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Gonadotropin-releasing hormone antagonists

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Hypothalamic gonadotropin-releasing hormone (GnRH) is a decapeptide that stimulates pituitary synthesis and secretion of gonadotropins and, therefore, gonadal hormones. GnRH antagonists, of which thousands have been formulated, inhibit the hormone from binding to its receptor, inducing a pharmacological hypophysectomy. Peptide derivations of GnRH and non-peptide compounds are both in clinical trials or approved for assisted reproduction. As these compounds reach the market, the use of antagonists might expand to treatment of other hormonally dependent diseases, hormonal male contraception and growth inhibition of extra-pituitary cancer cells expressing GnRH receptors.

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Abbreviations

BPH	benign prostatic hypertrophy
FSH	follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
IVF	*in vitro* fertilization
LH	luteinizing hormone

Introduction

Gonadotropin-releasing hormone (GnRH), or luteinizing hormone (LH)-releasing hormone, is a hypothalamic decapeptide that controls the reproductive axis [1] (Table 1). GnRH that is synthesized and released in a pulsatile manner from hypothalamic neurosecretory cells reaches pituitary cells by way of a specialized portal system, and regulates the synthesis and release of pituitary gonadotropins that, in turn, regulate steroidogenic and gametogenic functions of the gonads (Figure 1). This form of GnRH, called GnRH-I, is also found in extra-hypothalamic regions of the central nervous system, as well as in non-neuronal tissues. A form of GnRH that differs by three amino acids from GnRH-I, called GnRH-II, has been found in the brain of all vertebrates; its receptor is present in most tissues of the body [2,3••,4]. In the brain, GnRH-II robustly stimulates release of gonadotropins [5,6] and affects reproductive behavior [7•] (see also Update). GnRH-I and GnRH-II receptors have been found in several malignant tumors and cell-lines, where they are thought to participate in autocrine or paracrine growth inhibition [8].

Mechanism of action

Both GnRH agonists and antagonists suppress gonadal steroids and decrease gonad weight. Administration of GnRH agonists is accompanied by an initial gonadotropin and gonadal hormone surge known as a 'flare', delaying suppression by 7–14 days (Figure 1). Although GnRH agonist administration results in downregulation of GnRH receptors, the exact mechanism of suppression is unclear [9]. For example, a GnRH agonist decreased GnRH receptor mRNA in rats, whereas receptor protein levels remained elevated or increased [10•]. GnRH agonists also inhibited LH in male rats but not in female rats [10•,11] or women [12], which might reflect either an increase in the α-subunit of LH or immunologically active but biologically inactive LH molecules. These data suggest that GnRH agonist suppression is incomplete. Conversely, GnRH antagonists competitively block and inhibit GnRH-induced GnRH receptor gene expression, leading to immediate pituitary suppression [13]. GnRH membrane receptors are also immediately downregulated followed by an increase in the number of nuclear receptors, suggesting internalization and translocation of the receptors [14]; the ultimate fate of these receptors remains to be demonstrated.

Clinical use of GnRH antagonists

GnRH-I plays a rate-limiting role in reproductive processes; therefore, thousands of GnRH analogs have been generated because of their potential clinical utility. Currently, most GnRH antagonists are developed for the treatment of prostate cancer and for their utility in disrupting LH surges in the regulation of ovulation induction in women undergoing *in vitro* fertilization (IVF). Other important areas in which GnRH analogs could be utilized include benign gynecological disease [15,16], precocious puberty [17] and hormonal male contraception [18•] (Table 2). The initial hormonal flare following administration of a GnRH agonist might have adverse effects on hormone-dependent tumors [19••]. Use of a GnRH antagonist with immediate suppression of the gonadal axis therefore makes intuitive sense. Inhibition of tumor cell growth by GnRH analogs has been reported [20–22], and the importance of immediate 'pharmacological hypophysectomy' in hormone-dependent disease in both men and women has been noted

Table 1

Sequence of GnRH antagonist decapeptides compared with GnRH*.

Decapeptide	Amino acid sequence
GnRH	Pyro-Glu^1-His^2-Trp^3-Ser^4-Tyr^5-Gly^6-Leu^7-Arg^8-Pro^9-Gly^{10}-NH_2
Abarelix	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4**-N$^{\alpha}$MeTyr^5-DAsp^6-**Leu^7**-Ilys^8-**Pro^9**-DAla^{10}
Acyline	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4**-Aph(Ac)^5-DAph(Ac)^6-**Leu^7**-Ilys^8-**Pro^9**-DAla^{10}
Antarelix	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4**-Tyr^5-DHci^6-**Leu^7**-Ilys^8-**Pro^9**-DAla^{10}
Cetrorelix	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4-Tyr^5**-DCit^6-**Leu^7-Arg^8-Pro^9**-DAla^{10}
Degarelix	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4**-Aph(Hor)^5-D4Aph(Cbm)^6-**Leu^7**-Ilys^8-**Pro^9**-DAla^{10}
Ganirelix	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4-Tyr^5**-DHar(Et$_2$)^6-**Leu^7**-Har(Et$_2$)^8-**Pro^9**-DAla^{10}
Iturelix	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4**-NicLys^5-DNicLys^6-**Leu^7**-Ilys^8-**Pro^9**-DAla^{10}
Nal-Glu	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4**-Arg^5-Dglu(AA)^6-**Leu^7**-Ilys^8-**Pro^9**-DAla^{10}
Ornirelix	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4**-PicLys^5-D(6Anic)Orn^6-**Leu^7**-Ilys^8-**Pro^9**-DAla^{10}
Antide	Ac-DNal^1-DCpa^2-DPal^3-**Ser^4**-Lys^5(Nic)-Dlys^6(Nic)-**Leu^7**-Ilys^8-**Pro^9**-DAla^{10}

*Modified from [1]; see [1] for amino acid abbreviations. Bold font indicates an identical amino acid to the parent compound (i.e. GnRH).

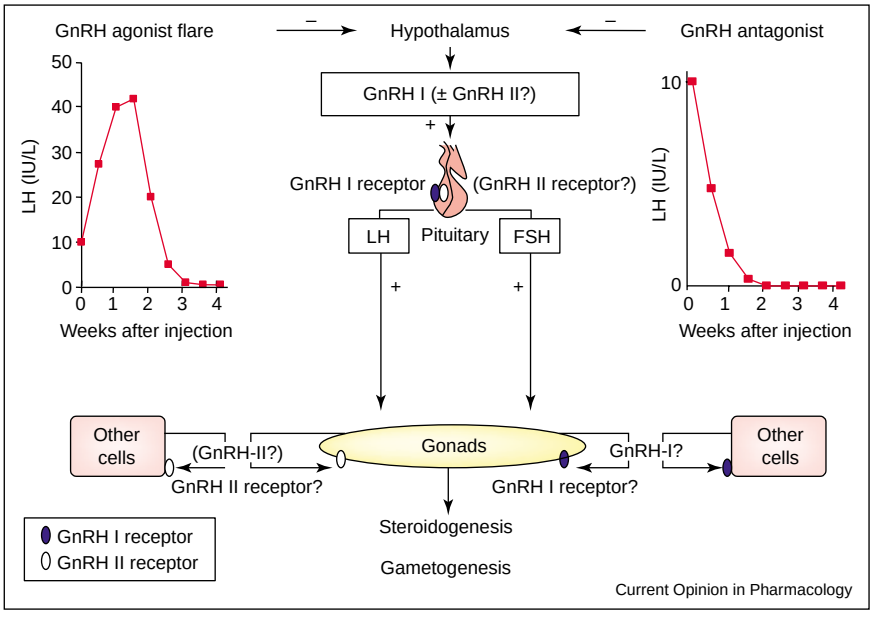
[23,24]. Because cells produce both GnRH and GnRH receptors to regulate growth, and GnRH agonist inhibition at GnRH-I receptors differs from that of antagonists, comparison of these two analogs on individual tumor growth requires further study [21].

Peptide GnRH antagonists

Early GnRH antagonist decapeptides, such as Nal-Glu [25] (Table 1), had a limited duration of action requiring daily subcutaneous injections, a histamine response at the site of injection and solubility limitations inducing nodule formation [26]. By changing the peptides at position five or six, newer decapeptides were developed (Table 1) with fewer side effects. Many of these decapeptides are being developed as depot preparations.

GnRH antagonists reported in the literature after 2001 are listed in Table 2. Abarelix has been formulated as an intramuscular depot injection (100 mg) given initially as a loading dose, and monthly thereafter. A 'New Drug Application' was submitted to the US Food and Drug Administration by Praecis Pharmaceuticals requesting approval to administer abarelix to men with prostate cancer for whom the flare and lag-time associated with

Figure 1



Gonadotropin-releasing hormones and their receptors. The hypothalamus produces GnRH-I and probably GnRH-II. GnRH-I binds to its receptor on the pituitary, stimulating synthesis and release of the gonadotropins LH and FSH. These then stimulate the synthesis of gonadal steroids and gametogenesis. GnRH agonists initially stimulate gonadotropins and gonadal steroids as shown, for example, by the flare in LH levels before suppression of the gonadal axis. GnRH antagonists immediately suppress gonadotropins and gonadal steroids. Both GnRH-I and GnRH-II receptors are found in the brain, as well as in peripheral tissues and cells. The role of GnRH-I receptors in the periphery is unclear but they might play a role in autocrine or paracrine growth regulation. The function of GnRH-II and its receptor is unknown. (Parentheses indicate an uncertain location, question marks indicate an uncertain function.)

Table 2

Decapeptides commercially available or in human trials.

Name	Manufacturer/ patent holder	Route	Trial phase	Approved indication	Clinically tested application	Preclinical applications	References
Decapeptides							
Abarelix (Plenaxis®)	Praecis	SC	Phase III	NA	Prostate cancer, endometriosis	NA	[28,52]
Acyline	NICHD/Salk Institute	SC	Phase II	NA	NA	Hormonal male contraception	[25,29,53]
Teverelix (Antarelix®)	Asta Medica AG/Europeptides	SC	Phase I	NA	NA	NA	NA
Cetrorelix (Cetrotide®)	Serono, ASTA Medica AG	SC	Commercially available	IVF	BPH, uterine fibroids, prostate cancer	Precocious puberty, gynecological cancer	[36,54–56]
Degarelix (FE200486)	Ferring Pharmaceuticals	SC	Phase II	NA	Prostate cancer	IVF, endometriosis	NA
Ganirelix (Antagon®, Orgalutran®)	Organon Inc	SC	Commercially available	IVF	Ovarian hyperstimulation syndrome, PCOS	NA	[57,58]
Iturelix® (Antide)	Serono	SC	Preclinical	NA	NA	leiomyoma	[59]
Orntide (Ornirelix®)	Oakwood Laboratories/ Tulane	SC	Phase I/II	NA	NA	NA	NA
Non-peptides							
CMPD1	Pfizer, Agouron	Oral	Preclinical	NA	NA	NA	NA
NBI-42902	Neurocrine Inc	Oral	Phase I	NA	NA	NA	NA
NOX 1255	NOXXON Pharma, Schering	SC	Preclinical	NA	NA	NA	NA
TAK-013	Takeda	Oral	Phase II	NA	NA	NA	NA

NA, not available; NICHD, National Institute of Child Health and Human Development; PCOS, polycystic ovarian syndrome; SC, subcutaneous.

GnRH agonists would not be appropriate; these include men at risk for severe complications from tumor growth. The market is limited to these men because of the immediate onset of systemic allergic reactions in a small subset of patients. In addition, testosterone levels fluctuated with abarelix administration, which was not seen with the depot form of the GnRH agonist Lupron, but which has been reported previously [27]. Praecis expects the FDA to complete its review by November 2003, and the company believes that the abarelix 'New Drug Application' will be approved for a defined subpopulation of advanced prostate cancer patients by the end of November 2003. Praecis is also seeking to market abarelix in Europe. In 170 men with prostate cancer, testosterone rapidly declined after the first injection of abarelix on day one, reaching castrate levels (< 5 nmol/L) in 95% of patients on day 29, which is comparable to 7.5 mg of Lupron Depot [28]. Fluctuating testosterone levels were not reported in this paper.

Acyline is a new GnRH antagonist that suppresses testosterone to castrate levels after injection of 75 μg/kg in healthy young men, maintaining suppression for 48 hours after a single injection [25]. Suppression of LH to less than 1 IU/L occurred within 24 hours, and follicle-stimulating hormone (FSH) reached nadir levels within 48 hours. These data were compared with published data for the GnRH antagonists Nal-Glu, Nal-Lys, cetrorelix and teverelix; acyline was found to be more potent and of longer duration than any of these antagonists. Histamine side effects were limited to a short-lived blush and mild itching at the injection site. A single dosage of 300 μg/kg suppressed testosterone below castrate levels for 15 days [29]. This dosage of acyline is approximately 21 mg for a 70 kg man, approximately a quarter of the abarelix dose, reflecting its longer half-life. Acyline is currently being formulated for depot administration to men for hormonal contraceptive regimens and for use in assisted reproduction.

Degarelix has high water solubility and a low histamine response in rat peritoneal mast cells compared with Nal-Glu, cetrorelix, ganirelix and abarelix [30]. In intact male rats, 2 mg/kg degarelix suppressed testosterone levels for up to 42 days compared with < 7 days for abarelix and ganirelix. In ovariectamized Rhesus monkeys, 2 mg/kg degarelix maintained suppression of LH for 79 days. Degarelix is currently in Phase II trials for prostate cancer and is under investigation for use in IVF and endometriosis. No reports from studies in humans are currently available in peer-reviewed journals.

Teverelix has been recently reviewed [31]; after a single 5 mg subcutaneous dose in healthy young men, teverelix inhibited LH and testosterone with similar efficacy and potency to acyline, but was slightly less potent at FSH

inhibition [32]. So far, it has only been tested in healthy men and is still in Phase I trials. A sustained formulation is currently under development for the treatment of prostate cancer, and clinical trials are expected for the treatment of breast and ovarian cancer. It might also be tested for use in benign prostatic hyperplasia (BPH), endometriosis and uterine fibroids.

Ornirelix® is being developed for treatment of prostate cancer as a depot formulation in microspheres secondary to its gelling properties as a native peptide [33]. It is currently available in one-, three-, four- and six-month depot preparations, with a 12-month depot preparation under development. In rats, 8.8 mg/kg subcutaneous injections of orntide formulated in microspheres suppressed testosterone levels below 5 ng/ml for approximately 150 days. Return to fertility was demonstrated at day 269 after injection [34]. Although not published, a Phase I trial demonstrated dose-dependent hormone suppression, with 4 mg of free peptide injected daily for seven days rendering all subjects castrate by day seven; testosterone levels returned to baseline 14 days after the last injection. Phase II trials are planned with the one-month depot formulation under an 'Investigational New Drug Application' filed in the US in April 2002.

Ganirelix and cetrorelix are both approved for use in the US, Europe and other countries for the prevention of premature LH surges in women undergoing IVF. Administration of ganirelix at 250 μg daily for seven days suppressed LH by 74% (four hours after injection), and FSH by 32% (16 hours after injection) [35]. This dose was also found to be the minimum effective dose at preventing LH surges when administered on day six after recombinant FSH administration. Higher doses decreased estradiol levels significantly below that required to achieve adequate implantation and pregnancy, although the mean number of good quality embryos obtained was not significantly different from the 250 μg dose. When compared with a GnRH agonist, the total treatment time with ganirelix was 18–21 days shorter, but the number of oocytes was significantly lower and ongoing pregnancy rates tended to be lower.

Cetrorelix is comparable in potency and efficacy to newer GnRH antagonists and has had data published on its efficacy and safety in men with symptomatic BPH [36]. In addition, 15 women with endometriosis all reported a symptom-free period when treated with 8 mg weekly doses of cetrorelix. Regression occurred in 60% of cases, and the degree of endometriosis declined from stage III to stage II [15,16]. Estrogen levels remained at approximately 50 pg/ml, without any apparent influence on disease regression. More potent GnRH antagonists will require 'add-back' therapy of estradiol and progestin to decrease side effects, especially bone loss associated with treatment longer than six months [37,38].

Cetrorelix is administered as multiple 250 μg doses or a single 3 mg dosage for the prevention of LH surges during oocyte induction protocols. Pregnancy rates were lower in a small study examining multiple cetrorelix injections, and the cost was felt to be prohibitive over a longer GnRH agonist protocol [39]. Sperm zona pellucida binding was also found to be lower following cetrorelix administration than after a GnRH agonist [40]. Because the cycle of injections for assisted reproduction is shorter with GnRH antagonists, the problem of lower pregnancy rates in some patients might be rectified by developing flexible antagonist regimens [41].

Iturelix® (also known as antide or Nal-Lys) has poor solubility, causing erythema and nodule formation at the site of injection [42]. The potency and efficacy of antide is comparable to acyline [25], which is probably associated with its high serum protein binding [43] and structure [44•]. Currently, antide is being formulated as a depot formulation in lipid microparticles [45]. In rats, single 2 mg injections of antide in several lipid microparticle formulations suppressed testosterone for up to 30 days. Iturelix® is being developed for the treatment of prostate cancer and BPH.

Non-peptide GnRH antagonists

The GnRH antagonist CMPD1 (or AG-045572) [46] is a compound developed for oral bioavailability that, when administered to intact male rats at a single dose of 100 mg/kg, maintained suppression of testosterone in the castrate range for 24 hours [47]. Unfortunately, work on this compound has been suspended and Pfizer/Agouron is waiting to see if there is a significant market for an orally available GnRH antagonist.

Another interesting oral compound that underwent initial development, called NOX 1255, is a spiegelmer that binds specifically to GnRH with high affinity and blocks its functional activity [48•]. Spiegelmers are mirror image, high-affinity oligonucleotide ligands composed of L-ribose or L-2′-deoxyribose units in which the chiral inversion results in high plasma stability. Although not a classic GnRH antagonist, subcutaneous injection of NOX 1255 in rats inhibits LH secretion, but with less potency than cetrorelix. There is no active program for this compound.

A non-peptide GnRH antagonist that is currently being pursued in human studies is NBI-42902; Phase I trials were initiated in November 2001 in Germany in 56 normal post-menopausal women, and in November 2002 in the US in healthy pre-menopausal women. These data have yet to be published in a peer-reviewed journal.

TAK-013, another non-peptide antagonist currently in clinical trials, is a nucleotide derivative with modifications to increase oral availability [49]. In castrated male monkeys, 30 mg/kg TAK-013 by oral gavage suppressed

plasma LH levels after 24 hours to approximately 11% of pretreatment values. Administration of 90 mg/kg/day TAK-013 for 80 days to female cynomolgus monkeys inhibited estradiol, progesterone, LH and menstrual cyclicity, although FSH was not suppressed and neither LH nor FSH was suppressed in female marmoset monkeys [50]. Phase I trials in humans are reported to be complete and Phase II trials are underway. TAK-013 has 220-fold greater antagonist potentcy at the human receptor [51]; it will be interesting to determine the dosage of TK-013 needed for long-term suppression of the gonadal axis in humans, and whether suppression of either gonadotropin will occur.

Conclusions

GnRH antagonists are clinically useful in prostate cancer and IVF because of their immediate suppression of gonadotropins and gonadal hormones. Their utility in cancer treatment, endometriosis, BPH and male contraception is still being explored but these areas might benefit from the continued development of depot formulations and oral GnRH antagonists. The function of GnRH receptors outside the brain remains unclear. If GnRH-I and its receptor do function to regulate growth, then the use of GnRH antagonists versus GnRH agonists in breast, uterine and gonadal cancer needs to be further explored. The effect of GnRH antagonists on the GnRH-II receptor is unknown. The receptor must first be demonstrated to be functional and its physiology in such diverse tissues outlined, perhaps by utilizing GnRH antagonists.

Update

GnRH-II was found to induce expression of LH and FSH in sheep but not in the presence of antide, a specific GnRH-I receptor antagonist [60]. GnRH-II did little to augment the activity of GnRH-I on gonadotropin secretion. A combination GnRH-I receptor antagonist/GnRH-II receptor agonist, 135-18, did not induce either gonadotropin, suggesting that GnRH-II acts primarily through the GnRH-I receptor but does little to augment GnRH-I activity on gonadotropins.

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