Regulation of Pituitary Gonadotropin-releasing Hormone Receptors by Pulsatile Gonadotropin-releasing Hormone Injections in Male Rats

Modulation by Testosterone

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Abstract. The pattern of the gonadotropin-releasing hormone (GnRH) stimulus is critically important in the regulation of pituitary gonadotropin secretion and continuous infusions down-regulate secretion while intermittent pulses maintain luteinizing hormone (LH) and follicle-stimulating hormone (FSH) responsiveness. We examined the effects of pulsatile GnRH administration on pituitary GnRH receptors (GnRH-R) and gonadotropin secretion in the presence of physiological concentrations of testosterone (T) to elucidate the mechanisms and sites of action of GnRH and T on the pituitary gonadotroph.

Castrate male rats received one, two, or four testosterone (T) implants (serum T concentrations of 1.1, 2.4, and 5.2 ng/ml, respectively) to suppress endogenous GnRH secretion. Subsequently, intracarotid pulse injections of GnRH (5–250 ng/pulse) or saline in controls were given every 30 min for 48 h, after which gonadotropin responses and pituitary GnRH-R were measured. In control rats, the T implants prevented the rise in GnRH-R that was seen in castrates (empty implant—600 fmol/mg protein) and maintained receptors at the level that was present in intact animals (300 fmol/mg). Pulsatile GnRH administration increased GnRH-R in castrate T-implanted rats, but the response was dependent on the serum T concentration. With one T implant, increasing GnRH doses per pulse stimulated GnRH-R in a linear manner and the maximum receptor concentration (703±99 fmol/mg) was seen after the 250 ng GnRH dose. In the presence of two T implants, GnRH-R was maximal (705±45 fmol/mg) after the 25-ng dose and higher doses did not increase receptors above control values. With four T implants, GnRH doses of 5 ng induced a maximum response, 17–50 ng/pulse did not increase GnRH-R, but receptors were again increased by the 250-ng dose (633±86 fmol/mg).

After 48 h of pulsatile GnRH administration there was no correlation between the number of GnRH-R and LH responses to GnRH. In rats with one or two T implants, LH responses were absent after all but the 250-ng doses. In contrast, LH responsiveness was not impaired in the presence of four implants. Thus, low dose GnRH pulses down-regulate LH secretion by an action at a post GnRH-R site, and this effect is regulated by testosterone. The results show that GnRH, given in a pulsatile manner, regulates its own receptor, and physiological increases in serum T produce a 50-fold increase in the sensitivity of GnRH-R stimulation by GnRH.

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Introduction

Secretion of the pituitary gonadotropin hormones, luteinizing hormone (LH)[1] and follicle-stimulating hormone (FSH), is controlled by the hypothalamic gonadotropin-releasing hormone (GnRH). In vivo, GnRH appears to be secreted in an

1. *Abbreviations used in this paper:* FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; GnRH-R, GnRH receptors; LH, luteinizing hormone; PE 50, polyethylene cannula; T, testosterone.

intermittent or pulsatile manner. This was initially suggested by studies which demonstrated pulsatile secretion of LH, and by inference GnRH, in normal men and in castrated rats (1, 2). Subsequent studies confirmed this observation in other species (3–5), and later direct measurement of GnRH in hypothalamic-portal blood provided direct confirmation of this suggestion. GnRH concentrations in portal blood were increased at the time of the proestrus gonadotropin surge and in castrate females (6, 7), and the variable pattern of the elevated hormone concentrations in 10–15-min collections suggested a pulsatile mode of secretion. Recently Clarke et al. (8) obtained simultaneous samples of portal and jugular venous blood in sheep and showed that each LH pulse was preceded by a pulse of GnRH. A pulsatile GnRH stimulus appears to be essential for gonadotropin secretion to be manifest. If GnRH is given to GnRH-deficient humans or hypothalamic lesioned monkeys in a pulsatile manner, the normal hormonal changes of puberty or the menstrual cycle can be reproduced (9–16). On the other hand, continuous infusion of GnRH to lesioned monkeys results in "down-regulation" of gonadotropin secretion, which is restored by reinstitution of a pulsatile stimulus (17). Similarly, "down-regulation" of LH secretion has been observed in man and in other animal species when constant infusions of GnRH or injections of long-acting GnRH agonists have been used (18–23). Thus, the pulsatile nature of GnRH secretion appears to be critical in determining LH and FSH responsiveness, and some evidence suggests that changes in frequency may be one mechanism by which steroid hormones exert feedback effects on the hypothalamic-pituitary axis. LH pulse frequency is increased in castrate animals and both progesterone and testosterone (T) can reduce LH pulse frequency in castrate sheep and rats, respectively (24–27).

These studies indicate the importance of the manner of GnRH delivery to the pituitary, but little information is available regarding the nature of the cellular mechanisms that are responsive to the stimulus. GnRH acts by an initial step of binding to a plasma membrane receptor (28, 29), and a considerable body of evidence has shown that GnRH is the main regulator of its own receptor on the gonadotroph. Castration results in an increase in GnRH receptors (GnRH-R), and this effect can be abolished by hypothalamic lesions, administration of GnRH anti-sera, or replacement of testosterone (30–33). These manipulations prevent the rise in GnRH after castration, and injections of GnRH given subcutaneously every 8 h increased GnRH-R in these animals (30, 32). Low dose infusions of GnRH can also increase GnRH-R, whereas high dose infusions reduce receptor number (34). These data clearly show the importance of GnRH in the regulation of its own receptor, but studies to date have not examined the effect of pulsatile administration of GnRH given in a manner to mimic the physiological mode of secretion. In this report we have examined the effects of pulsatile GnRH administration in castrate male rats replaced with testosterone. This model was selected as previous studies have shown that GnRH secretion is likely to be low in this model, as judged by infrequent LH pulses (27). Thus, the assessment of the effects of the administration of exogenous GnRH pulses should not be unduly complicated by endogenous GnRH secretion. In this manner we intended to delineate the effects of pulsatile GnRH in the modulation of GnRH-R with a view to determining if alterations in receptor number may be part of the mechanisms involved in changing pituitary responsiveness. In addition, by performing the pulsatile GnRH studies in the presence of varying concentrations of testosterone, we hoped to gain insight into the action of androgens at the pituitary level and to determine if part of the feedback action of steroids was exerted at the level of the pituitary cell and, more specifically, on the GnRH-R.

Methods

Experimental procedures. Adult Sprague-Dawley rats (275–300 g) were castrated under ether anesthesia and one, two, or four 20-mm silastic implants that contained testosterone were implanted subcutaneously. A polyethylene cannula (PE 50) with a silastic tip was placed in the right carotid artery for subsequent GnRH injections. Control animals were anesthetized and sham operated or sham implanted in an identical manner.

After surgery and recovery from anesthesia rats were placed in immobilization cages which allowed anterior mobility but restricted turning movements in order to prevent kinking or chewing of the cannula. The cannula was connected to a 10-ml syringe that was attached to a pump (Autosyringe AS-2C), and during the subsequent 48-h normal saline or different GnRH concentrations were delivered via the cannula as intermittent pulses at a frequency of every 30 min to mimic LH pulse frequency in castrate rats. GnRH was diluted in 0.1% bovine serum albumin (BSA) in normal saline and the syringes had been presoaked in 0.1% BSA for 24 h before each experiment. Syringes were refilled with a fresh GnRH solution every 12 h and the residual GnRH solution was assayed for GnRH as a check on the concentration delivered. At the end of this period, rats were decapitated 20 min after the last pulse injection and blood was collected for assay of LH, FSH, and testosterone. The anterior pituitary was removed, placed in 0.25 M sucrose, snap-frozen in ethanol-dry ice, and then stored at −70°C before use in the binding assay.

In initial studies we performed experiments to determine the maximum plasma levels of GnRH and the initial LH response to the different GnRH doses that were used in the subsequent pulsatile studies. This was done in acutely castrated rats where an additional PE 50 cannula was placed in the aorta. GnRH was injected 30 min after castration, and 1 min later blood was collected from the other PE 50 cannula for measurement of GnRH and 20 min later for measurement of LH. GnRH doses > 250 ng were not used in view of the plasma half life of GnRH (~3–4 min). Higher doses might not be completely cleared from the circulation within 30 min and this would alter the pulsatile nature of the GnRH signal.

Assessment of pituitary GnRH receptors. The affinity of the GnRH-R is unchanged in different physiological and experimental conditions (35–37) and changes in GnRH binding are due to changes in the number of receptors. Scatchard analysis of competition curves and saturation analysis produce identical estimates of GnRH binding capacity (36, 37), and in this study the number of GnRH-R was

determined by saturation analysis using D-Ala^6-des-Gly^{10} GnRH-ethylamide (D-Ala analog) as ligand. Details of the receptor assay have been reported previously (38). Briefly, single pituitaries were thawed on ice and homogenized in 2 ml of 0.25 M sucrose (pH 7.7). The homogenate was centrifuged at 10,800 *g* for 15 min, and the pellet was resuspended in Tris-HCl buffer (10 mM, pH 7.7) to a concentration of ~250 μg protein/ml. The homogenate (50 μg in 200 μl) was added to tubes that contained 100 μl assay buffer (10 mM Tris-HCl with 1 mM dithiothreitol and 0.1% BSA) and a near-saturating dose of D-Ala analogue (300 pg ^{125}I D-Ala analogue and 800 pg unlabeled D-Ala analog). After incubation (60 min on ice), tubes were centrifuged at 27,000 *g* (15 min at 4°C), the supernatant was aspirated, and the pellet was counted in a γ-spectrometer. GnRH binding capacity was expressed as femtomoles of analog bound per milligram of protein.

Hormone assays. Serum LH, and FSH were measured by radioimmunoassay as previously described (39, 40) using NIAMDD LH RPI and FSH RPI as standards. Serum T and GnRH were also measured by radioimmunoassay as described (41, 42).

Statistical analysis. The data were analyzed using analysis of variance or unpaired *t* test as appropriate.

Results

Determination of serum GnRH concentrations and LH responses to GnRH pulse injections. Preliminary studies were performed to determine the maximum serum concentrations achieved after a given dose of GnRH. GnRH in 200 μl of normal saline was injected as a single bolus into the carotid artery of groups of rats (4–6/group) within 30 min after castration. Blood (0.5 ml) was collected 1 min after the GnRH injection for GnRH assay and the volume replaced with normal saline. 20 min after the injection, the rats were decapitated and blood collected for LH assay. The serum GnRH concentrations that were obtained after different doses of GnRH are shown in Fig. 1, and the LH responses are correlated to the serum GnRH concentration in Fig. 2. A linear relationship between serum GnRH concentrations and dose injected was observed. Previous measurements of GnRH in pituitary stalk blood (6–8) reported concentrations in the 0–300 pg/ml range. Our data shows that similar concentrations were produced in peripheral blood by GnRH doses of <25 ng/pulse injection. LH responses increased with GnRH dose up to 25 ng. Thereafter, the LH response reached a plateau, and higher concentrations of GnRH did not increase LH release further. Similar experiments were performed in castrate rats with two T implants and identical LH responses to the 25- and 75-ng GnRH doses were observed.

Effect of T replacement in orchidectomized rats. In order to assess the effects of pulsatile GnRH injections on pituitary GnRH-R, initial experiments were performed to determine whether T implants could prevent the increase in serum LH, GnRH-R, and by inference hypothalamic GnRH secretion, in castrated rats. Rats were castrated and 1, 2, or 4 T implants were inserted subcutaneously. The animals then received N saline pulses which were given every 30 min for 48 h. At the end of this period, rats were decapitated and measurements of GnRH-R and serum hormones compared with those in adult

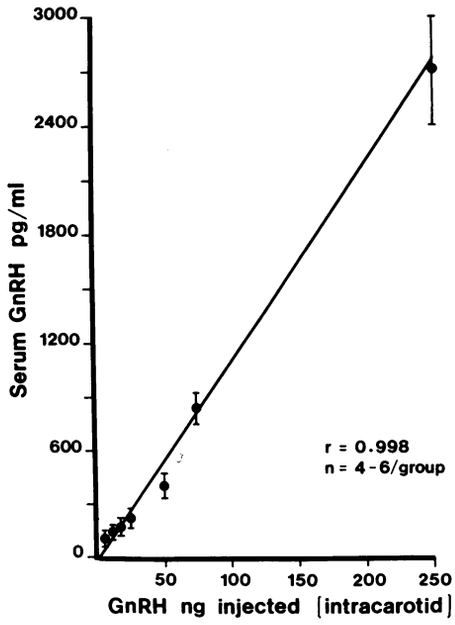

Figure 1. Serum GnRH concentrations obtained in serum 1 min after a pulse injection of GnRH. The dose of GnRH is shown on the horizontal axis. All rats were castrated with T implants. Mean±SE are shown.

intact male rats. The results are shown in Fig. 3. The castration responses were completely abolished by the T implants, which produced serum T concentrations of 1.15±0.04, 2.3±0.12, and 5.16±0.28 ng/ml (Mean±SE) for 1, 2, and 4 implants, respectively. Serum T in the rats with one or four implants was different to that in intact males ($P < 0.05$). These results agree with our earlier report (32) that testosterone prevents the postcastration rise in GnRH-R and also shows that constant

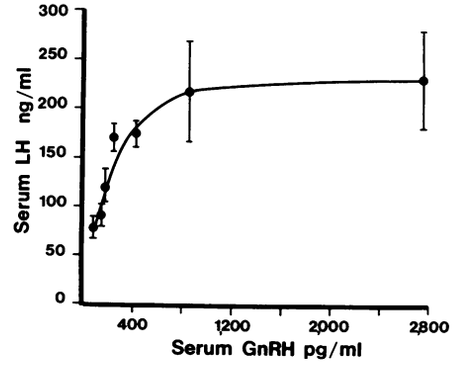

Figure 2. Correlation between serum GnRH concentrations (1 min after injection) and serum LH responses (20 min after the injection) in acutely castrated rats. Serum GnRH levels > 279 pg/ml (25-ng pulse injection) did not result in higher LH responses. Mean±SE are shown.

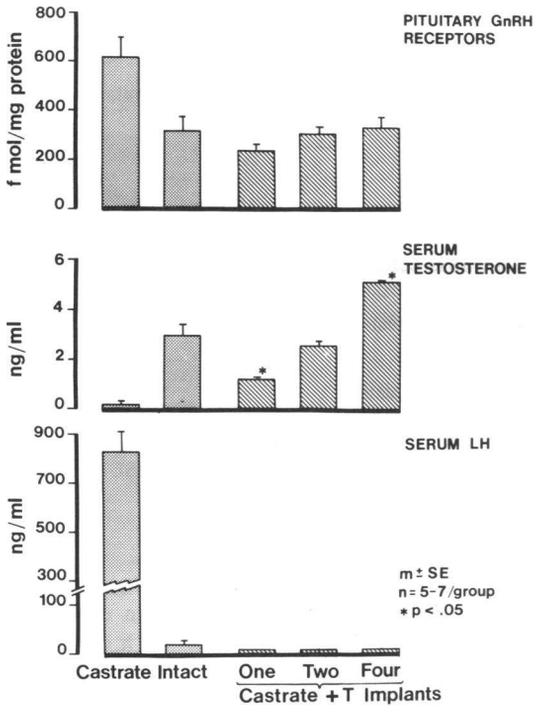

Figure 3. Pituitary GnRH-R, serum T, and LH in animals that were castrated and received one, two, or three T implants or empty implants. All groups were given injections of normal saline every 30 min for 48 h and decapitated 20 min after the last pulse.
* $P < 0.05$ compared with intact rats. In animals with T implants, LH was below the limits of assay detection (<12 ng/ml). Data are shown as Mean±SE.

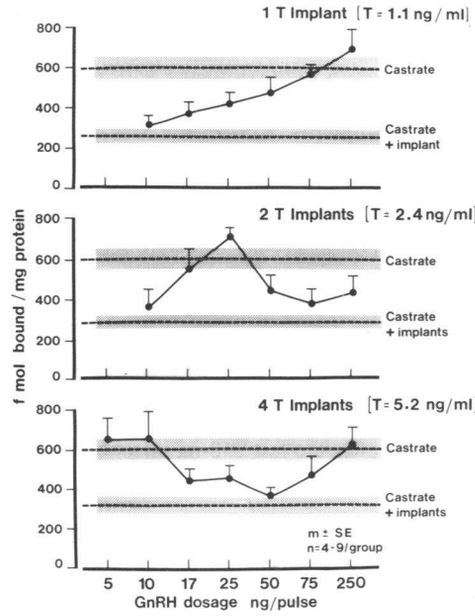

Figure 4. The effect of serum T on pituitary GnRH-R responses to different GnRH concentrations given as pulse injections every 30 min for 48 h. All animals that received GnRH were castrated and had T implants. The dotted line and hatched areas are (Mean±SE) for castrate (empty implant) or castrate plus T implants that received saline pulses. The serum T concentrations in rats with one, two, or four implants are shown in parentheses. Data are shown as Mean±SE (4–9 rats per group).

levels of testosterone, which are lower (one implant) than that present in intact males, also prevents the postcastration responses. Indeed, a constant serum T of 1.15 ng/ml reduced serum LH to values that were lower than that present in intact controls, which suggested that endogenous GnRH secretion was less than that present in intact males. This is supported by recent data (27) which showed markedly decreased frequency and amplitude of pulsatile LH secretion when serum T was maintained at ~1.6 ng/ml in castrated rats.

Pituitary GnRH receptor and gonadotropin responses to pulsatile GnRH and the effects of serum T concentration. Pituitary GnRH-R responses to different concentrations of GnRH given as intermittent pulses every 30 min for 48 h to castrate rats with T implants are shown in Fig. 4. Measurement of GnRH that remained in the syringe at the end of 12 h showed that the GnRH concentration was within 85–105% of the calculated dose for all doses tested.

A linear response was observed in rats with one T implant, and GnRH-R concentrations increased gradually with increasing GnRH doses. A dose of 17 ng of GnRH significantly increased receptors over saline injected controls ($P < 0.03$) and the 75- and 250-ng doses produced similar receptor concentrations to those present in castrate controls.

In the castrate plus two implant group, a biphasic receptor response to increasing GnRH dose was observed. GnRH, 17 ng/pulse, increased receptors to the range seen in castrate controls. Maximum receptor concentrations occurred in rats that received 25 ng/pulse, which was 10-fold less than that required to produce a maximum response in the presence of one T implant. GnRH doses > 50 significantly decreased ($P < 0.005$) receptor responses compared with the 25-ng dose, and 75 ng/pulse did not increase receptor concentration above that present in saline injected controls.

In the presence of higher T concentrations (four implants), a further increase in receptor sensitivity to GnRH stimulation was observed. Doses of 5 or 10 ng/pulse increased pituitary GnRH-R to the levels present in castrate controls. Similar to the observations in rats with two implants, higher doses (17–50 ng/pulse) resulted in lower GnRH-R, and receptor concentrations were not increased over saline controls by the 50 ng/pulses. However, in contrast to the results using two implants, the highest doses of GnRH, 75–250 ng/pulse, increased GnRH-R to the same levels as castrate controls.

Gonadotropin responses were measured at the end of the study (i.e., to the last GnRH pulse after 48 h) to examine the

effect of the intermittent GnRH pulses in the presence of different levels of testosterone on pituitary LH and FSH secretion. Serum LH responses were compared with responses to the first GnRH pulse injection and are shown in Fig. 5. In rats with one or two T implants, LH release was impaired after all but the highest GnRH doses at the end of the experiment. LH responses were variable after the 250 ng/ pulses and were not impaired in animals with two implants, but significantly lower responses ($P < 0.02$) were seen in rats with one T implant. In contrast, LH responses were not significantly reduced after 48 h of GnRH pulses in animals with the highest testosterone levels (four T implants). Serum FSH concentrations (not shown) were similar after the last GnRH pulse in all animals given GnRH doses of 10–75 ng/ pulse for 48 h (range 300–450 ng/ml). FSH levels were higher however, (701±42 ng/ml) after the last 250-ng injection in rats with two or four T implants ($P < 0.005$ compared with lower GnRH doses).

The lack of gonadotropin responses to GnRH at the end of 48 h may have been due to the constant levels of testosterone or have resulted from an effect of the preceding GnRH pulses. Two groups of castrated rats with two T implants received either 50 or 250 ng GnRH as a single pulse injection after 48 h of normal saline pulses. The results are shown in Table I and the responses are compared with those after the first and last GnRH injections in rats that received 50 or 250 ng/pulse every 30 min for 48 h. Using 50 ng GnRH/pulse, the LH response after 48 h of saline injections was not different to the initial LH response, which showed that the absence of LH secretion in rats that received 48 h of GnRH pulses was not due to the effects of the T implants. The down-regulation of LH responsiveness by GnRH pulses was not seen using 250 ng/pulse and LH responses were similar after 48 h of GnRH or saline injections.

Table I. Serum LH Responses to GnRH in Castrate T-implanted Rats before and after 48 H of GnRH or Saline Pulse Injections

GnRH dose	Serum LH (*ng/ml*) 50 ng/ pulse	250 ng/ pulse
First GnRH injection	175±10	231±53
Last GnRH injection (after 48 h of GnRH pulses)	<10	163±50
*Last GnRH injection (after 48 h of saline pulses)	199±23	201±17

All rats received two T implants.
* These animals received a single GnRH injection after 48 h of saline pulses that were given every 30 min. LH was measured in blood that was obtained 20 min after the GnRH injection. Mean±SE are shown for 4–6 rats per group.

In all the above studies, animals were killed after 48 h of GnRH pulse injections and the results might have reflected different time courses of receptor and gonadotropin responsiveness. To examine this question we studied the effects of two doses of GnRH, 25 and 75 ng/pulse, after 12, 24, and 48 h of injections in rats with two T implants. Results were compared with saline-injected controls and are shown in Fig. 6. 25-ng pulses of GnRH increased GnRH-R by 12 h ($P < 0.01$) and receptor concentrations were similar to those in

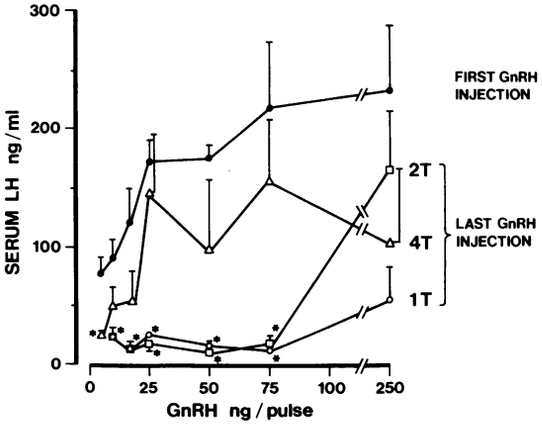

Figure 5. Serum LH responses after 48 h of GnRH pulse injections. Serum LH was measured 20 min after the last pulse of each GnRH dose that was given in castrate animals with (○) one T implant (1 T); (□) two T implants (2 T); or (△) four T implants (4 T). LH responses to the first pulse injection (●) of each GnRH dose are shown for comparison.
* $P < 0.05$ compared with the first GnRH injection. Data are shown as Mean±SE.

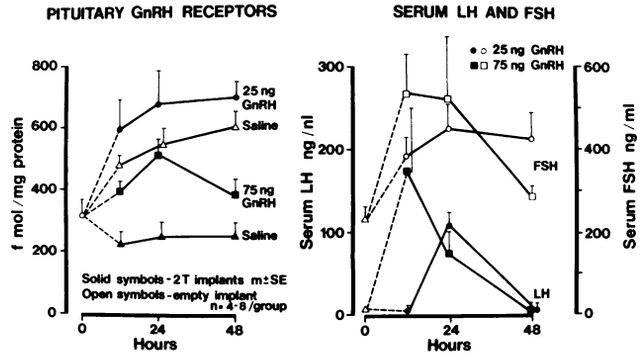

Figure 6. Castrate rats with two T implants. Time course of pituitary GnRH-R and serum gonadotropin responses to GnRH (25 [●] or 75 [■] ng/pulse) given for 48 h. Receptor concentrations are shown on the *left*, and LH (solid symbols) and FSH (open symbols) responses (20 min after the last injection) on the *right*. LH and FSH values from castrate plus T rats receiving saline pulses were not different at 12, 24, and 48 h and the mean is shown (▲, LH, △, FSH) at 0 time. Data are shown as Mean±SE (4–8 rats/group).

saline-injected (empty implant) controls throughout the duration of the experiment. In contrast, the 75-ng injections did not increase GnRH-R significantly at 12 h and receptor concentrations were only transiently increased at 24 h to the range present in castrate animals. Except for the 24-h time point receptor, concentrations were lower ($P < 0.03$) after the 75-ng dose when compared with rats that received 25 ng/pulse. The time course of serum LH and FSH responses (20 min after the last pulse) after 25- and 75-ng pulses of GnRH are also shown in Fig. 6. FSH responses showed little change with time though declined after 48 h of the highest dose ($P < 0.05$ cf, 12 and 24 h) pulses. In contrast, LH responses showed marked changes with time and LH responsiveness was only present at 24 h after 25-ng injections. Peak LH responses occurred at 12 h using 75-ng injections but thereafter declined and no response was present after 48 h.

Discussion

The number of pituitary GnRH-R varies in different physiological situations such as during the estrous cycle (37, 43, 44) and during puberty (36, 38). In most instances the number of pituitary GnRH-R was highest at times of maximal gonadotroph responsiveness to GnRH, such as on proestrus morning or at 15–20 d of age in maturing females. These data suggested that alterations in GnRH-R number is one of the mechanisms involved in the changing pituitary responsiveness to GnRH. Previous studies have shown that GnRH itself is a major factor controlling the number of its own receptor, and receptors are decreased after removal of endogenous GnRH secretion and are increased by administration of GnRH (32–34). In those experiments, however, GnRH was given either as infrequent injections (every 8 h) or by infusion of supraphysiological doses. As reviewed in the introduction, the pulsatile manner of the GnRH stimulus is critical for normal gonadotropin secretion. Thus the aim of the present study was to examine the effects of GnRH, given in a manner to approximate the in vivo situation, on modulation of pituitary GnRH-R.

In order to interpret the effects of exogenous GnRH administration we require a model where endogenous GnRH secretion is low or absent. Rats bearing hypothalamic lesions are one possible model, but the presence of hyperprolactinemia presents a potential problem, as elevated prolactin levels have been shown to inhibit the rise in GnRH-R after castration (45, 46). This may be due in part to a direct effect of prolactin at the pituitary level, as GnRH injections in hypothalamic lesioned rats do not increase GnRH-R to the levels seen in castrate controls (30, 47). Additionally, the absence of endorphins and enkephalins in lesioned animals may also be important, as opioid-active compounds have been shown to increase pituitary GnRH-R (48). To avoid these potential problems we chose the castrate-testosterone replaced rat as an experimental model. Previous studies have shown that T replacement prevents the rise in GnRH-R after castration (32, 33), which suggests that the postcastration rise in GnRH secretion is abolished. Other studies have indicated that constant levels of testosterone from a subcutaneous implant reduce endogenous GnRH secretion to a level below that seen in intact animals. In castrate rams, the frequency of LH secretory episodes was reduced to 0–2/24 h from 20/24 h in untreated castrates (26), and similar observations showing reduced LH pulse frequency after T implants have been made in castrate rats (27). These data suggested that endogenous GnRH secretion was markedly reduced by constant T concentrations within the physiological range. Our initial data in castrate-testosterone replaced animals receiving saline pulses supported this view and showed that both GnRH-R and serum LH were similar to or below values seen in intact animals. These observations provide the basis for using the castrate-testosterone implanted rat to examine the effects of pulsatile administration of GnRH in animals with an intact hypothalamus.

The results using this model with pulsatile delivery of GnRH show striking differences to previous data on GnRH modulation of GnRH-R. In the presence of a serum T at the lower end of the range found in intact rats (1.2 ng/ml), increasing doses of GnRH/pulse produced a dose-dependent increase in GnRH-R with a maximal response being similar to that present in castrate controls. However, the dose of GnRH/pulse required to produce this response produced peak serum GnRH levels of 1–3 ng/ml, which is higher than concentrations measured in stalk blood from castrate rats or monkeys (6, 7, 49, 50). Increasing the serum T concentration by as little as 1.3 ng/ml (two implants) resulted in a marked increase in the sensitivity of GnRH-R stimulation by GnRH (a 10-fold change), and maximum receptor numbers were produced by peak GnRH concentrations of ~250 pg/ml. Additionally, a biphasic response pattern was seen with higher doses of GnRH/pulse which produced a smaller increase in pituitary GnRH-R. A similar biphasic pattern was present in rats with higher T levels (four implants), but in these animals maximum receptor responsiveness to GnRH was produced by doses as low as 5 ng/pulse—a 50-fold increase in sensitivity to that seen in the presence of lower levels of testosterone. This biphasic pattern is similar to that reported after intravenous infusions of GnRH (34) where lower doses increased and higher doses decreased receptors in intact males. In that study, the higher doses of GnRH or GnRH analogues also decreased receptor number in castrate animals, which indicated that supra-physiological amounts of GnRH may reduce receptors even in the absence of testosterone. Our study design, using pulsatile delivery of GnRH, did not allow determination of the effects of higher GnRH doses, as this would have resulted in a loss of the intermittent stimulus. However, the results in the animals with the highest T levels suggest that the complete receptor response curve may be triphasic, and high doses of GnRH increase GnRH-R when T levels are elevated to the upper part of the normal intact male range.

These data show marked alterations in the sensitivity of receptor stimulation by GnRH, which is dependent upon the

ambient serum T concentration. To our knowledge, this is the first demonstration of a steroid hormone modulating a peptide hormone receptor response to homologous hormone.

Previous in vivo studies have shown reduced GnRH-R after administration of testosterone or estradiol, but the effects may have been mediated via decreased GnRH secretion. Similarly, the increase in GnRH-R after estradiol injections (51, 52) may have resulted from a transient positive effect of estradiol on GnRH secretion. Our data clearly show that steroids can alter GnRH-R by a direct action on the gonadotroph, but do not differentiate between the effects of testosterone or estradiol (formed by peripheral aromatization of testosterone). Similarly, the cellular mechanisms of T action are uncertain, and testosterone could increase GnRH-R synthesis or reduce receptor degradation. GnRH stimulation of GnRH-R requires protein and RNA synthesis (53), and can be mimicked by maneuvers that elevate intracellular calcium (54). Thus, testosterone could affect GnRH-R by enhancing receptor synthesis directly or by altering GnRH-stimulated calcium entry into gonadotrophs. Clarification of the mechanisms involved awaits further studies, but the data indicate a positive effect of testosterone on GnRH stimulation of GnRH-R.

This positive action on GnRH-R contrasts with the effects of testosterone on gonadotropin secretion, as both in vivo and in vitro studies have shown inhibition of LH release. This suggests that the site of T action on LH release is at a post–GnRH-R site, which is in agreement with our earlier studies in hypothalamic lesioned rats (30). This suggestion is supported by the absence of any correlation between the number of GnRH-R and GnRH induced LH release. Comparison of Figs. 4 and 5 shows that LH release was impaired when receptors were elevated (one and two T implants), and conversely, LH release was not reduced when receptor numbers were low (four implants, 25 and 50 ng/pulse). This divergence between receptor numbers and LH response contrasts with earlier in vivo studies (36–38), but has been clearly shown using cultured cells. Smith et al. (55) showed that exposure of pituitary cells to GnRH increased GnRH-R but reduced LH responsiveness within 12 h, and a similar dissociation between receptor number and LH secretion has been observed during a 6-h period (53). In keeping with these latter reports, the reduced LH secretion observed in the present study appears to be related to the effects of GnRH administration at a postreceptor locus rather than to the effects of testosterone (Table I). Previous studies have documented that continuous administration of GnRH can desensitize the pituitary (17–23), but our data show that this phenomenon also occurs after 48 h of low dose GnRH pulses given at a physiological frequency. In animals with lower serum T concentrations, no LH response occurred with doses of <75 ng GnRH/pulse, and the partial reduction in LH release after 250 ng/pulse is in keeping with the report that desensitization by GnRH is related to a shift in the dose-response curve to the right (56). GnRH desensitization induced by pulsatile GnRH takes longer than the 6–8 h reported for a constant infusion. Badger et al. (56) did not see reduced responses of cultured cells after nine hourly GnRH pulses, and our data show the effect occurs after 24–48 h. Interestingly, higher concentrations of testosterone appear to prevent desensitization by GnRH pulses, and no significant reduction of LH response occurred in the rats with four implants. No explanation for this effect of testosterone is evident at the present time, but the data show that testosterone exerts dose-related effects on the post receptor mechanisms involved in GnRH-induced desensitization of LH release in addition to its effect on GnRH-R.

In conclusion, these studies show that GnRH can increase its own receptor when given in a pulsatile manner in doses to approximate the presumed in vivo situation. The degree of receptor responsiveness to GnRH is dependent upon the circulating level of testosterone, and changes within the physiological range can markedly alter receptor dose-response curves to GnRH. The maximum number of receptors induced by GnRH is constant, and the effects of testosterone are exerted on the sensitivity of receptor stimulation by GnRH, which can be altered by up to 50-fold. Pituitary GnRH-R concentration and LH responsiveness to GnRH do not change in parallel. This shows that testosterone exerts at least two different effects on the pituitary gonadotroph. One is to alter GnRH-R responses to GnRH, and the other is to modify GnRH induced down-regulation of LH secretion by an action at a post–GnRH-R site.

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