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# A Single Dose of the Potent Gonadotropin-Releasing Hormone Antagonist Acyline Suppresses Gonadotropins and Testosterone for 2 Weeks in Healthy Young Men

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**Acyline is a novel GnRH antagonist that reliably inhibits gonadotropins and testosterone (T) levels in men for 48 h after a single dose up to 75 μg/kg. In this study we examined gonadotropin and T levels in 28 healthy young men administered acyline as single doses of 150 or 300 μg/kg or serial injections of 75 μg/kg. A single 300 μg/kg dose of acyline suppressed gonadotropins and T to castrate levels for 15 d (baseline, 21.1 ± 3.1; nadir, 1.95 ± 0.4 nmol/liter; mean ± SEM; $P < 0.05$). Serum acyline levels peaked 90 min after the injection of 300 μg/kg acyline to a maximum concentration of 112.4 ± 18 ng/ml (n = 7; $t_{1/2}$ = 4.9 d). Injections of 75 μg/kg acyline every 2 d for five doses suppressed gonadotropins for more than 20 d (nadir T, 1.06 ± 0.17 nmol/liter; $P < 0.05$ compared with baseline). Adverse events were mild and included erythema and pruritus at the injection site. Acyline, therefore, is one of the most potent peptide GnRH antagonists studied to date with minimal adverse events. A twice monthly injection of acyline could be used as a potent suppressor of the GnRH axis to advance the development of a hormonal male contraceptive or for treatment of hormonally dependent disease. (*J Clin Endocrinol Metab* 89: 5959–5965, 2004)**

GnRH IS A hypothalamic decapeptide that is synthesized and released in a pulsatile manner from hypothalamic neurosecretory cells and regulates the synthesis and release of pituitary gonadotropins that, in turn, regulate steroidogenic and gametogenic functions of the gonads. GnRH plays a rate-limiting role in reproductive processes, and thousands of GnRH analogs have been generated because of their potential clinical utility. Most GnRH analogs are employed in the treatment of prostate cancer and for their utility in the disruption of LH surges for the regulation of ovulation induction in women undergoing *in vitro* fertilization (1–3). Other clinical uses for GnRH analogs include endometriosis and other gynecological diseases (4, 5), precocious puberty (6), and hormonal male contraception (7, 8). Both GnRH agonists and antagonists suppress gonadotropins and gonadal steroids, but the administration of GnRH agonists is accompanied by an initial gonadotropin and gonadal hormone surge known as a flare, delaying suppression by 7–14 d (2). GnRH antagonists do not cause flare, because they competitively block and inhibit GnRH-induced GnRH receptor gene expression, leading to immediate pituitary suppression.

Acyline is a GnRH antagonist sponsored by the NICHD that reliably maintained suppression of gonadotropins and testosterone (T) for 48 h with doses up to 75 μg/kg by sc injection (9). We sought to determine whether larger or serial doses of acyline would suppress gonadotropins and T for sufficiently long periods of time to allow a depot formation of acyline to be conveniently used for clinical applications such as a male hormonal contraception.

We administered acyline as single doses of 150 and 300 μg/kg and as serial injections of 75 μg/kg and monitored serum gonadotropin and T levels for a period of 30 d or until serum gonadotropins and T returned to baseline levels.

Abbreviations: ACY 150, 150 μg/kg Acyline; T, testosterone.

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## Subjects and Methods

### *Acyline*

Acyline was originally synthesized by Jean Rivier at The Salk Institute (La Jolla, CA) (10) and is distributed by the NICHD. Acyline is prepared as a lyophilized powder (4.4 mg/vial) and is stored at −20 C. Acyline-lyophilized powder was suspended in bacteriostatic water to a final concentration of 2 mg/ml for injection into sc abdominal tissue. Serum levels of acyline were measured by RIA in a subset of subjects after the injection of 300 μg/kg acyline, using a specific antiserum and a proprietary peptide with authentic peptide standard (Woods Assay, Inc., Portland, OR) as described previously (11). The sensitivity of the assay for acyline was 0.35 ng/ml.

### *Subjects*

All study procedures involving human subjects were approved by the institutional review board at University of Washington and were performed at the University of Washington Clinical Research Center in accordance with institutional guidelines. Thirty-five men (age, 18–50 yr) were recruited by flyers posted on local college campus bulletin boards. All subjects were healthy, eugonadal men with normal medical histories and baseline physical examinations, including normal testicular size by Prader orchidometer and prostate size by digital rectal exam, serum chemistries, complete blood count, and gonadotropin and T levels. We excluded subjects who were current smokers, drank more than 7 oz

alcohol weekly, or had taken prescription medications or any sex steroid hormone within the last 6 months. Of these men, 34 were screened for study eligibility. Six men were excluded or withdrawn from the study after the screening process and before drug treatment: one man had low T levels, one man had abnormal hematology results at screening, one man had an elevated alanine aminotransferase test value, and two men had time constraints that prevented study participation. One subject was screened but did not begin the drug treatment phase, because recruitment for the study was complete.

Subjects were assigned to one of four groups (n = 7): group 1, single dose of 150 μg/kg acyline on d 0 (ACY 150); group 2, single dose of 300 μg/kg acyline on d 0 (ACY 300); group 3, 75 μg/kg acyline injections on d 0, 4, and 8 and placebo on d 2 and 6 (ACY 75×3); and group 4, 75 μg/kg acyline injections on d 0, 2, 4, 6, and 8 (ACY 75×5).

Acyline was administered by sc injection in the abdomen between 0700 and 1000 h. For ACY 150 and 300 groups, blood samples were obtained at 30, 60, 90, and 120 min; 3, 4, 6, 8, 12, and 24 h; and 2, 3, 4, 7, 9, 11, 13, 15, 17, 19, 21, 25, and 30 d after injection. For the serial injection groups ACY 75×3 and 75×5, blood samples were obtained on d 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 21, 25 and 30. All research subjects were monitored for 30 d with vital signs determined, and laboratory tests and physical exams performed.

### *Measurements*

*Hormones.* FSH, LH, and T levels were measured by immunofluorometric assay (Delfia, Wallac Oy, Turku, Finland). Samples from a given individual were measured in a single assay. The sensitivities of the assay for FSH and LH were 0.016 and 0.019 IU/liter, respectively. For low, mid, and high pooled values of 0.054, 1.04, and 20.8 IU/liter FSH, the intraassay coefficients of variation were 12%, 1.9%, and 2.9%, and the interassay coefficients of variation were 18%, 6.1%, and 4.1%, respectively. For low, mid, and high pooled values of 0.056, 0.95, and 15.6 IU/liter LH, the intraassay coefficients of variation were 6.5%, 3.9%, and 5.4%, and the interassay coefficients of variation were 21%, 8%, and 6.6%, respectively. The assay sensitivity for T was 0.5 nmol/liter. For low, mid, and high pooled values of 3.8, 10.6, and 24.4 nmol/liter T, the intraassay coefficients of variation were 9.6%, 5.2%, and 6.1%, and the interassay coefficients of variation were 12%, 8.2%, and 6.7%, respectively. If serum T levels had not returned to baseline within 30 d after the injection of acyline, subjects were requested to return for additional blood sampling until serum T levels had normalized.

*Serum laboratory tests.* Screening and monitoring laboratory tests for complete blood count, electrolytes, glucose (chemistry 7), calcium, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, bilirubin, and total protein were performed at the University of Washington.

### *Statistics*

FSH, LH, and T are expressed as the mean hormone level ± SEM. For statistical analysis, all hormone data were log-transformed and then back-transformed for ease of presentation. Comparisons of data within groups and between groups were analyzed with ANOVA (SigmaStat, SPSS, Inc., Chicago, IL). Evidence for change from baseline within a group was further analyzed at each time point by paired *t* tests. The area under the curve was calculated using the trapezoid rule from time zero to the last measured level without smoothing or curve fitting (PK Solutions, Summit Research, Montrose, CO). The half-life ($t_{1/2}$) was calculated from 3 d to the last measured level. For all comparisons, an $\alpha$ of 0.05 was considered significant.

## Results

### *Study population*

The 28 subjects enrolled in this study were eugonadal and lean by body mass index (Table 1). There were no significant differences in baseline parameters between groups.

### *Acyline administration*

Acyline was initially suspended in bacteriostatic water at a concentration of 8.8 mg/ml, which allowed acyline to be administered as a single, small volume injection at a concentration of 300 μg/kg (2.4-ml injection for a 70-kg man). Nodule formation at the sites of acyline injection (lingering for as long as 30 d postinjection) suggested that the drug might be forming a gel in the fat tissue in a volume large enough to be palpable, similar to other GnRH antagonists (12). Suppression of gonadotropins and T was also inconsistent (data not shown). Acyline was therefore administered as a 2.0 mg/ml solution in water that does not lead to palpable nodule formation when injected into sc tissue (9), but did require multiple injections for a dose of 150 or 300 μg/kg. A 70-kg man administered 300 μg/kg acyline at a concentration of 2.0 mg/ml would have an sc injection of 10.5 ml in four or five divided doses (2–3 ml/injection).

### *Single injections of acyline*

*Gonadotropins.* Baseline levels of FSH and LH are shown in Table 1. Both FSH and LH decreased rapidly after a single dose of 150 or 300 μg/kg acyline in all subjects (Fig. 1). In the ACY 150 group, FSH levels dropped significantly below baseline 8 h after injection, reaching a nadir at 3 d (1.06 ± 0.35 IU/liter), and remained significantly below baseline for 4 d after injection. LH levels decreased significantly below baseline by 1.5 h after injection, reached a nadir at 2 d (0.27 ± 0.05 IU/liter), and remained significantly below baseline for 3 d after injection.

In the ACY 300 group, FSH levels decreased significantly below baseline 1 h after injection and remained at these levels for 21 d, reaching a nadir on d 11 (0.26 ± 0.04 IU/liter). LH levels decreased significantly below baseline by 1.5 h after injection and reached a nadir at 2 d (0.17 ± 0.1 IU/liter), remaining significantly below baseline levels for 15 d. Gonadotropin levels tended to rebound above baseline by d 30 after the injection of acyline, but there was no significant difference between baseline and d 30 FSH or LH levels in the ACY 150 or ACY 300 groups.

*T.* Baseline levels of T for ACY 150 and ACY 300 are shown in Table 1. T rapidly decreased significantly below baseline

**TABLE 1.** Baseline parameters of subjects

| Group | ACY 150 | ACY 300 | ACY 75X3 | ACY 75X5 |
|---|---|---|---|---|
| n | 7 | 7 | 7 | 7 |
| Age (yr) | 26.6 ± 2.7 | 31.9 ± 3.8 | 30.9 ± 2.1 | 23.0 ± 1.8 |
| Body mass index (kg/m$^2$) | 26.1 ± 1.7 | 24.5 ± 0.7 | 25.6 ± 1.1 | 26.2 ± 1.6 |
| FSH (IU/liter) | 2.5 ± 0.3 | 2.7 ± 0.8 | 3.1 ± 0.5 | 2.0 ± 0.3 |
| LH (IU/liter) | 3.2 ± 0.5 | 3.3 ± 0.7 | 3.5 ± 0.2 | 4.0 ± 0.4 |
| T (nmol/liter) | 21.1 ± 3.1 | 21.6 ± 4.2 | 20.0 ± 2.6 | 26.1 ± 3.3 |

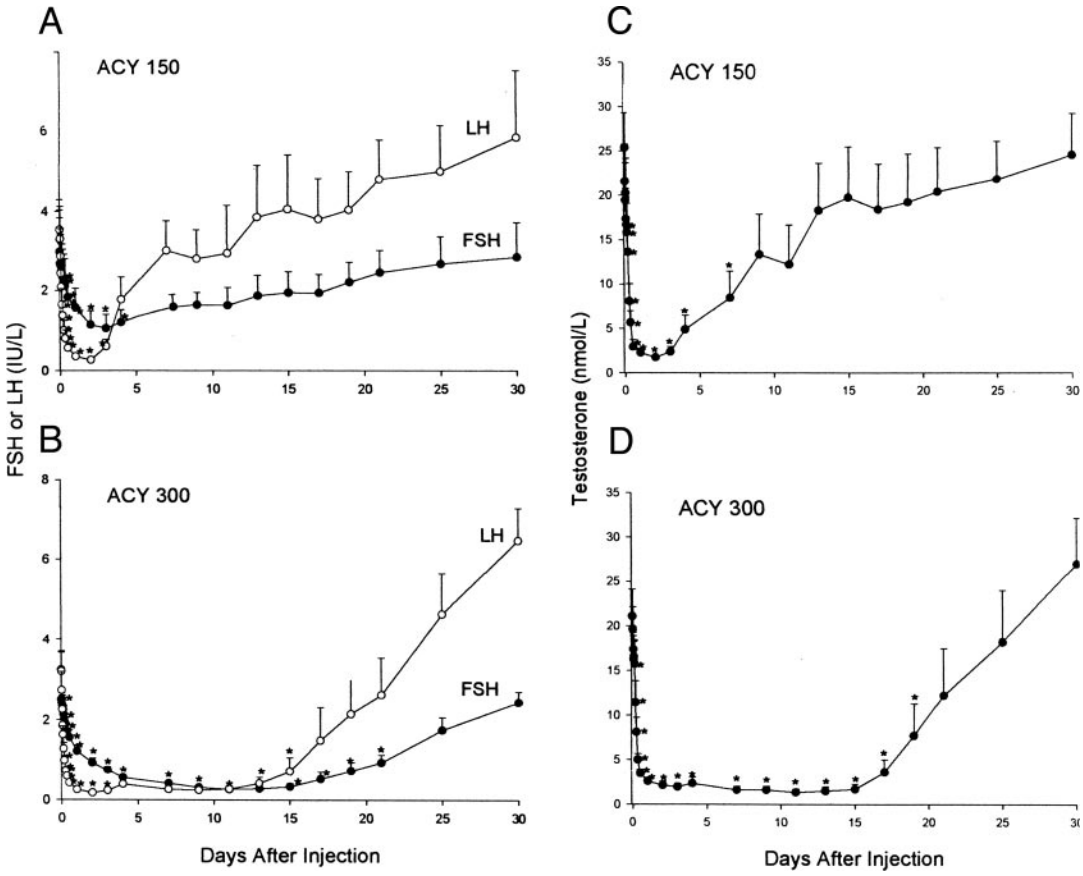


FIG. 1. Single injections of acyline rapidly suppress gonadotropins and T. Serum values of FSH (●) and LH (○) after 150 (A) and 300 (B) μg/kg acyline and serum values of T (●) after 150 (C) and 300 (D) μg/kg acyline are shown. Time is expressed as days after the first injection of acyline. Values (n = 7) are expressed as the mean ± SEM. *, $P < 0.05$ *vs.* baseline.

2 h after injection in all subjects after single acyline doses and decreased below castrate levels (5 nmol/liter) by 12 h after injection (Fig. 1). T levels reached a nadir in the ACY 150 group 2 d after injection (1.75 ± 0.28 nmol/liter) and began to rise but remained significantly below baseline through d 7 after injection. One subject's T level remained below baseline levels until d 37.

T levels reached a nadir on d 2 after injection in the ACY 300 group (1.95 ± 0.38 nmol/liter) and remained at approximately this same level through d 15. T levels began increasing on d 17 after injection, remaining significantly below baseline levels until d 19 after injection, but were not significantly different from baseline levels on d 21. One subject's T level remained below baseline levels until d 35. On the average, there was no significant difference between baseline and d 30 T levels in either group.

*Multiple injections of acyline*

*Gonadotropins.* In the ACY 75X3 group, FSH and LH levels decreased significantly below baseline on d 2 (Fig. 2, A and B). Gonadotropin levels increased before the next injection on d 4, but remained significantly below baseline, then decreased on d 6, reaching steady suppressed levels on d 10–18 after the third injection. The nadir level of FSH on d 18 was 0.39 ± 0.08 IU/liter, and the nadir level for LH was 0.16 ± 0.04 IU/liter on d 10. LH and FSH levels remained significantly below baseline through d 21 and 25, respectively, but the levels normalized and were not significantly different from baseline levels by d 30.

In the ACY 75X5 group, LH and FSH decreased significantly below baseline by d 2 (Fig. 2, C and D). LH levels remained significantly suppressed below baseline from d 2–16, with a nadir level on d 10 (0.07 ± 0.01 IU/liter). FSH levels gradually decreased to a nadir on d 12 (0.1 ± 0.01 IU/liter) and remained significantly below baseline on d 2–25.

*T.* In the ACY 75X3 group, T decreased significantly below baseline levels after the first acyline injection (Fig. 3A), with small rebound increases before the second and third injections. T levels reached a nadir on d 10 after the third injection (1.29 ± 0.22 nmol/liter), remained significantly below baseline levels from d 2–21, and returned to baseline by d 30. Two subjects' T levels remained below baseline on d 30, but returned to baseline levels by d 33 and 47, respectively.

In the ACY 75X5 group, T levels decreased rapidly and significantly below baseline, reaching a nadir on d 17 (1.06 ± 0.17 nmol/liter) after the first injection and remained significantly suppressed through d 25, returning to baseline levels by d 30. Four subjects' T levels remained significantly below baseline levels on d 30, returning to baseline levels on d 36, 37, 45, and 47, respectively.

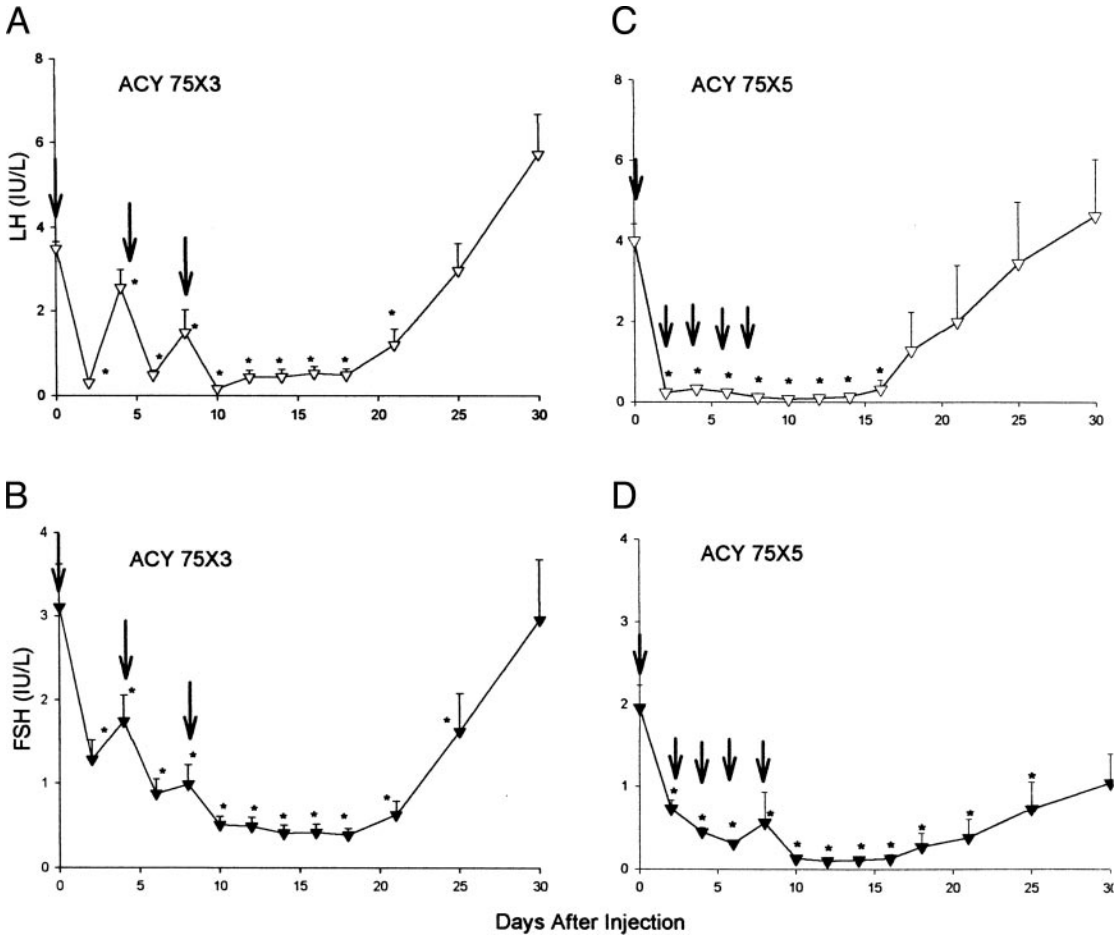


FIG. 2. Multiple injections of 75 μg/kg acyline can maintain suppression of gonadotropins for more than 20 d. Serum values of LH (A; △) and FSH (B; ▲) after three injections of 75 μg/kg acyline spaced 4 d apart (*arrows*) and LH (C) and FSH (D) levels after five injections of 75 μg/kg acyline spaced 2 d apart (*arrows*) are shown. Time is expressed as days after the first injection of acyline. Values (n = 7) are expressed as the mean ± SEM. *, $P < 0.05$ *vs.* baseline.

*Acyline pharmacokinetics*

Serum acyline levels were measured in all seven subjects in the ACY 300 group (Fig. 4). Acyline reached a maximum concentration of 112.4 ± 6.9 ng/ml 90 min after injection and remained significantly elevated above background through d 30. Blood levels of acyline dropped by 50% at 4.9 d.

*Adverse events and safety*

Skin reactions were the most common side-effect noted after sc acyline injections. A mild pink blush occurred at the site of injection in 22 of 28 subjects (78.5%), lasting up to 120 min. The blush occurred in 94% of injections in subjects who experienced it. Pruritus at the site of injection occurred in 75% of subjects, with an average score of 2.3 ± 0.1 arbitrary units of 5: 0 = no itch, 1 = barely noticeable, 2 = mild, 3 = moderate, 4 = severe, and five = the worst itch ever experienced. The pruritus persisted for approximately 40 min on the average. Nodules or deep induration were noted in three individuals, one in each of the groups receiving single acyline doses (although multiple injections), and persisted at 2 and 11 d; in one subject receiving serial injections, they persisted for 2 d. Bruising at the site of injection was also noted in 12 individuals (42.9%), although not at all injection sites. During the hypogonadal period (T, <5 nmol/liter), 10 subjects experienced decreased libido or fatigue (35.7%), and two experienced hot flashes or significant changes in mood/irritability (7.1%). One subject experienced myalgia without signs of infection, including fever. There were no significant changes in any chemistry parameter during the study, including aspartate aminotransferase and alanine aminotransferase. The hematocrit decreased slightly in all 28 subjects from an average baseline value of 42.5 ± 0.46% to 41.1 ± 0.49% ($P < 0.01$), remaining in the normal range (38–50%). The white blood cell and platelet counts were unaffected by acyline administration.

## Discussion

GnRH antagonists competitively block and inhibit GnRH-induced GnRH receptor gene expression, leading to immediate pituitary suppression (13) without the surge in gonadotropins and T seen after GnRH agonist administration (14). The GnRH antagonist, acyline, was developed to have greater potency and less histamine-mediated skin irritation than previous antagonists (10). A single injection of 300

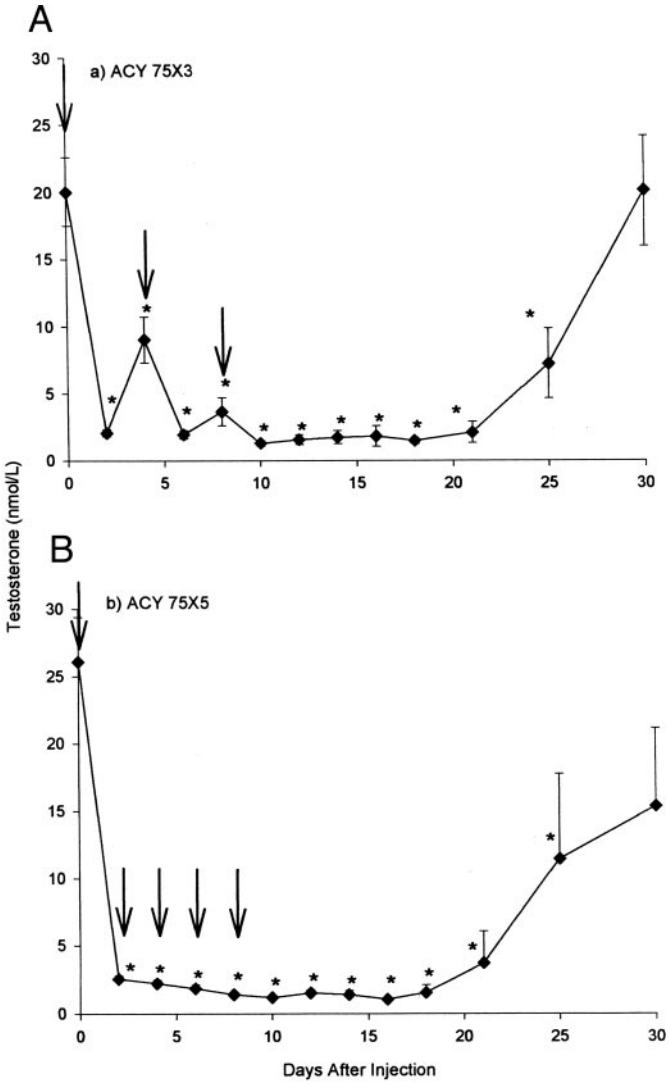


FIG. 3. Multiple injections of 75 $\mu$g/kg acyline can maintain suppression of T for more than 20 d. Serum values of T (♦) after three injections of 75 $\mu$g/kg acyline spaced 4 d apart (A, *arrows*) and five injections of 75 $\mu$g/kg acyline spaced 2 d apart (B, *arrows*). Time is expressed as days after the first injection of acyline. Values (n = 7) are expressed as the mean ± SEM. *, $P < 0.05$ *vs.* baseline.

$\mu$g/kg acyline in this study rapidly and significantly suppressed gonadotropins and T to castrate levels (T, <5 nmol/liter) for 15 d. Five injections of 75 $\mu$g/kg acyline spaced 2 d apart also immediately suppressed gonadotropins and T and maintained T within the castrate range for up to 21 d. Five injections of 75 $\mu$g/kg acyline might have suppressed the hypothalamic-pituitary-gonadal axis longer than the single 300 $\mu$g/kg dose, because the cumulative dosage administered was greater at 375 $\mu$g/kg. Alternatively, because 75 $\mu$g/kg is known to rapidly and effectively suppress gonadotropins and T for up to 48 h (9), serial injections of acyline every 2 d over an 8-d period might simply maintain that 48-h suppression. However, if the latter were true, then gonadotropins would be expected to begin returning to baseline approximately 48 h after the last injection. Instead, FSH and LH were still suppressed 8 d after the last of five injections of 75 $\mu$g/kg acyline.

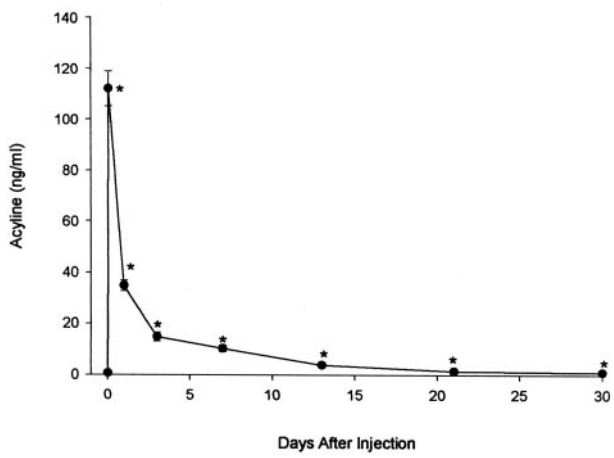


FIG. 4. Serum levels of acyline (nanograms per milliliter) remained significantly elevated for 30 d after sc injection of 300 $\mu$g/kg acyline in seven healthy young men. Values are the mean ± SEM after subtraction of 1 ng/ml background levels. *, $P < 0.05$ *vs.* baseline.

Cetrorelix is a GnRH antagonist that has similar potency to acyline (9, 15). Data examining the effect of cetrorelix on pituitary GnRH receptor expression and localization might help explain why serial injections of 75 $\mu$g/kg acyline induce longer suppression of gonadotropins than a single 300 $\mu$g/kg injection (15–18). GnRH receptors were significantly downregulated for at least 72 h in rat pituitaries, accompanied by suppression of serum LH and T, after a single injection of 100 $\mu$g cetrorelix (16). The lowest receptor level was found 6 h after the injection of cetrorelix, but a marked recovery in receptor number was observed at 48 h. A major decrease in the expression of mRNA for pituitary LH-releasing hormone receptors was also found after chronic treatment with cetrorelix (17). This suppression is not believed to be a direct effect on gene expression of the GnRH receptor, but due to the fact that GnRH antagonists prevent up-regulation of receptor mRNA expression induced by GnRH (18). These data suggest that the prolonged suppression of gonadotropins and T by serial injections of 75 $\mu$g/kg acyline is not only a reflection of the concentration of the antagonist at the level of the receptor, but that administration every 48 h might have prevented the up-regulation of GnRH receptor mRNA expression longer than the single dose of 300 $\mu$g/kg.

The question then becomes how is acyline best administered. Subjects and patients would probably prefer a single dose of sc acyline twice a month to serial injections. However, serial injections maintained suppression of gonadotropin and T levels longer than either single dose, as demonstrated by the four subjects whose T levels remained significantly below baseline more than 30 d after the start of the five injections. A minimal dose for a twice monthly injection might be 225 $\mu$g/kg, because gonadotropins and T were suppressed in the hypogonadal range for 14 d after three injections of 75 $\mu$g/kg acyline. Alternatively, a higher dose injection than 300 $\mu$g/kg might maintain acyline concentrations in the pituitary at a sufficiently high level to prevent up-regulation of GnRH receptor mRNA. The GnRH antagonist, abarelix, is administered as a 100-mg monthly injection to maintain suppression of serum gonadotropins and T (2). The amount of acyline administered to a 70-kg man in this

study as a 300 $\mu$g/kg injection was 21 mg, approximately 5 times less than the dose of abarelix. It is therefore possible that a single higher dose of acyline might suppress gonadotropins and T for a month. The only drawback is the volume of acyline that would need to be administered (10.5 ml). A depot formulation of acyline is currently under development by the NICHD.

Adverse side-effects with acyline injection were again minimal, similar to our previous study (9), and included a blush at the injection site and mild pruritus. In this study, however, there was more bruising at the site of injection. There was no pattern to the bruising; it did not occur more commonly for specific individual subjects and was not associated more often with individual nurses who administered the injections. We believe that the bruising probably reflects differences in the manner the injection was administered, rather than being a result of the acyline itself. Three sc nodules at the site of injection were noted in this study: two lasting for 2 d, and one lasting 11 d. Because nodule formation did not occur with every injection in these individuals, these nodules probably represent a tissue reaction to the injection, rather than a reaction to acyline itself.

Other adverse events that occurred during this study in the hypogonadal period were expected as a result of declining T levels. These included hot flashes, decreased libido, fatigue, and irritability, consistent with symptoms of male hypogonadism (19, 20). Because T is known to increase the production of erythropoietin (21, 22), and castration decreases hemoglobin levels (23), our data demonstrating a small, but significant, decrease in hematocrit within the normal range was predictable.

The amount of time for acyline levels in serum to decrease by half ($t_{1/2}$) in this study was 4.9 d, greater in length than the 28.3 h previously found (9). This calculated $t_{1/2}$ for acyline in serum does not fit the classical definition of a true $t_{1/2}$, because it reflects not only the time required for half the total amount of acyline to be cleared from the serum, but also the rate of entry of acyline into serum from the presumed sc depot. Nevertheless, this calculated $t_{1/2}$ allows us to compare data from different studies. The difference found in the $t_{1/2}$ values between the two studies probably reflects the increased number of subjects tested for acyline levels in this study (n = 7) *vs.* the former study (n = 4), the similarity in suppression of gonadotropins in the current study (only two of four subjects had suppression of gonadotropins and T for 7 d in the previous study), and the higher dosage of acyline administered in the current study. The long calculated $t_{1/2}$ of acyline might also represent the ability of acyline to bind to serum proteins, as previously discussed (9), or a prolonged time of entry from the sc tissue into the serum compartment secondary to increased volume of injections.

## Conclusion

Acyline is a likely candidate for use as a potent, long-lasting GnRH antagonist in the development of an effective male hormonal contraceptive regimen or for the treatment of sex steroid hormone-dependent syndromes, such as advanced severe endometriosis and prostate cancer. It is safe and potentially can be administered as a single dose, twice a month. Because exogenous long-acting T formulations also inhibit circulating gonadotropins levels and spermatogenesis, a combination of long-acting acyline plus T might make a male hormonal contraceptive regimen a safe, effective, and practical option.

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