

# From Contraception to Cancer: A Review of the Therapeutic Applications of LHRH Analogues as Antitumor Agents

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LHRH and its analogues produce profound antireproductive effects in both sexes of a variety of animal species. Although the *LHRH agonists* induce gonadotropin release, gonadal steroid secretion, ovulation, and spermatogenesis as an expression of their traditional pro-fertility pharmacologic profile, they paradoxically and characteristically cause predominant antifertility effects which have been extensively evaluated for potential contraceptive purposes. These agonists produce their antireproductive effects in both males and females by common mechanisms, ultimately resulting in disruption of pituitary-gonadal function, depression of steroidogenesis, and inhibition of target organs dependent on such gonadal support. Similar antireproductive effects have been observed with the *LHRH antagonists* which competitively inhibit LHRH-induced gonadotropin secretion resulting in reduced blood gonadal steroid levels. Use of the inhibitory properties has been extended to cancer therapy based on the ability of the LHRH analogues (particularly the agonists) to inhibit the growth of steroid-dependent (responsive) tumors (e.g., mammary, prostate) similar to that produced by gonadectomy and antisteroid treatments. The use of these peptides for selected hormone-sensitive tumors presents a novel pharmacotherapeutic application for this class of drug.

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## INTRODUCTION

The extensive pharmacologic evaluation of luteinizing hormone releasing hormone (LHRH) and its numerous analogues (agonists and antagonists) has clearly established the antireproductive properties of this class of compound [1-5]. The paradoxical antifertility activity and high potency of the agonists, in particular, are well-documented and have provided the basis for a new contraceptive approach in both females and males. However, it is only within the last few years that quantities of antagonists with sufficient potency have become available to permit their more detailed pharmacologic evaluation. A general scheme showing these relationships is presented in Fig. 1.

LHRH and its more potent congeners ("super" agonists) possess the traditional pro-fertility property of inducing pituitary follicle stimulating hormone (FSH) and luteinizing hormone (LH) release, which are, in turn, responsible for ovulation, spermatogenesis, and gonadal steroid secretion. However, using various dosing regimens and routes of administration in animal experiments and in clinical trials, these peptides invariably produced paradoxical antireproductive effects. This apparent contradictory but predictable contraceptive activity has been repeatedly demonstrated in a wide variety of studies [1-5]. Numerous synthetic analogues that

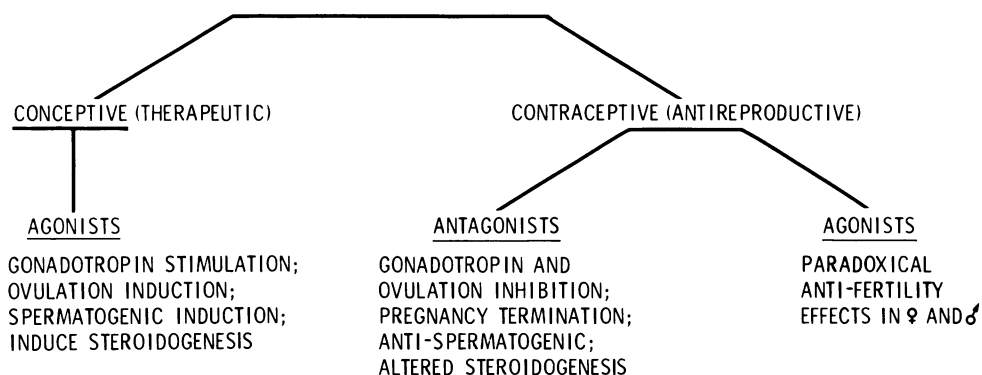


FIG. 1. LHRH analogues in fertility regulation.

have become available since LHRH was isolated, identified, and synthesized in 1971, have been used to demonstrate that the traditional agonist properties are characteristically correlated with and predictable of their antifertility effects. The ability of these compounds to inhibit or impede ovarian and testicular steroidogenesis, and consequently produce regression of the reproductive structures dependent on such steroid support, led to the concept that gonadal steroid-dependent (responsive) tumors might also be affected in a manner analogous to that produced by gonadectomy or by antiandrogenic or antiestrogenic drugs.

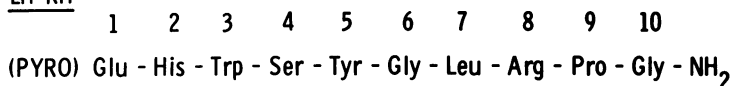
This review will describe, for the most part, the basic principles and developments supporting the paradoxical antireproductive effects of the LHRH agonists, current concepts regarding the mechanisms that are involved and the application of these properties as potential therapeutic antitumor agents. A brief reference will be made to the LHRH antagonists which also possess antireproductive properties but have been demonstrated only recently to produce antitumor effects.

#### LHRH AGONISTS PROPERTIES: TRADITIONAL AND PARADOXICAL ANTIFERTILITY EFFECTS

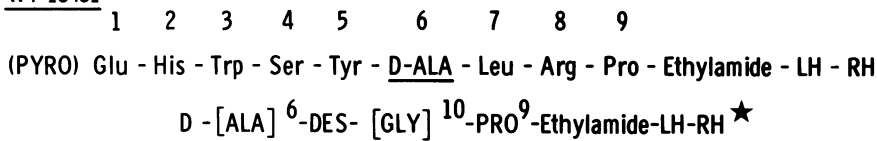
LHRH and its agonistic derivatives stimulate the pituitary-gonadal target organ axis under physiological and carefully regimented pharmacologic conditions in females and males.

In contrast, numerous studies [1] have demonstrated that the parent molecule, LHRH, was capable of terminating pregnancy when administered to inseminated rats either preimplantationally (claudogen test, days 1-7) or postimplantationally (interceptive test, days 7-12). This paradoxical antifertility effect gained prominence when it was demonstrated that several synthetic congeners produced similar effects at considerably lower doses. Subsequently, it was established that such antireproductive consequences were consistently associated with the ability of this class of peptide to release LH (and induce ovulation); these basic profertility effects served to identify and predict those LHRH agonists with contragestational (and eventually broad antireproductive) activity and enhanced potency. The amino acid sequence of LHRH and representative structural modifications that were made to yield some of the more potent agonists are shown in Fig. 2. The various agonists receiving significant attention and their dual properties (ovulation induction and postcoital contraception) from studies in the rat are listed in Table 1. Table 2 describes the general reproductive pharmacologic profile of the LHRH agonists, including mechanisms of

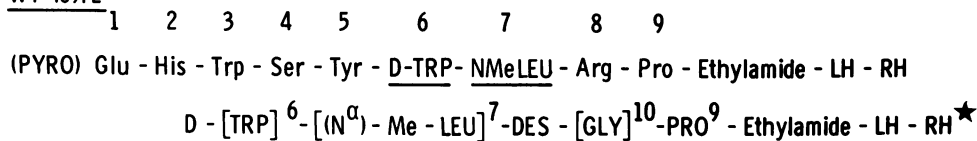
LH-RH



WY 18481



WY 40972



★ Abbreviated Structural Description

FIG. 2. Structure of LH-RH and exemplary agonists.

action, that have been reported in the animal and clinical literature. As noted in Table 2, these agents can also produce extra-pituitary effects, adding another dimension to their proposed mechanism(s) of action. Table 3 illustrates the laboratory observations with the Wyeth compound, Wy-40,972, which possesses a reproductive and safety profile characteristic of the LHRH agonist class.

The LHRH agonists, by impeding gonadal steroidogenesis, produce regression of

TABLE 1

The Agonist and Post-Coital Contraceptive Profile of the [D-Ala<sup>6</sup>]-LH-RH and [D-Trp<sup>6</sup>]-LH-RH Series

COMPOUND	AGONIST OVULATION INDUCTION MED <sub>100*</sub> , µg/RAT. I.V.	POST-COITAL CONTRACEPTION	
		CLAUDOGEN [MED <sub>100</sub> , µg/RAT/DAY, S.C.]	INTERCEPTIVE
LH-RH	5.0	200-300	200-500
D-Ala <sup>6</sup> -LH-RH	1.0	10	100
D-Ala <sup>6</sup> -Des-Gly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH	1.0	1.0	25-50
D-Ala <sup>6</sup> -N <sup>α</sup> -Me-Leu <sup>7</sup> -Des-Gly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH	0.10	1.0	1.0
D-Trp <sup>6</sup> -LH-RH	0.10	10	1.0-10
D-Trp <sup>6</sup> -Des-Gly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH	0.10	1.0	10
D-Trp <sup>6</sup> -N <sup>α</sup> -Me-Leu <sup>7</sup> -Des-Gly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH	0.10	1.0	~ 1.0
D-Ser(TBU) <sup>6</sup> -Des-Gly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH (Buserelin)	0.10 - 1.0	10	10

\* Approximate minimal effective 100% dose

NHET = ethylamide

TABLE 2  
Reproductive Pharmacologic Profile of LHRH Agonists

1. TRADITIONAL EFFECTS

- a) STIMULATE GONADOTROPIN SECRETION
- b) INDUCE OVULATION
- c) INDUCE SPERMATOGENESIS
- d) INDUCE STEROIDOGENESIS

2. PARADOXICAL ANTI-FERTILITY EFFECTS

- a) HYPERSECRETION OF PITUITARY LH AND FSH
- b) PITUITARY DESENSITIZATION WITH CHRONIC TREATMENT (DECREASED RESPONSIVENESS)
- c) PREMATURE OVULATION; INHIBITION OF OVULATION
- d) LUTEOLYSIS
- e) DOWNREGULATION OF GONADAL GONADOTROPIN RECEPTORS (DECREASED RECEPTOR NUMBERS)
- f) INTERFERENCE WITH GONADAL STEROIDOGENESIS - ACT EITHER DIRECTLY OR VIA LH RELEASE TO EFFECT ENZYMES IN STEROIDOGENIC PATHWAY (INHIBITION OF 17-HYDROXYLASES AND 17-DESMOLASES; STIMULATION OF REDUCTASES AND AROMATASES)
- g) TERMINATE PREGNANCY
- h) DISRUPT ESTROUS CYCLE; CAUSE OVARIAN AND UTERINE REGRESSION AND REDUCED FECUNDITY
- i) SHORTEN MENSTRUAL CYCLE; EARLY MENSTRUAL ONSET; AMENORRHEA
- j) PUBERTAL RETARDATION; INHIBITION OF PRECOCIOUS PUBERTY
- k) INHIBIT MALE REPRODUCTION - ANTISPERMATOGENIC; INHIBIT TESTOSTERONE PRODUCTION, MATING BEHAVIOR AND SECONDARY REPRODUCTIVE ORGANS
- l) PRODUCE EXTRAPITUITARY EFFECTS (IN ABSENCE OF HYPOPHYSIS) DIRECTLY AT LEVEL OF GONADS, SECONDARY REPRODUCTIVE TARGET TISSUES AND UTERUS/PLACENTA; PREGNANCY TERMINATION IN HYPOPHYSECTOMIZED ANIMAL

TABLE 3  
Profile of Wy-40,972

A. AS AN AGONIST

- 1. POTENT LHRH AGONIST AS DETERMINED BY GONADOTROPIN RELEASE AND OVULATION INDUCTION
- 2. EFFECTIVE BY 12 ROUTES OF ADMINISTRATION

B. AS AN ANTIFERTILITY AGENT

- 1. TERMINATES PREGNANCY PRE AND POST IMPLANTATIONALLY BY 6 ROUTES OF ADMINISTRATION
- 2. INDUCES LUTEOLYSIS
- 3. INHIBITS OVULATION; INTERFERES WITH STEROIDOGENESIS
- 4. DISRUPTS ESTROUS CYCLE
- 5. INHIBITS FEMALE PUBERTY
- 6. INHIBITS SPERMATOGENESIS AND ANDROGEN SECRETION

C. SAFETY

- 1. NO UNDESIRABLE SIDE EFFECTS AT MULTIPLES OF EFFICACIOUS DOSES UNDER CHRONIC REGIMENS
- 2. NO EFFECTS ON NON-REPRODUCTIVE SYSTEMS
- 3. WIDE THERAPEUTIC/SAFETY MARGIN
- 4. NO TERATOGENIC LIABILITY
- 5. ANTIFERTILITY EFFECTS ARE REVERSIBLE WITH RAPID RETURN TO NORMAL FERTILE STATES

steroid-supported reproductive tissues in both females and males. The sequelae of events and the proposed disruption of the steroidogenic pathway are visualized in Figs. 3 and 4, respectively. The entire antireproductive spectrum of effects is reversible, with the time course of recovery following cessation of treatment dependent upon the dose and the length of its administration. This aspect represents an important variable in designing dosing protocols to ensure significant tumor regression and to prevent its recrudescence should LHRH agonist treatment be withdrawn; this matter will be discussed more fully in a subsequent section.

Many of the aforementioned agonists are undergoing extensive clinical evaluation as contraceptives. The LH-releasing dose in humans of several of these compounds with various routes of administration are listed in Table 4. The contraceptive doses employed are generally multiples of the single LH-releasing dose which eventually lead to downregulation of gonadal gonadotropin receptors, impedance of steroidogenesis, and disruption of conceptive patterns. Table 5 summarizes the broader clinical applications of LHRH and analogues (both the agonists and the antagonists) that include their utility as diagnostic, therapeutic, and contraceptive agents. With regard to the LHRH antagonists, these particular derivatives of LHRH competitively inhibit the release of pituitary gonadotropins at the hypophysial level and also have been shown to act at a hypothalamic site, inhibiting the secretion of endogenous LHRH. Although it is only recently that antagonists of sufficient activity, potency, and quantity have become available for animal and clinical investigation, they are mentioned since they too can result in several of the effects observed with the agonists.

Interestingly, because the LHRH agonists (as well as the antagonists) can produce antireproductive effects through gonadotropic/steroidogenic hindrance, many disease processes that are dependent on these hormones for their support (e.g., hirsutism, precocious puberty, acne, endometriosis) become potential candidates for LHRH analogue therapy (Fig. 5).

#### ANTITUMOR EFFECTS OF LHRH ANALOGUES—ANIMAL STUDIES

##### *Agonists*

The marked inhibitory influences of the agonists on gonadal steroid-producing and gonadal steroid-dependent tissues led to consideration of their therapeutic util-

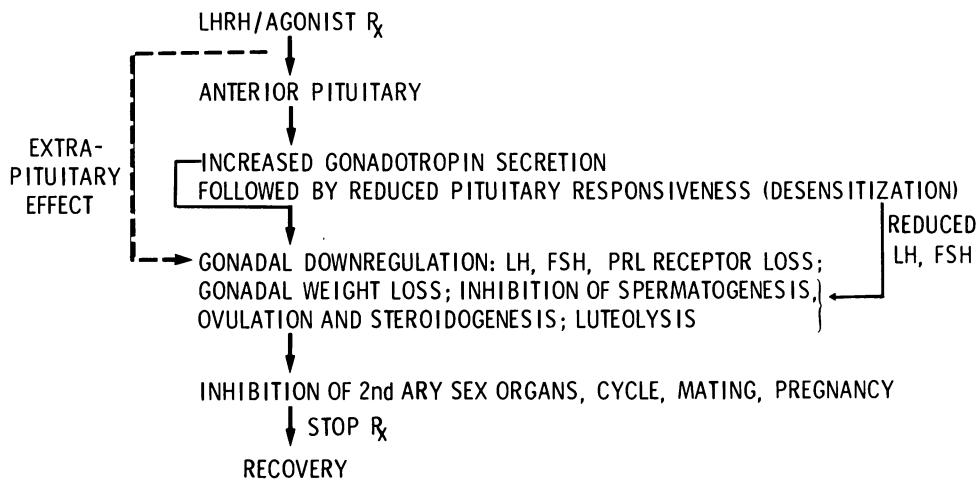


FIG. 3. Antireproductive effects of LHRH agonists.

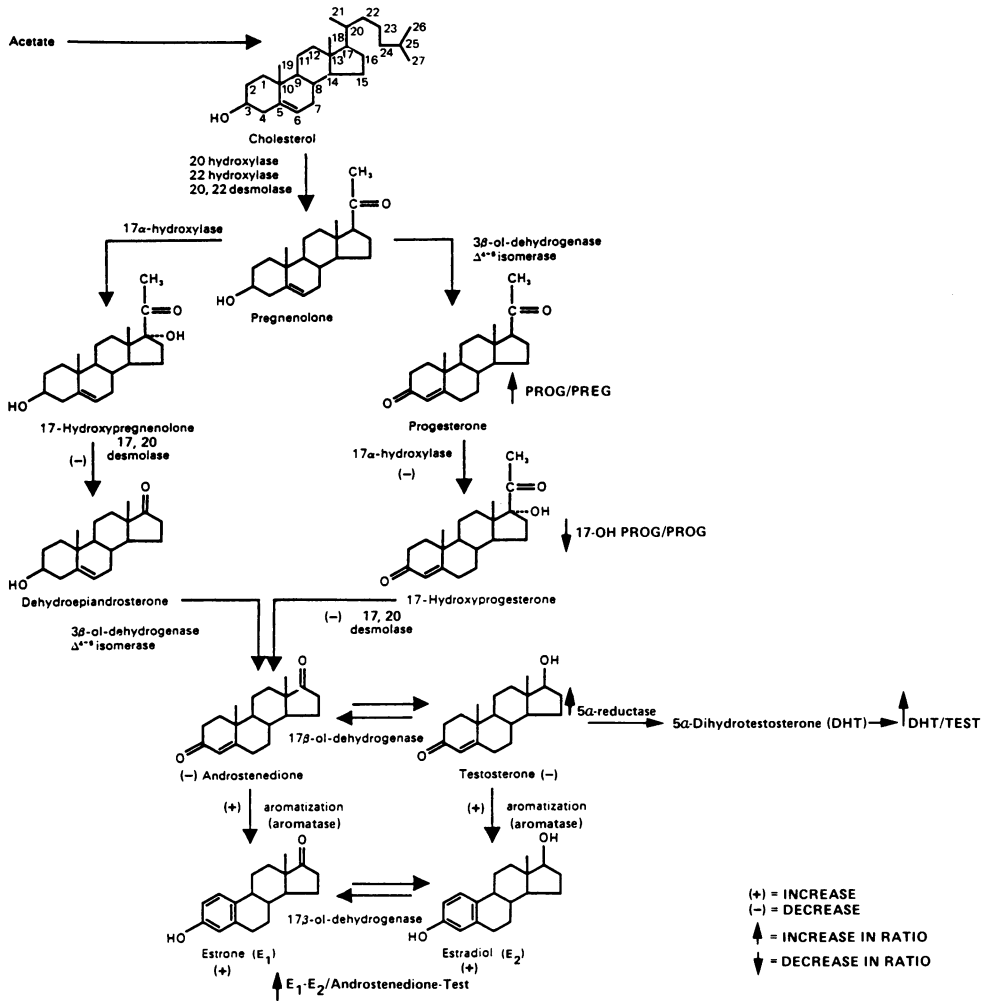


FIG. 4. Proposed LHRH agonist-induced gonadal inhibition: effect on the steroidogenic pathway.

ity as antitumor agents in a manner akin to that produced by antiestrogenic (e.g., Tamoxifen) [6] and antiandrogenic (e.g., Cyproterone acetate) [7] drugs or by gonadectomy. Several of the compounds (and their sponsors) that are being investigated both in animal tumor models and in clinical settings include: D-Trp<sup>6</sup>-LHRH (Schally); D-Trp<sup>6</sup>-DesGly<sup>10</sup>-Pro<sup>9</sup>-NH<sub>2</sub>-LHRH (Salk Institute); D-Trp<sup>6</sup>-N $\alpha$ -MeLeu<sup>7</sup>-DesGly<sup>10</sup>-Pro<sup>9</sup>-NH<sub>2</sub>-LHRH (Wyeth Labs., Wy-40,972); D-Leu<sup>6</sup>-DesGly<sup>10</sup>-Pro<sup>9</sup>-NH<sub>2</sub>-LHRH [Leuprolide; A43818 (Abbott Labs./Takeda Pharmaceuticals)]; D-Ser(TBU)<sup>6</sup>-AzGly<sup>10</sup>-LHRH (ICI Ltd., IC118630); D-Ala<sup>6</sup>-DesGly<sup>10</sup>-Pro<sup>9</sup>-NH<sub>2</sub>-LHRH (Schally); D-Ser(TBU)<sup>6</sup>-DesGly<sup>10</sup>-Pro<sup>9</sup>-NH<sub>2</sub>-LHRH [Buserelin; HOE 766 (Hoechst AG)].

a. *Female Reproductive Tumors* In 1976, reports by Johnson et al. [8] and De Sombre et al. [9] initially demonstrated that the LHRH agonist, Leuprolide, could cause the regression of a spontaneous mammary tumor or mammary tumors induced in rats by 7,12-dimethylbenz(a)anthracene (DMBA). During continuous treatment (about six weeks of daily subcutaneous administration) approximately 80

TABLE 4  
Effect of LH-RH and Agonists on LH Release in Humans by Various Routes of Administration

COMPOUND	SC	IM	PO	NASAL	VAGINAL	RECTAL
	┌ LH-RELEASING DOSE, µg/PERSON (Single Rx) ─┐					
LH-RH (GONADORELIN)	100-250	25	--	500; 1200-1300	--	--
DesGly <sup>10</sup> -Pro <sup>9</sup> -NHET-LHRH	100	--	--	--	--	--
D-Leu <sup>6</sup> -DesGly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH (LEUPROLIDE)	5-25	5-10	10,000	100	2000	2000
D-Trp <sup>6</sup> -DesGly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH	10	10	--	50~500	--	--
D-Ser(TBU) <sup>6</sup> -DesGly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH (Buserelin)	5	10	--	>200<400 10-100	--	--
D-Trp <sup>6</sup> -LH-RH	10	10	--	500	--	--
D-Ala <sup>6</sup> -DesGly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH	--	150	--	500	--	--
D-Phe <sup>6</sup> -DesGly <sup>10</sup> -Pro <sup>9</sup> -NHET-LH-RH	--	--	--	500	--	--
D-Trp <sup>6</sup> -DesGly <sup>10</sup> -N <sup>α</sup> MeLeu <sup>7</sup> -Pro <sup>9</sup> -NHET-LH-RH (WY-40972)	--	10	--	--	--	--

percent of the DMBA tumors regressed and, of these, 50 percent disappeared. Withdrawal of analogue for a period of four weeks led to tumor regrowth and to the appearance of new tumors. The antitumor effect of the peptide was equivalent to that of ovariectomy, suggesting that support of the tumor was estrogen-dependent.

The studies by Nicholson and colleagues [10-12] and Lamberts et al. [13] in the rat showed that the agonist, D-Ser(TBU)<sup>6</sup>-AzGly<sup>10</sup>-LHRH, also was capable of causing regression of DMBA-induced mammary tumors and of estrogen-induced transplantable prolactin (PRL)-secreting rat pituitary tumors (7315A), respectively. In these

TABLE 5  
Clinical Uses of LHRH and Analogues

1. DIAGNOSTIC

- A) EVALUATION OF INTEGRITY OF HYPOTHALAMIC-HYPOPHYSIAL-GONADAL AXIS IN REPRODUCTIVE HYPOFUNCTION WITH AGONISTS.
- B) PHARMACOLOGIC IDENTIFICATION: POTENCY AND ACTIVITY IDENTIFICATION IN NORMAL SUBJECTS WITH AGONISTS AND ANTAGONISTS.

2. THERAPEUTIC (UNDER INVESTIGATION)

STIMULATION OF REPRODUCTIVE FUNCTION WITH AGONISTS IN CLINICAL CONDITIONS SUCH AS AMENORRHEA, ANOVULATION, HYPOGONADISM, RETARDED PUBERTY, NON-DESCENDED TESTES, ETC.; USE OF AGONISTS AND ANTAGONISTS IN PRECOCIOUS PUBERTY; USE OF AGONISTS AND ANTAGONISTS IN GONADAL STEROID-DEPENDENT TUMORS; ENDOMETRIOSIS; HIRSUTISM; ACNE; CYSTIC OVARIAN DISEASE.

3. CONTRACEPTIVE (UNDER INVESTIGATION)

- A) INHIBITION OF OVULATION AND OF PREGNANCY WITH LH-RH ANTAGONISTS.
- B) INHIBITION OF OVULATION; LUTEOLYSIS, PREGNANCY TERMINATION WITH LHRH AGONISTS.
- C) INHIBITION OF SPERMATOGENESIS WITH LHRH AGONISTS AND ANTAGONISTS.

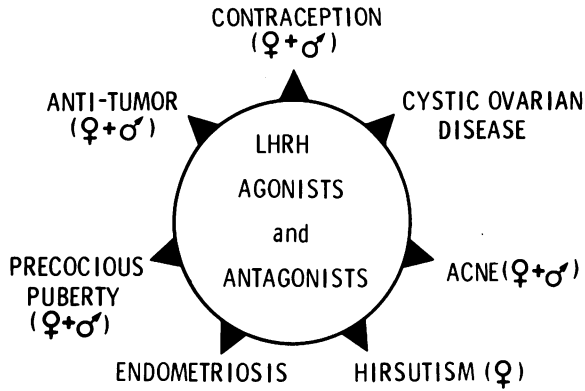


FIG. 5. Potential clinical applications of the antireproductive effects of LHRH analogues.

studies, the tumor-inhibiting effects of the compound were associated with a general reduction in blood levels of estradiol and prolactin; overall, the antitumor results were similar to those observed with Tamoxifen treatment or ovariectomy.

Corbin et al. [14] reported that the development of tumors in four-day-old hamsters inoculated with virulent mouse mammary tumor (MMT) cells was impeded by parenteral administration of either LHRH or D-Ala<sup>6</sup>-DesGly<sup>10</sup>-Pro<sup>9</sup>-NH<sub>2</sub>-LHRH (Wy-18,481) for a period of ten days (Fig. 6). Tumor size remained depressed during the five days following cessation of treatment. It is noteworthy that

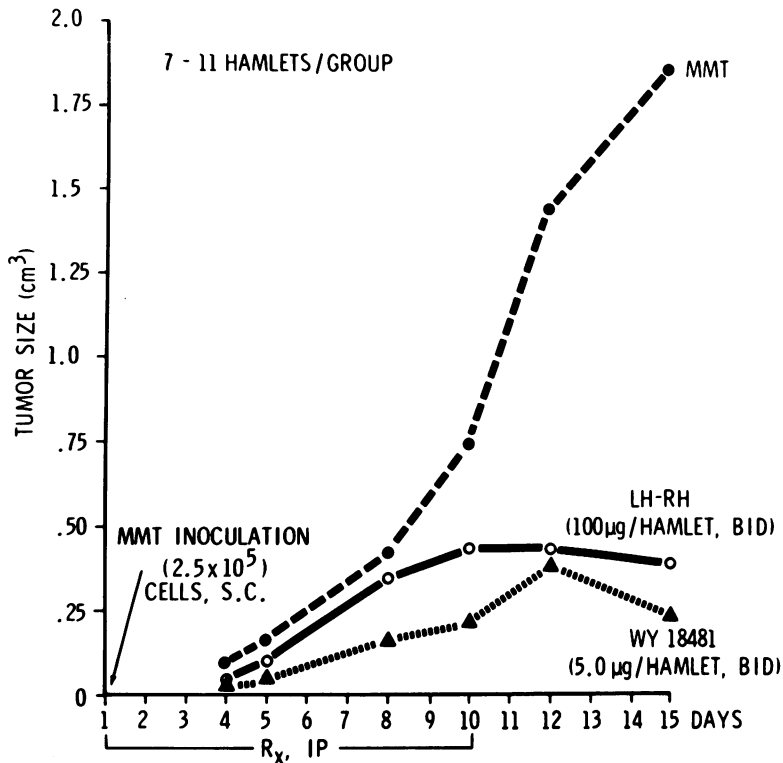


FIG. 6. Effect of LHRH or agonist on mouse mammary tumor (MMT) growth in neonatal hamsters (with permission from Raven Press, New York, 1980 [14]).



this tumor retardation occurred during suppression of the animal's own immune defense system, since these recipients were treated with antilymphocytic serum (ALS) on two occasions during the study. Thus, the peptides attenuated tumor growth under conditions which tended to promote maximal tumor growth. Kelly et al. [15] and Turcot-Lemay et al. [15a], utilizing the same LHRH agonist chronically, demonstrated similar effects on DMBA-induced tumors in rats, associated with significant increases in blood levels of LH and FSH, and a dramatic decline in progesterone and prolactin levels. These investigators suggested that LHRH agonists produce a "functional" castration, such effects being consistent with antifertility effects observed by others.

These collective results reinforce the concept that the reduction of the number of tumors and the tumor regression produced by chronic treatment with these peptides is analogous to that observed following ovariectomy or treatment with the antiestrogen, Tamoxifen [8-10]; furthermore, cessation of peptide treatment can lead to a recrudescence of the tumor, which upon reinitiation of treatment, once again is followed by tumor regression [9]. These data underline the requirement for uninterrupted or chronic intermittent administration of these compounds.

The predominant mechanism by which pharmacologic and sustained doses of LHRH and agonists produce their antireproductive manifestations is via initial hypersecretion of pituitary LH and subsequent downregulation of gonadal receptors for LH, FSH, and PRL (decreased receptor numbers), eventually followed, with chronic administration, by pituitary desensitization and overall inhibition of gonadal steroidogenesis. In contrast, the antisteroidal drugs (e.g., Tamoxifen, Cyproterone acetate) can act by blocking the effect of endogenous gonadal steroids directly at the target organ receptor, interfering with the cytoplasmic/nuclear steroid expression at the cellular level [6,7]. Thus, while the mechanisms of action of the LHRH agonists and those of the classical antiestrogens and antiandrogens are different, the end result is the same: interruption of steroid support for the tumor.

Additionally, there are data supporting extrapituitary, direct gonadal or direct gonadal target organ effects of these peptides [16]. In view of the possibility that LHRH agonists also may act directly on the tumor, we performed a dose-response study in which mouse mammary tumor cells were subjected, *in vitro*, to Wy-40,972 for seven days. The results (unpublished), described in Fig. 7, reveal a tendency toward a dose-related retardation in the increase of the viable cell population. However, after the fourth day of treatment, in spite of continuing exposure to the peptide, the number of viable cells reaches control levels. Because these cells grow so rapidly, relatively high doses of the agonist were required. The rapid growth pattern is further observed beyond the seventh day; a growth plateau eventually is reached followed by a rapid decline in the viable population and ensuing morbidity as the cells simply exhaust their limited environment. These preliminary results suggest the possibility of a direct effect of the agonist on the tumor.

In the studies reported by Rose and Pruitt [17,18] the LHRH agonist, Leuprolide, caused regression of rat mammary tumors induced by DMBA or by N-nitrosomethylurea (NMU); such tumors also responded favorably to ovariectomy or Tamoxifen treatment. Similar results were obtained by Danguy et al. [19] who also utilized Leuprolide in DMBA-induced tumor-bearing rats. In addition, these investigators showed that six weeks of treatment not only produced mammary tumor regression but also atrophy of pituitary lactotropes and decreases serum prolactin concentrations, the latter effect having been observed by several of the other investigators.

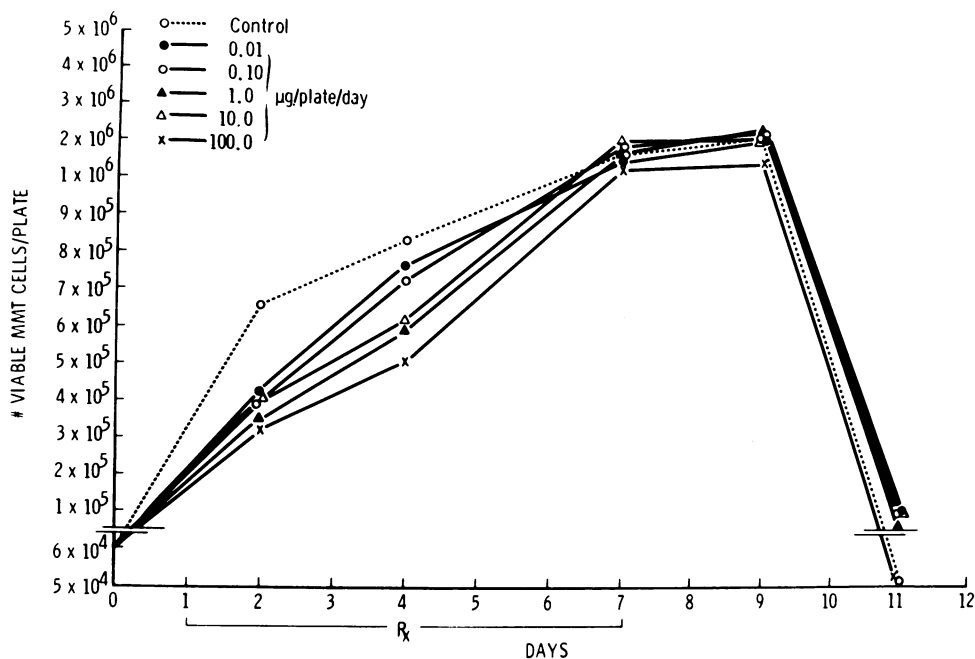


FIG. 7. Effect of LHRH agonist (Wy-40,972) on *in vitro* growth of MMT cells.

It is clear that treatment with several of the LHRH agonists produce their palliative effects on several types of experimentally induced tumors by minimizing or removing the supportive effect of estrogen, thereby mimicking the effects obtained with ovariectomy or antiestrogen therapy.

The role of prolactin in supporting mammary tumor growth and its modification by the agonists is an important point of consideration. Prolactin may have a direct effect on the tumor itself or act through its ability to increase estrogen receptor content or stimulate estrogen secretion. Thus, prolactin may play a permissive role in mammary tumor growth and that it is the estrogen (and perhaps other ovarian steroids) upon which the tumor depends. Furthermore, the degree to which prolactin plays a promotional or supportive role may be a function of the nature of the mammary tumor itself. The results of Danguy et al. [19] showed that while Leuprolide produced a greater suppression of serum prolactin levels than did ovariectomy, the peptide was less effective than ovariectomy in suppressing tumor growth. With regard to the Rose and Pruitt studies [17,18] the anti-DMBA-induced tumor effect of Leuprolide was impeded when estradiol benzoate was administered concomitantly. Moreover, when perphenazine (a phenothiazine which produces hyperprolactinemia) also was administered with Leuprolide, the antitumor efficacy of the latter likewise was impaired. It was suggested that the peptide produced its antitumor effect through initial inhibition of ovarian steroidogenesis with consequential hypoprolactinemia. In contrast, while Leuprolide caused regression of rat tumors induced by NMU, its effectiveness was inhibited by estradiol benzoate but not by perphenazine [18]. These investigators suggested that NMU-induced tumors are estrogen rather than prolactin-dependent, with the analogue producing a pharmacologic ovariectomy; additionally, they proposed that NMU-induced mammary tumors reflect the clinical disease state which is not responsive to prolactin inhibitors such as 2-Br- $\alpha$ -ergocryptine [20].

It is difficult to discern the degree to which estrogen or prolactin contributes to mammary tumor genesis and maintenance. DMBA-induced mammary tumors obviously are hormone-dependent and will regress following ovariectomy or hypophysectomy [21]. Kelly et al. [15] and Turcot-Lemay et al. [15a] showed that the LHRH agonist, D-Ala<sup>6</sup>-DesGly<sup>10</sup>-Pro<sup>9</sup>-NH<sub>2</sub>-LHRH, lowered the tumor concentrations of progesterone and prolactin receptors during the course of DMBA-mammary tumor growth retardation of intact rats; estradiol receptor levels were unaltered. However, ovariectomy alone was shown to lower the number of estradiol receptors in the tumor as well as those for progesterone and prolactin. Tsai et al. [22] also demonstrated that ovariectomy of rats bearing DMBA tumors produces a conspicuous loss of prolactin receptors within the regressing tumor. While both estradiol and prolactin influence DMBA tumor growth, prolactin appears to play the predominant role, since prolactin can, and estradiol cannot, induce tumor regrowth following regression due to hypophysectomy of DMBA-tumor bearing animals. The report of Arafah et al. [23] using hypophysectomized, hormone-replaced, DMBA-tumor rats concludes that prolactin is the major hormone precipitating the growth of this particular tumor. Such tumors are rich in prolactin, estradiol, and progesterone receptors; estrogen receptor levels decline following ovariectomy or treatment with legotrile mesylate, an inhibitor of prolactin secretion, and the estrogen receptors are restored in the tumor following either estradiol or prolactin administration. Furthermore, the reduced progesterone receptor levels within the tumor following ovariectomy return to control values with estradiol replacement, but not with prolactin; ovariectomy also lowers tumor prolactin receptor levels which are replenished by estradiol replacement but not with prolactin. These hormonal/receptor interactions are extremely complex and confound the choice of therapy where antihormone treatment might be appropriate based on tumor receptor analysis. It would appear though that tumors bearing gonadal-steroid receptors and/or prolactin receptors are the ones that are most amenable to antihormone intervention; however, the role of growth hormone must not be overlooked. For a more detailed coverage of these issues, the reader is referred to the publications of Arafah and others [23-31c]

*b. Male Reproductive Tumors* Numerous reports have demonstrated the antireproductive properties of LHRH and its agonists in the male [1,4,32-35]. The LHRH agonists have been shown to inhibit, gravimetrically, histologically, hormonally, germinally, and behaviorally, the reproductive status of the male, including humans [36].

Chronic administration of LHRH or agonists produce a loss of testicular LH and prolactin receptors, decreased androgen synthesis and blood levels, a reduction in the weight of the testes, testicular histologic disorganization, inhibition of spermatogenesis, and reduced weights of the sexual accessory apparatus [32,37]. Thus, the eventual decrease in androgen secretion observed in LHRH-agonist-treated animals provided the basis for the potential use of these peptides, either alone or as an adjunct to other therapies, in androgen-dependent pathologies in the human male.

Redding and Schally [38,39] evaluated the effect of D-Trp<sup>6</sup>-LHRH on the development of two different types of transplantable prostatic tumors in young male rats: (1) the Segaloff squamous cell tumor 11095 which can be induced by implanting methylcholanthrene into the ventral prostate, and (2) the spontaneous Dunning adenocarcinoma R3327. The former tumor is partially hormone-dependent and is influenced by castration or by estrogenic or progestational treatment; the latter is believed to represent the human model, requiring androgens for its maintenance.

Chronic treatment with the analogue for a period of 14-42 days produced qual-

itatively similar effects in both animal models. Predictably, the agonist significantly lowered serum levels of LH, prolactin, and testosterone, associated with reduction in the weights of the testes and ventral prostate. Serum progesterone levels were increased, as described by others [40,41]. Prostatic tumor weights were significantly decreased. With respect to the Dunning tumor, tumor volume was drastically diminished during exposure to the compound; the percentage increase in tumor volume was severely retarded as well as a lengthening of cell doubling time when compared to untreated control tumors.

### *Antagonists*

The preceding studies have dealt exclusively with the antitumor effects of the LHRH agonists. There are only a few studies relating to the antitumor effects of the antagonists. A recent report by Redding et al. [42] has demonstrated that newly developed potent LHRH antagonists likewise are capable of decreasing tumor size in animals. Historically, the LHRH antagonists required considerably greater chemical modification than the agonists to produce a series of potent molecules that would effectively compete with LHRH at the level of the pituitary receptor, thereby blocking the ability of LHRH to stimulate the secretion of LH and FSH. In animals and in limited human trials, the reproductive consequences of LHRH antagonist administration include inhibition of gonadotropin secretion, ovulation, and of pregnancy, disruption of testicular and accessory reproductive organ function and of gonadal steroid production [3,43].

Redding et al. [42] administered the LHRH antagonists, NAc-pF-D-Phe<sup>1</sup>, pCl-D-Phe<sup>2</sup>-D-Trp<sup>3,6</sup>, D-Ala<sup>10</sup>-LHRH, and NAc-pCl-D-Phe<sup>1,2</sup>, D-Trp<sup>3</sup>, D-Phe<sup>6</sup>, D-Ala<sup>10</sup>-LHRH, to rats bearing either the Segaloff squamous cell or the Dunning adenocarcinoma of the prostate. A daily subcutaneous dose of 50 µg/rat from 17–42 days produced the following overall effects: decreased weight of the whole prostate; depressed serum levels of LH, FSH, prolactin, and testosterone; increased serum progesterone levels; no effect on the weights of the anterior pituitary gland, testes, and adrenals.

Preliminary results from our laboratory (unpublished) reinforce the antitumor effect of the LHRH antagonists. Neonatal hamsters were inoculated with MMT cells (and ALS) as previously described [14] and intermittently treated parenterally with the LHRH antagonist, AC-dehydroPro<sup>1</sup>-pF-D-Phe<sup>2</sup>-D-Trp<sup>3,6</sup>-LHRH (Wy-44,599; obtained from the Salk Institute), over a period of ten days. The data in Fig. 8 reveal a dose-related retardation of tumor volume growth.

The available data suggest that both the LHRH agonists and the antagonists can inhibit the growth of androgen- and estrogen-dependent tumors by their ability, although by different mechanisms, to significantly depress gonadal steroidogenic function and perhaps, by an additional effect (of the agonist), directly on the tumor.

### ANTITUMOR EFFECTS OF LHRH AGONISTS—CLINICAL STUDIES

It is only recently that data have appeared in the clinical literature on the potential efficacy of the LHRH agonists as a tumor therapy. These studies represent an outgrowth from the earlier animal and human contraceptive investigations and more recent work in animal tumor models [1–5,32–37,44–46].

A preliminary study was carried out by Faure et al. [47,48] using either daily subcutaneous (50 µg for one to six months) or daily intranasal (200 and 500 µg for three to eight months) administration of Buserelin to patients with prostatic cancer. In-

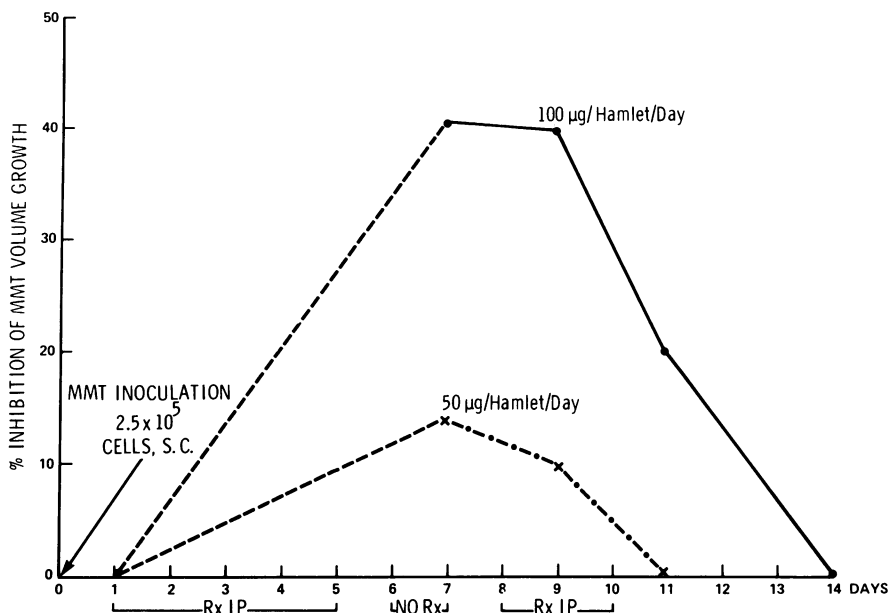


FIG. 8. Effect of LHRH antagonist (Wy-44,599) on mouse mammary tumor (MMT) growth in neonatal hamsters.

hibition of pituitary-gonadal function was observed: inhibition of LH responsiveness and decreased levels of serum testosterone and 17OH-progesterone. The decrease in these latter steroids was associated with no change in pregnenolone and progesterone, indicating that inhibition of androgen synthesis was due to blockade of 17-hydroxylase activity. Superficial physical examination of the patients suggested a reduction in tumor mass.

The utility of "medical castration" for treating endocrine-dependent neoplasias also has been explored by Tolis et al. [49,50], Harvey et al. [51], and Warner et al. [52-54] using Leuprolide and D-Trp<sup>6</sup>-LHRH in patients with prostatic carcinoma or breast cancer. In the preliminary Tolis study [49], daily subcutaneous administration of 1,000 µg of the former compound, or 50-100 µg of the latter, to males for 6-20 weeks, produced the predictable declines in LH, FSH, testosterone, dihydrotestosterone, and in estrone and estradiol. Decrease of prostatic mass and improvement of urinary outflow obstruction were documented. In the more detailed report Tolis et al. [50] treated ten geriatric patients with prostatic carcinoma (four with severe incapacitation due to pain; two with disseminated osteoblastic metastases; two with urinary flow obstruction) with either D-Trp<sup>6</sup>-LHRH (100 µg/day, subcutaneously) or Buserelin (50 µg/day, subcutaneously or 500 µg twice daily) for six weeks to 12 months. In general, patients experienced significant clinical improvement. In addition to achieving a "medical castration" (significant suppression of blood levels of testosterone and estradiol) there was relief of pain, decline in acid and alkaline phosphatase levels, improvement of urinary outflow obstruction and bone lesions (evidenced by isotopic bone imaging), and reduced prostatic mass (documented by ultrasonography). These results, although derived from a very small population, are encouraging for they indicate the potential use of the LHRH agonists in ameliorating the progress of a disabling prostatic malignancy.

Harvey et al. [51] and Warner and associates [52-54] carried out a series of studies

with Leuprolide on patients with postmenopausal advanced breast cancer or prostatic carcinoma. The 31 female subjects, most of whom had received prior therapy for metastatic mammary carcinoma and who were either estrogen receptor positive (ER+) or antihormone responsive, were administered a daily subcutaneous dose of 1.0 to 5.0 mg of the agonist for up to 30 weeks. Heterogeneous effects were obtained: 16 percent showed objective responses; 19 percent were stabilized, and 65 percent had disease progression. Remissions in the responder group lasted from 12 to 30 weeks. No side effects of the drug were obvious; however, it was not known what role the initial therapy played in influencing the outcome of the trial with the agonist.

In the male investigation, 57 patients with prostatic cancer were administered the analogue (1.0–10 mg/day, subcutaneously) for eleven weeks. An initial study in the androgen-dependent rat prostate tumor model (Noble adenocarcinoma) [55] demonstrated that chronic administration of the agonist produced decreased size of the prostate, seminal vesicle, and testes; reduction of blood levels of LH, FSH, and testosterone; inhibition of 17 $\alpha$ -hydroxylase and C-17,20 lyase activity; induction of 5 $\alpha$ -reductase and 3-keto-reductase activity and direct inhibition of testicular LH receptor numbers. The authors reported that the clinical data paralleled those derived from the animal study: gonadotropin levels were markedly suppressed and testosterone decreased to levels comparable to those found in the 31 castrate control subjects with prostate tumors. Additionally, dihydrotestosterone and 3 $\alpha$ -androstenediol values were reduced and objective tumor regression was observed. Based on these clinical results and the analogous antiandrogenic/antitumor effects in the animal model, these investigators emphasized the value of primary treatment of prostatic carcinoma employing the “medical castration” effect of the agonist as an alternative to surgical orchiectomy [54].

A generalized scheme of the possible mechanisms of action of LHRH agonists on tumor regression is depicted in Fig. 9.

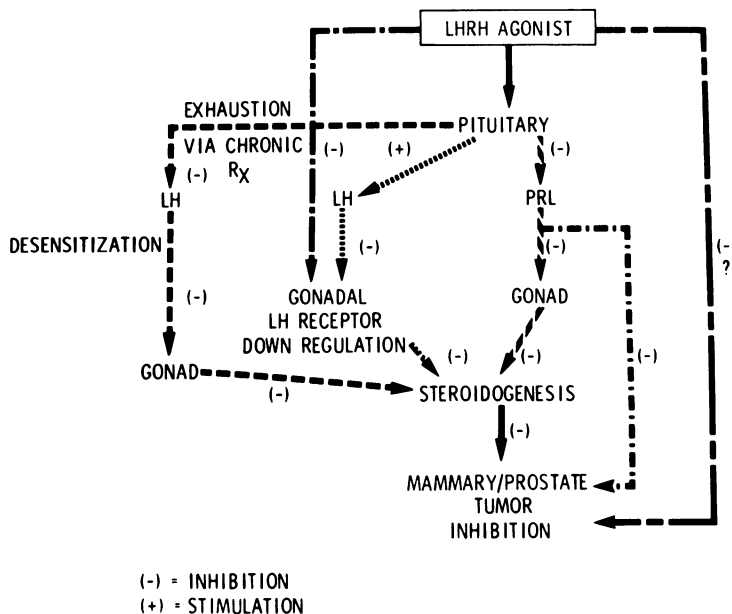


FIG. 9. Possible mechanisms of action of LHRH agonists on tumor regression.

## OVERVIEW

The animal studies reviewed unequivocally demonstrate the antireproductive and antitumor properties of the LHRH analogues providing justification for a detailed evaluation in selected oncologic settings. The limited clinical investigations described confirm the basic "medical castration" effects in patients with selected reproductive organ tumors that consistently and predictably have been observed in the preclinical studies.

The mechanisms by which the LHRH analogues inhibit tumor growth may be numerous. The major mechanism of the agonists appears to be via pituitary-gonadal downregulation and desensitization: the LHRH agonist induces initial gonadotropin hypersecretion that leads to gonadal gonadotropin receptor loss and reduced steroidogenesis; continuous agonist administration results in hypophysial desensitization and LHRH receptor downregulation leading to blunted secretion and decreased blood levels of gonadotropins producing a further suppression of steroidogenesis and removal of steroid support for the tumor. Additionally, the shift in steroidogenic patterns due to interference with the enzymes responsible for precursor conversion may lead to a decrease or an increase of a particular steroid (e.g., excess progesterone in the male).

The reduced serum levels of gonadotropins, prolactin, and the androgenic and estrogenic steroids are representative of "selective hypophysectomy" or of castration, procedures that can be effective in arresting the growth of particular tumors. Moreover, the agonist may have effects directly on the gonad since the testes and ovaries have been shown to possess LHRH receptors [16], or directly on the tumor itself (e.g., prostate), although there are conflicting data supporting the presence of LHRH receptors in secondary sex organs.

Of interest, however, is the report of Sundaram et al. [56] demonstrating that LHRH agonists could block the stimulatory effect of exogenous sex steroids on accessory reproductive organs in castrated and/or hypophysectomized male and female rats. These results suggested that the analogue could directly antagonize the effect of sex steroids at the target organ level.

Preliminary data on the LHRH antagonists indicate that these LHRH derivatives can also inhibit tumor growth. The mechanism of the LHRH antagonists is via competitive inhibition of pituitary LHRH receptors, preventing endogenous LHRH stimulation of pituitary gonadotrophs and gonadotropin secretion, subsequently leading to depressed gonadal steroidogenesis.

Several questions arise regarding the nature of the tumor that would be susceptible to LHRH analogue intervention, the dosing regimens, whether these peptides are to be administered solely or as adjuncts to established therapies, and routes of administration. The presence of high concentrations of gonadal steroid receptors (and perhaps even those for prolactin) in various neoplasias (e.g., estrogen receptor-positive) is used as a marker to judge if a tumor is a candidate for antihormone therapy [26]. In numerous instances, tumors are composed of heterogeneous cell populations, each subset possessing different physiologic properties, and varied metastatic potential and susceptibility to various modes of therapy [57]. Receptors for estrogen, androgen, and progestagen have been found in tumors of reproductive origin (e.g., mammary, endometrial, ovarian, prostatic); the presence of all or of some of these receptors in primary and metastatic tumor tissue has been utilized to determine if antihormone therapy would be appropriate. Tumor receptor concentrations may not only dictate the nature of the treatment but they can be of prognostic

value in terms of recurrence and survival, especially with regard to the presence of estrogen receptors in breast and endometrial carcinomas [27,58–61]. However, it should be realized that only approximately 50 percent of breast cancers that are deemed estrogen receptor positive respond to ablative or endocrine therapy; this perplexity complicates the view that an ER+ tumor condition can be clearly related to not only the hormonal factors that are supporting the tumor but also to its projected management [61a].

Non-reproductive organ tumors (e.g., renal cell carcinoma) or ectopic tumors (e.g., chorionic gonadotropin-secreting lung carcinoma), which may possess gonadal steroid receptors, also may benefit from antihormone/LHRH analogue therapy [62–64].

The possibility that the LHRH agonists may act directly on the tumor (i.e., extragonadal reproductive tissue) remains an intriguing proposition [16]. The *in vitro* study of Meyskens et al. [65] revealed that human melanoma tumor stem cells can specifically bind LH and that the gonadotropin also could suppress cell growth. These authors suggested that LH may modulate the growth of human melanoma cells and that control of LH levels may have clinical utility in this disease. Conceivably, LHRH agonists, per se, may have direct antitumor effects, by substituting for LH [16] and also by virtue of their ability to cause LH release under carefully controlled chronic regimens (i.e., pulsatile delivery in treatment of hypogonadotropic hypogonadal disorders), they may be of therapeutic value in a broader range of neoplastic diseases. However, the role of LH in breast cancer is unknown. Zumoff et al. [65a] have proposed that subnormal LH levels and its abnormal diurnal patterns may be associated with increased risk of breast cancer, subserved by hypothalamic dysfunction.

Limited clinical information exists on dosing regimens, use as adjunctive therapy, and routes of administration. However, it would appear from the available animal and human experiences that continuous treatment with the LHRH analogues would be required to maintain a condition of tumor abeyance; the animal investigations clearly indicate that interruption of treatment will lead to tumor recrudescence. Since antisteroidal and LHRH agonist interventions are not cytotoxic (i.e., tumoricidal), other traditional approaches (surgery, radiation, chemotherapy) might be required; the LHRH analogue would play an adjunctive, palliative role, perhaps minimizing the requirements for and the toxic side effects of radiation or chemotherapy, retarding metastases, or reducing tumor size to provide more favorable conditions for surgery.

The possibility that the LHRH agonists may provide a “sparing” effect on chemotherapeutic requirements and/or their damaging side effects has been suggested by Glode et al. [66]. Alkylating antineoplastic agents such as cyclophosphamide (Cytosan) cause severe damage to the rapidly dividing germinal epithelium of the gonads [66a]. In order to reduce the sensitivity of these highly proliferative cells to the deleterious effects of the chemotherapeutic agent, the pituitary-testicular axis of mice was suppressed by chronic administration with Leuprolide. While Cytosan alone produced tubular and epithelial disorganization and disrupted morphology, the addition of the LHRH agonist protected against these effects. Interestingly, mice treated with only the Leuprolide showed normal testicular cytoarchitecture. It has been reported that the male mouse is highly resistant to the antireproductive effects of LHRH agonists [67] at doses in considerable excess of those producing rapid and dramatic disruption of reproductive processes in the rat [1,32,33]. The mechanism



by which the agonist protects spermatogenesis at the level of the seminiferous tubule against the damaging effects of the cytotoxic agent is not certain; such effects may be manifested indirectly via gonadotropin suppression with low doses of the agonist, sparing germinal cell function, or directly on the testis itself. However, it should be noted that any extra-pituitary, direct testicular effect could be exerted only through the Leydig cells, since this is the only compartment possessing detectable LHRH receptors [68]. Irrespective of these confounding factors, the study of Glode et al. [66] suggests the potential ancillary antitumor use of LHRH agonists.

It has been proposed by Auclair et al. [37] that native LHRH could be used for benign prostatic hyperplasia, since LHRH has minimal and therefore less disruptive consequences on testicular tissue. On the other hand, the potent LHRH agonists would be employed, because of their more rapid, prominent, and protracted desensitizing and downregulatory effects on the pituitary-gonadal axis, for the more fulminating neoplasias (prostatic and breast carcinoma) where a "pharmacologic castration" would be desired.

The present modes of clinical administration include subcutaneous and nasal spray delivery. Although the LHRH agonists have been shown to be orally active, the large doses that would be required are impractical. In fact, some of the subcutaneous doses that have been used in the human breast and prostatic cancer studies are in the milligram range; even at these relatively high doses, the human mammary carcinoma trials, in contrast to the prostate studies, have not been encouraging. The nasal spray approach appears to be a reasonable alternative to traditional parenteral dosing methods, having a high degree of patient compliance that has been demonstrated in long-term clinical contraceptive evaluation of these peptides [1]. Another suggested mode of delivery is the transdermal one akin to that used for medicating angina patients with nitroglycerin.

## CONCLUSIONS

The antifertility properties of the LHRH analogues provide a novel approach to hormonotherapy of cancer. The LHRH agonists and the antagonists can disrupt pituitary-gonadal function and suppress steroidogenesis, resulting in removal of support for gonadal steroid-dependent reproductive structures. The collective animal and clinical data support the contention that these compounds (particularly the LHRH agonists) may be useful drugs for the treatment of gonadal-steroid dependent tumors or as therapeutic adjuncts to traditional procedures including surgical extirpation, chemotherapy, radiation, and antigonadal steroidal medication by an effect that is tantamount to a "pharmacologic castration."

The LHRH agonists may be of restricted therapeutic value in those cases of breast tumors that are overtly estrogen receptor-positive. By analogy, a similar view might apply to the treatment of prostatic tumors that would require an androgen (and/or estrogen) receptor-positive dimension [69-71]. However, neoplasias of a general reproductive target category, possessing additional receptors for prolactin, progesterone, LH, and LH-like hormones (e.g., HCG), may be responsive candidates for LHRH agonist intervention. Furthermore, because of the heterogeneous nature of tumor cell populations, non-reproductive neoplasias also possessing the above array of receptors may be likely candidates.

Finally, the role of growth hormone (GH) in supporting tumor growth should be noted. It has been suggested that elimination of prolactin and GH (because the latter is both growth-promoting and lactogenic and binds to prolactin receptors) may have

therapeutic utility in breast cancer [31,31c,72]. To this end, a combination of an LHRH agonist plus somatostatin (or an analogue) might be employed to produce a "medical hypophysectomy," simultaneously suppressing the secretion of gonadotropins, prolactin, gonadal steroids and growth hormone (DP Rose, personal communication).

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