Gonadotropin Releasing Hormone Antagonist Plus Testosterone: A Potential Male Contraceptive

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Although hormonal regimens provide effective, reversible contraception for women, no effective hormonal contraceptive regimen has yet been developed for men. Testosterone decreases gonadotropin secretion by exerting negative feedback at both the hypothalamus and pituitary (1–3), thereby inhibiting spermatogenesis. Administration of exogenous T causes azoospermia in only 50–70% of men, however (4). Agonist analogs of GnRH can inhibit gonadotropin secretion and gonadal function (5), but they do not consistently induce azoospermia in primates when given alone or in combination with androgen (6–9).

GnRH antagonists are synthetic analogs of GnRH that compete with endogenous GnRH for pituitary binding sites, thereby inhibiting the secretion of LH and FSH (5). In short-term studies in humans and in nonhuman primates, these antagonists reversibly suppress plasma levels of LH, FSH, T, and inhibin (10–12). When given without androgen replacement, GnRH antagonists can induce azoospermia in adult monkeys (13–15). An androgen must be administered with a GnRH antagonist in a contraceptive regimen to maintain the normal androgen milieu. However, in previous studies of concomitant antagonist and androgen administration in experimental animals, induction of azoospermia was inconsistent (15, 16). The present study was undertaken to determine the effects of daily injections of the GnRH antagonist, Detirelix, [N-Ac-D-Nal(2)1-DpCl-Phe2D-Trp3D-hArg(Et2)6-DAla10] GnRH, alone and in conjunction with simultaneous T replacement, on sperm production and on serum testosterone levels in adult male monkeys.
Experimental Procedures

Adult male monkeys, *Macaca fascicularis*, were housed under controlled conditions of temperature (21 ± 2°C) and light (on at 0600 h, off at 1800 h) in individual cages at the Regional Primate Center at the University of Washington. In addition to monkey chow, the animals received fresh fruit, chewable vitamins, and iron injections. The animals were aged 8 to 15 years (as assessed by dental radiographs). The GnRH antagonist (supplied courtesy of Drs. Brian Vickery and John J. Nestor of the Syntex Corporation, Palo Alto, CA) was dissolved at a concentration of 4µg/ml in a vehicle containing glacial acetic acid, benzyl alcohol, sodium hydroxide, and sterile water. The vehicle was supplied by the Syntex Corporation and contained 0.02M sodium acetate buffer, 0.9% benzyl alcohol preservative, and 0.02M glacial acetic acid. Antagonist was added to this solution and was filtered through a 0.8µm nucleopore filter. Aliquots of 20ml were frozen at -20°C until use. During the study period, either the antagonist or the vehicle was injected subcutaneously daily between 0800 h and 1200 h.

All animals in the experimental groups received Silastic capsule implants subcutaneously 5 days prior to the first injection of GnRH antagonist. The capsules were 0.33 cm ID × 0.46 cm OD and were 5.5 cm in length. These implants contained either crystalline testosterone or were empty, depending on the treatment regimen. Capsules were sterilized in Zephiran and rinsed in sterile saline before implantation. Implants were removed when injections of GnRH antagonist were completed.

Serum testosterone was measured by radioimmunoassay, using methods previously described (1). The minimum detectability of the assay was less than 0.35 nmol/L. The intra- and interassay coefficients of variation were 5.1% and 9.8%, respectively. GnRH antagonist levels were measured in groups 2, 3, and 4 by RIA at the Syntex Corporation. Seminal fluid was obtained by rectal electroejaculation. Sperm counts were performed in the Seminal Fluid Core Laboratory (C. Alvin Paulsen, Director) of the Population Center for Research in Reproduction.

All animals (n = 22) were studied for an initial 4-month control period during which baseline measurements were obtained. The animals were then divided into four groups: Group 1 (n = 5) received antagonist, 250µg/kg/day, plus sham implants, for 12 weeks. Group 2 (n = 5) received GnRH antagonist, 250µg/kg/day plus T via implants for 20 weeks. Group 3 (n = 5) received antagonist, 750µg/kg/day, plus T via implants for 16 weeks. Group 4 (n = 7) received vehicle alone for 20 weeks. Animals were monitored daily and observed for any physical or behavioral effects of the drug treatment. Throughout the control and experimental periods, seminal fluid, blood samples, and body weights were obtained every 2 weeks.