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Suppression of Plasma Gonadotropins and Testosterone in Adult Male Monkeys (*Macaca fascicularis*) by a Potent Inhibitory Analog of Gonadotropin-Releasing Hormone*

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ABSTRACT. The reduction of circulating levels of gonadotropins and testosterone is of value for the treatment of steroid-dependent neoplasms and the control of fertility. We tested the ability of single and multiple doses of the GnRH antagonist [*N*-Ac-D-Nal(2)[1], D-pCl-Phe[2], D-Trp[3], D-hArg(Et$_2$)[6], D-Ala[10]]GnRH (GnRH-Ant) to suppress levels of these hormones in intact and castrate adult male cynomolgus monkeys. In Exp I, single injections of the antagonist at doses of 0 (vehicle), 5, 50, 250, and 500 μg/kg BW were given to castrate and intact animals. In Exp II, daily injections of antagonist at doses of 0, 50, 100, or 250 μg/kg BW were given to intact animals for 21 days. Plasma levels of testosterone, FSH, and LH were determined by RIA, and in intact animals, LH levels were measured by bioassay. In Exp I, a single injection of 5 μg/kg BW or more of GnRH-Ant to castrate animals significantly reduced plasma LH and FSH by 4 h after injection ($P \leq 0.01$). Nadir levels (40% of preinjection control values) of LH and FSH were reached 8 and 24 h, respectively, after administration of 250 or 500 μg/kg BW, and these hormones remained significantly lower than preinjection values over at least 48 h ($P \leq 0.05$). A single injection of 50 μg/kg BW or more of antagonist to intact animals markedly reduced plasma LH and testosterone by 6 h after administration ($P \leq 0.05$), while 250 or 500 μg/kg BW antagonist maintained LH and testosterone levels below 30% ($P \leq 0.05$) of preinjection values for 24 h. In Exp II, daily injections of 250 μg/kg BW antagonist to intact animals resulted in near-castrate levels of plasma testosterone which were achieved by 24 h after the first injection of antagonist and persisted for the ensuing 20 days. Daily injections of 100 μg/kg BW or less of antagonist were ineffective in suppressing testosterone. Thus, this potent GnRH antagonist acutely and chronically lowers gonadotropin and testosterone levels in adult male cynomolgus monkeys. By virtue of its inhibitory effect, this antagonist is potentially useful as a therapeutic agent in clinical situations requiring long term suppression of testicular function, such as fertility control, the treatment of steroid-dependent neoplasms, and precocious puberty. (*J Clin Endocrinol Metab* **62:** 58, 1986)

SUPPRESSION of circulating levels of reproductive hormones is desirable in some clinical situations, such as the treatment of precocious puberty and steroid-dependent neoplasms and the control of male and female fertility. Synthetic inhibitory analogs of GnRH are being developed as potential therapeutic agents for use in these circumstances, and as a prelude to human trials have been tested for their ability to inhibit reproductive function in a variety of animal models (1). In the female rat, GnRH antagonists block cyclicity (2), prevent ovulation (3), and terminate pregnancy (2). In male rats, these compounds lower plasma levels of reproductive hormones, inhibit spermatogenesis, and reduce the weights of the testes and accessory sex organs (4). GnRH antagonists reduce the pulse amplitude, pulse frequency, and mean plasma levels of gonadotropins in intact female monkeys (5) and prevent the postcastration rise of gonadotropins in castrate female monkeys (6).

There is a relative dearth of information regarding the effects of these compounds in male nonhuman primates. High doses of one GnRH antagonist given to castrate male rhesus monkeys reduced circulating levels of gonadotropins for 24 h (7), and a different GnRH antagonist administered to intact male rhesus macaques reduced LH and testosterone levels for a similar period of time (8).

In the present study we examined acute and chronic effects of administration of the GnRH antagonist analog [*N*-Ac-D-Nal(2)[1], D-pCl-Phe[2], D-Trp[3], D-hArg(Et$_2$)[6], D-Ala[10]]GnRH (Syntex RS68439) to adult male macaques.

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We demonstrated previously that a single sc injection of this antagonist to intact or castrate male rats markedly reduced plasma LH and testosterone levels within 2 h after administration and sustained this suppression for at least 24 h (9). The initial phase of this study (Exp I) was designed to assess the effect of a single injection of antagonist on circulating levels of reproductive hormones in both castrate and intact monkeys. Since administration of this compound to men is likely to involve a regimen of single daily injections, we were particularly interested in identifying a dose of antagonist that would suppress testosterone levels for 24 h. The subsequent phase of the study (Exp II) was conducted to determine whether suppression of gonadotropins and testosterone in intact animals could be sustained for a period of weeks by daily injections of the antagonist.

Materials and Methods

Animals and surgical procedures

Sexually mature male crab-eating macaques, *Macaca fascicularis*, weighing 4.5–9.0 kg, were used in these experiments. The animals were housed in individual cages under controlled conditions of light (on at 0600 h; off at 1800 h), heat (25.5 ± 1 C), and relative humidity (65%). In addition to monkey chow, the animals received fresh fruit (twice weekly) and chewable vitamin and iron tablets (once weekly). Tap water was available *ad libitum*. Exp I was performed in castrate (n = 8) and intact (n = 7) animals. Exp II was performed in intact (n = 12) animals.

Surgery was performed under aseptic conditions on animals anesthetized with fluothane. Bilateral orchidectomy was followed by a 3-week recovery period, then by the insertion of an indwelling catheter (Silastic tubing; od, 0.04 in.; Dow-Corning, Midland, MI) into either a femoral or internal jugular vein. The distal portion of the catheter was exteriorized through a midscapular skin incision and passed through a protective housing to a sampling port mounted on top of the animal's cage. This arrangement provided access to the venous system of the unanesthetized animal, while allowing the animal freedom of movement within the cage. The catheter was kept patent by the infusion of 200 ml/day heparinized (3 USP units/ml) physiological saline.

Drugs

The GnRH antagonist (GnRH-Ant) used in this study was [*N*-Ac-D-Nal(2)[1], D-pCl-Phe[2], D-Trp[3], D-hArg(Et_2)[6], D-Ala[10]] GnRH (RS68439; Syntex Research, Palo Alto, CA). In Exp I, the compound was diluted in 0.15 M saline and kept frozen until used. In Exp II, the diluent was 0.02 M acetate buffer, pH 5.0, with 0.9% benzyl alcohol added as preservative; the solution was refrigerated until used.

Experimental design

Exp I: castrate animals. Administration of the antagonist was begun 1 week after insertion of the venous catheter. Each animal received a single bolus iv injection of GnRH-Ant at doses of 0 (vehicle; n = 8), 5 (n = 8), 50 (n = 8), 250 (n = 3), and 500 (n = 8) μg/kg BW; doses were given in pseudorandom order, separated by 1-week intervals. Three blood samples (1 ml/sample) were obtained at 30-min intervals beginning at −1, +4, +8, +24, +48, and +72 h relative to GnRH-Ant or vehicle administration; equal aliquots of plasma from each of the three samples were pooled for analysis of LH and FSH.

Exp I: intact animals. Each animal received single sc injections of antagonist at doses of 0 (vehicle; n = 7), 5 (n = 7), 50 (n = 7), 250 (n = 5), and 500 (n = 7) μg/kg BW administered in pseudorandom order at 1-week intervals. Blood samples were obtained at −0, +6, and +24 h relative to GnRH-Ant or vehicle administration and consisted of a single 5-ml sample drawn by venipuncture of a femoral, cephalic, or saphenous vein while maintaining the animal under sedation with 20–30 mg ketamine hydrochloride (Parke-Davis, Morris Plains, NJ).

Exp II: intact animals. Twelve intact animals received sc injections of a single daily dose of GnRH-Ant for 21 days. Injections were given between 1200–1300 h to animals anesthetized with ketamine, and a single 5-ml blood sample was drawn by venipuncture immediately before GnRH-Ant administration on days 0, 1, 7, 14, and 21. GnRH-Ant was administered at doses of 0 (vehicle), 50, 100, or 250 μg/kg BW; each dose was given to three animals.

Blood handling and hormone assays

Blood was centrifuged immediately after collection, and the plasma was stored at −20 C until analyzed for testosterone, LH, and FSH. Plasma testosterone levels in intact animals were determined by RIA, as described previously (10). The sensitivity of the testosterone assay was less than 0.1 ng/ml, and the intra- and interassay coefficients of variation (CVs) were 5.1% and 9.8%, respectively. Plasma levels of LH in castrate animals were measured by RIA, as described by Peckham and Tontala (11). The reference preparation was rhesus pituitary LH (WP-XV-20); purified cynomolgus pituitary LH was used for radioiodination, and the antiserum was rabbit anti-hCG (R13). The sensitivity of the assay was 64 ng/ml, and the interassay CV was 13.0% at 75% binding. Plasma LH levels in intact animals were measured with a mouse Leydig cell bioassay (12, 13), which had intra- and interassay CVs of 6% and 18%, respectively. The sensitivity of the assay was 0.3 μg/ml when samples were run at a 20-μl volume (Exp I) and 0.6 μg/ml when samples were run at a 10-μl volume (Exp II). The reference preparation was monkey pituitary gonadotropin LER 1909-2. FSH levels in castrate animals were determined by RIA with the method of Hodgen *et al.* (14). The reference preparation was monkey pituitary gonadotropin cyn-FSH-RP1. Human FSH was iodinated by the method of Greenwood *et al.* (15), and the primary antiserum was antiovine FSH (H-31), prepared in rabbits and used at a final dilution of 1:150,000. The interassay CV was 10% at a binding of 33%, and the sensitivity of the assay was 12.5 ng/ml. All samples from an individual animal were analyzed at the same time.

Data analysis

The amount of hormone present in the plasma at each postinjection time point was compared to the preinjection

control value for a particular animal, and the percentage of the control value was calculated. One-way analysis of variance was performed on log-normalized data and used in conjunction with a Student's one-tailed t test to identify significant ($P \leq 0.05$) decreases in hormone levels over time.

Results

Exp I: castrate animals

Plasma LH levels were markedly reduced by 4 h after a single injection of 5, 50, 250, or 500 μg/kg BW GnRH-Ant. LH levels reached nadir values by 8 h after administration of 250 or 500 μg/kg BW doses ($P \leq 0.05$), remained at these values for 24 h, and had returned to 85% of the preinjection values by 48 h (Fig. 1). LH levels were reduced ($P \leq 0.01$) for 8 h by 5 and 50 μg/kg BW GnRH-Ant and returned to 98% or more of control values by 24 h after injection. Vehicle injections did not result in significant suppression of LH at any time point.

Plasma FSH levels were reduced ($P \leq 0.5$) by 4 h after administration of each dose of antagonist (Fig. 2). Injections of 250 or 500 μg/kg BW GnRH-Ant maximally suppressed FSH levels for 24 h ($P \leq 0.01$), and 5 or 50 μg/kg BW GnRH-Ant suppressed FSH maximally for 8 h ($P \leq 0.01$). The maximal degree of FSH suppression was comparable to that of LH at each dose, but FSH levels recovered more slowly than did LH levels after administration of 50, 250, and 500 μg/kg BW GnRH-Ant. FSH levels were not significantly suppressed at any time after vehicle administration.

Exp I: intact animals

Plasma testosterone levels declined markedly ($P \leq 0.05$) 6 h after administration of 50, 250, or 500 μg/kg BW GnRH-Ant (Fig. 3). The two higher doses sustained this inhibition of testosterone to levels that were less than 30% of preinjection values at 24 h ($P \leq 0.05$). Neither vehicle nor 5 μg/kg BW GnRH-Ant significantly reduced plasma testosterone levels.

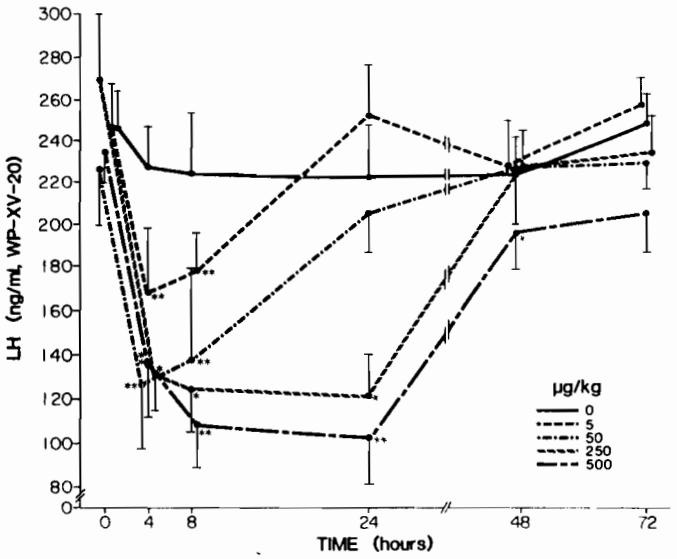

FIG. 1. Mean (±SEM) plasma LH levels in castrate animals before (0 h) and after iv injection of GnRH-Ant. *, $P \leq 0.05$; **, $P \leq 0.01$ (for GnRH-Ant treatment *vs.* preinjection value).

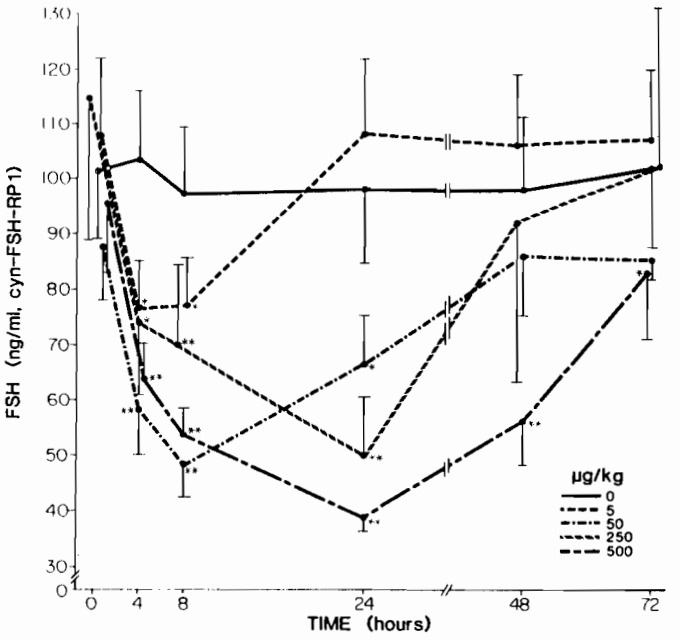

FIG. 2. Mean (±SEM) plasma FSH levels in castrate animals before (0 h) and after iv injection of GnRH-Ant. *, $P \leq 0.05$; **, $P \leq 0.01$ (for GnRH-Ant treatment *vs.* preinjection value).

Plasma LH levels decreased 6 h after administration of each dose of the antagonist (Fig. 4). Maximal inhibition was evident 24 h after administration of 250 or 500 μg/kg BW antagonist ($P \leq 0.05$), and doses of 5 and 50 μg/kg BW maintained a slight inhibition of LH levels for this period of time. In animals receiving vehicle, LH levels were elevated at 6 and 24 h, presumably because the samples were collected during an endogenously stimulated episode of LH release (31). Loss of a number of samples due to a laboratory accident precluded analysis of FSH values in intact animals.

Exp II

Plasma testosterone decreased to near-castrate levels 24 h after administration of 250 μg/kg BW GnRH-Ant ($P \leq 0.01$), and this suppression was sustained for the ensuing 20 days (Fig. 5). Daily injections of 0, 50, or 100 μg/kg BW GnRH-Ant did not suppress testosterone either acutely (24 h) or chronically (3 weeks).

Plasma LH levels were below the limit of detectability (0.6 μg/ml; samples run at a 10-μl volume) of the bioassay by 24 h after administration of 250 μg/kg BW GnRH-Ant and were also undetectable in samples obtained after 21 days of treatment (Fig. 6). Daily injections of vehicle did not lower plasma LH levels at either 24 h or 3 weeks.

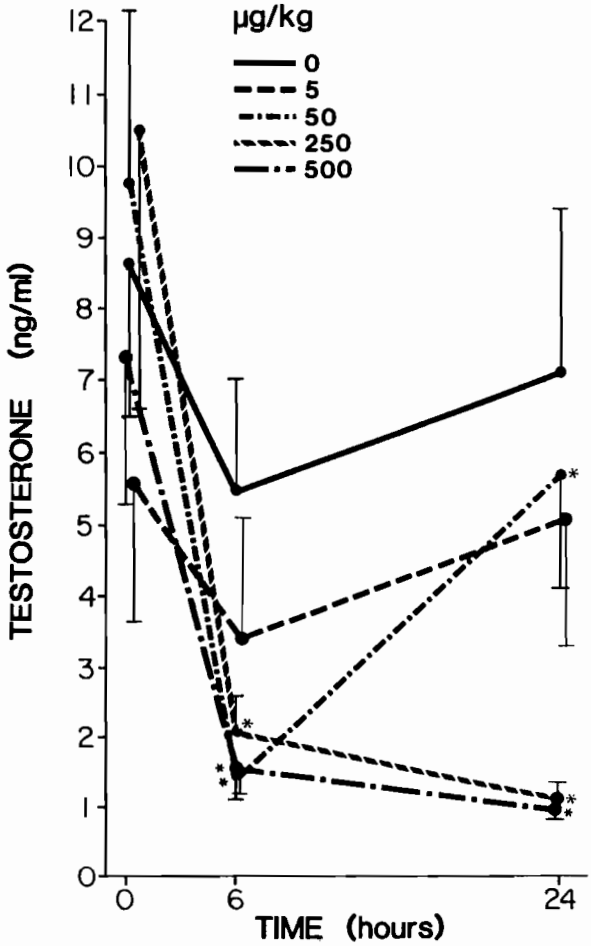

FIG. 3. Mean (±SEM) plasma testosterone levels in intact animals before (0 h) and after sc injection of GnRH-Ant. *, $P \leq 0.05$ (for GnRH-Ant treatment *vs.* preinjection value).

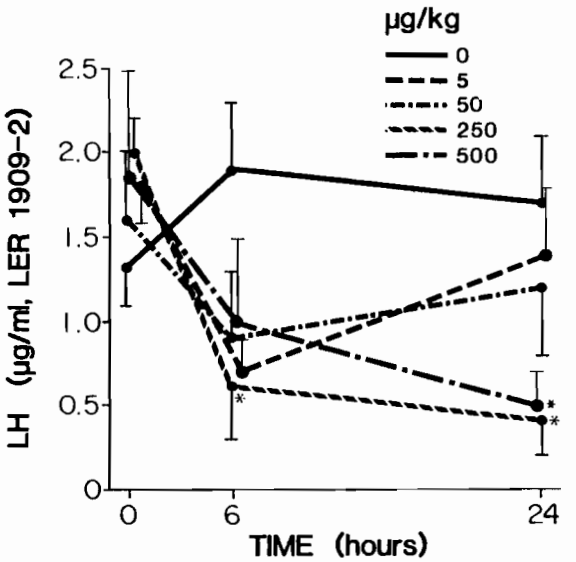

FIG. 4. Mean (±SEM) plasma LH levels in intact animals before (0 h) and after sc injection of GnRH-Ant. *, $P \leq 0.05$ (for GnRH-Ant treatment *vs.* preinjection value).

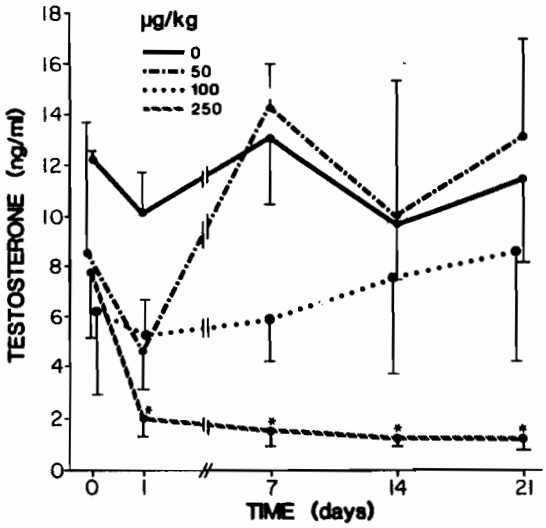

FIG. 5. Mean (±SEM) plasma testosterone levels in intact animals receiving daily sc injections of GnRH-Ant for 21 days. *, $P \leq 0.05$ (for GnRH-Ant treatment *vs.* preinjection value).

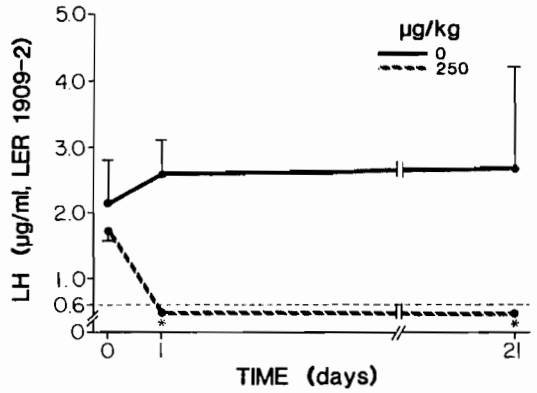

FIG. 6. Mean (±SEM) plasma LH levels in intact animals receiving daily sc injections of 0 or 250 µg/kg BW GnRH-Ant for 21 days. The *horizontal dashed line* represents the lower limit of detectability of the assay. *, $P \leq 0.05$ (for GnRH-Ant treatment *vs.* preinjection value).

Discussion

In the present study we evaluated the response of the reproductive axis to GnRH-Ant administration in both intact and castrate primates. The advantages of studying this compound in the castrate model include the fact that the pituitary (16) and hypothalamus (17) are released from inhibitory negative feedback effects normally exerted upon them by testicular hormones. This allows demonstration of the time course and degree of action of the antagonist specifically on pituitary function, without the confounding factors of changing gonadal feedback and possible direct gonadal effects of the peptide. Removal of the suppressive influence of testicular hormones results in circulating levels of gonadotropins an order of magnitude higher than those in intact animals (17) and increases the likelihood that antagonist-reduced gonadotropin levels will fall within the range of detectability of currently available assays for monkey gonadotropins.

A single injection of this potent GnRH antagonist produced a marked dose-related reduction of plasma LH and FSH levels in castrate animals within 4 h and sustained this effect for at least 24 h. FSH levels returned to preinjection values more slowly than did LH levels; although the mechanism whereby LH and FSH may be differentially sensitive to suppression is not clear, our observations are consistent with those made in ovariectomized monkeys that LH levels recovered from suppression by an antagonist 24 h earlier than did FSH levels (18).

A single injection of this compound reduced testosterone and LH levels in a dose-related manner for 24 h in intact animals, and daily injections of the compound for 21 days maintained suppression of LH and testosterone for that period of time. The fact that the degree of testosterone suppression effected by this compound was identical at 24 h and 21 days suggests that this primate system does not become refractory to the effects of the antagonist over a period of weeks.

Other workers tested this antagonist in castrate *Macaca fascicularis* and found that single injections of 200, 500, or 1000 μg/kg BW reduced plasma LH and FSH levels by 12 h and maximally suppressed both gonadotropins by 24 h after administration, and continuous administration of the compound to intact male monkeys for a period of months resulted in severe oligospermia or azoospermia (19). Although LH levels remained low for a longer period of time after single dose administration of 1000 μg/kg BW antagonist (96 h) than after 200 or 500 μg/kg BW antagonist (48–72 h), the maximal degree of LH suppression was the same for all three doses. Our data also indicate that 250 and 500 μg/kg BW doses suppress LH to the same extent; these observations identify the lowest dose of antagonist that maintains LH suppression when administered once daily. Other inhibitory analogs of GnRH also suppress gonadotropin secretion in monkeys; however, they differ in potency, which may in part reflect species differences in sensitivity. For example, administration of 200 μg/kg BW of the GnRH antagonist [Ac-Δ^3-Pro^1, *p*FDPhe^2, D-Trp^{3,6}]GnRH to castrate male rhesus monkeys reduced plasma LH, but had no effect on FSH levels (6). A similar dose (250 μg/kg BW) of the antagonist used in the present study reduced both gonadotropins for 48 h and had a more profound suppressive effect on LH levels. A single injection of 5 μg/kg BW of the GnRH antagonist [*N*-Ac-D-*p*-Cl-Phe^{1,2}, D-Trp^3, D-Arg^6, D-Ala^{10}]GnRH to intact male rhesus monkeys promptly reduced serum LH and testosterone levels (8) and maintained inhibition of testosterone for 24 h. In our animals, testosterone levels had returned to preinjection values 24 h after administration of 5 μg/kg BW antagonist.

Historically, the development of GnRH antagonists for use as antifertility and antitumor agents has been hampered by the low biological potency of these compounds (1, 20). In this regard, high potency GnRH agonists have offered more clinical promise. Whereas acute administration of these agonists results in an initial elevation of plasma gonadotropin and testosterone levels, with chronic treatment they paradoxically inhibit the reproductive axis in monkeys (21) and men (22). The mechanism of this suppressive effect is presumably down-regulation of pituitary GnRH receptors (23). Thus, although GnRH agonists have been used with success in treating men with prostatic cancer (24), the initial elevation of gonadotropins and testosterone resulting from agonist treatment can have serious clinical consequences, exacerbating some of the symptoms of the disease. The undesirable side-effects of agonist treatment increase the need for the development of clinically useful antagonist analogs (25).

GnRH antagonists exert their effect by competitively binding to pituitary GnRH receptors without eliciting a secretory response from the gonadotrope (20, 26). Antagonist binding does not appear to result in either receptor down-regulation or desensitization (20), and its suppressive effect on gonadotropin secretion is terminated with its dissociation from the receptor or degradation by membrane-bound peptidases (27). In some species, GnRH antagonists act on other glandular sites in addition to the pituitary. In the male rat, for example, suppression of testosterone (4) is most likely mediated by antagonist binding to both pituitary and testicular (28) receptors. There is no evidence supporting the presence of gonadal GnRH receptors in either monkeys (29) or man (30), implying that suppression of pituitary LH release is responsible for any decreases in testosterone effected by antagonists in these primate species.

The results of this and other studies demonstrate the ability of antagonist analogs of GnRH to suppress gonadotropin and testosterone levels for a period of days or weeks. The immediate decline of reproductive hormone levels after antagonist administration circumvents the problems associated with agonist treatment and supports the use of these compounds as therapeutic agents in clinical situations requiring long term suppression of gonadal function.

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