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Pharmacological Profile of a New, Potent, and Long-Acting Gonadotropin-Releasing Hormone Antagonist: Degarelix

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ABSTRACT

We describe the pharmacological profile in rats and monkeys of degarelix (FE200486), a member of a new class of long-acting gonadotropin-releasing hormone (GnRH) antagonists. At single subcutaneous injections of 0.3 to 10 $\mu\text{g}/\text{kg}$ in rats, degarelix produced a dose-dependent suppression of the pituitary-gonadal axis as revealed by the decrease in plasma luteinizing hormone (LH) and testosterone levels. Duration of LH suppression increased with the dose: in the rat, significant suppression of LH lasted 1, 2, and 7 days after a single subcutaneous injection of degarelix at 12.5, 50, or 200 $\mu\text{g}/\text{kg}$, respectively. Degarelix fully suppressed plasma LH and testosterone levels in the castrated and intact rats as well as in the ovariectomized rhesus monkey for more than 40 days after a single 2-mg/kg

subcutaneous injection. In comparative experiments, degarelix showed a longer duration of action than the recently developed GnRH antagonists abarelix, ganirelix, cetrorelix, and azaline B. The *in vivo* mechanism of action of degarelix was consistent with competitive antagonism, and the prolonged action of degarelix was paralleled by continued presence of radioimmunoassayable degarelix in the general circulation. In contrast to cetrorelix and similarly to ganirelix and abarelix, degarelix had only weak histamine-releasing properties *in vitro*. These results demonstrate that the unique and favorable pharmacological properties of degarelix make it an ideal candidate for the management of sex steroid-dependent pathologies requiring long-term inhibition of the gonadotropic axis.

The pulsatile secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus and its binding to membrane GnRH receptors located on the cell surface of the pituitary gonadotropes lead to release of gonadotropins. Subsequently, these hormones, luteinizing hormone (LH) and follicle-stimulating hormone, stimulate steroidogenesis and regulate gametogenesis through activation of cognate receptors in the gonads.

Blockade of the GnRH receptor by GnRH antagonists produces a rapid and effective suppression of gonadotropin release and therefore gonadal steroids secretion in human males (Jockenhövel et al., 1988; Salameh et al., 1991; Bagatell et al., 1993, 1995; Klingmüller et al., 1993). Therefore, such antagonists have been recognized as potential drugs for the management of sex steroid-dependent pathologies, such as prostate cancer and endometriosis. These conditions are currently managed with long-acting preparations of GnRH superagonists that suppress sex steroids through desensitization of the pituitary-gonadal axis (Conn and Crowley, 1991). Despite the advantages of GnRH antagonists over agonists in suppressing serum gonadal steroids, many of the peptidic GnRH antagonists investigated so far show histamine-releasing properties

and/or solubility limitations that affect their clinical usefulness or even preclude their development as drugs (Bagatell et al., 1993, 1995; Hutchison et al., 1999).

We have developed a new series of potent and long-acting competitive antagonists for the GnRH receptor of the general formula, Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph/Amf (P)-D-4Aph/Amf (Q)-Leu-Ilys-Pro-D-Ala-NH₂, showing high water solubility and low histamine-releasing properties (Jiang et al., 2001). The aim of the present study was to characterize the *in vitro* and *in vivo* pharmacological profile of a selected representative of this series, degarelix (FE200486: Ac-D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph (L-hydroxyrotyl)-D-4Aph (carbamoyl)-Leu-Ilys-Pro-D-Ala-NH₂) in comparison with other recently developed GnRH antagonists such as abarelix (Cook and Sheridan, 2000), ganirelix (Gillies et al., 2000), cetrorelix (Reissmann et al., 2000), and azaline B (Rivier et al., 1995b).

Materials and Methods

Animals. Male Sprague-Dawley rats, intact or castrated, were purchased from Iffa-Credo (L'Arbresle, France) at 6 to 8 weeks of age and housed in a temperature-, humidity-, and light-controlled room

ABBREVIATIONS: GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; Aph, aminophenylalanine; Amf, aminomethylphenylalanine; RIA, radioimmunoassay; ANOVA, analysis of variance.

and were given free access to food and water. Experimental protocols concerning the use of laboratory animals were reviewed by the University of Geneva School of Medicine Ethical Committee for Animal Experimentation and approved by the State of Geneva Veterinary Office. Ovariectomized female rhesus monkeys 8 to 10 years of age were housed in individual cages at the Oregon Regional Primate Research Center. The animals were fed Purina Lab Diet (high-protein monkey diet #5047; Purina, St. Louis, MO) and received approximately 125 to 175 g twice daily at around 8:00 AM and 3:00 PM and fruit two or three times per week. Animals had free access to water. Experimental protocols concerning the use of laboratory animals followed the guidelines for animal experimentation in Oregon Regional Primate Research Center.

Experimental Procedure. Rats weighed 200 to 300 g at the initiation of the study. Antagonists were injected either subcutaneously in the scapular region or intravenously using a jugular catheter. After jugular catheter implantation, rats were allowed to recover for at least 24 h in individual cages with food and water available ad libitum. Blood sampling (200–250 μ l) was performed through the jugular catheter or at the tail tip and blood was collected in heparinized tubes (30 IU/ml). Plasma was extracted by centrifugation at 3000 rpm for 10 min then stored at -20°C until determination of LH or testosterone.

Rhesus monkeys had a range of weight during study of 5.0 to 6.7 kg. Antagonists were injected subcutaneously in the right hip. Blood samples of 2.5 ml were collected from the saphenous vein, into unheparinized Vacutainer tubes, placed on wet ice immediately after collection, and then refrigerated for storage and separation. The samples were spun the next day in a refrigerated centrifuge at 8°C at 2500 rpm for 15 min.

Determination of LH Levels. LH levels in plasma samples were determined by standard RIA techniques with reagents prepared by Dr. A. F. Parlow (Pituitary Hormone and Antisera Center, Harbor-UCLA Medical Center, Torrance, CA) and provided by the National Institute of Diabetes and Digestive and Kidney Diseases (Bethesda, MD), and a commercially obtained secondary antiserum. National Institute of Diabetes and Digestive and Kidney Diseases anti-rat LH S11 sera were used. Values are expressed in terms of RP-3 reference standard. For each experiment, all plasma samples (vehicle control and tested antagonists) were measured in the same RIA. Monkey serum LH was determined by RIA with standard LH, LH antisera, and iodination grade LH obtained from Dr. A. F. Parlow.

Determination of Plasma Testosterone Levels. Rat plasma testosterone was determined using an RIA kit purchased from Diagnostic System Laboratories (Webster, TX).

Determination of Serum Degarelix Levels. Serum degarelix was determined by standard RIA techniques with antibodies raised in rabbits, highly specific for degarelix, and nonreactive with various natural hormones, including GnRH (Woods Assay, Portland, OR).

Histamine Release from Rat Peritoneal Mast Cells. The effects of GnRH antagonists on histamine release from rat peritoneal mast cells was assayed using the procedure described by Hakanson et al. (1972). Briefly, mast cells were isolated from rat abdominal cavity, suspended in heparinized balanced salt solution with bovine albumin, pH 7.2, and then purified on a Percoll gradient. Mast cells were incubated at 37°C for 2 min with the GnRH antagonists and their vehicle. After the incubation, the samples were clarified by centrifugation, and extracellular (supernatant) and intracellular (lysed mast cell pellet) histamine was determined by spectrofluorometric measurement. The ability of a compound to induce histamine release is given by the following formula: activity (%) = $100 \times (\text{CIHR}/\text{corr TH})$, where compound-induced histamine release (CIHR) = extracellular histamine – spontaneous histamine release (not compound-related) and corrected total histamine (corr TH) = total histamine – spontaneous histamine release.

EC_{50} values (concentration causing a half-maximal stimulation) were determined by nonlinear regression analysis of the concentra-

tion-response curves. These parameters were obtained by Hill equation curve fitting.

Drugs. Degarelix and the reference GnRH antagonists abarelix, azaline B, cetrorelix, ganirelix, and Nal-Glu were synthesized at Ferring Research Inc. (San Diego, CA) according to published protocols. For in vivo studies, all antagonists were dissolved in a 5% mannitol solution. For in vitro studies, all antagonists were dissolved in water (histamine release assay).

Statistical Analysis. In the rat studies, untransformed hormone data were analyzed by Kruskal-Wallis one-way ANOVA on ranks or by a two-way repeated measures ANOVA (comparison between duration of action after subcutaneous or intravenous administration). After a significant ANOVA, statistical analysis continued by either Student-Newman-Keuls or Dunn's multiple comparison procedures. For the dose-response studies with degarelix and abarelix on testosterone, a Dunnett's multiple comparison procedure versus vehicle mean was performed. Differences between organ weights were measured by the Student's *t* test or by the Mann-Whitney rank sum test when normality test failed. In the monkey studies, untransformed hormone data were analyzed by one-way repeated measures analysis of variance followed by Tukey's multiple comparisons versus pretreatment mean, or when normality or equal variance test failed by Friedman's repeated measures analysis of variance on ranks followed by Dunnett's multiple comparison procedure versus pretreatment mean. Differences were considered statistically significant when *p* was smaller than 0.05. All statistics were performed using SigmaStat 2.0 software (Jandel Scientific, San Rafael, CA).

Results

Suppression of Plasma LH Levels in Castrated Male Rat by Degarelix. When injected s.c. at doses ranging from 0.3 to 10 $\mu\text{g}/\text{kg}$, degarelix produced a dose-dependent and reversible decrease in plasma LH levels with a minimal effective dose of 3 $\mu\text{g}/\text{kg}$. At 10 $\mu\text{g}/\text{kg}$, degarelix produced a significant suppression of plasma LH up to 12 h postinjection (Fig. 1).

Comparison of Duration of Action of Degarelix and Abarelix after Subcutaneous Administration in Castrated Rat. Degarelix and abarelix were given at doses of 12.5, 50, and 200 $\mu\text{g}/\text{kg}$, and plasma samples were collected for the next 7 days (Fig. 2). The results obtained with degarelix indicated that for all doses, onset of action and effi-

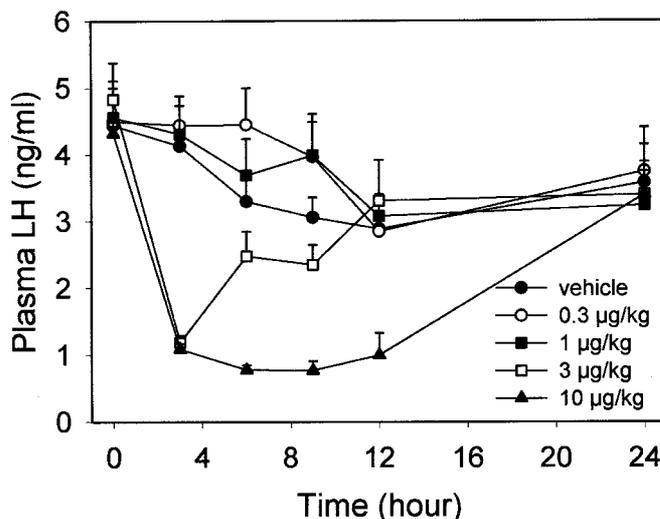


Fig. 1. Effects on plasma LH levels in the castrated rat of degarelix administered at 0.3 to 10 $\mu\text{g}/\text{kg}$ s.c. in 5% mannitol (0.4 ml/kg). Results are mean ($n = 5-8$) \pm S.E.M.

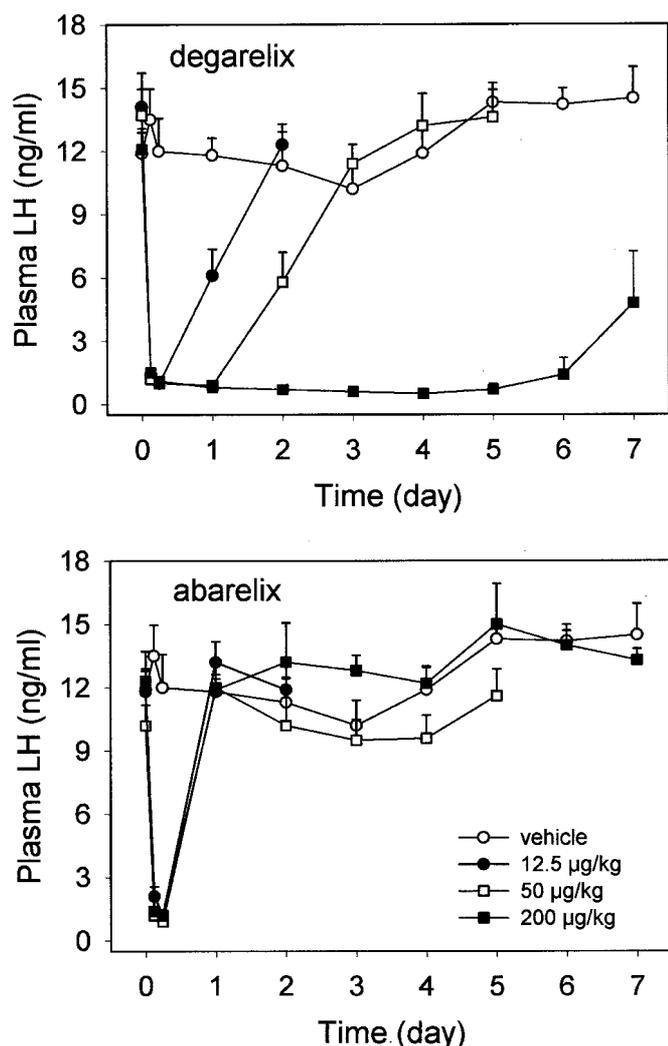


Fig. 2. Duration of LH suppression induced in the castrated rat by degarelix (top) and abarelix (bottom) administered at 12.5, 50, and 200 µg/kg s.c. in 5% mannitol (0.4 ml/kg). Results are mean ($n = 6$) \pm S.E.M.

cacy were identical. Significant suppression of LH was achieved within 3 h, maximal suppression was achieved within 6 h, and at the latter time point there was no difference in plasma LH between the three treated groups: plasma LH levels were 12.0 ± 1.57 , 1.0 ± 0.04 , 1.0 ± 0.11 , and 1.1 ± 0.11 ng/ml in rats injected with vehicle or degarelix at 12.5, 50, and 200 µg/kg, respectively. The duration of LH suppression increased with the dose: plasma LH levels in the degarelix-treated rats were significantly different from vehicle-injected rats up to 24 h, 48 h, and day 7 post-treatment for the doses of 12.5, 50, and 200 µg/kg, respectively ($p < 0.05$ for all doses versus the vehicle group). Similarly to degarelix, abarelix produced a significant suppression of LH at hour 3 and a maximal suppression at hour 6 post-treatment. At this latter time point, there was no difference in efficacy between the three doses of abarelix: plasma LH levels were 12.0 ± 1.57 , 1.2 ± 0.18 , 0.9 ± 0.04 , and 1.2 ± 0.15 ng/ml in rats injected with vehicle or abarelix at 12.5, 50, and 200 µg/kg, respectively ($p < 0.05$ for all doses versus the vehicle group). The duration of LH suppression by abarelix did not increase with the dose: at all doses examined, the plasma LH levels returned to control values by 24 h after injection. The dura-

tion of LH suppression by the 12.5-, 50-, and 200-µg/kg doses of degarelix were significantly longer than the duration of LH suppression evoked by the same doses of abarelix.

In a parallel study, measurements of plasma degarelix levels indicated that for the 50- and 200-µg/kg doses, $t_{1/2}$ of absorption values were 4 and 30 min, T_{max} values were 1 and 5 h, and apparent plasma disappearance $t_{1/2}$ values were 12 and 67 h, respectively.

Comparison of Subcutaneous and Intravenous Administration in Castrated Rat. The duration of LH suppression induced by abarelix, cetrorelix, azaline B, and degarelix after s.c. and i.v. injections at 200 µg/kg was compared. For both routes of administration, abarelix suppressed LH for 12 h. In contrast, significant differences in the duration of LH suppression produced by i.v. and s.c. administration were observed after injections of cetrorelix, azaline B, and degarelix. After i.v. injections, azaline B suppressed LH for 24 h, whereas cetrorelix and degarelix did so for 12 h. After s.c. injections, maximal LH suppression was maintained for 2, 3, and 6 days for cetrorelix, azaline B, and degarelix, respectively (Fig. 3).

Duration of Action of Degarelix at 2 mg/kg s.c. in Castrated Rat: Comparison with Azaline B. To investigate the efficacy of a high dose and concentration of degarelix, castrated male rats were treated with 2 mg/kg degarelix prepared in 5% mannitol and injected at a volume of 20 µl/rat, yielding a mean concentration of 29 mg/ml. The same dosing procedure was used for azaline B and the duration of LH suppression in response to these two antagonists was compared. Both compounds had similar onset of action and efficacy (Fig. 4): plasma LH levels at 6 h post-treatment were 11.2 ± 1.9 , 1.5 ± 0.08 , and 1.5 ± 0.20 ng/ml for the vehicle-, azaline B-, and degarelix-treated groups, respectively ($p < 0.05$ for both antagonists versus the vehicle group). The two compounds differed in their duration of action: azaline B suppressed LH up to day 14 post-treatment (at this time point plasma LH levels were 13.1 ± 1.3 , 0.29 ± 0.06 , and 0.19 ± 0.02 ng/ml for vehicle, azaline B, or de-

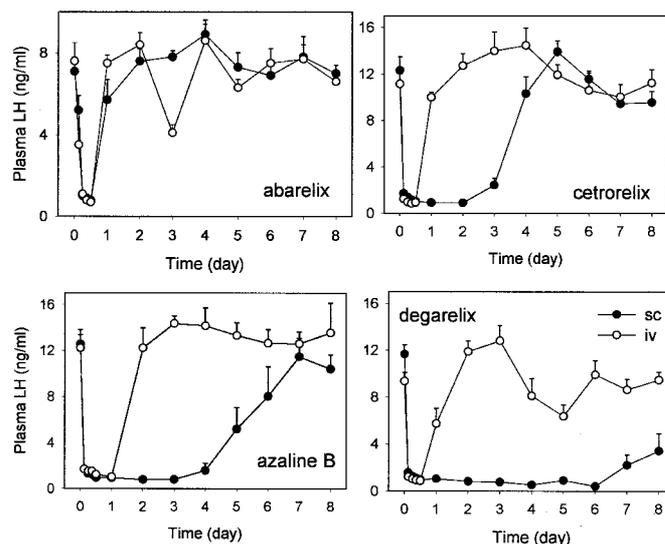


Fig. 3. Duration of LH suppression induced in the castrated rat by degarelix, abarelix, azaline B, and cetrorelix administered at 200 µg/kg s.c. or i.v. in 5% mannitol (0.4 ml/kg). Results are mean ($n = 5-6$) \pm S.E.M.

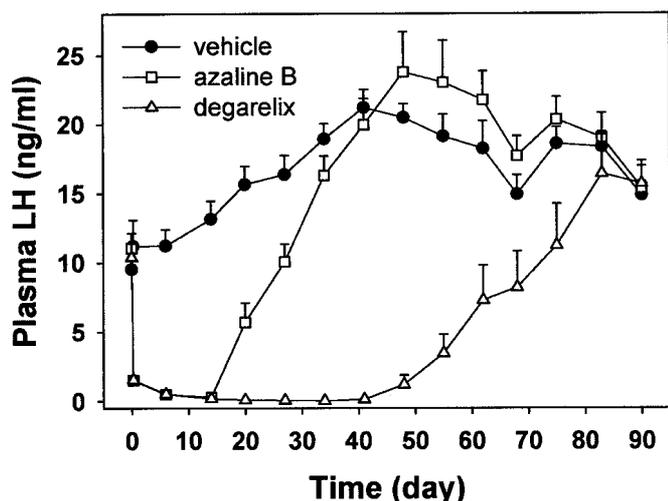


Fig. 4. Duration of LH suppression induced in the castrated rat by degarelix and azaline B administered at 2 mg/kg s.c. in 5% mannitol (20 μ l/animal). Results are mean ($n = 6-7$) \pm S.E.M.

garelix, respectively; $p < 0.05$ for both antagonists versus the vehicle group), whereas degarelix maintained significant suppression of LH up to day 55 post-treatment (at this time point plasma LH levels were 19.1 ± 1.5 , 23.0 ± 3.0 , and 3.4 ± 1.3 ng/ml for vehicle, azaline B, or degarelix, respectively; $p < 0.05$ for both degarelix versus the vehicle and versus the azaline B-treated groups). The suppression of LH induced by both compounds was reversible. Measurements of plasma degarelix levels indicated that absorption half-life value was 2 min, T_{max} value was 6 h, and apparent plasma disappearance $t_{1/2}$ value was 214 h, i.e., approximately 9 days.

Suppression of Plasma Testosterone Levels in Intact Male Rat by Degarelix in Comparison with Abarelix.

The potency of degarelix and abarelix in suppressing testosterone was compared after s.c. injections. Plasma testosterone levels were measured 6 h after injections. At doses ranging from 0.3 to 10 μ g/kg, degarelix produced a dose-dependent decrease in plasma testosterone levels with a minimal effective dose of 1 μ g/kg. Within the same range of doses, abarelix also produced a dose-dependent decrease in plasma testosterone with a minimal effective dose of 3 μ g/kg (Fig. 5).

Efficacy and Duration of Action of 2 mg/kg s.c. Degarelix in Intact Male Rat in Comparison with Azaline B, Ganirelix, Abarelix, and Surgical Castration.

Intact male rats were treated with a dose of 2 mg/kg degarelix prepared in 5% mannitol and injected at a volume of 20 μ l/rat. The same dosing procedure was applied for azaline B, ganirelix, and abarelix. Additionally, a group of rats was surgically castrated at day 0 to provide comparison with castrated levels of testosterone. At day 1 postinjection all antagonists suppressed plasma testosterone levels with similar efficacy: testosterone levels were 4431 ± 1546 , 61 ± 8 , 51 ± 9 , 83 ± 10 , and 51 ± 8 pg/ml in the vehicle-, degarelix-, azaline B-, ganirelix-, and abarelix-treated groups, respectively ($p < 0.05$ for all antagonists versus vehicle-treated group). At this time point, plasma testosterone levels in surgically castrated rats were 3.6 ± 0.4 pg/ml ($p < 0.05$ versus vehicle and versus antagonists-treated groups). At day 7 post-treatment, testosterone levels in rats treated with degarelix or surgically castrated were not significantly different (4523 ± 1284 , 8 ± 2 , and 3.7 ± 1.3 pg/ml in the vehicle,

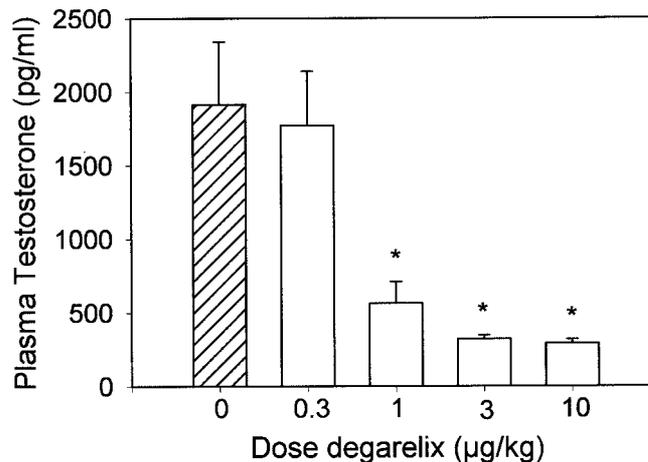
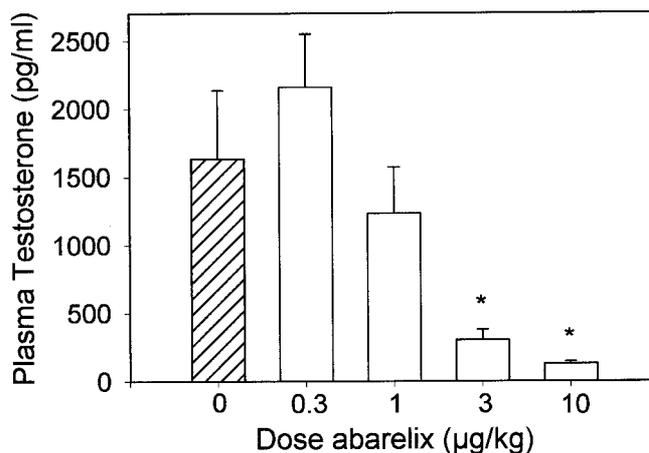


Fig. 5. Plasma testosterone levels in the intact rat 6 h after injection of degarelix or abarelix administered at 0.3 to 10 μ g/kg s.c. in 5% mannitol (0.4 ml/kg). Results are mean ($n = 8$) \pm S.E.M.

degarelix, and castrated groups, respectively), testosterone levels in the abarelix- and ganirelix-treated rats (2038 ± 475 and 2310 ± 462 pg/ml, respectively) were already returning to control values ($p > 0.05$ versus control level), and testosterone levels in rats treated with azaline B (539 ± 254 pg/ml) were significantly lower than control levels yet significantly higher than measured in castrated animals. Azaline B maintained below-normal levels of testosterone up to day 14, whereas only degarelix was capable of suppressing testosterone to castrated levels for an extended period, up to day 42, after which time testosterone increased gradually to reach normal values by day 70 to 83 (Fig. 6).

Effects of Degarelix on Sex Steroid-Dependent Organs in Rat. Intact male rats received a 2-mg/kg single s.c. injection of degarelix; 45 and 102 days later, prostate, seminal vesicles, and testes were weighed and compared with vehicle-injected controls. As shown in Table 1, 45 days after injection an 88, 95, and 86% reduction in prostate, seminal vesicles, and testes weight, respectively, was observed after treatment with degarelix. At 102 days after injection, prostate and seminal vesicles weight in the degarelix-treated group was still significantly reduced by 35 and 29%, respectively, whereas testes weight had returned to control values.

Functional Antagonism of Rat Pituitary-Gonadal Axis by Degarelix. To further explore the mechanism of action of degarelix in vivo, two groups of intact rats received

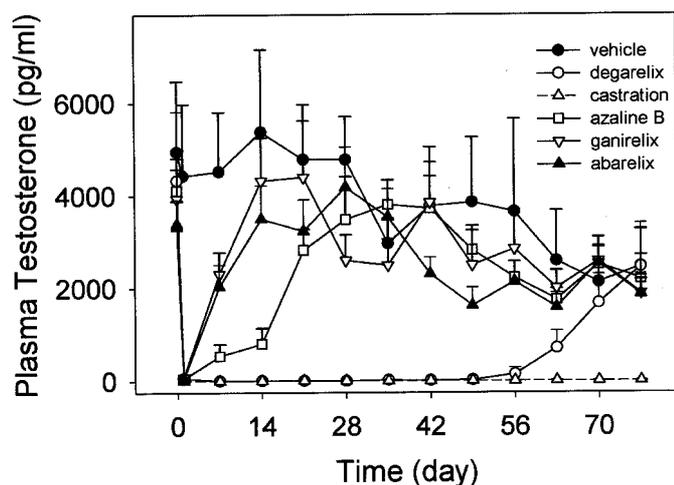


Fig. 6. Efficacy and duration of testosterone suppression induced in the intact rat by degarelix, abarelix, azaline B, and ganirelix administered at 2 mg/kg s.c. in 5% mannitol (20 μ l/animal), in comparison with surgical castration. Results are mean ($n = 8$) \pm S.E.M.

either a single s.c. injection of 2 mg/kg degarelix or vehicle. At days 7, 15, and 42 postinjection, independent groups of rats were injected intravenously with increasing doses of GnRH. The LH and testosterone responses to GnRH in rats pretreated with vehicle or degarelix are shown in Fig. 7. At days 7, 15, and 42 post-treatment, GnRH stimulated a dose-dependent increase in plasma LH levels in both groups. Dose-response curves in rats pretreated with degarelix were shifted rightward compared with those obtained in vehicle-pretreated rats. In the latter group, plasma testosterone also increased as a function of the dose of GnRH. However, in rats pretreated with degarelix, a significant testosterone response to GnRH was observed at day 7 only.

Pharmacodynamics and Serum Levels of Degarelix in Ovariectomized Rhesus Monkey. At the tested doses of 0.045, 0.2, and 2 mg/kg s.c., degarelix decreased serum LH levels in the ovariectomized monkey. When compared with pretreatment values, significant suppression of LH lasted 2, 7, or 79 days after injection of the 0.045-, 0.2-, or 2-mg/kg dose of degarelix, respectively (Fig. 8). Serum levels of FE200486 increased with the dose. After a 0.045- or 0.2-mg/kg injection, degarelix is rapidly absorbed; serum levels of degarelix peaked at 1 or 3 h postinjection to reach concentrations of 39 or 80 ng/ml, respectively. Apparent serum disappearance half-life was estimated to be 80 and 193 h for the 0.045- or 0.2-mg/kg dose. Injected at 2 mg/kg, degarelix is less rapidly absorbed: serum degarelix levels peaked at 24 h postinjection to reach a concentration of 249 ng/ml. Afterward, serum concentrations of degarelix declined gradually over time to reach 1.6 ng/ml at day 41 (where maximal suppression of LH was still observable) and less than 0.1 ng/ml at day 101, the time of complete recovery.

Histamine-Releasing Activity of Degarelix in Comparison with Other GnRH Antagonists. All antagonists assayed produced a concentration-dependent increase in histamine release from rat peritoneal mast cells (Fig. 9). The relative order of potency in stimulating histamine release was Nal-Glu ($EC_{50} = 0.5$ μ g/ml) > cetrorelix ($EC_{50} = 1.3$ μ g/ml) > ganirelix ($EC_{50} = 11$ μ g/ml) > azaline B ($EC_{50} = 19$ μ g/ml) > abarelix ($EC_{50} = 100$ μ g/ml) > degarelix ($EC_{50} = 170$ μ g/ml).

Discussion

Our interest in developing GnRH antagonists was stimulated by the need for improved therapeutics for the management of sex steroid-dependent pathologies such as uterine leiomyoma, endometriosis, and prostate and gynecological cancers. Such pathologies are currently managed with long-acting (1 or 3 months) formulations of GnRH agonists that initially stimulate pituitary LH and follicle-stimulating hormone release as well as gonadal steroids, and then, after 2 to 4 weeks, desensitize the gonadotrophs, leading to suppression of gonadotropin and sex steroids. However, in clinical situations where an immediate suppression of the gonadotropins is required, the major disadvantage of agonists is their initial stimulatory effect on hormone release that may lead to a transient flare-up of the disease (Kahan et al., 1984). In this context, competitive GnRH antagonists are expected to have significant clinical advantages on the basis of the avoidance of this initial stimulation of gonadotropin release and a faster onset of action (Balmaceda et al., 1983; Cetel et al., 1983). However, incorporation of sufficient quantities of antagonist into a formulation that would allow a slow release of the molecule at a concentration sufficient to antagonize the effects of endogenous GnRH for an extended period (e.g., 1 month or more) has been and remains a technical challenge. We designed new antagonists for the GnRH receptor with the expectations that they would be longer acting and would not need to rely on slow-release formulations to maintain suppression of the pituitary-gonadal axis during at least a month. The present studies report on the activity in both intact and castrated animal models of a new competitive GnRH antagonist, degarelix, alone and in comparison with other GnRH antagonists that had experienced clinical development: abarelix, azaline B, cetrorelix, and ganirelix. The data presented in the current study show that degarelix has a unique property of long duration of action when formulated in a vehicle as simple as mannitol and displays a good safety margin with regard to histamine-releasing properties.

In castrated and intact rats, the minimal dose of degarelix required to suppress plasma LH or testosterone levels appears to lie between 1 and 3 μ g/kg. Abarelix was found to decrease plasma testosterone *in vivo* with comparable potency to degarelix, consistent with their similar *in vitro* potency at the GnRH receptor (Jiang et al., 2001). When the durations of LH suppression induced by abarelix and degarelix were compared, degarelix, but not abarelix, exhibited a dose-dependent increase in duration of action. Increasing the dose of degarelix from 12.5 to 200 μ g/kg did not further enhance the efficacy of the compound but increased the duration of LH suppression from 1 to 7 days. These data suggest that duration of action is not so much dependent on potency per se but instead could be due to unique pharmacokinetic properties: slow absorption from the subcutaneous site of injection, binding to plasma proteins, and decreased plasma clearance and/or enzymatic stability. For example, the GnRH antagonist antide has significant binding to serum proteins (Danforth et al., 1990), which has been used to explain its long circulatory half-life and long duration of LH suppression after intravenous or subcutaneous administration. On the other hand, *in situ* depot formation after subcutaneous administration and slow release from this depot have been suggested to occur with several GnRH antagonists (Chan et

TABLE 1

Effects of degarelix (2 mg/kg s.c. in 5% mannitol, 20 μ l/animal) on prostate, seminal vesicles, and testes weight (mg)

Treatment	Day 45 Post-Treatment			Day 102 Post-Treatment		
	Prostate	Seminal Vesicles	Testes	Prostate	Seminal Vesicles	Testes
Vehicle	510 \pm 30	1280 \pm 60	3620 \pm 110	779 \pm 97	1684 \pm 55	3830 \pm 120
Degarelix	60 \pm 19**	54 \pm 2**	510 \pm 19**	499 \pm 40*	1193 \pm 69**	3344 \pm 182

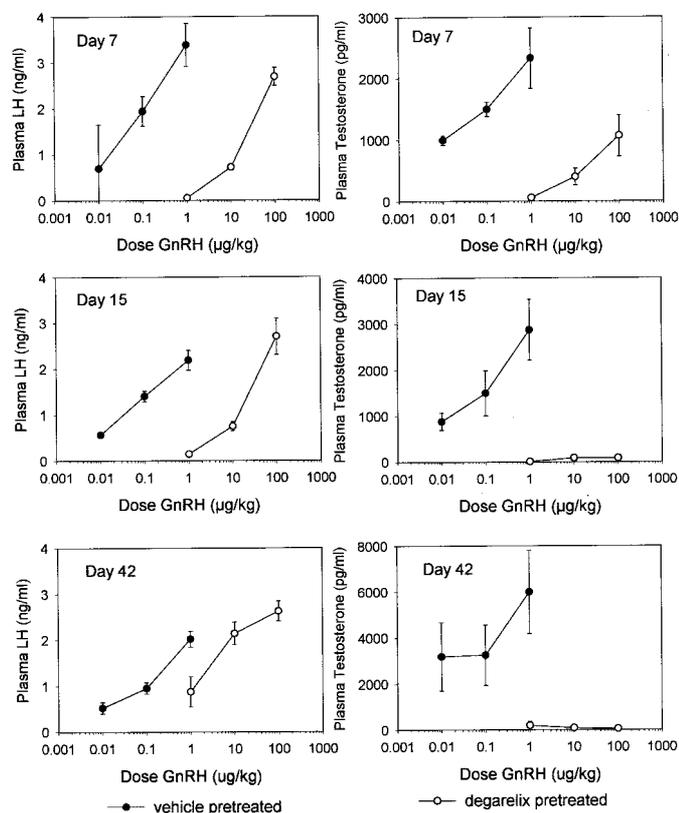
** $p < 0.001$; * $p < 0.05$.

Fig. 7. Plasma LH and testosterone levels in response to i.v. administration of GnRH in rats 7, 15, or 42 days after treatment with degarelix administered at 2 mg s.c. in 5% mannitol (20 μ l/animal). Results are mean ($n = 4-6$) \pm S.E.M.

al., 1991; Daghenghi, 1995; Shangold et al., 1995; Pechstein et al., 2000). To elucidate the parameters involved in the long duration of action of degarelix, we compared the duration of LH suppression induced by degarelix injected intravenously or subcutaneously and observed that degarelix exhibited a longer duration of LH suppression after subcutaneous injection, suggesting that slow release of degarelix occurred from a spontaneous subcutaneous depot. This property was shared by cetrorelix and azaline B but not by abarelix.

The ability of peptidic GnRH antagonists to form subcutaneous depot could rely on their propensity to form gels, which could be increased in the subcutaneous environment (Gray et al., 1994; Muller et al., 1994; Rivier et al., 1995a). For example, azaline B forms a gel when injected subcutaneously in rats in 5% aqueous mannitol solutions at concentrations from 5 to 20 mg/ml (Gray et al., 1994). Because gel formation is concentration-dependent, the results obtained with a 2-mg/kg dose of azaline B in rats suggest that increasing the concentration of this antagonist beyond a certain point affects its bioavailability. In the clinic, formulation issues

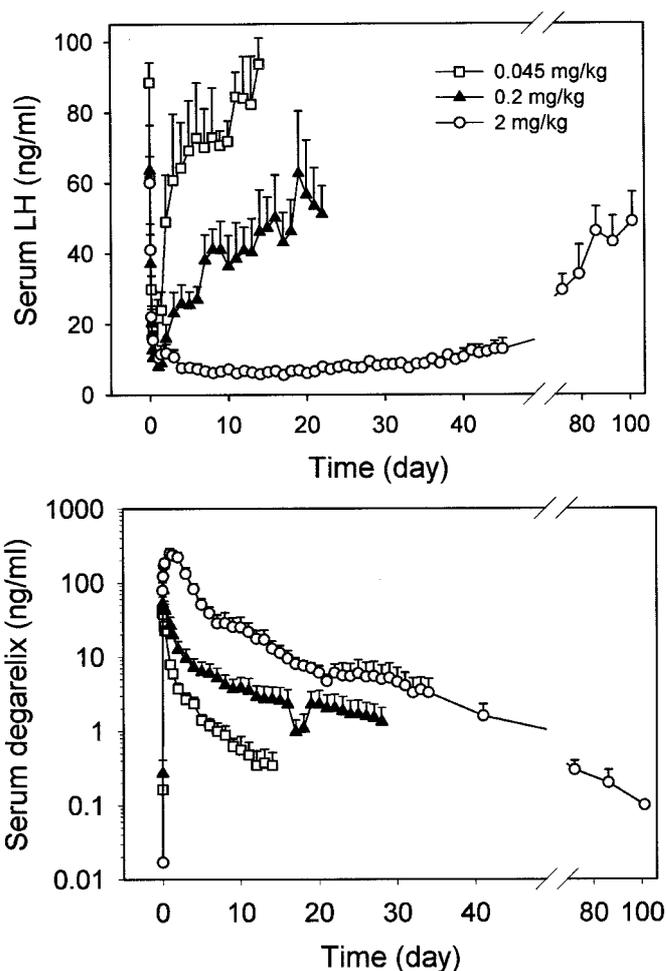


Fig. 8. Effects on serum LH levels in the ovariectomized rhesus monkey of degarelix administered at 45 and 200 μ g/kg s.c. in 5% mannitol (0.1 ml/kg) and 2 mg/kg s.c. in 5% mannitol (80 μ l/kg). Results are mean ($n = 3-4$) \pm S.E.M.

prevented azaline B from consistently suppressing testosterone to therapeutic ranges in humans (Hutchison et al., 1999). In contrast, increasing the dose and concentration of degarelix resulted in a marked increase in duration of LH and testosterone suppression: more than 40 days at 2 mg/kg in the rat and monkey, suggesting that the properties of resorption and bioavailability of degarelix from the subcutaneous depot are less dependent on concentration than in the case of azaline B. As indicated by the serum kinetics of degarelix in the monkey as in the rat, the prolonged action of degarelix on pituitary LH secretion after a single injection is apparently due to the continued presence of degarelix in circulation. This sustained presence of degarelix in the general circulation probably reflects its slow entry into circulation from the subcutaneous depot.

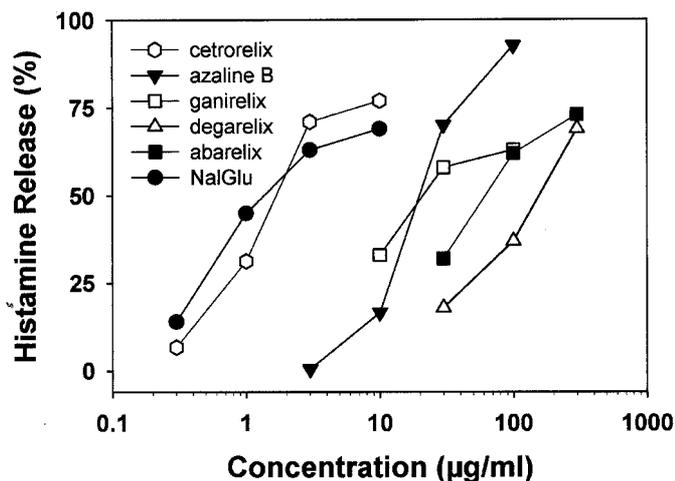


Fig. 9. Effects of degarelix, Nal-Glu, cetorelix, ganirelix, azaline B, and abarelix on histamine release from rat peritoneal mast cells. Each point represents the mean ($n = 3$).

Inhibition of gonadotropin secretion in response to the administration of a competitive GnRH antagonist is based on its ability to block the receptor, precluding substantial occupation and stimulation by endogenous GnRH. Consistent with a competitive antagonism mechanism of action, the dose-response curves for GnRH-stimulated LH release in rats pretreated 7, 15, or 42 days before with degarelix were shifted rightward compared with that obtained in vehicle-pretreated rats. This displacement was more pronounced at 7 days and decreased through days 15 and 42 in parallel with lower degarelix concentrations at the receptors. Seven days after pretreatment with degarelix, the LH surge induced by exogenous administration of GnRH stimulated testosterone release, indicating that the testosterone-producing Leydig cells were still functional at this time. However, 15 and 42 days after pretreatment with degarelix, GnRH stimulated LH but not testosterone release. Testosterone production by the Leydig cell depends on the action of LH exerted through its homologous receptor (Dufau, 1988) and LH is also required to maintain the fully differentiated structures and function of Leydig cells (Ewing and Zirkin, 1983). LH deprivation has been shown to result in the loss of the testosterone-secreting capacity of Leydig cells, with this loss of function being associated with atrophic alterations in cell morphology and decreased activities of some steroidogenic enzymes (Keeney et al., 1988; Russell et al., 1992). It is therefore likely that the loss of testosterone response in rats 15 and 42 days after injection of degarelix is secondary to long-term deprivation of LH and the consequent decline in testosterone-secreting capacity of the testis. Suppressing actions of degarelix on plasma LH, testosterone, and sex steroid-dependent organs were reversible over time, indicating that degarelix did not induce irreversible change to the reproductive axis.

Earlier developed GnRH antagonists were found to cause histamine release from mast cells (Schmidt et al., 1984; Hook et al., 1985), resulting in transient edema and systemic or local anaphylactoid reactions in clinical tests. For example, administration in humans of the GnRH antagonist Nal-Glu results in local erythema, pruritus, and subcutaneous nodule formation at the injection site (Bagatell et al., 1995). In our

study, degarelix was found to have the lowest propensity to release histamine of the antagonists tested with an EC_{50} value of 170 $\mu\text{g}/\text{ml}$. Considering the potency of degarelix for suppressing pituitary LH release in vitro and testosterone in vivo, it is expected that the safety margin of degarelix regarding histamine release potential will be superior to any GnRH antagonists developed so far.

In conclusion, degarelix is a new GnRH antagonist producing rapid and long-lasting suppression of the pituitary gonadal axis in rats and nonhuman primates. These data provide a compelling profile of degarelix as a potential candidate for the clinical management of sex steroid-dependent pathologies where long-term chemical castration is warranted.

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