0021-972X/89/6901-0043$02.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 69, No. 1
Printed in U.S.A.

A Comparison of the Suppressive Effects of Testosterone and a Potent New Gonadotropin-Releasing Hormone Antagonist on Gonadotropin and Inhibin Levels in Normal Men*

CARRIE J. BAGATELL, ROBERT I. McLACHLAN, DAVID M. DE KRETSER, HENRY G. BURGER, WYLIE W. VALE, JEAN E. RIVIER, AND WILLIAM J. BREMNER

Medical Service, Veterans Administration Medical Center, Department of Medicine and Population Center for Research in Reproduction, University of Washington School of Medicine, Seattle, Washington 98108; the Department of Anatomy, Monash University and Medical Research Centre, Prince Henry's Hospital, Melbourne, Victoria, Australia; and Salk Institute, La Jolla, California 92138

ABSTRACT. GnRH antagonists have been developed in large part because of their potential use as contraceptive agents, particularly in men. Specifically, it was hoped that GnRH antagonists combined with testosterone (T) would be a more effective contraceptive regimen than T alone. We compared the suppressive effects of a potent GnRH antagonist, Nal-Glu [AcD2NaL^1,D4ClPhe^2,D3Pal^3,Arg^5,DGlu^6(AA),DAla^{10}-GnRH], and of T together and separately on serum and urinary gonadotropin and serum inhibin levels in normal men. Ten-day courses of Nal-Glu (75 μg/kg; Nal-Glu alone), 200 mg testosterone enanthate, im, on days 0 and 7 (T alone), and the combination (Nal-Glu + T) were given to nine men. Serum gonadotropin and inhibin concentrations decreased after 1–2 days of Nal-Glu administration, while gonadotropin suppression occurred more slowly after T alone. Serum T fell to 30% of baseline values during Nal-Glu administration. The combination of Nal-Glu + T was more effective in suppressing serum LH, FSH, and inhibin than was either Nal-Glu alone or T alone. All hormone levels returned to baseline levels within 2.5 weeks after the end of the three regimens. We conclude that the Nal-Glu GnRH antagonist effectively inhibits gonadotropin, inhibin, and sex steroid secretion when given daily for 10 days and that the administration of Nal-Glu + T results in more complete gonadotropin and gonadal suppression than that produced by either agent given alone. These results encourage further investigation of the combination of a GnRH antagonist and T as a male contraceptive regimen and of the antagonist alone as a treatment for hormone-dependent neoplasia. (*J Clin Endocrinol Metab* **69:** 43, 1989)

GNRH antagonists are synthetic analogs of GnRH that compete with endogenous GnRH for pituitary binding sites and thereby suppress gonadotropin and gonadal steroid secretion (1). The antagonists have a different mechanism of action than testosterone (T), which suppresses gonadotropin secretion by inhibiting the GnRH pulse generator (2) and may be a negative modulator at the pituitary level as well (3, 4). GnRH agonists, GnRH antagonists, and exogenously administered T have all been tested as potential male contraceptive agents. GnRH agonists combined with T inconsistently suppress spermatogenesis in normal men; no more than 50–70% of men become azoospermic (5). Similarly, exogenous T causes azoospermia in only 50–70% of normal men (6). GnRH antagonists have been reported to suppress spermatogenesis in rats (7) and primates (8, 9), although their long term efficacy in men is untested.

Nal-Glu [AcD2Nal^1,D4ClPhe^2,D3Pal^3,Arg^5,DGlu^6 (AA), DAla^{10}-GnRH] is a potent GnRH antagonist that inhibits gonadotropin and sex steroid secretion in men for 24 h after a single injection (10, 11). Since Nal-Glu and T inhibit gonadotropin secretion by separate mechanisms, we hypothesized that simultaneous administration of Nal-Glu and T might suppress gonadotropin secretion and, therefore, inhibit gonadal function more completely than either compound given alone. To test this hypothesis, we administered the Nal-Glu antagonist alone, T alone, and the combination of Nal-Glu plus T, each for 10 days, to nine normal men in three randomly ordered experimental periods. Gonadotropins were measured in serum and urine, and gonadal function was assessed by

Received October 4, 1988.
Address all correspondence and requests for reprints to: Carrie J. Bagatell, M.D., Seattle Veterans Administration Medical Center, Medical Service (111), 1660 South Columbian Way, Seattle, Washington 98108.
* This work was supported in part by NIH Grant P-50-HD-12629, the V.A., and the Clinical Research Center at the University of Washington, supported by NIH Grant RR-37.

measurement of serum levels of inhibin, a Sertoli cell product (12, 13).

Materials and Methods

Subjects

Nine normal men, aged 21–35 yr, were studied. All were of normal body weight, and none smoked or abused alcohol, took any medications, or was an elite athlete. All men signed a consent form approved by the University of Washington Human Subjects Committee.

Experimental protocol

Each man received three different treatment regimens. These were: 1) Nal-Glu alone (75 μg/kg in vehicle) daily for 10 days plus im injections of 1 mL sesame oil on days 0 and 7 (Nal-Glu alone); 2) T enanthate (Delatestryl, E. R. Squibb and Sons, Inc., Princeton, NJ; 200 mg in 1 mL sesame oil) im on days 0 and 7 plus 0.6 mL 150 mmol/L NaCl, sc, daily for 10 days (T alone); and 3) Nal-Glu antagonist (75 μg/kg in vehicle) sc daily for 10 days plus T enanthate (200 mg) im on days 0 and 7 (Nal-Glu + T). The 75 μg/kg Nal-Glu dose was chosen on the basis of preliminary experiments demonstrating that a single injection of this amount decreased serum T levels for more than 24 h (11).

Each man received the treatments in random order and without knowledge of which treatment he was receiving. Blood samples were drawn on days 0, 1, 2, 3, 4, 7, 8, and 9 of treatment and 2 and 7 days posttreatment (days 11 and 16). In each man blood samples were drawn at approximately the same time of day and immediately before the day's injection(s). The men also collected 3-h urine samples for measurement of urinary gonadotropins on day 7. A minimum of 18 days (2.5 weeks) elapsed between the last injection of one regimen and the first injection of the next regimen.

Antagonist preparation

The antagonist was dissolved in bacteriostatic water containing 4 g/L mannitol (vehicle), diluted to a concentration of 10 mg/mL, and then, under sterile conditions, passed through a 0.2-μm filter into sterile 5-mL vials, which were stored at −20 C.

Hormone assays

Serum LH and FSH RIAs. Serum LH and FSH were measured by RIAs described previously (14). The LH reference standard (LER 907) and antibody (antihuman LH, batch 2) were supplied by the National Hormone and Pituitary Program. The tracer was purified hCG radioiodinated with ^{125}I using chloramine-T (15). The sensitivity of the assay was 2.0 μg/L, and the intra- and interassay coefficients of variation (CVs) were 5.5% and 8.4%, respectively. FSH reference standard LER 907 and antibody (antihuman FSH, batch 5) also were supplied by the National Hormone and Pituitary program. The tracer was FSH HS-1 radioiodinated with ^{125}I using chloramine-T (15). The sensitivity of the assay was 21 μg/L, and the intra- and interassay CVs were 7.3% and 9.7%, respectively. All samples from an individual man were analyzed in duplicate in the same LH and FSH assays. The results of both assays were calculated using the computer program of Burger *et al.* (16).

Urinary LH and FSH RIAs. Urinary LH and FSH excretion was measured by RIA after acetone precipitation of urine (17) as previously described (14).

Serum inhibin. Serum inhibin was measured using a heterologous double antibody RIA which used an antiserum to purified 31K bovine follicular fluid inhibin and the same antigen labeled with ^{125}I as tracer (18). The assay standard was a partially purified human follicular fluid inhibin standard preparation of defined bioactivity (19). Serial dilutions of serum gave dose-response lines similar to that of the standard. Bovine activin-A, Mullerian inhibitory substance, and porcine and human transforming growth factor-β had less than 1% cross-reactivity in this assay, as did free α and β inhibin subunits obtained by reduction and alkylation of 31K inhibin. The sensitivity (ED_{10}) of the assay was 100 U/L, and the ED_{50} was 660 U/L. The within-assay CUs in the upper, mid, and lower portions of the Standard Curve were 5.8%, 3.4%, and 1.8%, respectively. The between assay CV was 10.2% from 14 assays.

Serum T and estradiol (E_2) RIAs. The RIAs for serum T and E_2 were described previously (20). The reagents were provided by the WHO Matched Reagent Program. The hormones were separated from serum by ether extraction, and separation of bound from free hormone was accomplished by dextran-coated charcoal. The assay sensitivity was 0.35 nmol/L for T and 44.0 nmol/L for E_2. The intra- and interassay variations were 5.1% and 9.5%, respectively, for T and 5.8% and 8.2%, respectively, for E_2.

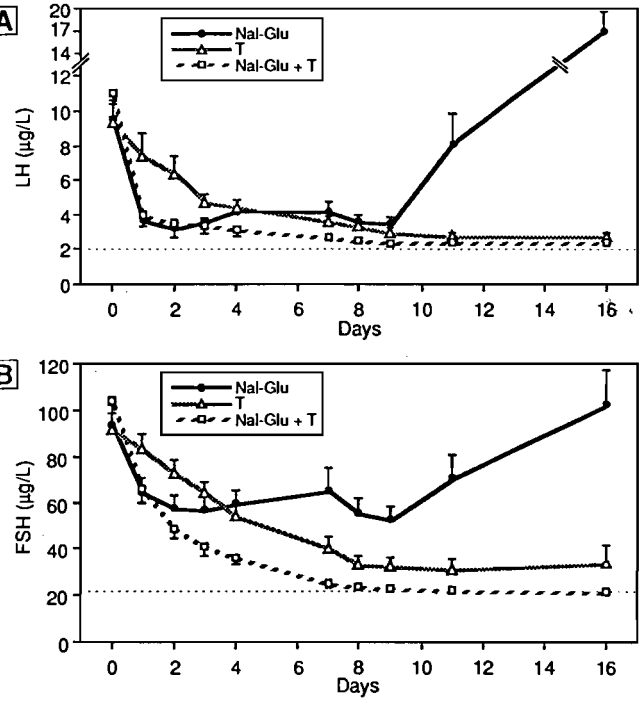
Statistical analysis

Analysis of variance with repeated measures was used to detect significant differences over time within each regimen and between regimens. Student's paired *t* test was used to examine differences between Nal-Glu + T and T or Nal-Glu alone and to compare the day 0 and day 9 results within treatments. In all calculations, hormone levels above or below the limit of detectability of the assay are computed as that particular upper or lower value. All data are expressed as the mean ± SE. $P < 0.05$ was considered significant.

Results

Serum LH

Administration of Nal-Glu alone caused a rapid decline in mean serum LH from 9.5 ± 1.1 to a nadir of 3.2 ± 0.1 μg/L on day 3 (36.1% of baseline; Fig. 1A). It then remained at about this level until Nal-Glu was discontinued, after which it increased quickly, reaching the baseline level by day 11 and increasing further to 17.1 ± 2.4 μg/L on day 16 (195.6% of baseline). Administration of T alone caused a slow progressive decline in serum LH, which, in contrast with Nal-Glu alone, declined even



G. 1. Mean (±SE) serum immunoreactive LH and FSH levels during lministration of Nal-Glu antagonist (75 μg/kg, sc) alone, T (200 mg,) alone, and Nal-Glu + T in nine normal men. A, LH; B, FSH. – – Minimum detectable level.

rther between days 9 and 16 [day 9, 3.0 ± 0.2 μg/L 5.7% of baseline; $P < 0.01$); day 16, 2.8 ± 0.2 μg/L 5.4% of baseline)]. Administration of Nal-Glu + T used a rapid decline in serum LH from 12.0 ± 1.3 to 5 ± 0.5 μg/L on day 2; by day 9 (the last treatment ay) the mean LH level was lower during the Nal-Glu + regimen (2.3 ± 0.2 μg/L) than during either treatment ith Nal-Glu alone (3.5 ± 0.4; $P < 0.05$) or T alone (3.0 0.2 μg/L; $P = 0.07$). Serum LH remained low on day 6 after Nal-Glu + T (2.4 ± 0.1; 21.5% of baseline). After l regimens, serum LH returned to baseline by the eginning of the next injection period 2.5 weeks later.

erum FSH

With Nal-Glu alone, serum FSH decreased from 93 ± μg/L to a nadir of 53 ± 5 μg/L on day 9 (57.4% of aseline; $P < 0.001$; Fig. 1B) and then increased to 102 14 μg/L (110.8% of baseline) on day 16. Administration T alone resulted in a gradual decrease in serum FSH om a baseline value of 92 ± 6 to 32 ± 4 μg/L on day 9 5.2% of baseline; $P < 0.001$). Administration of Nal-lu + T also resulted in a decrease in serum FSH from 4 ± 11 to 23 ± 1 μg/L on day 9 (23.5% of baseline; P 0.001). Nal-Glu + T led to a lower mean FSH level on ay 9 than did Nal-Glu alone ($P < 0.001$) or T alone (P 0.05). This relationship was true in all nine men. erum FSH remained low on day 16 during both T-containing regimens and returned to baseline within 2.5 weeks in all three groups.

Urinary LH and FSH excretion

Urinary LH excretion was 177 ± 31 mIU/h during Nal-Glu + T, 341 ± 63 mIU/h during Nal-Glu alone ($P < 0.05$), and 262 ± 67 mIU/h during T alone (P = NS; Fig. 2). Urinary FSH excretion was also lower (72 ± 18 mIU/hr) during Nal-Glu + T, but this value was not significantly lower than those during T alone or Nal-Glu alone.

Serum inhibin

During Nal-Glu alone, serum inhibin decreased from 707 ± 79 U/L to a nadir of 373 ± 30 U/L on day 4 (54.4% of baseline; Fig. 3) and remained low throughout the injection period. During T alone, serum inhibin levels fell progressively to 355 U/L on day 9 (47.4% of baseline; $P < 0.01$) and remained low on day 16 (318 ± 31 U/L: 44.8% of baseline). With Nal-Glu + T, serum inhibin

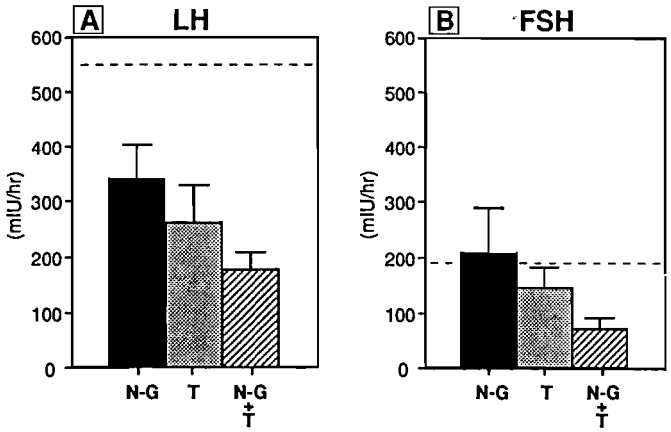


FIG. 2. Mean (±SE) urinary LH and FSH excretion on day 7 during administration of Nal-Glu antagonist (N-G; 75 μg/kg, sc) alone, T (200 mg im) alone, and Nal-Glu + T in nine normal men. A, Urinary LH; B, urinary FSH. – – –, lower limit of normal range for the assay.

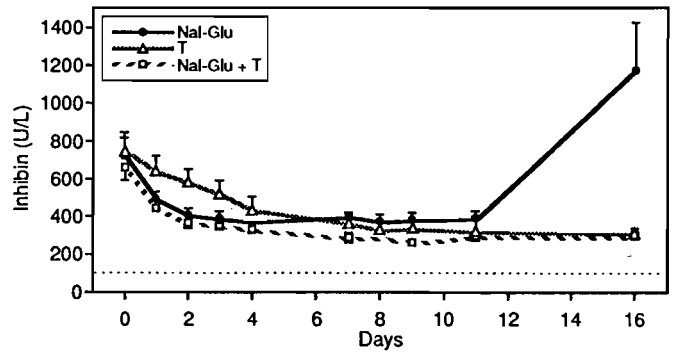


FIG. 3. Mean (±SE) serum inhibin levels during administration of Nal-Glu antagonist (75 μg/kg, sc) alone, T (200 mg, im) alone, and Nal-Glu + testosterone in nine normal men. – – –, Minimum detectable level.

also declined progressively to 275 ± 34 U/L on day 9 (43.0% of baseline; $P < 0.001$) and remained low on day 16 (313 ± 35 U/L; 49.2% of baseline). On day 9 serum inhibin was significantly lower during Nal-Glu + T than during Nal-Glu alone ($P < 0.01$) or T alone ($P < 0.05$).

Serum T and E_2

Serum T and E_2 levels decreased profoundly within 48 h during administration of Nal-Glu alone and remained low thereafter (Fig. 4). On day 9, serum T was 3.8 ± 1.0 nmol/L (31.6% of baseline; $P < 0.001$), and serum E_2 was 56.1 ± 7.7 pmol/L (60.3% of baseline; $P < 0.05$); seven of the nine men had E_2 levels near or below the limit of detectability (44.0 pmol/L). Serum T and E_2 levels returned to the normal range by day 16. Serum T and E_2 values increased transiently after administration of T alone. The peak serum T and E_2 values after the first T injection were 35.9 ± 3.5 nmol/L and 178.7 ± 23.4 pmol/L on day 2, respectively. After the second T injection on day 7, both T and E_2 rose again, reaching peaks of 38.7 ± 4.5 nmol/L and 189.7 ± 27.5 pmol/L, respectively. Both serum T and, to a much lesser extent, E_2 increased in response to Nal-Glu + T. The peak T and E_2 values after the first T injection occurred on day 3 and were 28.4 ± 2.1 nmol/L and 109.4 ± 12.8 pmol/L, respectively. After the second injection of T on day 7, both hormones rose again, reaching peaks of 42.6 ± 2.4 nmol/L and 198.2 ± 11.7 pmol/L, respectively; near-baseline values were achieved by day 16.

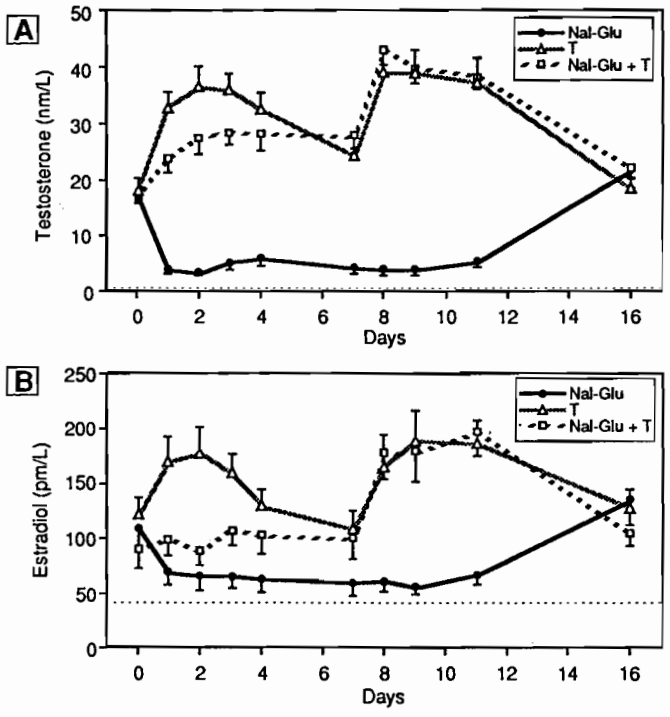


FIG. 4. Mean (±SE) serum T and E_2 levels during administration of Nal-Glu antagonist (75 μg/kg, sc) alone, T (200 mg, im) alone, and Nal-Glu + T in nine normal men. A, T; B, E_2. – – –, minimum detectable level.

Side-effects

All of the men noted occasional temporary erythema and discomfort at the site of injections after Nal-Glu administration, but not after saline placebo. Most developed nontender sc nodules of up to 1–2 cm in diameter at the injection sites. These were more prominent in the leaner men and were still palpable 2–3 weeks postinjection, but resolved during the next several weeks. No man withdrew from the study because of local side-effects.

None of the men reported decreased libido or altered sexual function after receiving T alone or Nal-Glu + T. Approximately half of the men noted difficulty achieving erections and/or decreased libido during Nal-Glu alone. All changes in sexual function or behavior reversed after antagonist injections ended.

Discussion

We compared the short term suppressive effects of Nal-Glu alone, T alone and Nal-Glu + T on pituitary and testicular function in normal men. The combination of Nal-Glu plus T was more effective than either Nal-Glu alone or T alone in suppressing serum and urinary LH and FSH levels and reducing testicular function, as assessed by serum inhibin levels. These results imply that the combination of Nal-Glu + T may be more effective than previous regimens designed for hormonal male contraception.

Daily Nal-Glu administration caused the rapid suppression of gonadotropins, inhibin, and gonadal steroids; maximal or near-maximal suppression occurred within 48 h after the injections began, and the suppression was sustained throughout the injection period. After the Nal-Glu injections ended, serum gonadotropin, inhibin, and T levels rapidly returned to baseline values; thus, pituitary and gonadal suppression was fully reversible. Serum LH declined to 36.3% of the baseline value during Nal-Glu alone; this suppression was greater than that found by other investigators (21, 22) using other antagonists, but was similar that achieved with a single 5-mg dose of the Nal-Glu antagonist (10, 11). The nadir value of serum FSH (56.4% of baseline) was similar to that found in previous single and multiple dose antagonist studies (21–23). Measurement of immunoreactive FSH may not be an accurate indicator of biologically active hormone, however; serum bioactive FSH has been reported to decrease to 21% of baseline after a single dose of the antagonist RS-68439, while immunoreactive FSH decreased to 50% of baseline (24). In addition, Dahl *et al.* (25) reported that the immunoreactive FSH present after GnRH antagonist administration to women con-

tains both agonist and antagonist FSH forms. Therefore, long term administration of the GnRH antagonist may have a more profound effect on spermatogenesis than would be predicted on the basis of the decrease in immunoreactive FSH alone. The rapidity and completeness of gonadotropin and steroid suppression with Nal-Glu alone suggest that this compound may be potentially useful in the treatment of prostatic cancer.

The Nal-Glu-induced declines in serum LH and FSH were accompanied by an immediate and sustained decline in T and E_2 levels. In contrast, Pavlou *et al.* (22) found that the 4F antagonist, administered to normal men at a dosage of 100 μg/kg BW every 6 h for 7 days, allowed partial escape from T suppression on days 4 and 5. The Nal-Glu antagonist has a greater affinity for pituitary binding sites than does 4F and is less easily displaced by native GnRH. A possible explanation for the escape phenomenon is that the 4F antagonist was displaced from its pituitary binding sites as a result of increased endogenous GnRH secretion in response to declining serum T levels. Although serum T and gonadotropin levels rose slightly on day 4 or 5 of Nal-Glu administration, complete escape did not occur. Serum T and especially E_2 levels increased less after the injection of Nal-Glu + T than after the first injection of T alone, probably due to the suppressive action of Nal-Glu on gonadotropins and thereby on gonadal steroids. The reason for the much lesser rise in E_2 is not clear, although it is possible that Nal-Glu has effects on the aromatase enzyme complex and, therefore, impairs conversion of T to E_2.

The combination of Nal-Glu + T resulted in greater suppression of serum and urinary gonadotropins than did either agent alone. The enhanced suppression was probably due to several factors. Since T has negative feedback effects directly at the pituitary level (3, 4), the combined regimen inhibits gonadotropin secretion by the independent actions of the antagonist and T. In addition, T has well documented negative feedback effects at the hypothalamus (2). T-mediated inhibition of endogenous GnRH secretion might result in additional inhibition of gonadotropin secretion, even in the presence of antagonist. Thus, both hypothalamic and pituitary effects of T may contribute to the enhanced efficacy of the combination regimen.

We used a supraphysiological dose of T (200 mg/week), as this dose has been demonstrated to suppress gonadotropin secretion in contraceptive trials (6). Since T and Nal-Glu probably induce gonadotropin suppression by different mechanisms, lower doses of T may be equally effective in enhancing the suppression induced by Nal-Glu. Similarly, if our combination regimen effectively suppresses spermatogenesis in men, a regimen using a GnRH antagonist plus physiological or slightly supraphysiological doses of T should also be evaluated.

We cannot predict whether long term administration of the combined regimen will consistently suppress spermatogenesis in men. Nevertheless, several of our findings suggest that the combined regimen may be an effective contraceptive agent. First, after 7 days of the Nal-Glu antagonist plus T, urinary gonadotropin excretion was lower than in men who had received T in doses up to 300 mg/week for 6 months (Matsumoto, A.M., unpublished data). Azoospermia was achieved in approximately 50–60% of the men in that study; it is likely that more men would become azoospermic on an antagonist + T regimen. Secondly, serum FSH was suppressed to nearly undetectable levels on the combination regimen. Since FSH administration to T-suppressed normal men can stimulate sperm production (26), the degree of FSH suppression achieved may be an important determinant of the effectiveness of any potential contraceptive agent. Lastly, serum inhibin levels declined in a pattern similar to that of LH and FSH; suppression was most complete with the combined regimen and was fully reversible. Since inhibin is a Sertoli cell product (12, 13) and is, thus, one index of seminiferous tubule function, our results imply that testicular function as well as gonadotropin secretion are more fully suppressed with Nal-Glu + T than with either single agent alone.

In conclusion, our data show that the Nal-Glu GnRH antagonist given daily for 10 days caused sustained suppression of serum T gonadotropins, and inhibin in normal men. When Nal-Glu was combined with T, suppression of gonadotropins and inhibin was consistently more profound than with either agent given alone. Whether these differences persist during longer treatment periods and whether the antagonist combined with T will reliably induce azoospermia in men is not known, but our results suggest that GnRH antagonists in combination with T have considerable promise as male contraceptive agents.

Acknowledgements

We thank Mr. Arlen Sarkissians, Ms. Florida Flor, and Ms. Dorothy McGuiness for their technical assistance. Dr. Laurence M. Demers and the staff at the Milton S. Hershey Medical Center (Hershey, PA) performed the urinary gonadotropin measurements. We appreciate the work of Ms. Liza Noonan in the biostatistical analysis and the assistance of Ms. Maxine Pollock in the preparation of the manuscript.

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