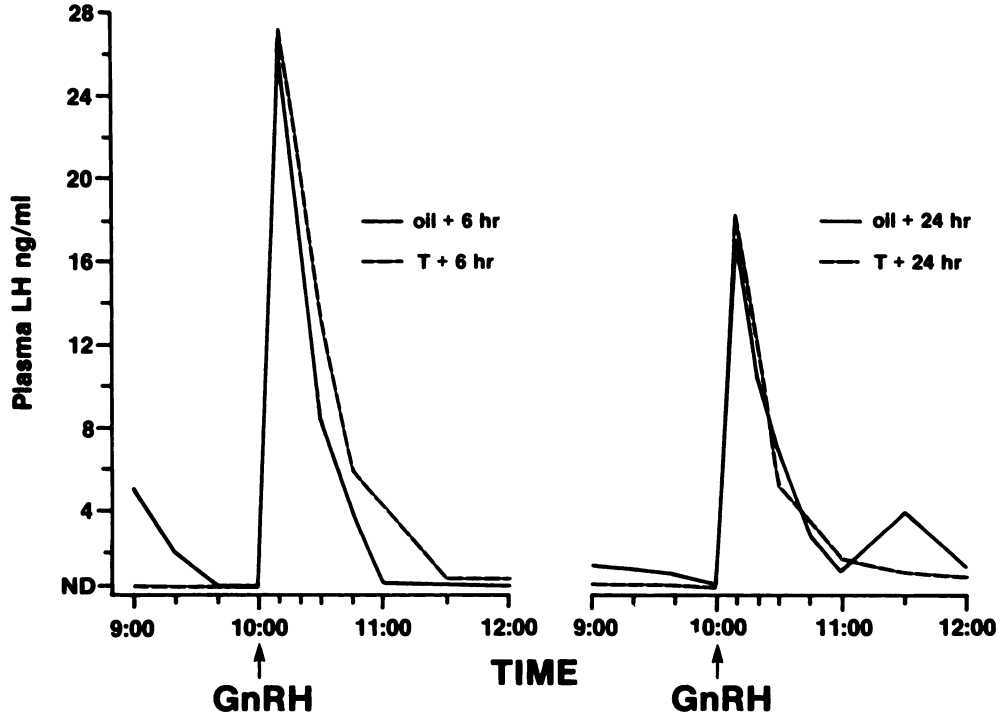

Fig. 1. Response of plasma LH to GnRH (50 ng/kg) following either testosterone (T) (500 μg/kg) or oil pretreatment at 6 and at 24 hours in an individual dog.

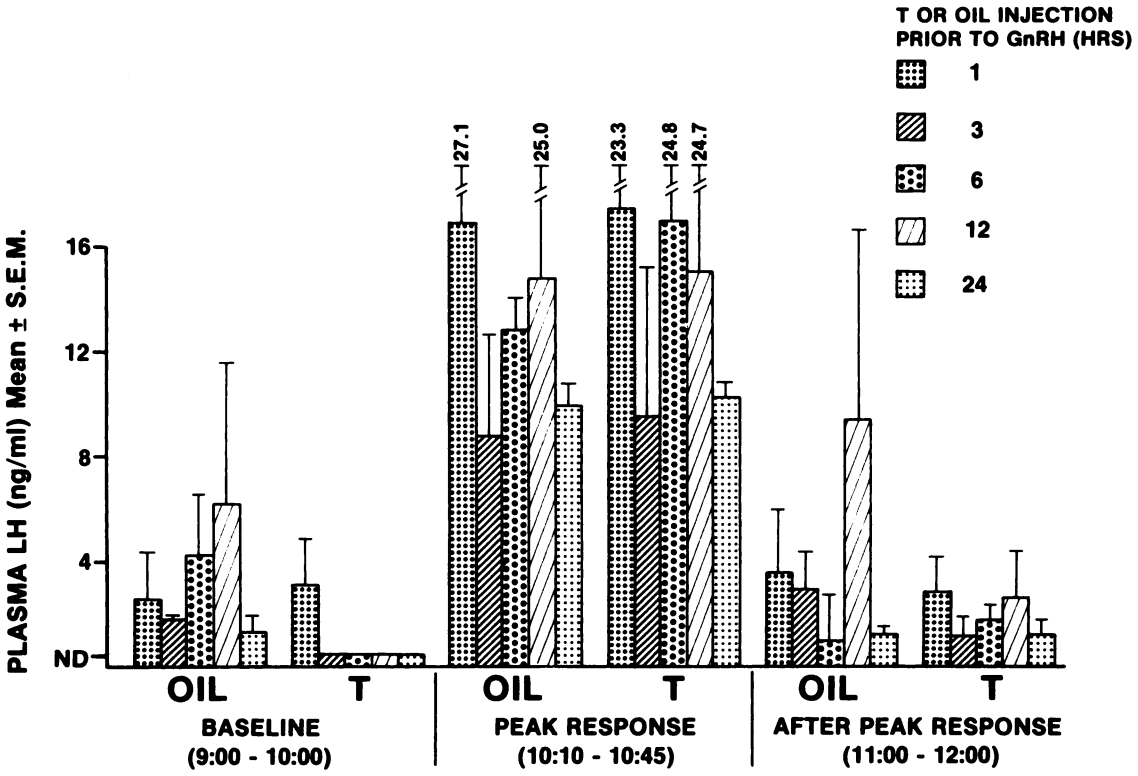

Fig. 2. Response of plasma LH to GnRH (50 ng/kg) following either testosterone (T) (500 μg/kg) or oil pretreatment at 1, 3, 6, 12, and 24 hours.

prompt rise (~5–10 minutes) in the plasma concentrations of LH. Our finding of a dose-response relationship of plasma LH to GnRH agrees with previous work in the dog (Boyns et al, 1972). However, the doses of GnRH used in the latter study were not based on body weight. Furthermore, our doses of GnRH (5 to 250 ng/kg) were lower than those used in the previous study (0.5 to 7.5 μg/dog) and this may account for the statistical overlap in the responses to different low doses. The 50, 125, and 250 doses of GnRH showed no differences and probably represent a plateau in the effect of GnRH on plasma LH.

In Experiment 2, our dose of GnRH (50 ng/kg) was similar to that used by Jones and Boyns (1976) in their study of the effect of steroid pretreatment on pituitary responsiveness to GnRH. Our dose of GnRH was selected to cause an increase in the concentration of plasma LH which would approximate the pulsatile release of this hormone observed in the eugonadal male dog (DePalatis et al, 1978). The dose of androgen chosen for this study was also based on our previous experience in which we characterized the resulting concentrations of both plasma T and LH following a single 500 μg/kg dose of T in the eugonadal male dog (Falvo et al, 1979). In that study we found an inhibition of LH to nondetectable (ND) levels within 140 minutes of T treatment, and the level of LH remained ND until 21 to 24 hours post injection. We also observed that T concentrations rose significantly by 60 minutes, and by 12 hours had returned to pretreatment values. These previous data explain why the plasma concentrations of LH were unchanged in the dogs pretreated with T at 1 hour and also why the plasma concentrations of LH were reduced in dogs treated with T at 3, 6, 12, and 24 hours (Fig. 2).

In the peak response period (Fig. 2) following GnRH, neither oil nor T pretreatment had any effect on the release of LH from the pituitary following GnRH administration. However, there was some indication of either reduced or heightened sensitivity of the pituitary response to GnRH depending on when the dogs were injected with either oil or T. This indicated that the stress of the oil or T injections may have affected the response to GnRH. However, the responses of LH to GnRH in each individual dog after both oil and T pretreatment were, without exception, similar at all time periods tested (Fig. 2). Therefore, under the conditions of this study, direct androgen feedback on the pituitary was not observed. However, since the 50 ng/kg dose of GnRH did cause a maximal release of LH, it still leaves open the question of whether this could have masked the direct effect of T on the pituitary.

The question of direct pituitary feedback has been studied in various species. In the dog, various doses (2.5–25.0 mg) of T, dihydrotestosterone, or other 5α-reduced metabolites of T did not affect the response of pituitary LH to GnRH (Jones and Boyns, 1974). However, pretreatment with 50 μg of estradiol at 60 or 165 minutes, but not at 15 minutes, did inhibit the GnRH-induced LH release (Jones and Boyns, 1976). In the eugonadal human male, T infusions, which raised the plasma concentrations of T to twice normal levels, did not affect the GnRH-induced LH release (Santen, 1975; Winters et al, 1979); but if these high T levels were maintained for four weeks, the response was eventually reduced (Caminos-Torres et al, 1977). Studies in the ram have shown that large doses of T-propionate can suppress the GnRH-induced LH release (Hopkinson et al, 1974; Galloway and Pelletier, 1975); but the resulting circulating levels of T were not measured, and thus these observations may represent a pharmacologic rather than a physiologic effect. In the orchidectomized rat, evidence has been obtained to suggest a dual feedback action of T on LH regulation. An initial inhibition of the GnRH-induced LH release due to hypothalamic inhibition was followed by suppression of pituitary responsiveness, most probably caused by the direct action of T on the pituitary (Cheung and Davidson, 1977). Furthermore, in orchidectomized rats with unilateral intrapituitary implants of T-propionate, Kingsley and Bogdanove (1973) have shown that androgens can exert feedback action directly at the pituitary level *in vivo*.

In conclusion, the present study and former studies in dogs and men have shown that T pretreatment does not have an acute effect on the response of LH to GnRH. However, in men, inhibition may be observed if T treatment is maintained for a prolonged period of time. Thus, it appears that the physiologic role of T or its metabolites in the control of LH secretion is mediated through the hypothalamus where they influence GnRH synthesis and/or release. Further studies are necessary to differentiate between direct and indirect actions of T on the hypothalamic-pituitary unit in the control of LH secretion.

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