Dose effects of the gonadotropin-releasing hormone antagonist, Nal-Glu, combined with testosterone enanthate on gonadotropin levels in normal men

Carrie J. Bagatell, M.D.†‡
Jean E. Rivier, Ph.D.§
William J. Bremner, M.D., Ph.D.¶

University of Washington School of Medicine, Seattle, Washington, and Salk Institute, La Jolla, California

Objective: To test the hypothesis that over a 4-week treatment period, Nal-Glu GnRH antagonist ([Ac-D2-Nal1, D4Cl-Phe5, D9PAl10, Arg2, DGlu4 [AA], DAla10] GnRH) at a dose of 200 μg/kg per day SC would suppress levels of immunologically active and biologically active LH and FSH more completely than a dose of 100 μg/kg per day.

Design: Placebo controlled clinical study.

Setting: A university community.

Subjects: Thirty normal male volunteers.

Interventions: We administered Nal-Glu at doses of 0, 100, and 200 μg/kg body weight per day in combination with T enanthate, 50 mg IM weekly, to separate groups of men (9 or 10 men per group) for 4 weeks.

Results: Serum levels of immunologically active and biologically active gonadotropins were suppressed similarly in both groups of men who received Nal-Glu; this suppression was significantly greater than in the men who received placebo + T. Local side effects were more severe in the Nal-Glu 200 μg/kg per day group.

Conclusions: Administration of Nal-Glu in combination with T suppresses gonadotropins more completely than does T alone, but at doses > 100 μg/kg, gonadotropins are not suppressed additionally with larger doses of Nal-Glu. Subjects experienced greater local discomfort and side effects with the higher dosage. These findings suggest that dosages of Nal-Glu of >100 μg/kg per day may have no advantage over the 100-μg/kg dose in a male contraceptive regimen.

Key Words: Gonadotropin-releasing hormone, gonadotropins, male contraception, GnRH antagonist, testosterone

Human spermatogenesis is dependent on gonadotropin stimulation (1–3); therefore, efforts to develop a hormonal means of contraception for men have focused on regimens that suppress gonadotropin secretion. Administration of high-dose T causes profound gonadotropin suppression, but azoospermia develops in only 50% to 70% of white subjects (4–6). A recent World Health Organization trial has shown that among men who achieve azoospermia, the contraceptive efficacy of the method is nearly

† Reprint requests: Carrie J. Bagatell, M.D., Endocrinology (111), Department of Veterans Affairs Medical Center, 1660 South Columbian Way, Seattle, Washington 98108 (FAX: 206–764–2689).
§ Salk Institute.
¶ Department of Medicine and Population Center for Research in Reproduction, University of Washington School of Medicine.

Bagatell et al. Dose effects of GnRH-a + T in men 139
100% (7). However, because it would be desirable to induce azoospermia in a higher proportion of men, combination regimens using T and other hormonal agents have been explored under a variety of paradigms.

Gonadotropin-releasing hormone antagonists (GnRH-a) are synthetic analogues of GnRH that compete with endogenous GnRH for pituitary binding sites, thereby causing immediate suppression of gonadotropin secretion (8). Gonadal steroid production is also severely inhibited. When GnRH-a are given on a daily basis, suppression of gonadotropins and gonadal products is sustained throughout the duration of treatment; when treatment is ended, hormone levels return to baseline within 1 to 2 weeks (9–11). Because GnRH-a and T suppress gonadotropins by different mechanisms, the combination of antagonists combined with T have been explored as contraceptive regimens in monkeys (12–15) and in men (16, 17).

Two groups of investigators (16, 17) have found that a combination of the GnRH antagonist Nal-Glu [(AcD2Nal1, D4CIPhe2, D3Pa15, Arg2, DGLu6 [AA], DALal10] GnRH) plus T induced azoospermia in nearly 90% of their subjects. We also have tested recently the ability of Nal-Glu combined with T enanthate, 200 mg/wk, to suppress spermatogenesis in healthy men (18). We found that 7 of 10 men became azoospermic on this regimen. At the same time, we administered placebo Nal-Glu injections together with T to another group of men, and we found that 6 of 9 men became azoospermic. Thus, in our paradigm, the combination of Nal-Glu and T was somewhat less successful in inducing azoospermia than in other paradigms.

There are several possible explanations for this apparent disparity. First, it is possible we did not use an adequate dose of Nal-Glu. Pavlou et al. (16) found that two of their subjects (whose weights were greater than the other men) required a dose of Nal-Glu of 20 mg/d (approximately 200 μg/kg per day) to suppress gonadotropins and spermatogenesis adequately. Second, we used a higher dose of T than did the other investigators, and it is possible that the dose we used caused some stimulation of spermatogenesis in certain subjects. We also initiated T injections on the first day of treatment, whereas the other investigators delayed T replacement for 2 weeks. Finally, it is possible that some of our men responded more slowly than average to the treatment regimen and that had we extended the duration of the treatment period, more men would have become azoospermic.

In this study, we have tested the first of these possibilities. We hypothesized that when combined with T enanthate at a dose of 50 mg/wk, administration of Nal-Glu at a dose of 200 μg/kg per day would suppress gonadotropins in normal men more effectively than would Nal-Glu at doses of 0 or 100 μg/kg per day. We administered each dosage of antagonist to separate groups of men together with a dosage of T that does not independently suppress gonadotropins fully (6). Because gonadotropin suppression is necessary for suppression of spermatogenesis, we used levels of immunologically active and biologically active gonadotropins as our endpoints.

MATERIALS AND METHODS

Subjects

Thirty healthy men, aged 19 to 38 years, enrolled in the study. All of the men had normal medical histories, physical examinations, and screening laboratory studies. All of the men had acceptable responses to intradermal skin testing with 10 μg Nal-Glu. An acceptable skin test is considered to be no more than the formation of a wheal with surrounding erythema, without pseudopod formation. Their mean weight was 77.3 ± 1.8 (mean ± SE) kg (range 64.6 to 110.6 kg). None of the men were smokers, and none abused alcohol. All of the men signed informed consents that were approved by the Human Subjects Committee of the University of Washington. One man developed a large pruritic wheal after the first injection of Nal-Glu and therefore withdrew from the study. This wheal regressed over the succeeding few days. Thus, 29 men completed the protocol.

Clinical Protocol

The study consisted of a 2-week baseline period, a 4-week treatment period, and a 6-week post-treatment period. During the pretreatment and treatment periods, blood samples were drawn twice weekly for analysis of hormone levels. All blood samples were drawn before the day’s injections were administered. During the post-treatment period, blood samples were drawn twice weekly for the first 3 weeks, and a final sample was drawn after 6 weeks of recovery. Subjects were interviewed by one of the investigators weekly.

After the baseline period, each subject was assigned randomly to receive [1] Nal-Glu, 100 μg/kg per day SC, plus T enanthate, 50 mg IM weekly (Nal-Glu 100); [2] Nal-Glu, 200 μg/kg per day SC, plus T enanthate, 50 mg IM weekly (Nal-Glu 200) or [3] saline placebo, 0.6 to 1.0 mL SC daily, plus T enanthate, 50 mg IM weekly (placebo). The doses of Nal-Glu were chosen based on the results of our earlier multiple-dose studies using this antagonist (18) as well as the study of Pavlou et al. (16). To separate
the effects of Nal-Glu on gonadotropins from those of T, we used a dose of T (50 mg IM weekly) that would not suppress gonadotropins fully (6): The study was double-blind.

Drug Preparation

The Nal-Glu GnRH-a was dissolved in bacteriostatic water containing 4% mannitol, diluted to a concentration of 10 mg/mL, and then under sterile conditions passed through a 0.2-μm filter into sterile vials and stored at −20°C until used. All of the subjects were taught to self-administer their injections. Subjects received a new vial of antagonist each week and refrigerated each vial between injections. To ensure compliance, subjects returned their empty vials of drug each week. Testosterone enanthate (Schoen Pharmaceuticals, Port Washington, NY) was administered in a dosage of 50 mg (0.5 mL) weekly by nursing staff at the Clinical Research Center at the University of Washington.

Hormone Assays

Serum T levels were measured by RIA using reagents from the World Health Organization matched Reagent Program by methods previously described (19). Testosterone was separated from serum by ether extraction; bound and free hormone were separated by dextran-coated charcoal. The assay sensitivity was 0.1 ng/mL (0.35 nmol/L) the interassay and intra-assay variabilities were 4.1% and 8.1%, respectively. Serum levels of immunologically active LH and FSH (i-LH and i-FSH) were measured by a sensitive fluorimunoassay (DELFIA, Wallac Inc., Gaithersburg, MD). The limits of detectability of each assay was 0.02 mIU/mL (conversion factor to SI unit, 1.00 for both). The interassay variabilities for i-LH were 9.5%, 4.7%, and 7.6% for the low, medium, and high pools, respectively. The interassay variabilities for i-FSH were 20%, 4.6%, and 3.3% for the low, medium, and high pools, respectively. The intra-assay variabilities for i-LH were 7.2%, 2.8%, and 3.5%, for the low, medium, and high pools, respectively. Serum levels of i-LH and i-FSH were also measured using an immunoradiometric assay (MAIAclone, Serono Laboratories, Geneva, Switzerland) as described previously (20).

Serum levels of biologically active LH (bio-LH) were measured in each man in samples collected at the first pretreatment visit, at the last visit of the treatment period, and during the third week of the post-treatment period. Levels of bio-LH were measured in vitro using the MA-10 cell line of mouse Leydig tumor cells, which secrete P in response to LH stimulation, by methods recently described (21). All samples were run in duplicate at a volume of 10 μL. The intra-assay and interassay coefficients of variation (CVs) were 10.0% and 16.0%, respectively. Biologically active FSH (bio-FSH) was measured in vitro using a modification (22) of the methods of Jia and Hsueh (23). This assay measures FSH production from granulosa cells obtained from immature diethylstilbestrol-treated Sprague-Dawley rats. The intra-assay and interassay CVs were 10.0% and 14.0%, respectively.

Statistical Analysis

For each parameter, each man’s pretreatment data were meaned; these values were then meaned to give the group mean value. Differences over time within each group and between the groups were determined using two-factor analysis of variance (ANOVA) with repeated measures. Within each group, the presence of differences among the gonadotropin and T values were determined by one-way ANOVA with repeated measures, followed by the Fischer protected least-significant difference post-hoc test.

RESULTS

Serum T

Serum T levels were similar among the three groups during the pretreatment period (Fig. 1). After injections began, T levels in both groups of men receiving Nal-Glu were in the baseline range 2 days after each T injection, but they were suppressed to 50% to 70% of baseline (P < 0.05) at the end of each week. Mean serum T levels in these two groups were

![Figure 1](image-url)
similar during the treatment period. After injections ended, T levels in both groups remained suppressed below the baseline range for 1 week and then slowly normalized. During treatment, mean serum T levels in the placebo group increased by 25% to 30% \((P < 0.05)\) on day 2 after each T injection, returning to the baseline range after 7 days. At the end of each week, mean serum T levels were significantly higher in the placebo group than in the other two groups.

Serum i-LH

During the pretreatment period, serum i-LH levels were similar among the three groups of men (Fig. 2, top). Within 2 days after the initial injection of T combined with Nal-Glu at either dosage, serum i-LH levels were suppressed to approximately 10% of baseline \((P < 0.05)\), and they remained suppressed until 7 days after injections ended. There were no statistically significant differences in mean i-LH levels during the treatment period in the two groups that received Nal-Glu, although recovery was slightly slower in Nal-Glu 200 group. In the placebo + T group, i-LH levels were more suppressed on day 2 of each week than on day 7 (45% to 60% of baseline on day 2 versus 72% to 82% on day 7), presumably due to the effect of the preceding T injection. Significant suppression of mean i-LH occurred on day 2 of weeks 2, 3, and 4 of treatment. Baseline i-LH levels were re-established approximately 1 week after the end of treatment. Throughout the treatment period, mean i-LH levels in this group were significantly higher (less suppressed compared with baseline) than in the other two groups. Serum i-LH levels measured in the MAIAclone assay were similar to those measured in the Delfia assay, although the former assay was not able to detect some of the lowest i-LH values, which were all detectable in the Delfia assay.

Serum i-FSH

Mean pretreatment i-FSH levels were not different among the treatment groups (Fig. 2, bottom). In both groups of men who received Nal-Glu, serum i-FSH levels were markedly suppressed within 2 days after the first injections; after 7 days, they were suppressed further, to 20% to 25% of baseline \((P < 0.05)\), and they remained suppressed throughout the treatment period. Baseline levels were re-achieved approximately 1 week after injections ended in the group who received Nal-Glu 100 and after 2 to 3 weeks of recovery in the men who received Nal-Glu 200. There were no statistical or clinical differences in mean i-FSH levels between the two groups during the treatment period. In men who received placebo + T, mean serum i-FSH levels decreased slowly over the first 2 weeks of the treatment period; by the 2nd day of week 2, i-FSH levels were significantly lower than baseline, and they remained so until 1 week after treatment ended. However, as with serum i-LH, mean i-FSH levels in this group were significantly higher (less suppressed compared with baseline) throughout the treatment period than in the other groups. Serum i-FSH levels measured in the MAIAclone assay were similar to those measured in the Delfia assay, although the former assay was not able to detect some of the lowest i-FSH values, which were all detectable in the Delfia assay.

Serum bio-LH and bio-FSH

Mean levels of bio-LH were similar in the three groups during the baseline period (Fig. 3, top). During the treatment period, bio-LH levels were suppressed significantly in all three groups, but the decrease in the placebo + T group \((26% \pm 6\%)\) was significantly less than in the two groups that received Nal-Glu \((50% \pm 5\% \) and \(47% \pm 3\% \) for Nal-Glu 100 and Nal-Glu 200, respectively). The degree of suppression in the latter two groups did not differ statistically. During the recovery period, mean bio-LH levels recovered by 3 weeks, and mean levels in the two groups that received Nal-Glu were slightly
Figure 3  Serum levels of bio-LH (top) and bio-FSH (bottom) during the pretreatment, treatment, and post-treatment period in each group of men. □, placebo; ■, 100 μg/kg Nal-Glu; ■■, 200 μg/kg Nal-Glu.

greater during recovery than during the pretreatment period.

Mean levels of bio-FSH were similar in the groups during the baseline period (Fig. 3, bottom). During treatment, bio-FSH levels were suppressed significantly in each group, but the degree of suppression (40% to 45% decrease) was not different among the groups. Bio-FSH levels returned to baseline during the recovery period in all three groups.

Side Effects

All of the men who received Nal-Glu experienced some degree of erythema and induration at the SC injection sites. The magnitude of these effects varied considerably from man to man and depended in part on adiposity (those with more abdominal adipose tissue generally experienced fewer effects). However, the men who received the 200 μg/kg per day dose of Nal-Glu had more pronounced local effects, including more discomfort, larger areas of cutaneous erythema after injection, and larger areas of subcutaneous induration, with slower resolution after the end of injections. Nine of the 10 men who received 200 μg/kg per day complained of erythema, pain after injections, and induration, whereas only 4 of the 9 men who received 100 μg/kg per day had these complaints. One man who received placebo complained of pain after injections, although he had minimal erythema and no induration. One man in each of the three groups complained of decreased libido toward the end of the 4-week treatment period; in each case, the symptoms resolved after 2 to 3 weeks of recovery.

There was a trend toward a slight (<1 kg) weight loss during treatment in all groups, but this trend was not statistically significant. Baseline weights were re-established by the end of the recovery period. One man in the Nal-Glu 200 group experienced worsening acne, which responded to Retin A (Ortho-McNeil Pharmaceutical, Raritan, NJ) therapy and had largely resolved by the end of the recovery period.

DISCUSSION

We administered the GnRH-a, Nal-Glu, at doses of 0, 100, and 200 μg/kg per day, together with a nonsuppressive dose of T, to healthy young men for 28 days. We hypothesized that the higher antagonist dosage would suppress gonadotropins more completely than would the lower dosage. Our data demonstrate that either dosage of antagonist, combined with T, suppresses gonadotropins more fully than does T alone. However, although the higher Nal-Glu dose had a more long-lasting effect on suppression of gonadotropins, the effects of the two antagonist doses on serum levels of both immunologically and biologically active LH and FSH during the treatment period were similar. The men who received the higher antagonist dose generally experienced greater local discomfort from the injections. These results suggest that in the design of male contraceptive regimens that include GnRH-a of potency similar to Nal-Glu, an antagonist dosage of approximately 100 μg/kg per day may be optimal.

The dose–response relationships of GnRH-a and gonadotropins have most often been investigated in the setting of single injections of antagonist, without T replacement, in men (9, 10) or in nonhuman primates (24, 25). Most of these studies have demonstrated enhanced suppression of LH and/or FSH with increasing antagonist doses. However, greater incremental suppression generally occurred when small antagonist doses (5 to 50 μg/kg) were increased to moderate ones (75 to 200 μg/kg); less incremental suppression occurred when moderate doses were increased to larger doses. Because T also partially suppresses gonadotropins, Nal-Glu in the dosages we used, combined with T, was presumably on the flat portion of the dose–response curve.

In the study of Pavlova et al. (16), Nal-Glu was administered to normal volunteers on a daily basis at a dose of 10 mg/d, and T enanthate was replaced at a dose of 25 mg/wk starting with the 3rd week of treatment. In that study, two volunteers, whose body weights were >100 kg each, failed to reach oligospermia by 10 weeks, and their daily dosage was in-
creased from 10 mg (approximately 100 μg/kg per day) to 20 mg (approximately 200 μg/kg per day). In one of the subjects, serum LH and FSH were suppressed to undetectable and <0.3 mIU/mL, respectively, and azoospermia developed. In the other man, serum LH and FSH levels decreased moderately, and his sperm count decreased, although azoospermia was not achieved. These findings suggest that the higher dosage of Nal-Glu enhanced suppression of gonadotropins and spermatogenesis. In our study, we did not evaluate spermatogenesis, but our data showing similar suppression of LH and FSH by Nal-Glu doses of 100 and 200 μg/kg per day are not consistent with the findings of Pavlou et al. (16). Possible explanations of the discrepancy in our results include the following. [1] In the Pavlou study, the two subjects received Nal-Glu at a dose of 10 mg/d for 10 weeks before their dosage was increased. It is possible that these men were slow responders and that the decrement in gonadotropins and sperm counts would have been seen regardless of whether the dose of Nal-Glu was increased. [2] The subjects in the study of Pavlou et al. (16) received half the dosage of T that men in our study received, and their gonadotropins were presumably less fully suppressed than in our men at any given dose of antagonist. [3] In our study, different groups of men received the two different Nal-Glu doses, whereas in the study of Pavlou et al. (16), and in many of the single-dose studies, each subject served as his own control (9, 10, 24, 25). It is also possible that if we had given each man each of the regimens, we might have seen more gonadotropin suppression with the higher Nal-Glu dosage.

Most previous studies in which levels of immunologically and biologically active gonadotropins have been measured have shown a greater decrease in biologically active hormone than in immunologically active hormone (9, 10). In contrast, in this study, with the exception of the FSH response in men who received placebo + T, immunologically active gonadotropin levels were suppressed to a greater degree than were levels of biologically active gonadotropins. The reasons underlying this occurrence are not clear, but it is likely that the development of new more sensitive assays for immunologically active gonadotropins allows lower levels of immunologically active hormone to be measured, and thus the calculated decreases in i-LH and i-FSH are proportionately greater than in older studies. We have no obvious explanation for why bio-FSH levels in men who received placebo + T were suppressed to the same extent as in the men who received Nal-Glu, whereas i-FSH levels were suppressed less in men who received placebo than in men who received Nal-Glu. The same phenomenon did not occur with serum bio-

LH. That is, both bio-LH and i-LH levels were suppressed less in men who received placebo than in men who received Nal-Glu. The differential suppression of bio-LH and bio-FSH by T deserves further study.

In summary, we administered a dose of T enanthate that did not fully suppress gonadotropins (50 mg IM weekly) to groups of normal men, together with 0 (placebo), 100, or 200 μg/kg Nal-Glu SC daily for 4 weeks. We found that serum gonadotropin levels were suppressed similarly in both groups of men who got Nal-Glu; this suppression was significantly greater than in the men who received placebo + T. Addition of Nal-Glu therefore suppresses gonadotropins to a lower level than those found with T alone. However, at doses > 100 μg/kg, gonadotropins are not suppressed additionally with larger doses of Nal-Glu. In addition, subjects experienced greater local discomfort and side effects with the higher dosage. These findings suggest that in future studies, dosages of Nal-Glu > 100 μg/kg per day may have no advantage over the 100-μg/kg dose when combined with the administration of T in male contraception studies.

Acknowledgments. Nal-Glu was synthesized at the Salk Institute (under contract N01-HD-0-2968 with the National Institutes of Health) and made available by the Contraceptive Development Branch, Center for Population Research, National Institute of Child Health and Human Development, Bethesda, Maryland. We appreciate the technical assistance of Elaine Post, B.A., Kristine Dahl, Ph.D., Arlen Sarkissian, B.S., Ms. Dorothy McGuinness, and Elizabeth Van Gaver, B.S.

REFERENCES

7. World Health Organization task force on methods for the regulation of male fertility. Contraceptive efficacy of testos-


Note: Additional references are available upon request from the author.