

Effect of Very High Dose D-Leucine⁶-Gonadotropin-releasing Hormone Proethylamide on the Hypothalamic-Pituitary Testicular Axis in Patients with Prostatic Cancer

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ABSTRACT Potent synthetic analogs of gonadotropin-releasing hormone produce paradoxical anti-reproductive effects when administered chronically. These compounds are minimally toxic and may exhibit no plateau of the dose-response curve even at very high doses. These considerations served as the basis for our systematic evaluation of [D-leucine⁶-desarginine-glycine-NH₂¹⁰]gonadotropin-releasing hormone (GnRH-A) proethylamide in the very high dose range (i.e., 10-fold larger amounts than previously used). In rats given the analog for 12 wk, prostate, testis, and seminal vesicle weights were suppressed to a greater extent with 200 μ g q.d. than with 40 μ g q.d. ($P < 0.01$ prostate, <0.01 testis, <0.01 seminal vesicles), indicating dose-response effects in the very high dose range. 200 μ g of [D-Leu⁶-des-Gly-NH₂¹⁰]-GnRH-A consistently suppressed luteinizing hormone (LH) values at 6 and 12 wk (basal 71 ± 9.5 ; 6 wk 34 ± 3.8 ; 12 wk 28 ± 5 ng/ml) whereas 40 μ g suppressed LH variably (basal 33 ± 3.8 ; 6 wk 17 ± 3.9 ; 12 wk 32 ± 5.2). Testosterone fell to 15 ± 2.4 and 19 ± 2.0 ng/100 ml in response to 200 μ g q.d. and to 27 ± 6.4 and 22 ± 7.4 ng/100 ml with the 40- μ g dose.

These findings in the rodent prompted treatment of stage D prostate cancer patients with similarly high doses of [D-Leu⁶-des-Gly-NH₂¹⁰]-GnRH-A. After treatment for 11 wk with 1,000 or 10,000 μ g/d of the analog, testosterone and dihydrotestosterone levels transiently rose and then fell into the surgically castrate range (testosterone 19 ± 4.4 ng/100 ml [D-Leu⁶-des-

Gly-NH₂¹⁰]-GnRH-A vs. surgically castrate 11 ± 0.9 ng/100 ml, $P = \text{NS}$; dihydrotestosterone 15 ± 1.7 ng/100 ml GnRH-A vs. surgically castrate 15 ± 4.1 ng/100 ml, $P = \text{NS}$). However, unlike the chronic stimulatory effect on the pituitary at lower doses, very high dose therapy resulted in profound suppression of plasma and urine LH. Plasma levels fell to the limit of assay detectability, whereas the more sensitive urinary assay detected prepubertal levels of excretion (i.e., 64 ± 8.4 mIU/h). The highly sensitive rat interstitial cell testosterone bioassay for LH also demonstrated a marked decline in LH to undetectable levels in 17/19 subjects. Clinical results with [D-Leu⁶-des-Gly-NH₂¹⁰]-GnRH-A simulate those achieved by surgical castration in men with prostatic cancer as suggested by available preliminary data.

INTRODUCTION

70,000 new cases of prostate cancer are diagnosed annually in the United States. 85% of these present with disseminated lesions and ~75% are hormone dependent (1-3). Surgical castration effectively lowers androgen levels and produces tumor regression in these patients. Because this procedure is unacceptable to many men, several investigative groups have attempted to develop medical alternatives to castration. Accelerated death rates induced by cardiovascular and/or thromboembolic disease related to high-dose estrogen administration limited the usefulness of this form of "medical castration" (4). Lower doses of estrogen produce tumor regression without cardiovascular complications but may be less effective than castration. Progestational agents alone are only transiently effective at reducing testosterone (5, 6), whereas

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the recent introduction of antiandrogens (7) or combination of estrogen with progestins shows promise for a sustained reduction of testosterone levels lasting up to 16 mo in one study (8).

Studies in a variety of species utilizing highly potent (superagonist) analogs of gonadotropin-releasing hormone (GnRH-A)¹ suggested an alternative approach to medical castration. Chronic administration of these compounds produces paradoxical atrophy of reproductive tissues. [D-Leu⁶-des-Gly-NH₂¹⁰]-GnRH proethylamide (D-Leu⁶-GnRH) is a superagonist analog with 12–20 times greater luteinizing hormone (LH) and follicle-stimulating hormone (FSH) releasing activity than that of GnRH itself (9). Studies in rats (10–15) demonstrated inhibition of testosterone secretion, reduction of testicular LH receptors, and prostate regression with chronic administration of this compound. This ability to induce “reproductive atrophy” pharmacologically with this agent has suggested its potential usefulness in androgen-dependent neoplasia.

Prior studies in the rodent focused upon the inhibitory activities of 0.005–5 µg of GnRH-A. Because these compounds are devoid of toxicity at very high doses, we initiated systematic studies to evaluate the effects of even higher doses of D-Leu⁶-GnRH (40–200 µg daily) in rats bearing androgen-dependent prostate tumors and then extended these investigations using comparable doses on a milligram per square meter basis to men with prostatic carcinoma (16). This report details the profound inhibition of gonadotropins and androgens observed with very high-dose GnRH-A therapy in these studies.

METHODS

Rodent studies

Groups of animals. Three groups of 25 adult male Noble rats bearing subcutaneous prostate tumor implants (17, 18) included: Group 1 was castrate (animals were surgically castrated 2 wk before treatment); group 2 received 20 µg of D-Leu⁶-GnRH twice daily subcutaneously for 12 wk; and group 3 received 100 µg of D-Leu⁶-GnRH twice daily subcutaneously for 12 wk. Animals in group 1 weighed 319±25 g; group 2, 300±10 g; and group 3, 290±9.2 g.

Blood for measurement of serum LH, testosterone, and prolactin was obtained basally in each of 25 animals in each group. Further blood was drawn for hormone measurements again at 3, 6, and 12 wk of therapy, 12 h subsequent to previous drug administration. To reduce the number of assays necessary and to increase sample size, the serum from pairs of animals in each group were pooled before analysis. Animals were killed at 12 wk and the testes, prostates, and seminal vesicles weighed.

¹ *Abbreviations used in this paper:* DHT, dihydrotestosterone; FSH, follicle-stimulating hormone; GnRH-A, gonadotropin-releasing hormone analog; LH, luteinizing hormone; PRL, prolactin; RICT, rat interstitial cell testosterone.

Patient studies. 38 noncastrate and 23 surgically castrate men with histologically confirmed stage D prostatic adenocarcinoma were included in this prospective multicenter clinical trial. In each of the surgically castrate patients, at least 1 mo had elapsed since orchiectomy before study entry. Patients were randomly assigned to receive either 1,000 or 10,000 µg of D-Leu⁶-GnRH s.c. on a daily basis. The participating investigators (see Acknowledgments) fully explained the purpose of the study and obtained written informed consent after describing the discomforts, risks, side effects, and potential benefits of this therapeutic approach and other therapeutic options available.

Patients were hospitalized for 3 d for initial study measurements and disease staging. An initial blood sample was obtained between 7 and 9 a.m., after which either 1,000 or 10,000 µg of D-Leu⁶-GnRH s.c. was administered daily. Blood samples were drawn just before and at 4 and 8 h after the first D-Leu⁶-GnRH dose (day 1), 24 h after the second dose (72 h), and 4 and 8 h after the third dose (76 and 80 h) as shown on Figs. 2 and 3. Subsequent blood samples were obtained 24 h after previous drug administration on days 7, 14, and wk 4–5, 6–7, 10–11. At each time interval, measurements of FSH, LH, prolactin (Prl), testosterone, dihydrotestosterone (DHT), and cortisol were made in all patients. A subgroup consisting of the first 12 noncastrate patients entered into the study had additional measurements for progesterone, 17α-hydroxyprogesterone, and androstenedione. Limited amounts of plasma precluded measurement of each hormone of each patient. In addition, 3-h timed urines were obtained during chronic therapy (i.e., at least 1 mo) in six men for measurement of LH and FSH by radioimmunoassay (RIA). Biologically active LH was measured (in 19 castrate and 9 intact men) before treatment and again during the 6th to 11th wk of drug administration.

Hormone assays. Serum LH, FSH, and Prl were measured in rodents (19) and in patients by RIA as previously described (20, 21). To enhance assay sensitivity, we also used RIA of urinary LH and FSH, which involved an extraction and 40-fold concentration of urine before quantitation using the same reagents as for the plasma RIA (22). The rat interstitial cell testosterone (RICT) assay for LH of Dufau et al. (23) was used to measure biologically active LH in serum. This assay has a limit of sensitivity of 0.4 mIU/ml and a bioassay/RIA potency ratio of 3.95±0.97 (SD, n = 168). Assay precision, sensitivity, normal ranges, specificity and physiologic studies using this assay have been previously published (23, 24). A direct solid-phase assay was used to measure plasma cortisol (25). RIA of testosterone and DHT used plasma extraction, LH-20 column chromatography, and then specific RIA (26). Measurement of androstenedione involved ether extraction of 1–3 ml of plasma, celite chromatography, and then RIA by methods used extensively in prior studies (27). Plasma progesterone and 17α-hydroxyprogesterone were assayed after extraction and column chromatography on LH-20 using specific antisera.

With the amounts of plasma extracted, the working sensitivities of the steroid RIA used included testosterone, 10 ng/100 ml; DHT, 10 ng/100 ml; progesterone, 0.1 ng/ml; and 17α-hydroxyprogesterone, 0.1 ng/ml. The sensitivities of the human LH, FSH, and Prl RIA reflecting 85% binding on the assay standard curves included: LH, 8 mIU/ml; FSH, 4 mIU/ml; and Prl, 5 ng/ml. For the rat LH assay 18 ng/ml of RP-1 was the amount that yielded 85% binding on the standard curve. The sensitivities of the urinary LH and FSH assays depended upon the volumes extracted and volume of urinary output but averaged 10 mIU/h for both LH and FSH assays.

Data analysis. Unpaired *t* tests were used to compare parameters between each treatment group. Basal levels of LH and Prl were unexpectedly variable among the three treatment groups (Table I). As a consequence, paired *t* tests were used to evaluate the statistical significance of the degree of suppressibility of various hormone levels when compared with basal values. Since the number of patients evaluated at each time point varied, unpaired *t* tests were used in a similar fashion for the human data. Since the experimental design required multiple comparisons between basal and treatment values, we used the Bonferroni method of analyzing multiple comparisons (28). With this highly conservative technique, the usual *P* value necessary for statistical significance is divided by the number of comparisons made and thus one arrives at a lower and more stringent *P* value. Thus, for the rodent studies *P* < 0.05 is divided by the three comparisons made (i.e., basal vs. 3, 6, and 12 wk), which equals a required *P* value of <0.017 for statistical significance. In addition, where appropriate, analysis of variance was undertaken to evaluate overall differences between initial values and those between 2 and 11 wk in patients treated with the D-Leu⁶-GnRH. When hormone levels were undetectable in various assays, results were expressed as the lower limit of detectability for statistical purposes.

RESULTS

Rodent studies

LH. The administration of very high dose D-Leu⁶-GnRH (40 and 200 µg daily) caused an initial increase in plasma LH concentrations at 3 wk followed by later suppression at 6 and 12 wk (Table I). 40 µg daily of D-Leu⁶-GnRH increased LH from 33±3.8 (SEM) to 230±18 ng/ml (*P* < 0.01) at 2 wk, whereas 200 µg exerted a less pronounced stimulatory effect (basal, 71±9.5 ng/ml; 3 wk, 98±6.5 ng/ml [*P* < 0.01]). During 6–12 wk of treatment, LH consistently fell below basal

values in animals receiving 200 µg daily. This hormone declined significantly only at week 6 in those given 40 µg daily. In the castrate animals, a sevenfold rise in LH from 77±18 basally to 460±60 ng/ml at 12 wk was observed.

Testosterone. The levels of this steroid increased slightly but not significantly at 3 wk and then fell in response to both high doses of D-Leu⁶-GnRH at 6–12 wk (Table I). At the highest dose (i.e., 200 µg daily), D-Leu⁶-GnRH suppressed testosterone to 15±2.4 and 19±2.0 ng/100 ml (*P* < 0.001 and *P* < 0.01, respectively) at 6 and 12 wk. By comparison, castration reduced testosterone to 11±1.7 and 8.8±1.0 ng/100 ml (*P* < 0.001 and *P* < 0.001) at the same time points. At the 12th wk, testosterone levels were notably significantly higher in the GnRH-A-treated animals (*P* < 0.02 for 40 µg; *P* < 0.001 for 200 µg) than after castration. The levels observed after drug or castration were an order of magnitude lower than the testosterone concentrations before treatment, which ranged from 90±12 to 100±25 ng/100 ml.

Prl. All animals exhibited similar Prl levels basally and during the 12 wk of observation (Table I). However, prolactin transiently increased during the third and sixth weeks of drug administration (Table I).

Organ weights. Measurement of androgen target organ weights provides a bioassay of the effects of high-dose GnRH-A administration. In animals receiving the 40 µg daily D-Leu⁶-GnRH dose prostate weight was 0.26±0.08 (SD) g and in those given 200 µg daily, 0.16±0.09 g (Fig. 1). This reflected a significant dose-response effect (*P* < 0.01, 40 vs. 200 µg daily). In comparison, castration reduced the weight of this organ

TABLE I
Effect of D-Leu⁶-GnRH on Rodent Plasma Hormone Levels

Hormone	Treatment group	Week on treatment			
		0	3	6	12
LH (ng/ml)	Castrate	77±18	490±185	520±81‡	460±60‡
	D-Leu ⁶ 40§	33±3.8	230±18	17±3.9°	32±5.2
	D-Leu ⁶ 200	71±9.5	98±6.5°	34±3.8°	28±5.0‡
Testosterone (ng/100 ml)	Castrate	100±25	9.8±1.3°	11±1.7‡	8.8±1.0‡
	D-Leu ⁶ 40§	100±22	130±20	27±6.4°	22±7.4‡
	D-Leu ⁶ 200	90±12	100±6.6	15±2.4	19±2.0°
Prolactin (ng/ml)	Castrate	72±9.1	48±9.4	67±15	73±6.7
	D-Leu ⁶ 40§	48±3.5	101±10‡	89±9.4‡	59±6.9
	D-Leu ⁶ 200	64±6.3	100±8.9°	100±13°	77±13

° *P* < 0.01.

‡ *P* < 0.001.

§ D-Leu⁶-GnRH, 20 µg b.i.d.

|| D-Leu⁶-GnRH, 100 µg b.i.d.

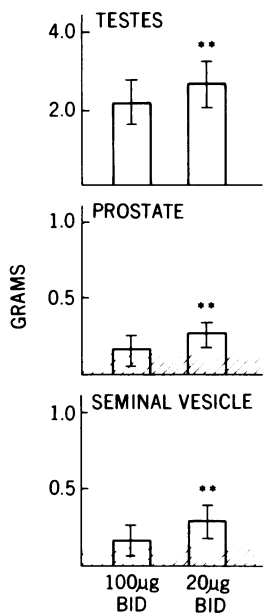


FIGURE 1 Testis prostate and seminal vesicle weight in animals receiving 20 and 100 μg of D-Leu⁶-GnRH proethylamide s.c. twice daily (i.e., 40 and 200 μg total daily dose). Cross-hatched area represents mean \pm SEM for castrated animals. This was different than either treatment dose ($P < 0.001$). ** $P < 0.01$.

(0.08 \pm 0.05 g) to a greater extent than either the 40 μg ($P < 0.01$) or the 200 μg ($P < 0.005$) doses of D-Leu⁶-GnRH. Dose-response effects were also noted in the seminal vesicles and testis as well (Fig. 1).

Human studies

Dose-response data. Based on the rat data, we chose to initiate clinical studies with comparable amounts of D-Leu⁶-GnRH (i.e., 1,000 and 10,000 μg) on a milligrams per square meter basis (16). In contrast to findings in the rat, no dose-response differences were observed in the level of any hormone between patients receiving 1,000 or 10,000 μg D-Leu⁶-GnRH daily. For example, Fig. 2 illustrates testosterone levels expressed as percentage of control values (to facilitate comparison) in patients receiving 1,000 and 10,000 $\mu\text{g}/\text{d}$. Both doses produced an initial rise in testosterone lasting 1 wk followed by suppression to a maximum of 6% of basal values thereafter. At no time point are the responses to either dose statistically different. Consequently, for all additional data analysis, the 1,000- and 10,000- μg dose groups were pooled and dose-response data were omitted for other hormones.

LH and FSH levels. In both the castrate and noncastrate men, LH initially rose to peak values 8 h after the first dose. For castrate men, the increase was from

71 \pm 8.4 (SEM) mIU/ml basally, to 127 \pm 14 mIU/ml ($P < 0.001$), whereas the noncastrate men exhibited an increment from 28 \pm 3.5 to 97 \pm 9.9 ($P < 0.001$) (Fig. 3) mIU/ml, $P < 0.001$) and 2 wk for the noncastrate men (13 \pm 0.9, $P < 0.001$). Thereafter, the titers of this gonadotropin in both groups fell further to values that bordered assay sensitivity (i.e., 85% binding on the assay standard curve) for the remainder of the 11 wk.

FSH levels similarly rose in both castrate and intact men and peaked 8 h after the initial injection. The first significant suppression occurred at 72 h in the castrate group and 2 wk in the noncastrate group. Thereafter, both groups fell further to the levels of borderline assay detectability.

To determine accurately the degree of gonadotropin suppression, highly sensitive methods of quantitating low levels of gonadotropins are necessary. For this purpose, we used 40-fold concentrates of urine extracts, a method extensively validated for this purpose (22). In a subgroup of six intact men, urine LH levels were 64 \pm 8.4 mIU/h (mean \pm SEM) during chronic treatment with D-Leu⁶-GnRH (Fig. 4). These levels, actually prepubertal, are nearly 20-fold lower than the mean urinary LH for adult men (i.e., 1,022 \pm 67 mIU/h) and 10-fold below the lower level of the adult male normal range (i.e., 560–2,500 mIU/h). Urine FSH levels during D-Leu⁶-GnRH therapy were more variable in individual patients but the mean excretion (209 \pm 68 mIU/h) was below the mean adult male level of 489 \pm 46 mIU/h and near the lower limit of normal of 190 mIU/h.

Biologic LH activity (RICT). To assess the inhibition of biologically active LH by D-Leu⁶-GnRH, a

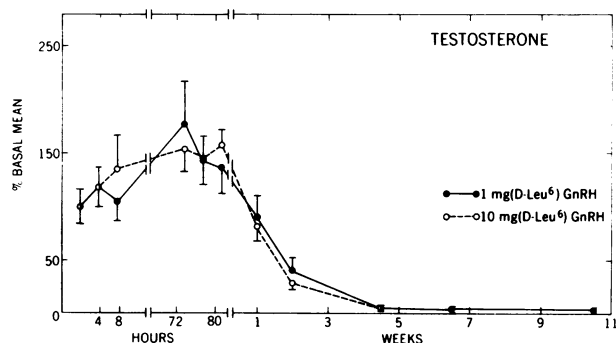


FIGURE 2 Dose-response effects of 1,000 and 10,000 μg of D-Leu⁶-GnRH on human serum testosterone expressed as percentage of basal mean values. Data points represent the mean and SEM. D-Leu⁶-GnRH was administered subcutaneously daily. Blood samples were drawn just before, and at 4 and 8 h after the first D-Leu⁶-GnRH dose (day 1), 24 h after the second dose (72 h), and 4 and 8 h after the third dose (76 and 80 h) as shown on Figs. 2 and 3. Subsequent blood samples were obtained 24 h after previous drug administration on days 7, 14, wk 4–5, 6–7, 10–11.

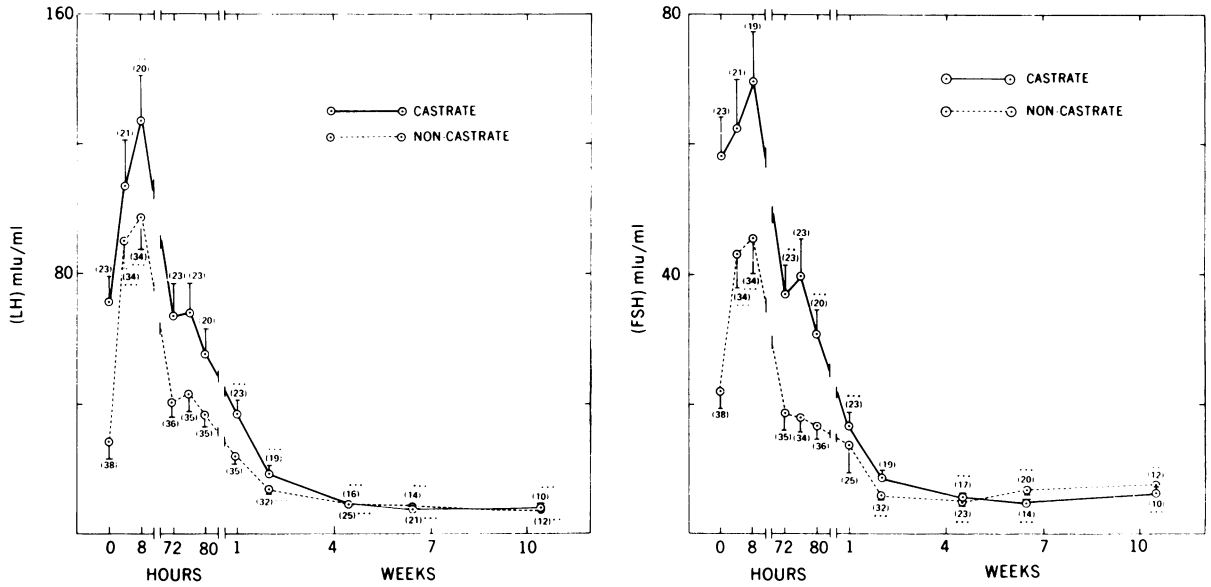


FIGURE 3 Serum LH and FSH levels by RIA in castrate and noncastrate men. The data points represent the mean and SEM determinations. The numbers in parentheses represent the numbers of individuals included in each data point. The lines are broken to indicate the change in scale on the ordinate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. D-Leu⁶-GnRH was administered subcutaneously daily. Sampling schedule was same as in Fig. 2.

subset of 9 castrate and 19 intact men (Fig. 5) were selected for study based upon availability of serum. Of note is the elevation in basal LH levels in 5/19 noncastrate men, both by RIA (normal 7.9 ± 4.4 [SD]) and RICT (normal 41.4 ± 15.1 [SD], 168 samples, 7 subjects). These findings of increased LH are consistent with a variety of data in support of elevated LH in noncastrate, older men (29–35), although one recent study suggested otherwise (36). Of additional interest is the fact that 3/9 castrate men had normal LH levels by RICT (but not by RIA). This may reflect the lowered B/I ratios that occur after castration (37). These observations will be expanded and discussed fully in an additional publication.

In castrate men, 4/9 LH levels were suppressed to undetectable levels (i.e., < 0.4 mIU/ml). The other five men suppressed LH levels markedly (i.e., 5–20-fold) but remained detectable. In comparison, 17/19 of the noncastrate men suppressed LH levels below the level of assay detectability, whereas the other two fell 15–50-fold.

Acute on chronic responses. In other species, the pituitary continues to respond to each subsequent dose of GnRH-A, even though overall gonadotropin suppression 24 h after each dose is observed (“acute on chronic” effect) (38–41). To assess this phenomenon, we compared the acute LH and FSH increments during the first with that during the third daily injection of D-Leu⁶-GnRH. Surprisingly, by the third day,

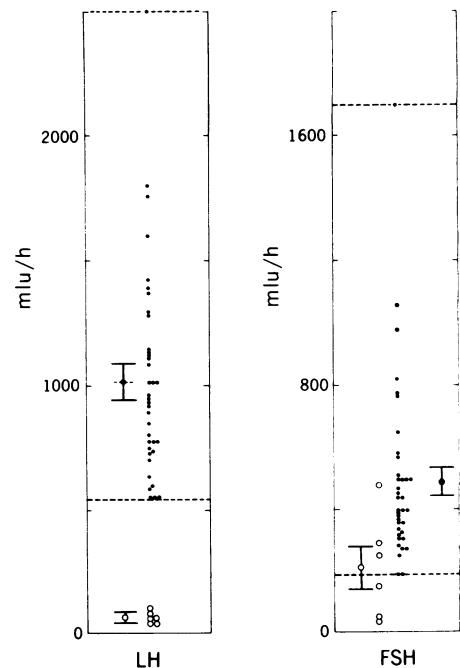


FIGURE 4 Measurement of urine LH and FSH by RIA of 40-fold concentrations of urinary extracts. Data points represent control levels in untreated normal men (●) and values measured during the chronic administration (>1 mo) of D-Leu⁶-GnRH (○). Bars represent means \pm SEM; dashed lines represent normal ranges.

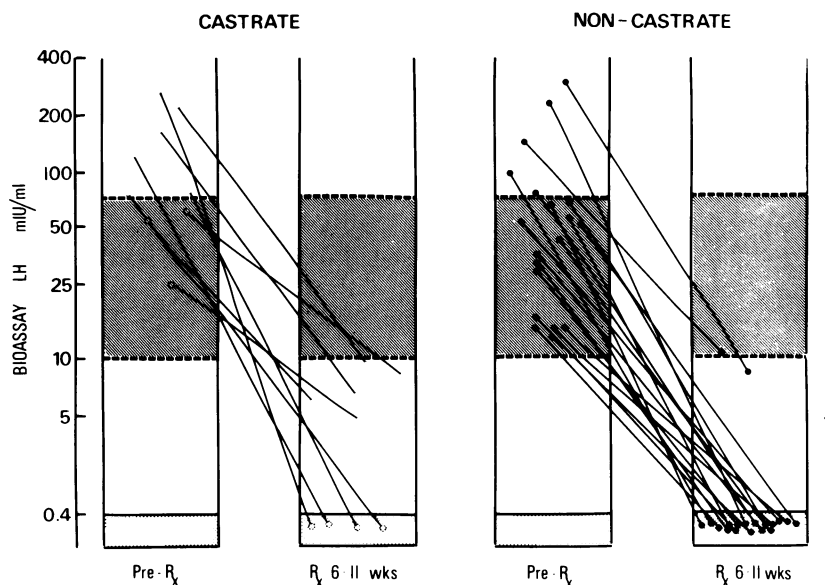


FIGURE 5 Interstitial cell LH bioassay of serum LH in castrate and noncastrate men. The cross-hatched area represents the normal range in men ages 20-45 yr. The dotted area below represents the undetectable range of $<0.4\text{ mIU/ml}$.

the acute on chronic stimulatory effect of $\text{D-Leu}^6\text{-GnRH}$ on LH and FSH was completely abolished by high-dose GnRH-A administration (Fig. 6, FSH data not shown).

Testicular steroids. Only response data from noncastrate patients are presented since no effects of $\text{D-Leu}^6\text{-GnRH}$ were observed in castrate men (Fig. 7).

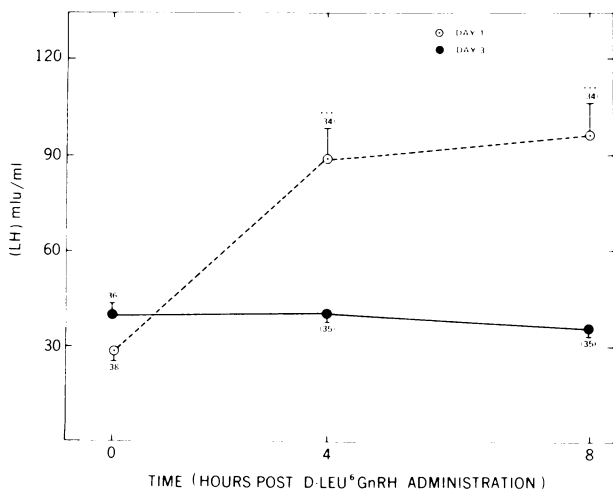


FIGURE 6 Acute on chronic effect of $\text{D-Leu}^6\text{-GnRH}$ on LH levels. The open circles represent LH levels in intact men receiving $\text{D-Leu}^6\text{-GnRH}$ for the first injection. The solid circles represent LH levels in men following the fourth daily subcutaneous injection of the same doses.

In intact men, testosterone levels rose initially from $323\pm 37\text{ ng/100 ml}$ to $461\pm 51\text{ ng/100 ml}$ at 8 h ($P < 0.01$) and then fell to suppressed levels ($114\pm 26\text{ ng/100 ml}$) by 2 wk. Thereafter, testosterone declined further to $19\pm 4.4\text{ ng/100 ml}$ ($P < 0.001$) at 10-11 wk, a reduction of 94% over basal. These levels approximated those observed basally ($11\pm 0.9\text{ ng/100 ml}$) in 25 castrate men before therapy. DHT concentrations paralleled those of testosterone, increasing initially from $49\pm 4.8\text{ ng/100 ml}$ to $87\pm 17\text{ ng/100 ml}$ at 72 h ($P = 0.01$), and then fell to $28\pm 4.5\text{ ng/100 ml}$ ($P < 0.01$) at 2 wk. By 10-11 wk of treatment, the levels fell further to $15\pm 1.7\text{ ng/100 ml}$ ($P < 0.01$), a 70% reduction over basal values. This represented a level identical to castrate values in men studied under basal conditions ($15\pm 4.1\text{ ng/100 ml}$, $n = 23$).

Steroids of mixed gonadal and adrenal origin. In a subgroup of intact men, 17α -hydroxyprogesterone, androstenedione, and progesterone were measured during $\text{D-Leu}^6\text{-GnRH}$ therapy. All steroids originate from both adrenal and testicular sources, although the testis source of 17α -hydroxyprogesterone predominates (42). Although there was a tendency for an initial rise, no statistically significant changes occurred during the first 80 h after initial injection. At 6 wk of treatment, androstenedione decreased from $0.8\pm 0.13\text{ ng/ml}$ to $0.3\pm 0.08\text{ ng/ml}$ and 17 -hydroxyprogesterone declined similarly. These decrements, however, did not reach statistical significance because the Bonferroni correction for multiple comparisons required sig-

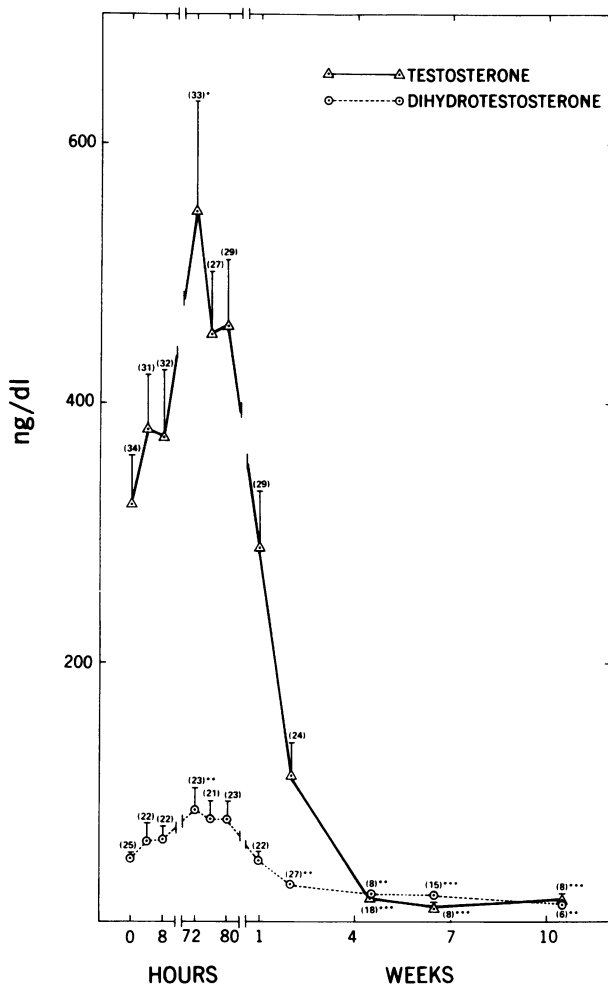


FIGURE 7 Testosterone and dihydrotestosterone levels in men receiving D-Leu⁶-GnRH superagonist analog over 11 wk. The numbers within the parentheses represent the numbers making up each data point. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. D-Leu⁶-GnRH was administered subcutaneously daily. Sampling schedule same as in Fig. 2.

nificance levels of *P* < 0.01. Use of analysis of variance to compare basal values with those observed after 2 wk of therapy also did not reveal statistically significant differences for androstenedione and 17-hydroxyprogesterone (Fig. 8).

Initial analysis of progesterone levels revealed that all values approached assay sensitivity. Consequently, samples from 10 men before treatment and during the second to eleventh weeks of treatment were pooled to allow large extraction volumes (i.e., 2 ml). With this method of analysis values remained <0.1 ng/ml before and during treatment.

Prolactin and cortisol levels. Analysis of basal and posttreatment levels of prolactin and cortisol revealed

no statistically significant differences at any time between groups (data not shown).

DISCUSSION

Potent GnRH-A produce prostatic atrophy and reduction of testosterone levels in a variety of species (38-41, 43-45). Reports of these paradoxical actions stimulated several ongoing studies of GnRH-A as potential treatment strategies for androgen-dependent prostatic carcinoma in man (46-48). We first questioned what doses of analog should be chosen for initial studies in patients. Unpublished data in rats (research files, Abbott Laboratories) suggested that very high doses of GnRH-A (i.e., doses an order of magnitude greater than previously used) might produce greater suppression of prostatic weight than lower doses. Our rodent data (Fig. 1) demonstrated significantly greater suppression of prostate, seminal vesicle, and testis weight with the 200 μg than with the 40 μg daily dose of D-Leu⁶-GnRH. This surprising dose-response effect with very high doses supported the potential usefulness of such large amounts of GnRH-A in patients. A major margin of patient safety was predicted since no toxicity occurred with amounts >10 mg/kg per d for 32 d or lower doses administered for up to 2 y in rats. Thus, for the patient trials, we chose GnRH-A doses (i.e., 1,000-10,000 μg/d) equivalent to the amounts used in rats when corrected for square meter differences (16). These doses are in striking contrast with the much lower amounts used chronically in three recently published preliminary studies of GnRH-A administration in prostate cancer patients (46-48). It is pertinent, then, to consider whether LH and androgen suppression were greater in the present study than in those using lower GnRH-A doses. Our data indicate a 75-90% suppression of plasma LH and FSH to the limit of detectability of the respective RIA. By comparison, Faure et al. (48) found no inhibition and Borgmann et al. (47) a 60% reduction in three patients. LH levels were not reported in the study of Tolis et al. (46). These data support the possibility that LH suppression may be greater in patients receiving 1,000-10,000 μg of GnRH-A daily as reported in the present study.

Since the GnRH-A exert significant direct gonadal effects in some species, it may not be necessary to suppress LH fully to achieve castrate levels of testosterone in noncastrate men treated with GnRH-A. To examine this possibility, we also compared the published levels of testosterone in men given lower amounts of GnRH-A with those in subjects receiving 1,000-10,000 μg daily (Table II). As shown in Table II, the data of Faure et al. (48) and Tolis et al. (46) suggest that lower amounts of GnRH-A than used in the current study

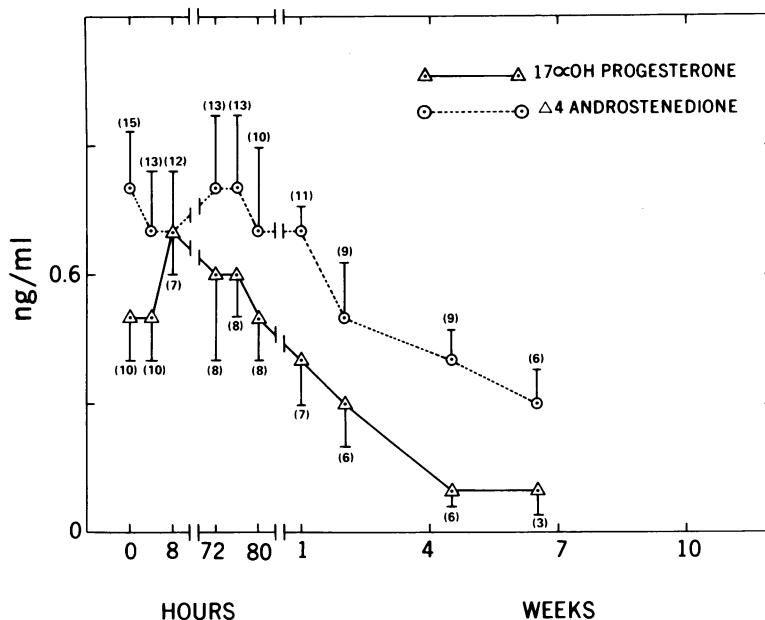


FIGURE 8 Levels of 17α -hydroxyprogesterone and androstenedione in selected patients during administration of $D\text{-Leu}^6\text{-GnRH}$. $D\text{-Leu}^6\text{-GnRH}$ was administered subcutaneously daily. Sampling schedule same as in Fig. 2.

do not suppress testosterone to castrate levels. The approach of Borgmann et al. (47) to use very high doses, initially and lower doses chronically did appear to lower testosterone to castrate levels in three patients. This is of great interest and requires examination in a larger study.

Based upon the above data, use of very high dose GnRH-A in man is nontoxic and produces a profound suppression of LH to prepubertal (Figs. 4 and 5) and testosterone to castrate levels. However, it requires emphasis that our study has not firmly established the requirement for such large amounts of drug. The relative efficacy of very high doses and of lower amounts can only be inferred currently by comparing published data. It will now be necessary to conduct full dose-response studies in man to determine the maximal doses required. This issue is particularly important since lower doses can be administered much more practicably by the nasal spray route. Since only 1–4% of drug is absorbed by this method of delivery (51, 52), administration of very high amounts intranasally is not feasible.

Based upon rodent studies, previous investigators emphasized the importance of the direct gonadal effects of GnRH-A in producing reproductive atrophy. These conclusions relied heavily on the observations that rodent testes contain GnRH receptors (53) and analog effects can be demonstrated in hypophysecto-

mized animals (13, 54–56). In addition, GnRH-A do not suppress basal LH levels in intact male rats during chronic treatment. With a variety of regimens (i.e., $D\text{-Leu}^6\text{-GnRH}$, 2–200 ng [57]; $D\text{-Ala}^6\text{-GnRH}$; 100 ng–1 μg [10, 58, 59]; $D\text{-Tryp}^6\text{-GnRH}$, 0.001–1 μg [12]; Buserelin, 0.034–34 μg , or GnRH itself, 0.034–340 $\mu\text{g}/\text{d}$ [11]), basal LH was unchanged or increased in intact animals, while suppression occurred exclusively in castrates. Only with very high dose GnRH (i.e., 200 μg q.d.; this study) has inhibition of basal LH been demonstrated during chronic treatment in intact male rats. The biological importance of this additional effect of GnRH-A is suggested by the organ weight and testosterone data in this study (Fig. 1, Table 1). Prostatic seminal vesicle and testis weight as well as testosterone decreased to a significantly greater extent in the rats (i.e., 200 μg q.d. group) whose LH was consistently suppressed during 6–12 wk of therapy than in those with variable LH inhibition (i.e., the 40 μg q.d. group) (Table I).

The recent data of Clayton (11) provide a possible mechanistic explanation for the LH suppression observed only at the very high dose level in the rodent. He demonstrated a dose-related, biphasic action of GnRH-A on pituitary GnRH receptor content. Low-dose GnRH-A infusion (i.e., 0.34 $\mu\text{g}/\text{d}$ per rat) stimulated GnRH receptors; intermediate doses (i.e., 3.4 μg) produced no effects; and very high doses (34 μg)

TABLE II
Clinical Studies of GnRH-A in the Treatment of Prostatic Carcinoma

Investigative group	Number of subjects	Analog used	Dose and route	Analog (49, 50) potency LH-RH = 1	Treatment duration	Testosterone at studies end
					wk	ng/dl
Faure et al. (48)	8	Buserelin*	50 µg s.c. daily	20-50	4-30	128±56
Tolis et al. (46)	10	D-Trp ⁶ GnRH Buserelin*	100 µg s.c. daily 50 µg s.c. daily or 500 µg i.n.† b.i.d.	26-50 20-50	16	65±15‡
Borgmann et al. (47)	3	Buserelin*	1 mg b.i.d. s.c. × 3-6 d, then 0.4-1.2 mg/d i.n.†	20-50	24	<25
Warner et al. (current study)	38	D-Leu ⁶ GnRH	1 or 10 mg daily	12-20	11	19±4.4

* [D-Ser(TBU)⁶, des-Gly-NH₂¹⁰]LH-RH ethylamide.

† i.n. absorption = 1-4% (51-52).

‡ Reflects pooled data from both analogs not differentiated.

inhibited receptor content in the pituitaries of intact male rats. Thus, chronic administration of very high doses of GnRH-A may be necessary to down-regulate GnRH receptors and to suppress LH during chronic administration in the intact male rat. In addition, such doses may be required to produce the profound reduction in urinary LH and plasma LH bioactivity observed in patients in this study.

The data presented serve to highlight the well-recognized species differences regarding the mechanisms whereby the GnRH-A produce reproductive atrophy (10). In the rat, GnRH-A inhibit testicular function by a predominately testicular effect with a lesser inhibition of gonadotropin secretion. In contrast, the major effect in men is to suppress LH released by the pituitary.

The rationale for treatment of prostatic carcinoma with GnRH-A is to mimic the biologic effects of surgical castration (60). Even with very high doses of GnRH-A, testosterone levels and reproductive organ weight did not decrease to the extent produced by castration in our rodent studies (Table I, Fig. 1). Previous investigators have not identified this lack of complete reproductive atrophy since castrate animals were not included as controls. In our patient studies (Fig. 7), testosterone levels did fall to the castrate range (19±4.4 ng/dl GnRH-A, 11±0.9 ng/dl castration, *P* = NS) but organ weights were not available for comparative analysis. In general, these data suggest that complete inhibition of the pituitary Leydig cell axis is more easily achieved in man than in the rodent. Nonetheless, further study of the comparative biologic effects of GnRH-A vs. castration in men is necessary. In particular, the relative efficacy of castration and

GnRH-A on prostatic tumor growth in patients as a biologic endpoint of androgen suppression is required.

In this regard, the prostate cancer response data in patients from this trial have now been published and support our contention that very high dose GnRH-A treatment mimics the clinical effects of surgical castration (61, 62). In 57 patients completing >10 wk of therapy, all with Stage D prostatic carcinoma, responses according to the National Prostatic Cancer Project criteria (63) were as follows: (a) In 30 noncastrate patients who had received no previous hormonal therapy, 22/30 (74%) objectively responded and 7/30 (23%) were without change. (b) Of 27 intact (i.e., noncastrate) patients who had received prior hormonal therapy, 5 (19%) experienced objective tumor regression and 9/27 (33%) no change. These responses are similar to those expected after surgical castration.

It is of interest that disease flare occurred in 4.4% of patients (61, 62) treated with D-Leu⁶-GnRH for prostate cancer. Flare of disease is probably related to the acute testosterone rise observed upon initiation of GnRH-A treatment. The transient nature of this acute androgen increment following chronic agonist treatment was first shown by Faure et al. (48) and is consistent with our data. Tumor stimulation or flare on this basis should be equally transient. Thus, an acute increase in testosterone upon each chronic injection (acute on chronic phenomenon) as has been reported in rats (40), rams (39), and monkeys (38, 41) is not seen in man.

Findings of low toxicity with dramatic hormonal and clinical results using very high doses of GnRH-A make it incumbent upon other investigators to explore this upper end of the dose-response range in other

treatment settings. Currently, the antireproductive properties of GnRH-A are being used for precocious puberty (64, 65), contraception (66), gonadal sparing during chemotherapy (67), nongonadal tumors (49, 68), endometriosis (69), combination hormonal therapy (70), hirsutism, and acne as well as other nontumorous conditions (71).

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