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Gonadotropin-Releasing Hormone: Physiological and Therapeutic Aspects, Agonists and Antagonists

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Abstract. Rapid advances in the understanding of gonadotropin-releasing hormone (GnRH) actions and in development of new agonist and antagonist analogues of GnRH have taken place over the past several years. As a result, new compounds are now available to treat patients with various disorders of reproductive function. Used as probes of biologic processes, these compounds will also allow a greater depth of understanding of basic biologic processes.

Introduction

The structure of gonadotropin-releasing hormone (GnRH) as a decapeptide was identified and its synthesis accomplished in the early 1970s by the independent research teams of Schally and Guillemin [1]. With development of potent and long-acting GnRH agonist analogs (GnRH-A), paradoxical inhibition of gonadotropin secretion was observed during administration of these substances. The potential for exploiting this paradoxical effect was quickly recognized as a means for therapy of individuals with endocrine-dependent tumors or with estrogen- and testosterone-dependent benign disorders [2]. In rapid succession, animal studies demonstrated the regression of hormone-dependent breast and prostate tumors with these agents and clinical trials confirmed these actions in patients [3]. While the exact molecular mechanisms whereby the GnRH agonist analogs inhibit LH and FSH are incompletely understood, the general inhibitory actions are amply documented. More recently, potent antagonist analogs of GnRH have been synthesized [1]. The compounds are currently entering clinical trial. In the present overview, we will detail the physiologic mechanisms of GnRH action and review basic and clinical information regarding the agonists and antagonists of GnRH. We have chosen to use data from our own studies to illustrate pertinent points even though data from a variety of published sources were often available.

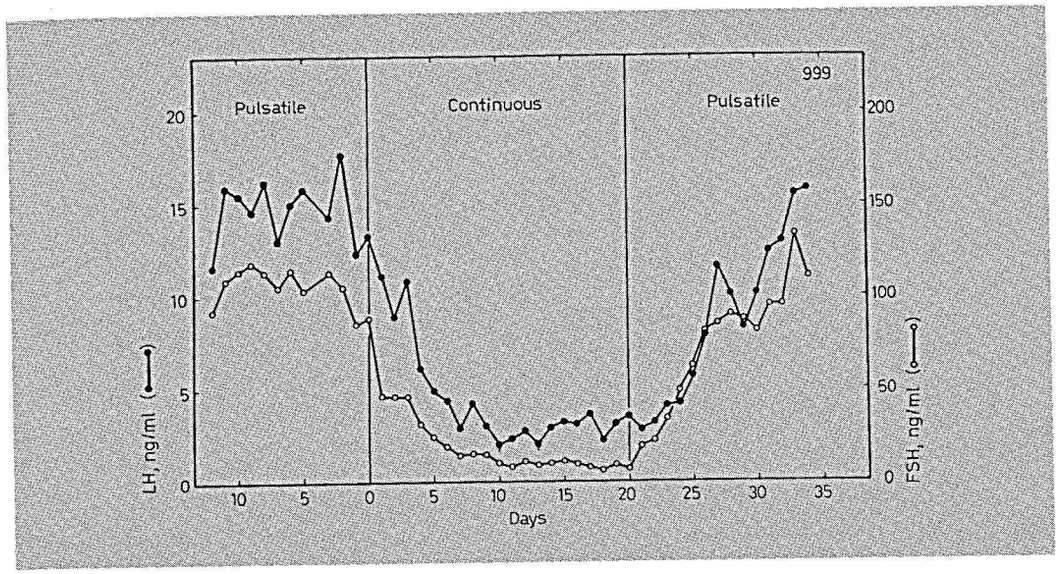

Fig. 1. LH and FSH released during pulsatile vs. continuous administration of native GnRH in castrate female monkeys. Radiofrequency lesions in the hypothalamus destroyed the production of endogenous LHRH pulses and 'disconnected' the hypothalamus from the pituitary. Steroidal feedback on the pituitary was prevented by surgical oophorectomy. Gonadotropin release was maintained by pulsatile GnRH stimulation (1 μg/min for 6 min every hour) but suppressed during continuous infusion [from 4, with permission].

Pituitary Desensitization to Native GnRH or GnRH-A

Under normal physiologic conditions, one or several regions of the hypothalamus generate pulses of GnRH at intervals of approximately 2 h. This interval varies according to physiological conditions such as the menstrual cycle and the menopause. These 'boluses' of GnRH travel to the pituitary by the portal veins, bind to GnRH receptors, and stimulate discrete pulses of LH. Exposure of the pituitary to constant amounts of GnRH rather than pulsatile secretion desensitizes the pituitary to further GnRH stimulation and causes paradoxic inhibition of LH and FSH secretion (fig. 1) [4]. The process of pituitary desensitization is a subject of intense current investigation [5, 6]. Constant administration of GnRH can reduce the concentration of GnRH receptors in the pituitary by a process of down-regulation. However, in experimental systems, the reduction in receptor number is transient [5, 6]. For this reason, several investigators believe that constant exposure to GnRH also induces postreceptor effects to suppress gonadotropin release.

To understand the potential sites of inhibitory action of GnRH, it is necessary to review the steps involved in detail. The events which normally occur after initial binding of GnRH to its receptor (fig. 2) are becoming increasingly understood [5, 6]. Receptor microaggregation occurs shortly after binding of GnRH to its receptor on the go-

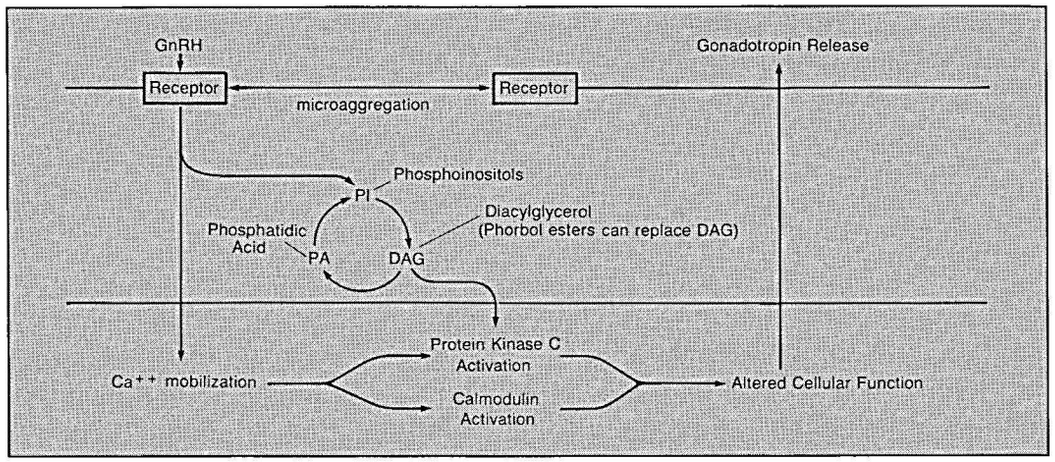

Fig. 2. Summary of likely GnRH stimulatory events on the pituitary gonadotropin-producing cells. GnRH interacts with its plasma membrane receptor and causes receptor microaggregation. The activated receptor stimulates hydrolysis of inositol phospholipids (PI) and prompts an influx of intracellular calcium (Ca^{++}). The mobilization of Ca^{++} activates calmodulin (CAM) while the diacylglycerols (DAG) produced in the hydrolysis of inositol phospholipids activate protein kinase C (PKC). These two enzymes, CAM and PKC, exert synergistic effects via as yet unidentified mechanisms to provoke gonadotropin release [from 5, 6, with permission].

nadotrope. Internalization of the GnRH receptor complex into the cell follows shortly thereafter [7]. Calcium then enters the cell from extracellular stores and binds to calmodulin, a calcium-binding protein [8, 9]. At the same time, the enzyme, protein kinase C, is activated. These two events are necessary for and precede LH and FSH release. At the same time, a modulating system is activated. GnRH receptor-binding initiates hydrolysis of phosphoinositol and formation of diacylglycerols. Measurement of ^{3}H-inositol phosphates (^{3}H-IP) provides an indirect assessment of diacylglycerol formation. Exposure of pituitary cultures to GnRH induces a dose-response (i.e. 10^{-10} to 10^{-6} *M* GnRH) related stimulation of ^{3}H-IP accumulation. This effect is likely to be related causally to LH release since GnRH antagonists reduce ^{3}H-IP accumulation and LH release in a parallel fashion. Diacylglycerol, in turn, further activates protein kinase C. In a recycling mechanism, the diacylglycerols are converted into phosphatidic acid and then back to phosphoinositol. Of interest is the fact that the phorbol esters, a major class of tumor promoters, can completely substitute for the diacylglycerols in this process. Recent evidence indicates that arachidonic acid is also involved in a coordinated fashion with the protein kinase C-dependent pathways [10].

Which specific steps are inhibited when GnRH is infused constantly over a prolonged period is currently unknown. These postreceptor inhibitory actions could perhaps involve events mediated by the calcium, protein kinase C, or arachidonic acid pathways. Work is in progress to identify precisely these mechanisms. Notwithstanding the steps involved, the highly potent agonist analogs of GnRH (superagonist analogs) appear to exert suppressive actions similar to those of constant GnRH infusions (fig. 2). The prolonged half-life of clearance after subcutaneous injection and, more importantly, the longer duration of binding of superagonist analogs to the GnRH receptor produce a state analogous to that of constant GnRH receptor occupancy [11]. This effect desensitizes the pituitary to further GnRH stimulation and causes paradoxic inhibition of LH and FSH secretion. A variety of highly potent GnRH analogs are now available [1].

Table I. Superagonist analogs[1]

Position	Substitution	Mechanism
10	ethylamide	increased receptor-binding affinity
	α-aza-gly	decreased activity post-proline cleavage enzyme (postulated)
6	*D*-amino acids	resistance to enzymatic degradation
	hydrophobic amino acids	longer plasma clearance

[1] Data summarized from Karten and Rivier [1].

Structure Activity Relationships of GnRH-A

Several structure activity relationships were identified during attempts to synthesize long-acting and potent GnRH analogs (table I). The major site of metabolic degradation of native GnRH is at the sixth amino acid position. Insertion of dextrorotatory for levoamino acids at this position enhances biologic activity. A refinement is the substitution of hydrophobic amino acid molecules at the sixth position, such as *D*-Nal(2)[6], which further enhances biopotency. Enhanced receptor binding of the GnRH analogs also increases biopotency. When in the state requiring least thermal energy, GnRH is folded into a C-shape with the 1 and 10 amino acids in an optimal position for binding to the GnRH receptor. Substitution of the 10th amino acid of native GnRH with proethylamide increases the affinity of binding for the receptor and biopotency of the GnRH analog. This modification also retards the rate of dissociation of the GnRH analog from its receptor. Structural modifications such as α-aza-gly substitution also result in prolonged plasma clearance of the GnRH analogs when compared to the native material [1]. The half-life of Zoladex® (an α-azo-glyl compound) after subcutaneous injection, for example, is 4.5 h (vs. <1 h for native GnRH). Other compounds, such as nafarelin, bind to plasma proteins which further retard their rate of biologic disappearance [1].

GnRH-A Bioavailability

Another important factor for efficacy of the GnRH analogs is their bioavailability. The rates and degree of absorption of the GnRH analogs are critical, since sustained GnRH receptor occupancy is required for the process of receptor or postreceptor desensitization. The subcutaneous route of administration allows $>94\%$ absorption and sustained plasma levels. In contrast, with currently available GnRH analog preparations, only 2–5% of the administered dose is absorbed through the intranasal route. This

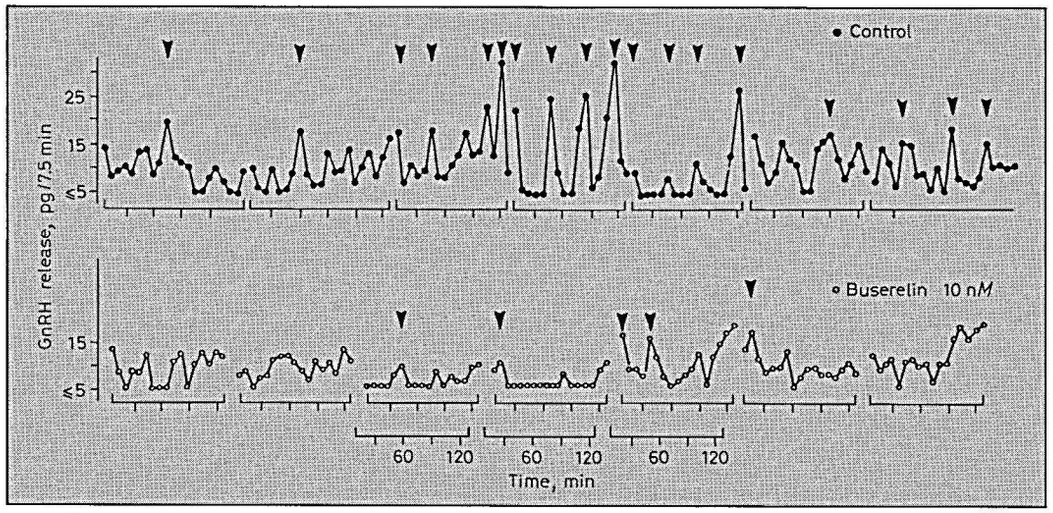

Fig. 3. Demonstration of episodic GnRH release in vitro from explants of male rat hypothalamus. The arrows represent pulses of GnRH secretion as detected by the PULSAR program. The lower panel illustrates the reduction of pulses induced by addition of Buserelin® to the medium. [Modified from Endocrinology, *121:* 993, 1987, with permission.]

problem prevents complete inhibition of the gonadotropins and sex steroids in most patients receiving intranasal preparations [12]. A promising new approach is the development of very long-acting biodegradable preparations. Sustained plasma levels may be achieved over a 1- to 3-month period with these formulations. Studies with one such preparation, Zoladex®, demonstrated the persistence of plasma levels for at least 1 month following implantation. A moderate degree of fluctuation in plasma levels was detected by the specific radioimmunoassay method used. Nonetheless, therapeutic concentrations were uniformly maintained over this time period. Of the formulations undergoing development, biodegradable ones such as Zoladex®, Decapeptyl®, and others appear most promising for patient acceptance and ease of administration [1, 13].

Additional Actions of GnRH Agonists

Recent evidence suggests that the GnRH superagonists may also inhibit GnRH release through an ultrashort loop feedback mechanism [14]. Originally proposed 25 years ago, ultrashort loop feedback involves the inhibition of GnRH release by the hypothalamus in response to increasing local GnRH levels. Bourguignon et al. [14] studied the ultrashort loop feedback effects of GnRH superagonists on GnRH secretion in a rat hypothalamic explant system. In this in vitro system, GnRH is secreted as episodic pulses which can be objectively identified by standard pulse analysis programs (fig. 3). The superagonist analog, Buserelin®, markedly diminishes the frequency of significant episodic discharges. In addition, the amount of GnRH secreted can be reduced in a dose-dependent

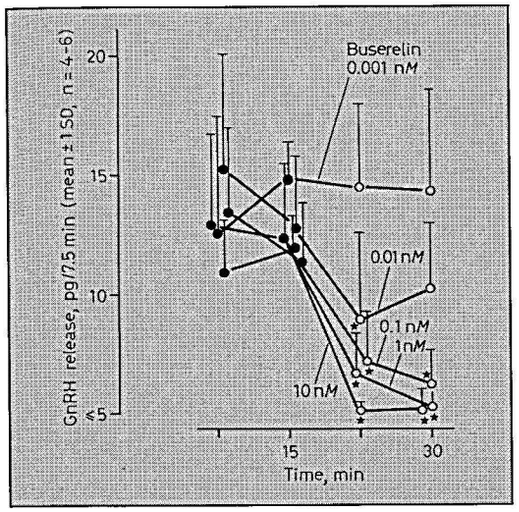

Fig. 4. Mean secretory levels of GnRH in explants of male rat hypothalamus in vitro. The vehicle was present in the medium for the first 15 min of the experiment. Thereafter, medium was changed to include Buserelin® in doses ranging from 0.001 to 10 n*M*. GnRH values represent mean and SD of 6 hypothalami. Asterisks indicate $p < 0.05$. [Modified from Endocrinology, *121:* 993, 1987, with permission.]

fashion with increasing amounts of Buserelin® from 0.01 to 10 n*M* (fig. 4). This is consistent with the in vivo inhibition of LH pulse frequency after intraventricular administration of a GnRH agonist in the rat [15]. We do not yet know the hypothalamic site of GnRH autofeedback and its significance in vivo. It should be noted that the autoregulatory effect of GnRH is unlikely to be the major mechanism responsible for pulse generation [16]. Further experiments are now required to fully delineate the exact physiologic significance of the autoregulatory effect of GnRH. A possible ultrashort loop action of GnRH superagonists at the hypothalamic level does not seem to represent an important mechanism during therapy since pulsatile administration of GnRH during treatment with the agonists does not result in detectable gonadotropic responses [17].

It has been suggested that the GnRH superagonist analogs may act at extra pituitary sites. In a number of species, specific binding sites for GnRH and its analogs are present in the ovary and testis [18]. Direct inhibitory effects of GnRH on steroidogenesis through blockade of the C_{17-20}-lyase and 17α-hydroxylase enzymes can be demonstrated in these animals. However, while such mechanisms are likely in the rat, human testes and ovary do not contain high affinity GnRH receptors and direct gonadal effects are likely to play a minimal role [19]. This does not exclude the paracrine significance of intragonadal GnRH-like peptides which might interact with lower affinity binding sites.

Two laboratories recently demonstrated specific GnRH agonist binding sites in breast tumor tissues [20, 21]. In addition, the mRNA for GnRH has been shown to be present in breast cancer cells in culture [22]. Miller et al. [21] presented biologic evidence supporting a direct antitumor effect of the GnRH analogs. They detected inhibition of growth of MCF-7 human breast cancer cells in culture when exposed to concentrations of GnRH analogs in the range of 10^{-9} to 10^{-7} *M*. These effects were unrelated to nonspecific toxicity since addition of GnRH antagonist analogs rescued cells from the inhibitory effects of the agonists.

Hormonal Data in Patients Treated with GnRH Agonists

Our initial experience with the GnRH-A involved men with stage D (metastatic) prostate cancer [23, 24]. These men entered into

trials to receive GnRH analogs either as their first hormonal therapy, as secondary therapy after the use of diethylstilbestrol, or after castration. Drug doses represented an extrapolation on a $\mu g/m^2$ basis from those studied extensively in rats. The protocol compared doses of 1, 10 or 20 mg of *D*-Leu^6-GnRH proethylamide (Leuprolide) given by daily subcutaneous injection. As expected, an initial phase of LH and testosterone stimulation occurred before pituitary desensitization and gonadotropin suppression followed. The first subcutaneous dose induced a 4-fold rise in plasma LH levels after 8 h. By the third daily injection (i.e., from 72 to 80 h), this stimulatory effect disappeared. After 2–4 weeks of continuous daily therapy, the gonadotropins were suppressed. Plasma testosterone concentrations followed a similar pattern, i.e., a 2-fold stimulation during the first week followed by a 95% suppression below basal level thereafter. We could discern no dose-response difference between the 1, 10 and 20 mg daily dosages. The hormonal effects did not differ in previously untreated and in diethylstilbestrol-pretreated patients. Both testosterone and dihydrotestosterone fell to levels (i.e., 19 ± 4.4 and 15 ± 1.7 ng/100 ml) indistinguishable from those measured in 30 castrate patients. During continuous therapy for up to 5 years, no escape from LH, testosterone, or dihydrotestosterone suppression occurred (fig. 5). Of interest was the partial return of FSH toward basal levels, a finding which probably has no practical relevance regarding therapy of prostate cancer (but is discussed later).

While these data evolved, questions regarding a direct effect of the GnRH agonists on the testes arose. A number of investigators had demonstrated GnRH receptors on rat testis and established a direct testicular inhibitory effect of GnRH on testosterone production in that species [18]. Of further interest was the finding of large molecular weight peptides (i.e., GnRH-pp) in rat testes which possessed GnRH receptor-binding affinity and biologic actions. Taken together, these studies suggested the possibility that the GnRN-A might directly inhibit testosterone production in men with prostate cancer. Three lines of evidence, however, argued strongly against such an effect in men. Firstly, the presence of gonadal GnRH receptors is highly species-dependent and the human testis, as opposed to rat testis, does not have high affinity GnRH-binding proteins [18]. Secondly, testicular responsiveness to LH or its analog, hCG, is not affected by long-term, high-dose GnRH agonist treatment in man [19]. Finally, the degree of LH suppression observed during GnRH-A therapy is adequate to explain the 95% inhibition of testicular androgens [23, 24].

It should be emphasized that demonstration of a profound degree of LH suppression with GnRH analogs requires use of a highly sensitive plasma LH assay. Routine LH radioimmunoassays are not sufficiently sensitive to demonstrate greater than a 5- to 10-fold suppression of plasma LH. In contrast, the in vitro LH bioassay of Dufau and coworkers [23] is 10-fold more sensitive than currently available plasma radioimmunoassays, and furthermore, is specific for biologically active LH. Using this assay, we demonstrated a 95% or greater suppression of plasma LH during chronic therapy in men receiving *D*-Leu^6-GnRH proethylamide (fig. 6). Unexpectedly, we also found that the GnRH analog lowered the ratio of biologic to immunologic LH activity in noncastrate as well as in castrate men with prostate cancer (fig. 7) [23–25]. Additional measure-

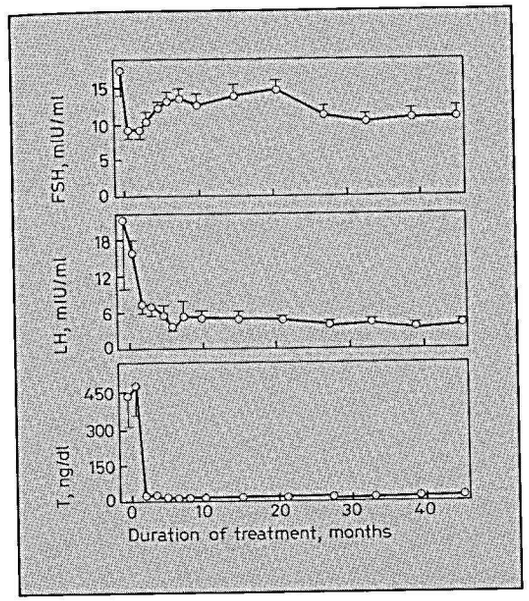

Fig. 5. Plasma concentrations of FSH, LH and testosterone in men with prostatic carcinoma receiving *D*-Leu^6-GnRH proethylamide (Leuprolide®) for up to 5 years. Data represent results in 8 men receiving therapy for at least 4 years (standard error bars for testosterone during chronic therapy are omitted because they generally fit within the symbols indicating mean level).

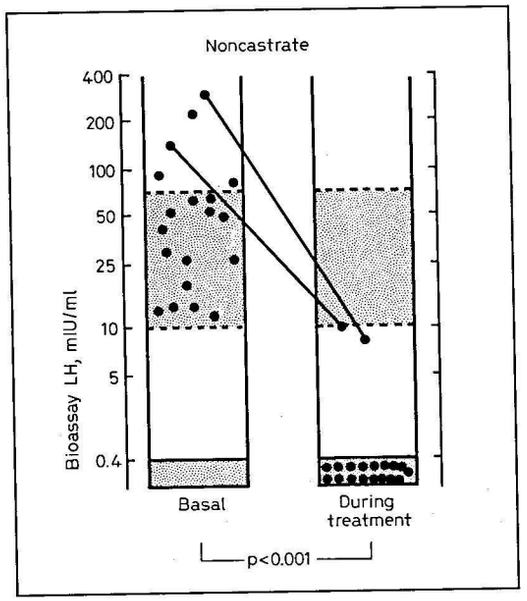

Fig. 6. Bioassay (rat interstitial cell testosterone bioassay) of LH in men measured prior to treatment and in a single sample obtained during the 6th to 11th week of treatment. Values less than 0.4 mIU/ml are undetectable. The lines connect the basal and treatment values in the 2 individuals whose LH levels remained measurable during therapy. In the remaining patients, LH fell to undetectable titers during treatment [from 23, with permission].

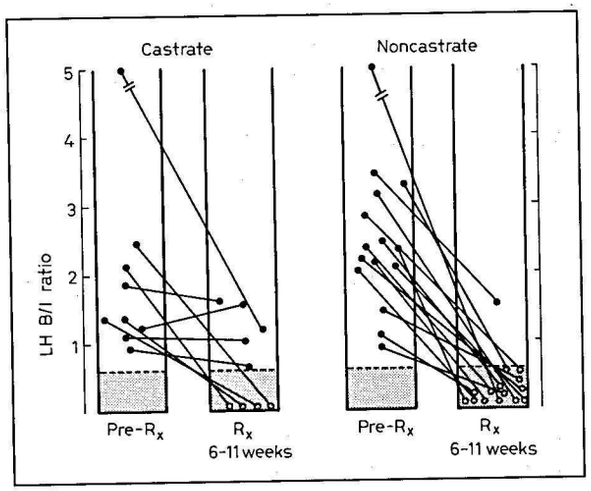

Fig. 7. The ratio of biologic to immunologically detectable LH (LH B/I ratio) prior to the initiation of treatment and during the 6th to 11th weeks of treatment in men with prostatic carcinoma. The left panel illustrates men who had been treated by surgical castration for at least 1 month prior to treatment with *D*-Leu^6-GnRH proethylamide (Leuprolide®). The right panel illustrates men who had intact testes at the time that Leuprolide® therapy was initiated.

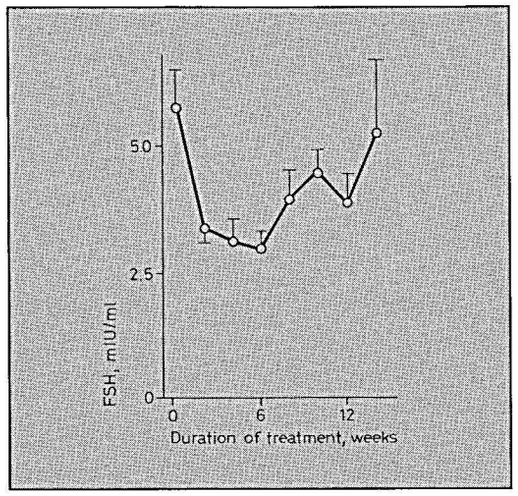

Fig. 8. Levels of FSH in normal men receiving 500 µg of *D*-Trp^6-GnRH by constant subcutaneous infusion. Values represent mean and standard error of the mean [from 28, with permission].

ments in urine also supported the conclusion of marked suppression of LH [23]. A radioimmunoassay utilizing 40-fold concentrates of urine detected mean levels of urinary LH during GnRH agonist therapy which were 50-fold suppressed below normal adult levels. Considered together, these three lines of evidence indicate that the GnRH agonists profoundly suppress LH as a means of inhibiting testosterone. Consequently, this effect does not result from a primary action on Leydig cells whereas a possible direct action on spermatogenesis has not been fully ruled out.

Not all studies have demonstrated a fall in radioimmunoassayable LH during administration of GnRH superagonist analogs, whereas the fall in biologically active LH has been uniformly shown [3, 26]. A partial explanation for this dichotomy is the rise in the α-subunit of LH during GnRH superagonist analog therapy. Although α-subunit LH cannot be measured as biologically active LH in standard bioassays, it is detected in some but not in all radioimmunoassays as intact LH. To date, we have no definite explanation for the increased α-subunit secretion during GnRH agonist treatment. It is noteworthy that experimental or pathologic conditions such as anencephaly or study of the pituitary gland in vitro are also associated with an increased release or secretion of α-subunit [27]. These similar alterations of α-subunit after anatomical as well as biochemical perturbations of the hypothalamic-pituitary axis suggest that the pituitary has an autonomous property for secreting α-subunit which diminishes when under the inhibitory control of the hypothalamus.

The effects of GnRH superagonist analogs on FSH secretion in men have been controversial. Most studies have demonstrated an initial inhibition of FSH with a nadir at 6–12 weeks after continuation of therapy. In our studies, FSH increased back toward but not completely to baseline values during chronic administration (fig. 5) [24]. This 'escape' phenomenon did not appear to be related to an insufficient dose of GnRH-A since a similar effect was observed in men receiving either 1 or 10 mg of *D*-Leu^6-GnRH proethylamide daily. A recent study by Pavlou et al. [28] demonstrated a similar FSH escape in normal men receiving 500 µg daily of *D*-Trp-GnRH proethylamide by subcutaneous infusion over a 12-week period (fig. 8). We observed a similar phenomenon in girls with central precocious puberty treated with Buserelin® intranasally [29]. Their mean (± SD) serum level of FSH (n = 7) before therapy, after 1–3 months, and 12 months of Buserelin® administration were respectively 6.0 ± 1.1, 2.6 ± 0.8 and 4.2 ±

1.7 mIU/ml. Other investigators have been unable to demonstrate FSH escape during administration of GnRH-A long term to men [13, 30]. The recent data of Hsueh and co-workers [31] may provide an explanation for these conflicting results. Their group utilized a granulosa cell biossay for FSH in conjunction with an FSH radioimmunoassay to measure biologic to immunologic ratios in men receiving a GnRH antagonist. They demonstrated a prompt decrease in FSH B/I ratio within 6 h of administration of the antagonist. FSH levels measured by bioassay fell promptly whereas those detected by radioimmunoassay did not. Perhaps the 'escape' in FSH observed in our studies reflects an alteration in the FSH molecule which renders it more easily detectable by our radioimmunoassay. Investigators using other radioimmunoassays may not detect this alteration. Alternatively, it is conceivable that the progressive alterations of spermatogenesis during therapy might effect the negative feedback that gonadal inhibin might exert directly at the pituitary level. This hypothesis could now be evaluated with the specific inhibin radioimmunoassays which are available for clinical studies. In addition, the possible FSH stimulatory activity of an inhibin subunit (activin) [32]might also be considered if the secretion of activin would turn out to be increased during superagonist analog therapy.

The phenomenon of FSH escape, if observed by bioassay as well as by radioimmunoassay, could be practically important [33]. If the indication for GnRH analog therapy is to produce azoospermia in men as a form of contraception, a lack of suppression of biologically active FSH could allow sperm production to continue. It is of interest that, in the majority of men treated to date, it has been impossible to achieve azoospermia during superagonist analog GnRH therapy [33].

The hormonal effects of high-dose GnRH analogs in women have also been fully documented. Our data will be used to illustrate the degree of suppression [34, 35]. We studied a group of premenopausal women receiving *D*-Leu^6-GnRH as a potential treatment for breast cancer. Both plasma LH and FSH transiently rose during the initial 4 days of therapy before falling to suppressed levels. Patterns of inhibition strikingly paralleled those observed in men with prostate cancer. Estradiol levels varied markedly during the first week of treatment but fell to postmenopausal levels after 4 weeks and remained at these concentrations thereafter (fig. 9). The lack of a consistent early rise (hormonal flare) in estradiol differed from the uniform increase in testosterone observed in men with prostate cancer during the same time period. While this finding was initially unexpected, a study by Fraser and Sandow [36] provides a plausible explanation. Initiation of GnRH superagonist analog administration during the follicular phase of the menstrual cycle in the monkey reproducibly initiated prolonged rises in estradiol. Administration during the luteal phase, just prior to menses, promptly lowered estradiol after only a transient increase. Thus, the variability of initial estradiol responses observed in our patients may have reflected the fact that they began treatment at variable times during their menstrual cycle. Systematic studies are now required to substantiate this conclusion.

The decline in estradiol observed during treatment induced complete cessation of menses in all 19 women receiving drug for over 10 weeks [34, 35]. However, 9 women

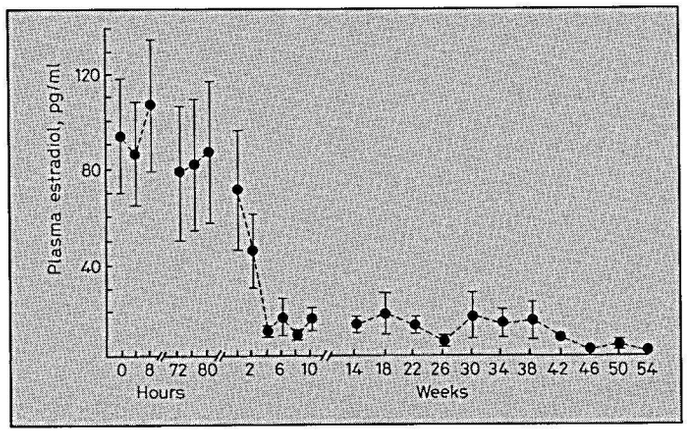

Fig. 9. Plasma estradiol levels in premenopausal patients with advanced breast cancer treated chronically with Leuprolide®. Values represent mean ± SEM [from 34, with permission].

reported one menstrual cycle before becoming amenorrheic and 5 patients had two cycles. The lack of ovulatory function in these women was documented by an absence of LH peaks or transient estradiol elevations and, more importantly, by complete suppression of progesterone levels [37]. Although blood samples were not obtained from patients at hourly intervals, it is unlikely that minor increases of LH or estradiol occurred after each daily injection of *D*-Leu^6-GnRH proethylamide. In another group of premenopausal patients studied by Meldrum and co-workers [3], no rises in estrogen or estradiol occurred during an every 4 h blood sampling protocol. Thus, it would appear that complete ovarian blockade can be accomplished by GnRH agonist administration.

In premenopausal women, estrone and estrone sulfate originate from the adrenal as well as the ovary. As a reflection of this fact, the levels of estrone and estrone sulfate fell to a lesser extent during GnRH-A therapy than did estradiol. Nonetheless, both estrogens decreased to levels routinely measured by our group [38] in postmenopausal women with breast cancer. In a similar fashion, another steroid of combined adrenal and ovarian origin, androstenedione, decreased to the same extent as estrone (data not shown).

The long-term use of GnRH agonists in patients with central precocious puberty produced arrest or regression of secondary sex characteristics. Moreover, this therapy decreased height velocity and bone maturation to within normal prepubertal limits. This latter effect, which was usually not achieved with classical therapy such as medroxyprogesterone acetate or cyproterone, suggests the potential improvement of adult height [39, 40]. However, final stature has not yet been attained in our patients to confirm this hypothesis. In addition, pediatric endocrinologists are awaiting substantial evidence that pituitary gonadal function is completely reversible after the arrest of treatment. Therefore, studies on final adult height and potential for fertility in the patients treated for precocious puberty are required during the next several years. To date, our experience has been based predominantly on the short-term response to treatment and the

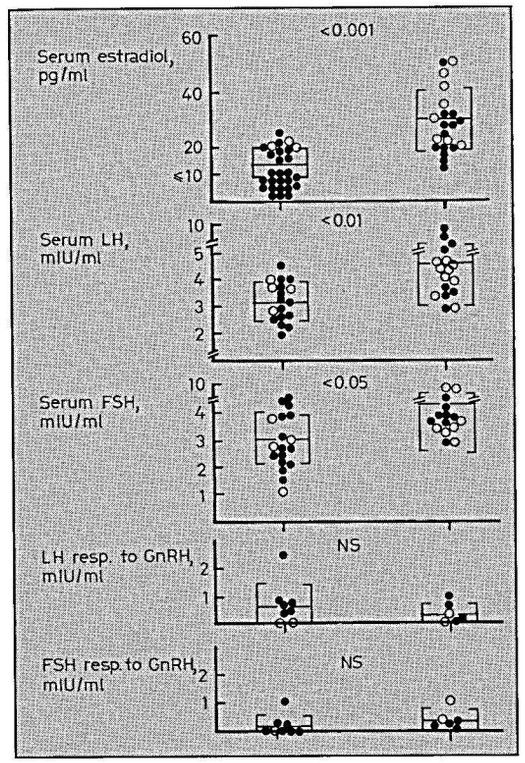

Fig. 10. Serum estradiol, basal gonadotropins, and response to GnRH (25 μg/m^2) observed at 6–12 months of Buserelin® therapy in girls with central precocious puberty (●) or idiopathic short stature (○). The patients have been arbitrarily separated into two groups according to their suppressed (left panel) or nonsuppressed (right panel) estradiol level. Mean ± SD are also shown in both groups [from 41, with permission].

monitoring of its efficacy. By giving Buserelin® intranasally in children (see below) we have used a suboptimal dosage in some patients who demonstrated pubertal levels of gonadal steroids during therapy [41]. However, all of these patients had been found to have abolished gonadotropin responses to GnRH. These results indicated that responsiveness to low doses of GnRH is an overly sensitive index of pituitary gonadal suppression by the GnRH superagonist (fig. 10). Recent observations suggest that study of gonadotropin responses to the superagonist analogs, rather than low-dose GnRH, provides a better index of treatment efficacy [42].

GnRH-A Formulations

Current regimens of GnRH-A require daily subcutaneous injection. Alternative routes would be more acceptable to patients. Formulation of the GnRH-A as nasal sprays eliminates the need for daily injections. Currently available formulations have undergone extensive testing [1, 29, 43]. Unfortunately, the results indicate that a maximum of only 5% of the GnRH-A is absorbed by the nasal route. To allow absorption of sufficient analog into the systemic circulation, a 6-times daily schedule of intranasal administration or the use of high doses is required. Even with 1.5 mg of analog given daily at this frequency, a significantly greater suppression of testosterone was observed with the subcutaneous regimen of Leuprolide® than with intranasal Buserelin® [25]. The different responses to treatment may also involve the differences in nature and potency of the agonists. We found, using intranasal buserelin at 1,200 μg/day [29, 41], that this compound exerted a less inhibitory effect than that reported using Nafarelin® 1,200 μg/day [44]. Although the nasal route has not yet been optimized with respect to drug absorption, it appears likely that other routes of administration will ultimately be preferable.

A number of investigators have developed methods to bind GnRH-A to biode-

Table II. Maximum antiovulatory potencies of GnRH antagonists for a given number of amino acid substitutions [from 1, with permission]

1 2 3 4 5 6 7 8 9 10
⟨Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

GnRH antagonists		total anovulatory dose, μg (ED_{100})
number of substitutions	position(s) of substitution(s)	
1	2	–[a]
2	2, 6	6,000[b]
3	2, 3, 6	750[c]
4	1, 2, 3, 6	1[d]
5	1, 2, 3, 6, 10	0.5[e]
6	1, 2, 3, 6, 7, 10	0.5[f]

[a] [*D*-Phe²]GnRH.
[b] [4-F-*D*-Phe²-Ala⁶]GnRH.
[c] [*D*-Phe², Pro³, *D*-Trp⁶]GnRH.
[d] [Ac-2-*D*-Nal¹, 4-F-*D*-Phe², *D*-Trp³, *D*-Arg⁶]GnRH.
[e] [Ac-2-*D*-Nal¹, α-Me-4-Cl-*D*-Phe², *D*-Trp³, *D*-Arg⁶, *D*-Ala¹⁰]GnRH.
[f] [Ac-2-*D*-Nal¹, 4-Cl-*D*-Phe², 3-*D*-Pal³, *D*-Arg⁶, Trp⁷, *D*-Ala¹⁰]GnRH.

gradable polymers as a means of allowing long-term sustained release. In an example of this approach, Zoladex® (*D*-Ser(+Bu)⁶-AzGly¹⁰-LHRH) is homogeneously dispersed in rods of *DL*-lactide-co-glycolide polymer [13, 45]. Insertion under the skin by trocar once monthly allows continuous maintenance of therapeutic levels of drug. A monthly dose of 3.6 mg suppresses testosterone to castrate levels, causes no local problems at the implantation site, and appears active in prostate cancer patients. Other analogous formulations for nafarelin, *D*-Trp⁶-LHRH, and *D*-Leu⁶-GnRH proethylamide are also being investigated [1]. *D*-Trp⁶-LHRH in microcapsules has already been shown to be useful in precocious puberty [46]. These approaches appear promising as a means to achieve highly selective testicular suppression with a minimum of problems of patient acceptance and compliance. Additional studies are also required to determine the optimal dosage in patients with central precocious puberty. Although maximal doses can be used in prostate cancer patients who require a medical castration, it is possible that, in children, suboptimal doses could result in low but incompletely suppressed sex steroid secretion. This might allow preservation of growth-promoting effects of sex steroids without concomitant progression of sexual characteristics.

GnRH Antagonists

Recent research attention has been directed toward development of potent antagonist analogs of GnRH. In contrast to the history of agonist analog development, it has been difficult ot synthesize highly potent antagonists. The structure activity relationships are highly complex [1]. It appears, as shown in table II, that substitution of GnRH at multiple sites is necessary to produce highly potent compounds. Several of these agents are now undergoing extensive study in animal models and in patients. These drugs are not associated with a transient rise in gonadotropin secretion as is seen with the potent superagonist analogs. The new antagonists are extremely long acting since the rate of dissociation of receptor-bound antagonists from pituitary particles and cells is retarded in comparison with that of the agonists. The antagonists cause a prolonged reduction in available GnRH receptor sites, probably attributable to persistent antagonist receptor occupancy [1].

A number of clinical studies detail the effects of antagonist analogs on animals and in patients. The most recently published data involve use of the 4-F antagonist [Ac-3-Pro¹,4-F-*D*-Phe²,*D*-Trp³,⁶-LHRH] administered by constant infusion to normal men [47]. The levels of serum FSH, LH and testosterone fell immediately upon infusion of this antagonist (13.3 μg/kg/h s.c.). Notably, an escape toward baseline then occurred over the ensuing 72 h. A similar escape occurred in men receiving 100 μg/kg s.c. every 6 h for 7 days. These, and observations with other antagonists [48], raise caution regarding the possibility that endogenous GnRH may rise sufficiently in men receiving antagonist analogs to overcome the blocking effect of these synthetic peptides. Further studies will be required to fully understand the therapeutic potential of the antagonists in patients.

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