

Acyline: The First Study in Humans of a Potent, New Gonadotropin-Releasing Hormone Antagonist

KAREN L. HERBST, BRADLEY D. ANAWALT, JOHN K. AMORY, AND WILLIAM J. BREMNER

Department of Medicine, University of Washington (K.L.H., B.D.A., J.K.A., W.J.B.) and Medical Service, Department of Veteran Affairs, Puget Sound Health Care System (B.D.A.), Seattle, Washington 98195

Acyline is a novel GnRH antagonist found in animal studies to be a potent suppressor of circulating gonadotropin and testosterone (T) levels. We conducted the first study of acyline administration to humans. Eight healthy, eugonadal young men were administered a series of acyline injections (0, 2.5, 7.5, 25, and 75 $\mu\text{g}/\text{kg}$), each injection separated by at least 10 d. Serum FSH, LH, and T levels were measured for 7 d after injections. Acyline suppressed FSH, LH, and T levels in a dose-dependent fashion. Maximal suppression occurred after injection of 75 $\mu\text{g}/\text{kg}$ acyline, which suppressed FSH to $46.9 \pm 2.5\%$, LH to $12.4 \pm 2.2\%$, and T to $13.4 \pm 1.4\%$ of baseline levels,

maintaining suppression for over 48 h. Serum acyline levels peaked at 1 h at 18.9 ± 0.9 ng/ml, remained significantly elevated above background 7 d after injection, and returned to background levels by 14–17 d after injection. Side-effects at the site of injection were limited to infrequent blush and pruritus that resolved within 90 min of injection. Higher doses of acyline might be effective as depot injections for long-lasting gonadotropin suppression in hormone-dependent diseases and for use in male hormonal contraception regimens. (*J Clin Endocrinol Metab* 87: 3215–3220, 2002)

GnRH IS A hypothalamic peptide that stimulates the release of FSH and LH by the pituitary. GnRH antagonists are peptide analogs of GnRH that competitively inhibit the action of GnRH at pituitary receptor sites (1). They produce immediate and sustained declines in gonadotropin and sex steroid levels and therefore have potential as treatment for hormone-dependent conditions and as contraceptive agents. Although previously studied antagonists have effectively suppressed circulating gonadotropin levels, most have histamine-releasing local effects, including pruritic rashes, and have short half-lives that require daily injection (2, 3). Some newer antagonists are not generally available for research (4–6). GnRH agonists also suppress the gonadal axis, but can cause an initial flare in circulating gonadotropin and testosterone (T) levels that can last for days to weeks (5). In addition, depot injections of GnRH agonists may result in small surges in circulating gonadotropin and T levels over time after the initial flare (7). Efforts have therefore continued to develop an effective, safe, and convenient GnRH antagonist.

Acyline is a GnRH antagonist that has not been studied previously in humans. It was found in animal studies to be at least 4 times more effective at suppression and maintenance of suppression of gonadotropins and T than previous GnRH antagonists (8) (Contraceptive and Reproductive Health Branch, Center for Population Research, NICHD, unpublished data). In adult male rhesus monkeys, acyline was more potent than azaline B, Nal-Glu, and lystide. No significant adverse effects were observed in these animal studies. We tested the hypothesis that acyline would effectively and safely suppress circulating levels of gonadotropins and T in healthy, eugonadal young men.

Abbreviations: T, Testosterone.

Materials and Methods

Acyline

Acyline was originally synthesized by Jean Rivier at The Salk Institute (9) and is being distributed by the NICHD. Acyline is prepared as a lyophilized powder at a concentration of 4.4 mg/vial and is stored at -20 C. In this study the acyline powder was suspended in 2.2 ml bacteriostatic water and used within 1 h of reconstitution. There was no evidence of gel formation after reconstitution in water. All placebo injections consisted of bacteriostatic water equal to the smallest volume of acyline injected into each subject (0.08–1.3 ml). In comparison, the largest volume of acyline injected ranged from 2.28–3.67 ml for the 75 $\mu\text{g}/\text{kg}$ dose. Serum levels of acyline were measured in a subset of subjects after injection of 75 $\mu\text{g}/\text{kg}$ acyline by RIA using a specific antiserum and a proprietary peptide, with authentic peptide standard (Woods Assay, Inc., Portland, OR) as described previously (10). The sensitivity of the assay for acyline was 0.35 ng/ml.

Subjects

Nine men, aged 20–39 yr, were recruited by posted flyers on local college campus bulletin boards. All subjects were healthy, eugonadal men with normal baseline physical examinations and medical histories, including testicular size by Prader orchidometer and prostate size by digital rectal exam, serum chemistries, complete blood count, and hormone levels. Subjects who smoked or had an alcohol intake greater than 7 ounces weekly, prescription medication use, or involvement in a male contraceptive study within the last 6 months were excluded from the study. Eight subjects completed the study; one subject withdrew from the study for personal reasons. All subjects were given a test dose (0.1 ml of a 2.2 mg/ml solution) of acyline intradermally during the screening period to assess allergic reactions. Subjects had minimal or no reactions to the test dose. As an additional safety precaution, all subjects were admitted to the Clinical Research Center at University of Washington for 24 h after each acyline injection. Each admission was separated by at least 10 d. During each admission blood was drawn 30 min before and immediately before the injection of acyline. Acyline or placebo was administered by sc injection in the abdomen between 0700–1000 h. Blood samples were obtained at 30, 60, 90, and 120 min; 3, 4, 6, 8, 12, and 24 h; and 2, 3, 4, and 7 d after injection. The initial two subjects received injections beginning with placebo, followed (during subsequent admissions) by escalating doses of acyline at 2.5, 7.5, 25, and 75 $\mu\text{g}/\text{kg}$. After determining that acyline caused minimal local effects and

no clinically systemic adverse effects in these first two subjects, the next three subjects were randomized until it was noted that the first subject's gonadotropin and T levels remained suppressed longer than 7 d after the 75 $\mu\text{g}/\text{kg}$ dose. Subsequent injections in all subjects were nonrandomized and in escalating dosage. In four of eight subjects, serum gonadotropin and T levels remained suppressed for 7 d after the injection of 75 $\mu\text{g}/\text{kg}$ acyline, and these four subjects had serial blood samples drawn until hormone levels returned to baseline. Screening laboratory studies were repeated on d 7 after each injection. A nurse or physician closely monitored all subjects after each acyline or placebo injection. A physician examined all subjects at screening and on d 7 after injections. All study procedures involving human subjects were approved by the institutional review board at University of Washington and were performed at University of Washington Clinical Research Center in accordance with institutional guidelines.

Measurements

FSH, LH, and T levels were measured by immunofluorometric assay (Delfia, Wallac, Inc., Turku, Finland). Samples from a given individual were run in one assay. The sensitivities of the assay for FSH and LH were 0.016 and 0.019 IU/liter, respectively. The intraassay coefficient of variation was 2.9%, and the interassay coefficient of variation was 6.1% for a mid-range of pooled FSH values of 0.96 IU/liter. The intraassay coefficient of variation was 3.2%, and the interassay coefficient of variation was 12.5% for a mid range of pooled LH values of 1.2 IU/liter. The assay sensitivity for T was 0.5 nmol/liter. The T intraassay coefficient of variation was 4.4%, and the T interassay coefficient of variation was 7.3% for a mean of mid-range pooled values of 11.4 nmol/liter. Subjects were asked to return for additional hormone measurements if T levels had not returned to baseline within 7 d after injection of acyline.

Screening and monitoring for complete blood count, electrolytes and glucose (chemistry 7), calcium, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, bilirubin, and total protein were performed at Department of Laboratory Medicine, University of Washington.

Statistics

We analyzed the data from eight subjects who completed the entire protocol and excluded a single subject who dropped out of the study because of scheduling conflicts. FSH, LH, and T were expressed as the mean hormone level \pm SEM. Differences between groups were compared by two-way ANOVA for repeated measures. Data points were evaluated by ANOVAs at each time point and were analyzed *post hoc* using Duncan's comparison measures. $P < 0.05$ was considered significant.

Results

Gonadotropins

Higher doses of acyline suppressed gonadotropins to lower levels and maintained suppression for longer time intervals up to 48 h in a dose-dependent manner (Fig. 1, A–D). Mean levels of FSH began to decline within 1 h from a baseline of 2.7 ± 0.1 IU/liter and were maximally suppressed by 24 h after injection of 2.5 and 7.5 $\mu\text{g}/\text{kg}$ acyline and by 48 h after injection of 25 and 75 $\mu\text{g}/\text{kg}$ acyline. FSH was maximally suppressed to $45.1 \pm 2.5\%$ of baseline after injection of the highest dose of acyline. Mean FSH levels returned to baseline within 168 h (7 d). LH levels were maximally suppressed from a baseline level of 4.2 ± 0.5 IU/liter to $9.8 \pm 2.2\%$ of baseline by 48 h after administration of 75 $\mu\text{g}/\text{kg}$ acyline; maximal suppression of LH at lower doses occurred within 12 h. Mean LH levels returned to baseline by 96 h after acyline injection for all doses.

T

Mean serum T levels dropped within 1 h from a baseline level of 20 ± 0.2 nmol/liter and remained suppressed for at

least 12 h after injection of acyline for all doses (Fig. 1, E and F). T levels were suppressed even longer after injection of 75 $\mu\text{g}/\text{kg}$ acyline to $13.3 \pm 1.3\%$ of baseline for 48 h, equal to the time LH remained suppressed at this dose (Fig. 1C). For all doses, mean serum T levels returned to baseline by 168 h (7 d), except after injection of the 75 $\mu\text{g}/\text{kg}$ dose of acyline, when the mean T level remained significantly below baseline for 7 d. Seven days after injection of 75 $\mu\text{g}/\text{kg}$ acyline, T levels had returned to baseline in half of the subjects. In three of the four subjects whose serum T levels were persistently low 7 d after acyline injection, T levels returned to baseline between 14–22 d after injection. The fourth subject terminated the study after the d 7 sample was obtained.

Serum acyline levels

Acyline levels were determined in four subjects after injection of 75 $\mu\text{g}/\text{kg}$ acyline, two in whom T levels returned to baseline by 7 d after injection of acyline and two in whom T stayed suppressed below baseline levels beyond 7 d. Three of these subjects received a dose of 25 $\mu\text{g}/\text{kg}$ acyline 14 d before injection of the 75 $\mu\text{g}/\text{kg}$ dose. One individual was naive to sc acyline injections before the 75 $\mu\text{g}/\text{kg}$ dose. Acyline levels reached an average peak level of 18.9 ± 0.9 ng/ml in serum between 1–5 h after injection and remained elevated above background at an average level of 0.9 ± 0.2 ng/ml for 7 d in all four subjects (Fig. 2). The half-life of acyline in serum was 28.3 ± 4.2 h. Acyline levels returned to background levels by 7 d after injection in two subjects whose T levels returned to baseline 7 d after injection of acyline and at 14 and 17 d after injection of acyline in two subjects, respectively, whose T levels had not returned to baseline by 7 d after injection of acyline. There was no significant difference in acyline levels between subjects at any time point after injection of 75 $\mu\text{g}/\text{kg}$ acyline.

Adverse effects and safety

Skin reactions were the only clinical side-effect noted with sc acyline injections. A mild pink blush occurred at the site of injection after 50–100% of injections in all subjects. The blush generally correlated with the volume of injection, was observed within 5–10 min of injection, and faded within 90 min. One placebo injection induced a blush lasting 30 min. The greatest dermatological effects of acyline involved four subjects who had mild swelling at the site of injection (1–1.5 cm) that disappeared within 90 min of injection. One subject reported soreness in the area of injection for 2 d after the largest acyline injection. A mild pruritus at the site of acyline administration was reported by five of the subjects, constituting 36% of the acyline injections administered during this study, and all resolved without intervention by 90 min after the injection. No nodular induration was found after any of the acyline injections during this study.

There were no changes in heart rate, blood pressure, weight, body mass index, serum chemistries, hematocrit, testicular size, or prostate size in any of the subjects throughout the study. No subject experienced dizziness, bronchospasm, shortness of breath, or edema during the study.

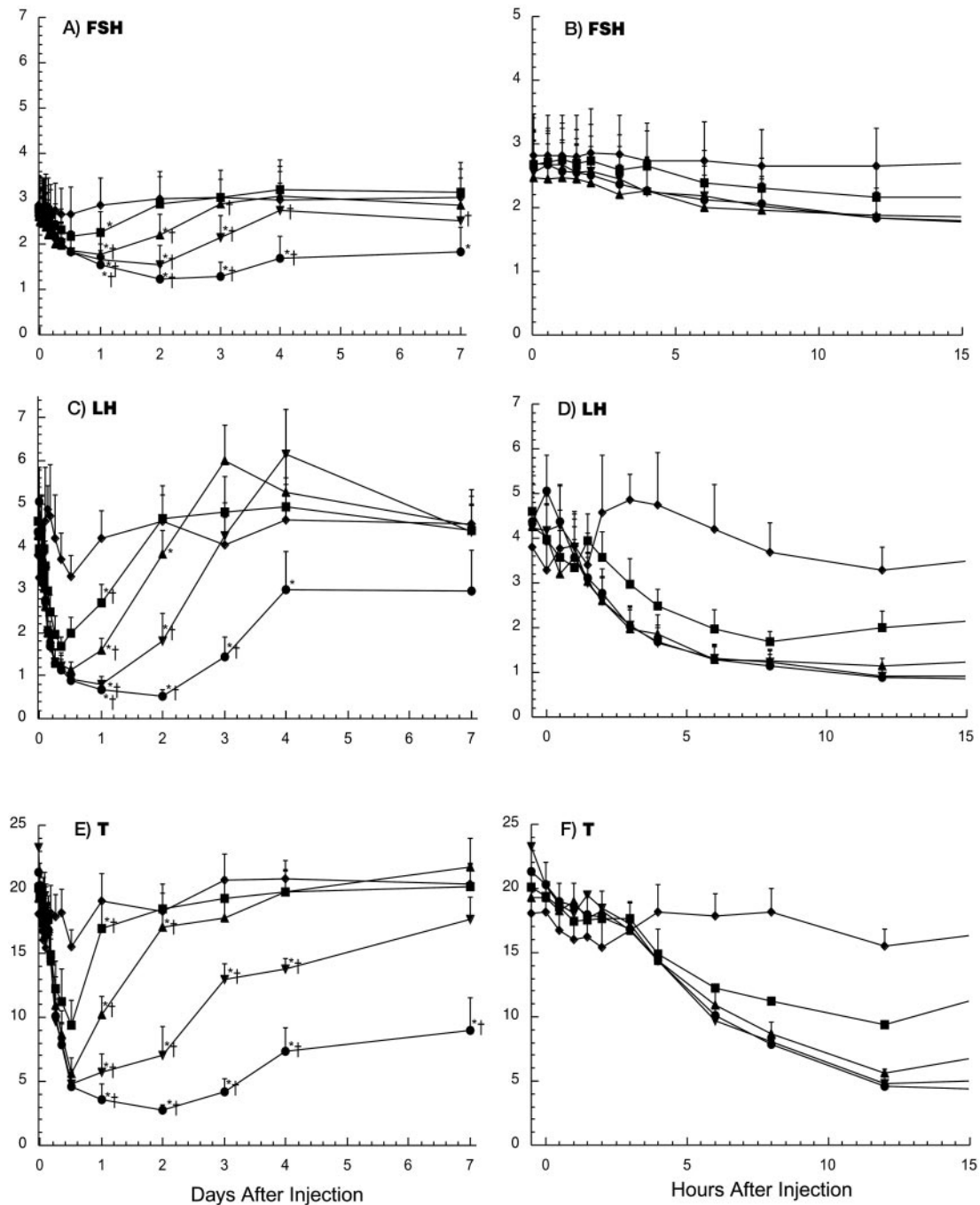


FIG. 1. Acyline is more effective than placebo at suppressing serum levels of FSH, LH, and T in a dose-dependent manner in normal young men ($n = 8$). Serum values of FSH (A and B), LH (C and D), or T (E and F) after injection of acyline at $2.5 \mu\text{g}/\text{kg}$ (■), $7.5 \mu\text{g}/\text{kg}$ (▲), $25 \mu\text{g}/\text{kg}$ (▼), and $75 \mu\text{g}/\text{kg}$ (●) or placebo (◆). Values are the mean \pm SEM. A, C, and E represent time points in days after injection; B, D, and F represent time points in hours after injection of acyline. The y-axis of B is smaller than that of A to separate time points. *, $P < 0.05$ vs. placebo; †, $P < 0.05$ below baseline (by ANOVA).

Discussion

The GnRH antagonist, acyline, was developed to have greater potency and less histamine skin irritation than previous antagonists. We have demonstrated that acyline is highly effective in suppressing gonadotropins and T in a dose-dependent manner for up to 48 h after injection in healthy, eugonadal young men. Circulating gonadotropins

and T were suppressed rapidly within 1 h of injection and returned to baseline within 7 d after injection, except for T levels that remained suppressed beyond 7 d for four of the eight subjects.

We have used two other GnRH antagonists in previous studies, Nal-Glu and Nal-Lys, and can make some general statements about their relative effects on gonadotropins and

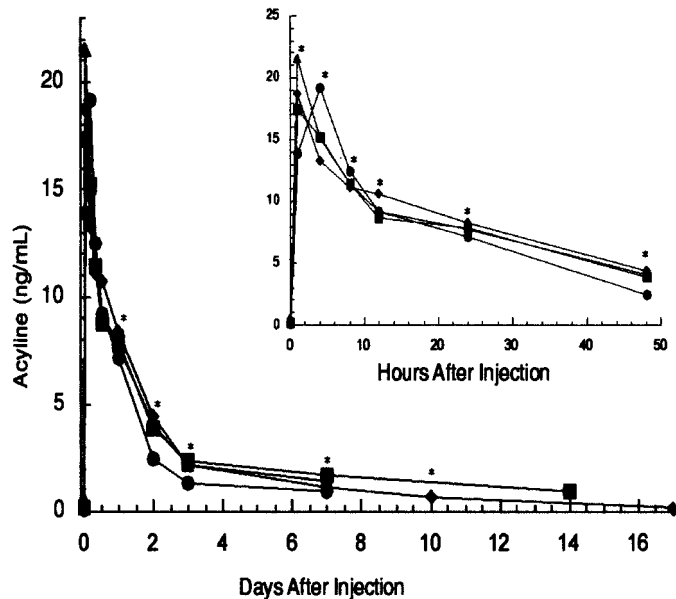


FIG. 2. Acyline serum levels rise within 1 h after injection of 75 $\mu\text{g}/\text{kg}$ acyline and remain elevated for 7 d ($n = 4$). The data represent two subjects whose T remained suppressed at 7 d after injection (\blacklozenge and \blacksquare) and two whose levels of T had returned to baseline by 7 d after injection of acyline (\blacktriangle and \bullet). The inset represents early time points in hours. *, $P < 0.05$ vs. background.

T compared with the effects of acyline. Comparisons between the GnRH antagonists were made after raw data were changed to percent baseline values, as hormonal assay sensitivity and/or testing were different between the studies (2, 3). Both 25 and 75 $\mu\text{g}/\text{kg}$ acyline suppressed LH and FSH more completely than either Nal-Glu or Nal-Lys administered at the same dose (Table 1). None of the GnRH antagonists suppressed FSH to less than 45% of baseline. Delayed suppression of FSH by GnRH antagonists has been discussed previously (3, 4). FSH requires a longer period of time for suppression to occur than LH; therefore, these studies on acyline are not long enough to evaluate the full effect of a GnRH antagonist on long-term suppression of FSH. In a longer study of Nal-Glu, FSH levels reached a nadir of 48% of baseline after 9 d after administration of 75 $\mu\text{g}/\text{kg}$ Nal-Glu daily for 10 d (3), similar to levels reached after a single injection of acyline at the same dose. FSH levels were suppressed below the limits of detection 21 d after daily administration of 5 mg Nal-Glu (11), but only in four subjects. Bioactive FSH levels fell to a greater extent than immunoreactive FSH levels (3), suggesting that a bioassay may be a more accurate measure of early suppression of FSH.

Time to maximal suppression of gonadotropins and T and time of maintenance of suppression were longer for acyline at all doses *vs.* either earlier GnRH antagonist. At a dose of 25 $\mu\text{g}/\text{kg}$, acyline suppressed T to a significantly lower level than either Nal-Lys or Nal-Glu, and maintained suppression for 48 h, whereas Nal-Lys and Nal-Glu did not maintain T suppression beyond 12 h (Table 1). At a higher dose of 75 $\mu\text{g}/\text{kg}$, acyline maintained suppression of T to a significantly lower level and for a longer period of time than Nal-Glu. Pavlou *et al.* (12) demonstrated suppression of T for 48 h after

the sc injection of 20 mg Nal-Glu in healthy men, a dose approximately equal to 285 $\mu\text{g}/\text{kg}$ acyline, a much larger dose than that used in this study, supporting the greater potency of acyline.

Prolonged levels of acyline in serum might in part account for its greater potency and suppression of gonadotropins and T compared with Nal-Glu. Serum Nal-Glu levels were measured after injection of 5 mg Nal-Glu into healthy eugonadal young men (12), approximately equal to the amount of acyline when a dose of 75 $\mu\text{g}/\text{kg}$ was injected into a 70 kg subject in our study (5.25 mg). Serum Nal-Glu levels reached a peak 50 min after injection and declined linearly thereafter with a half-life of 12.8 ± 2.7 h, approximately half that of acyline.

Serum levels of acyline and Nal-Lys reached similar peak levels, had similar half-lives, and remained significantly elevated above baseline for 14 d, suggesting similar kinetics in serum (2). The serum kinetics of Nal-Lys and acyline demonstrated a biphasic decline, different from the kinetics of Nal-Glu. This biphasic decline was also seen in monkeys after the iv injection of Nal-Lys, with an apparent terminal half-life of 6.5 d (13). Importantly, Nal-Lys was associated with serum proteins, including a 66-kDa protein that has been associated with other GnRH analogs (14, 15). The similar kinetics of Nal-Lys and acyline in serum, therefore, suggest that protein binding may also protect acyline from rapid elimination. That acyline appears to have similar disposal kinetics as Nal-Lys yet is more potent than Nal-Lys suggests that acyline is more potent than Nal-Lys at the receptor level in humans (8). Alternatively, because the antibody used to assay for the presence of acyline in serum recognizes only a single epitope (four to six amino acids; Woods Assay, Inc., unpublished data), a partial and perhaps inactive breakdown product of acyline may be detected rather than the complete molecule. The prolonged effect of acyline in suppression of LH and T would then be secondary to a potent effect of acyline at the receptor level.

Serum acyline levels could also be prolonged secondary to gel formation in the sc fat at the injection site, limiting entry into serum because some GnRH antagonists readily form gels in saline (8, 9, 16, 17) (Bagatell, C. J., and W. J. Bremner, unpublished observations). In animals, these gel pellets can be dissected out from the injection site before they resolve, demonstrating gel formation *in vivo* (Contraceptive and Reproductive Health Branch, Center for Population Research, NICHD, unpublished data). A continuous supply of acyline from an sc gel depot in the subjects might explain why some of the subjects had T levels that remained suppressed longer than 7 d after injection and others did not. There was, however, no palpable gel or nodule at the site of injection of acyline at any dose in any of the subjects. Acyline levels were also not significantly different between men whose T levels remained suppressed at 7 d and those whose levels did not; therefore, gel formation is unlikely to account for the majority of differential responses among these men.

Cetrorelix is a GnRH antagonist tested in healthy young men in Europe (4). A dose of 5.0 mg cetrorelix, comparable to an injection of 75 $\mu\text{g}/\text{kg}$ acyline in a 70-kg man (5.25 mg), suppressed LH from a baseline of approximately 2.7 IU/liter to a nadir of 0.6 ± 0.1 IU/liter and maintained suppression for at least 24 h. LH levels were back to baseline within 48 h.

TABLE 1. Acyline is more potent than Nal-Glu or Nal-Lys in suppressing gonadotropin and T levels (expressed as percentage of baseline \pm SEM)

	Acyline (25 μ g/kg)	Nal-Glu (25 μ g/kg)	Nal-Lys (25 μ g/kg)	Acyline (75 μ g/kg)	Nal-Glu (75 μ g/kg)
FSH	54.1 \pm 4.7 ^a	70.7 \pm 2.5	81 \pm 5.8	45.1 \pm 2.5 ^a	69.5 \pm 1.5
LH	18.0 \pm 2.4 ^a	45.3 \pm 8.5	52.1 \pm 5.1	9.8 \pm 2.2 ^a	38.1 \pm 7.8
T	21.4 \pm 2.2 ^a	33.8 \pm 6.2	66.3 \pm 7.1	13.3 \pm 1.3 ^a	18.6 \pm 1.7
Time to nadir	12 h	8 h	12 h	48 h	8 h
Nadir duration	48 h	12 h	12 h	>168 h	24 h

^a $P < 0.05$ compared with Nal-Glu and Nal-Lys.

Acyline suppressed LH to similar nadir levels at 48 h, equal to the time that LH levels had already returned to baseline for cetrorelix. FSH levels were suppressed to 65% of baseline values at 16 h after injection of cetrorelix (1.8 \pm 0.4 IU/liter), which did not reach statistical significance. Acyline suppressed FSH to 45% of baseline at 48 h and maintained suppression for up to 72 h, clearly different from the effect of cetrorelix. Maximal T suppression from a baseline of approximately 23 nmol/liter was seen at 12 h after injection of 5.0 mg cetrorelix (2.2 \pm 4.4 nmol/liter) and had reached normal serum concentrations at 48 h. T levels did not reach nadir levels until 48 h after injection of 75 μ g/kg acyline and remained below normal serum levels at 7 d after injection. Similar suppression of gonadotropins and T as with cetrorelix were also seen with teverelix (6), although FSH suppression was greater and suppression of T longer (33 h) with teverelix than with cetrorelix. Acyline is clearly more potent and long lasting than either cetrorelix or teverelix.

Acyline was administered safely to men in this study and caused no clinically significant systemic effects. There were minimal skin changes at the sites of acyline injection in all subjects, but no changes in hematocrit, serum chemistries, blood pressure, heart rate, weight, body mass index, prostate size, or testicular size. The effects of acyline on gonadotropins were fully reversible, and both FSH and LH levels normalized within 7 d after acyline injection.

Over 30 yr of work have failed to produce a commercially available, reversible, hormonal method of contraception in men that completely suppresses spermatogenesis with minimal adverse effects. Azoospermia induced by high dosage T provides effective contraception, but only 50–70% of non-Asian men become azoospermic during the administration of weekly im injections of T esters (19–21). The search for a more effective regimen focused on combinations of exogenous T plus progestogen agonists or GnRH analogs (22–28). A combination of T plus a GnRH antagonist was highly effective at suppression of spermatogenesis (29), but was limited by adverse dermatological effects of the GnRH antagonist, Nal-Glu (2). A safe GnRH antagonist such as acyline that has high potency and a long duration of action make it a good candidate for combination with other hormones in male contraceptive studies.

A GnRH antagonist that can rapidly and effectively inhibit gonadotropins and T with minimal side-effects and that does not have to be given as a daily injection is important not only in male contraception but also in treatment of benign prostatic hypertrophy and prostate cancer (30) endometriosis (31), infertility (32, 33), ovarian cancer (34, 35), possibly precocious puberty, polycystic ovarian syndrome as well as the study of obesity, and the basic science of the gonadotropin

axis (36). GnRH agonists are known to cause a surge in gonadotropins after administration that, for example, would be undesirable in metastatic prostate cancer. Further studies on higher doses or multiple injections of acyline may improve its duration of action, allowing for decreased intervals between injections, making acyline even more acceptable to patients.

Conclusion

Acyline is a new potent GnRH antagonist that might be useful as a safe and convenient compound in a depot formulation for suppression of the gonadal axis in male hormonal contraceptive regimens and for treatment of sex steroid hormone-dependent diseases.

Acknowledgments

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Address all correspondence and requests for reprints to: Karen L. Herbst, M.D., Ph.D., Box 357138, Department of Medicine, Division of Metabolism, University of Washington, Seattle, Washington 98195. E-mail: kherbst@u.washington.edu.

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