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RECENT DEVELOPMENTS IN FEMALE CONTRACEPTION: LHRH

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Summary—During the last few years new approaches to female contraception based on LHRH and its analogs have been developed. The physiological significance of pulsatile LHRH release and its stimulation of the pituitary has been elucidated by recent studies in rhesus monkey. Immunization against LHRH results in complete inhibition of reproductive function in animals and may find as a useful method of long-term fertility control in domestic animals. Clinical studies have utilized this knowledge to treat infertile hypogonadal women with chronic intermittent low-dose of LHRH. The superactive stimulatory LHRH analogs, used to treat infertility, paradoxically proved to have antifertility effects. They induce desensitization of the processes responsible for gonadotropic and gonadal hormone secretion, mediated by specific LHRH receptors in the pituitary and gonad. While contraceptive effectiveness of luteolytic approach remains to be proven, inhibition of ovulation by intranasal LHRH analog administration or continuous LHRH infusion by programmed minipumps seem to provide safe and effective contraception in women.

INTRODUCTION

Gonadotropin releasing hormone (LHRH) was the second of the neurohumoral agents postulated by Harris[1] more than three decades ago to mediate hypothalamic control of anterior pituitary function. Demonstration of the existence of a factor in the hypothalamic extracts which causes pituitary to release LH and subsequent ovulation by McCann[2] in the early sixties marked the beginning of an intense search for the identification of LHRH. In the race for its isolation, characterization and synthesis Schally[3] and Guillemin[4] became the first to decipher the ancient, unuttered language in which the brain says to the body:

"Keep warm (TRH), Reproduce (LHRH) and Grow no more (SRIF)"

As early as 1971, Schally *et al.* speculated that the following approaches to fertility control could be considered: (a) disruption of the normal menstrual cycle by administration of LHRH early in the cycle, (b) pinpointing of ovulation for standardization of the rhythm method, (c) an immunological approach with the use of antiserum to LHRH for controlling ovulation and (d) use of synthetic inhibitors of LHRH. LHRH has been used extensively as tool in neuroendocrine control of fertility research. Early attempts to use this decapeptide clinically for the treatment of reproductive disorders supposed to be due to an inadequate secretion of endogenous LHRH, were of only limited success. Effective therapeutic use had to await further progress in the understanding of the physiologic mechanisms that control gonadotropin secretion and gonadal function. The demonstration that the *pattern* of hypophysiotropic stimulation is of critical importance in this respect and the elucidation of the physiologic significance of pulsatile gonadotropin secretion have provided the rational basis for the efficient use of synthetic LHRH in the treatment of LHRH deficiency. These findings have also furthered the understanding of the seemingly paradoxical antifertility effects of long-acting LHRH analogues initially designed to compensate for the short action of the parent decapeptide and thus to simplify treatment of infertility. During the last few years the antireproductive effects of LHRH and its agonistic analogs have been extensively studied. Recent clinical trials have demonstrated that LHRH agonists can be used as safe and effective contraception in the human female [5, 35].

ANALOGS OF LHRH

The amino acid sequence of LHRH and some of its synthetic agonistic and antagonistic analogs are shown in Table 1. Studies on the relationship between structure and biological activity showed that the amino acids histidine and tryptophan in positions 2 and 3 seemed to play a functional role in the biological activity of LHRH. Modifications in these positions decreased or abolished LHRH activity. The amino acids in position 1 and 4 to 10 appeared to be involved only in the binding to receptors. Glycine in position 6 and 10 seemed to be most critical for preserving conformation [6, 7].

Potent stimulatory analogs of LHRH with prolonged biological activity were developed to increase the therapeutic usefulness of LHRH. Replacement of the C-terminal glycineamide with ethylamide resulted in analogs which were more active than LHRH [8]. Incorporation of a D-amino acid instead of glycine in position 6 increased the biological LHRH activity [9]. A combination of both these modifications within the same molecule resulted in a peptide with greatly

Table 1. Structures of LHRH; agonistic and antagonistic analogs

LHRH	pGlu - His - Trp - Ser - Tyr - Gly - Leu - Arg - Pro - Gly-NH_2
	1 2 3 4 5 6 7 8 9 10
Agonists	
(des Gly-NH_2^{10})-LHRH ethylamide	——— NH-CH_2CH_3
D-(Ala^6)-LHRH	——— D-Ala ———
(D-Ala^6-des-Gly-NH_2^{10})-LHRH ethylamide	——— D-Ala ——— NH-CH_2CH_3
(D-Trp^6-des-Gly-NH_2^{10})-LHRH ethylamide	——— D-Trp ——— NH-CH_2CH_3
[D-Ser(TBU)^6 des-Gly-NH_2^{10}]-LHRH ethylamide	——— D-Ser(TBU) ——— NH-CH_2CH_3
Antagonists	
(D-Phe^2)-LHRH	——— D-Phe ———
(D-Phe^2, -D-Ala^6)-LHRH	——— D-Phe ——— D-Ala ———
(D-Phe^2, -Pro^3, -D-Phe^6)-LHRH	——— D-Phe-Pro ——— D-Phe ———
(D-Phe^2, D-Trp^3-D-Phe^6)-LHRH	——— D-Phe-D-Trp ——— D-Phe ———
(D-$pGlu^1$, D-Phe^2, D-$Trp^{3,6}$)-LHRH	D-pGlu ——— D-Phe-D-Trp ——— D-Trp ———

enhanced gonadotropin releasing activity [10]. The most potent stimulatory LHRH analogs have biological potencies of more than 100 times that of LHRH [7]. The enhanced biological activity of the potent LHRH agonists is due to their resistance to inactivation and to increased pituitary binding. Peptidases present in the hypothalamus and anterior pituitary cleave LHRH primarily at the Gly^6-Leu^7 and Pro^9-Gly-NH_2^{10} positions [11, 12]. The endopeptidases in the brain are blocked by the substitution with D-aminoacids at position 6 and by replacement of glycine on position 10 by ethylamide [12]. LHRH agonists have greater affinity for the LHRH receptors than LHRH, and their relative binding potencies closely parallel their biological potencies [13].

ANTAGONISTIC ANALOGS

Large numbers of analogs have been synthesized with the aim of obtaining LHRH antagonists which could form the basis of new birth control methods [6, 14]. Inhibitory analogs of LHRH would compete with endogenous LHRH for pituitary receptor sites but would exhibit little or no intrinsic LHRH activity. Structures of some LHRH antagonists are shown in Table 1. Several antagonistic analogs have been synthesized and shown to be active in animals. Inhibition of the preovulatory gonadotropin surge, blocking of ovulation and prevention of pregnancy have been demonstrated in rodents treated with LHRH antagonists [15]. However, the LHRH antagonists are still not powerful enough to be used clinically [5]. Successful immunization against LHRH results in complete inhibition of reproductive function in female and male animals. A problem with immunization methods is the individual variation in antibody response and the necessity to use adjuvants during immunization. However, LHRH immunization may find a role as a method of long-term fertility control in domestic animals [16].

ANTIFERTILITY EFFECTS OF LHRH AND ITS AGONISTIC ANALOGS

The preimplantational antifertility effect of pharmacological doses of LHRH in rats was discovered by Corbin *et al.* [15, 17]. LHRH analog (D-Phe^2, D-Ala^6)-LHRH was without activity when administered to rats during the first 7 days of pregnancy, whereas synthetic LHRH terminated pregnancy. Chronic treatment with LHRH during the prenidatory period was found to delay or completely inhibit implantation in pregnant rats [18, 19, 20].

Superactive agonistic analogs of LHRH proved to be even more potent than LHRH in exerting pre- and postimplantational antifertility effects in pregnant rats. The effect of LHRH and its agonistic analogs on pregnancy termination is presented in Tables 2 and 3. It is clearly evident that all the analogs were capable of interfering with or terminating pregnancy when administered either prior to implantation (day 1–7) or following implantation (day 7–12). Analogs III and IV proved to be the most potent under both dose regimens. Similar results have been confirmed by several others.

LHRH induced increases in serum LH and decreased prolactin concentration had disrupted the normal hormonal preimplantation pattern [20]. Rivier *et al.*[21] proposed that the low secretion of progesterone was due to the combined loss of gonadal LH receptors and a fall in pituitary LH responsiveness to LHRH. Postimplantation antipregnancy effect of (D-Ala^6, EA^{10}) LHRH was accompanied by an early and almost complete inhibition of ovarian LH/hCG and FSH receptor levels [22]. Treatment with estradiol and progesterone alone or in combination could reverse the pre- and postnidatory antifertility effects of LHRH and its agonists [23]. In primates, early luteal phase infusion of the agonistic analog (D-Trp^6)-LHRH failed to show any luteolytic effect [24]. However, postovulatory implantation of the agonist (D-Trp^6-EA^{10})-LHRH and (D-Ala^6-EA^{10})-LHRH was recently found to shorten the intermenstrual interval

Table 2. Postcoital contraceptive effect of LHRH agonists in the rat

Group	Total *N*	Total daily dose μg/rat (subcutaneous)	Rx schedule day of pregnancy	% Inhibition of pregnancy	Average no. of implantation sites, normal/total sites
Control:	24	—	1–7	0	11.2/11.4
I: LHRH	10	100	1–7	60	6.2/7.4
	8	500	1–7	100	0/7.4
II: D-Ala^6-des-Gly^{10}-Pro^9-LHRH ethylamide	8	0.01	1–7	36	10.4/10.8
	6	1.0	1–7	100	0/0
III: D-Ala^6-(*N*)-Me-Leu^7-des-Gly^{10}-Pro^9-LHRH ethylamide	7	0.01	1–7	0	9.2/10.2
	6	1.0	1–7	100	0/0.4
IV: D-Trp^6-(*N*)-Me-Leu^7-des-Gly^{10}-Pro^9-LHRH ethylamide	10	0.01	1–7	0	10.4/11.2
	12	1.0	1–7	100	0/7.6

*Based on no. of rats pregnant/total *N*.

Table 3. Postcoital contraceptive effect of LHRH agonists in the rat

Group	Total *N*	Total daily dose μg/rat (subcutaneous)	Rx schedule day of pregnancy	% Inhibition of pregnancy	Average no. of implantation sites, normal/total sites
Control	18	—	7–12	2.2	10.4/11.2
I: LHRH	10	100	7–12	46	7.2/8.4
	6	500	7–12	98	0.2/6.6
II: D-Ala^6-des-Gly^{10}-Pro^6 LHRH ethylamide	6	0.10	7–12	0	11.2/12.0
	6	1.0	7–12	48	6.0/8.4
III: D-Ala^6-(*N*)-Me-Leu^7-des Gly^{10}-Pro^9 LHRH ethylamide	7	0.10	7–12	24	10.2/12
	6	1.0	7–12	100	0/0
IV: D-Trp^6-(*N*)-Me-Leu^7-des Gly^{10}-Pro^9 LHRH ethylamide	5	0.10	7–12	20	9.8/11.2
	8	1.0	7–12	90	4/10

*Based on number of rats pregnant/total *N*.

and to decrease progesterone secretion during the luteal phase of baboons [25]. LHRH agonist implantation also suppressed the rise in progesterone stimulated by exogenous hCG during the luteal phase of non-pregnant rhesus monkeys [26].

Several mechanisms of action have been proposed to explain the paradoxical anti-reproductive effects exerted by LHRH and its agonists in animals. These include: (1) desensitization of the pituitary gonadotropin release; (2) down regulation of gonadal gonadotropin receptors and desensitization of steroidogenic responses in the ovary or testis; and (3) direct inhibitory actions of LHRH and its agonists on the reproductive organs independent of the pituitary [5]. Extrapituitary actions of LHRH and LHRH agonists may explain, at least in part, some of the paradoxical antifertility effects of LHRH and its agonistic analogs in experimental animals.

CLINICAL STUDIES

The superactive agonistic analogs of LHRH were originally developed for stimulation of fertility. Induction of both follicular maturation and ovulation had been achieved in women with amenorrhea by chronic intermittent therapy with high doses of LHRH [27]. However, during chronic treatment of amenorrheic women with (D-Ser (TBU)^6-EA^{10})-LHRH the gonadotropin responses markedly decreased, the initial estradiol elevations were not sustained and failed to induce follicular maturation and ovulation [28]. The observation of paradoxically decreasing gonadotropin responses in women during chronic treatment with superactive LHRH analog prompted studies in normally ovulating women. Administration of 5 μg [D-Ser(TBU)^6-EA^{10}]-LHRH daily, beginning within the first 3 days of the menstrual bleeding, for 22–30 days led to inhibition of ovulation. During the first day of treatment with LHRH analog evoked great LH and FSH release, with subsequent decrease in gonadotropin responses. There were no preovulatory gonadotropin surge and progesterone increases consistent with normal ovulation. This finding suggested a new approach to birth control in the human female [29]. Further evaluation of the potential contraceptive utility of the LHRH agonist has shown that daily intranasal administration seems to provide safe, effective and highly acceptable method of contraception [30].

Another potential approach to fertility control in women is induction of luteolysis with LHRH agonist. Possible luteolytic effects of LHRH in normal women were reported by Lemay *et al.*[31]. Administration of 5, s.c., 250 μg doses of LHRH at 4 h intervals during the luteal phase of the menstrual cycle shortened the luteal phase from 1 to 4 days in 16 out of 17 cycles. The serum progesterone levels were decreased and the

maximum sensitivity to this treatment was on days 6–9 of the luteal phase. Intranasal administration of the analog [D-Ser(TBU)^6, EA^{10}]-LHRH was found to induce luteolysis in normal women who received 2 doses (500 μg) of nasal drops on 1 day between days 4 and 9 after the LH peak. The treament shortened the luteal phase by 0.5 to 4.5 days, reduced the plasma progesterone levels and normal cycles occurred immediately after treatment [32]. These results were confirmed by Bergquist *et al.*[33] who administered the same agonist by nasal spray into 5 women on each of 2 successive days of the midluteal phase. Exogenous hCG (1500 i.u. daily for 10 days) in three women, however, increased basal progesterone levels and prevented luteolysis induced by the LHRH agonist. Several possible mechanisms of luteolytic effects of LHRH and its agonists have been proposed [see Ref. 5].

A serious drawback to the use of LHRH agonists for induction of luteolysis in women is the fact that the timing of LHRH administration seems to be critical for its effectiveness. LHRH treatment in the early luteal phase is less effective in shortening the luteal phase than midluteal phase treatment [31]. The irregularity of "regular" menstrual cycles makes it difficult for the women to administer the LHRH agonist exactly during the critical time period for effective luteolysis. A more serious problem is the fact that both exogenous and endogenous hCG can overcome the luteolytic effect of LHRH agonists [33, 34]. These findings raises doubts about the effectiveness of LHRH agonists as post ovulatory contraceptive agents. However, recent studies with intranasal administration of a more potent superagonist nafarelin (D-(Nal)2^6-GnRH, Syntex) produced consistent inhibition of ovulation and seems to be a promising new lead to contraception in women [35].

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REFERENCES

1. Harris G. W.: *Neural Control of the Pituitary Gland.* Edward Arnold, London (1955).
2. McCann S. M., Taleisnik S. and Friedman H. M.: LH-releasing activity in hypothalamic extracts. *Proc. Soc. exp. Biol. Med.* **104** (1960) 432–434.
3. Schally A. V., Arimura A., Baba Y., Nair R. M. G., Matsuo H., Redding T. W., Debeljuk L. and White W. F.: Isolation and properties of the FSH and LH releasing hormone. *Biochem. biophys. Res. Commun.* **43** (1971) 393–399.
4. Guillemin R. and Burgus R.: The hormones of the hypothalamus. *Sci. Am.* **227** (1972) 24–33.
5. Nillius S. J.: New approaches to female contraception: LHRH. In *Endocrine Mechanisms in Fertility Regulation* (Edited by G. Bengiano and I. Dilzfalusy). Raven Press, New York (1983) pp. 163–193.
6. Schally A. V., Kastin A. J. and Coy D. H.: LH-releasing hormone and its analogues, recent basic and clinical investigations. *Int. J. Fert.* **21** (1976) 1–30.
7. Coy D. H. and Schally A. V.: Gonadotropin releasing hormone analogues. *Ann. Clin. Res.* **10** (1978) 139–144.
8. Fujino M., Kobayashi S., Obayashi M., Shinagawa S., Fukuda T., Kitada C., Nakayama R., Yamazaki I., White W. F. and Rippel R. M.: Structure activity relationship in the C-terminal part of the luteinizing hormone releasing hormone, LHRH. *Biochem. biophys. Res. Commun.* **49** (1972) 863–869.
9. Monahan M. W., Amoss M. S., Anderson H. A. and Vale W.: Synthetic analogs of the hypothalamic luteinizing hormone releasing factor with increased agonist or antagonist properties. *Biochemistry* **12** (1973) 4616–4620.
10. Coy D. H., Coy E. J., Schally A. V., Martinez V. J., Hirotsu Y. and Arimura A.: Synthesis and biological properties of (D-Ala^6-des-Gly-NH$_2$-10) LHRH ethylamide, apeptide with greatly enhanced LH and FSH releasing activity. *Biochem. biophys. Res. Commun* **57** (1974) 335–340.
11. Koch Y., Baram T., Chobsieng P. and Fridkin M.: Enzymic degradation of luteinizing hormone-releasing hormone (LH–RH) by hypothalamic tissue. *Biochem. biophys. Res. Commun.* **61** (1974) 95–103.
12. Marks N. and Stem F.: Enzymatic mechanisms for the inactivation of luteinizing hormone releasing hormone (LH–RH). *Biochem. biophys. Res. Commun.* **61** (1974) 1458–1463.
13. Perrin M. H., Vaughan J. M., Rivier J. E. and Vale W. W.: Radioligand assay for gonadotropin releasing hormone: relative potencies of agonists and antagonists. *Endocrinology* **106** (1980) 1289–1296.
14. Schally A. V.: Aspects of hypothalamic regulation of the pituitary gland: its implications for the control of reproductive processes. *Science* **202** (1978) 18–28.
15. Corbin A. and Beattie C. W.: Inhibition of the preovulatory proestrous gonadotropin surge, ovulation and pregnancy with a peptide analog of LHRH. *Endocr. Res. Commun.* **2** (1975) 1–23.
16. Fraser H. M.: Inhibition of reproductive function by antibodies of luteinizing hormone releasing hormone. In *Immunological Aspects of Reproduction and Fertility* (Edited by J. P. Hearn). MTP Press, Lancaster (1980) pp. 143–171.
17. Corbin A., Beattie C. W., Jones R. and Bex F.: Peptide contraception: antifertility properties of LHRH analogues. *Int. J. Gynec. Obstet.* **16** (1979) 359–372.
18. Beattie C. W. and Corbin A.: Pre- and post-coital contraceptive activity of LH–RH in the rat. *Biol. Reprod.* **16** (1977) 333–339.
19. Jones R. C., Cole G. and Koch K.: Antiimplantational effects of luteinizing hormone releasing hormone (LRH) in the rat. *Int. J. Fert.* **24** (1979) 202–205.
20. Nagesh Babu G. and Vijayan E.: Antifertility actions of luteinizing hormone releasing hormone (LHRH) in rats, *Ind. J. Expt. Biol.* **21** (1983) 408–409.
21. Rivier C., Rivier J. and Vale W.: Chronic effects of [D-Trp^6-Pro^9-N-Et] LHRH on reproductive processes in the female rat. *Endocrinology* **105** (1978) 229–235.
22. Kledzik G. S., Cusan L., Auclair C., Kelly P. A. and Labrie F.: Inhibitory effect of a luteinizing hormone releasing hormone agonist on rat ovarian LH and FSH receptor levels during pregnancy. *Fert. Steril.* **29** (1978) 560–564.
23. Jones R. C.: Reversal of the antiimplantational effects of luteinizing hormone releasing hormone (LRH) by estradiol 17β and progesterone, alone and in sequence, *Contraception* **19** (1979) 239–245.
24. Hagino N., Nakamoto O., Kunz Y., Arimura A., Coy D. H. and Schally A. V.: Effect of D-Trp^6-LHRH on the pituitary gonadal axis during the luteal phase in the baboon. *Acta endocr., Copenh.* **91** (1979) 217–223.
25. Vickery B. H. and McRae G.: Effects of continuous treatment with superagonists of LHRH when initiated

at different times of the menstrual cycle in female baboons. *Int. J. Fert.* **25** (1980) 171–178.
26. McRae G., Vickery B. H. and Stevens V.: Suppressive effect of LHRH agonist analogs on luteal and placental function in primates. *Endocrinology* **106** (1980) 523A.
27. Nillius S. J.: Gonadotropin releasing hormone for induction of ovulation in women. In *Human Ovulation* (Edited by E. S. E. Hafez). Elsevier/North Holland, Amsterdam (1979) pp. 385–404.
28. Skarin G., Nillius S. J. and Wide L.: Chronic effects of the stimulatory luteinizing hormone releasing hormone analog D-Ser (TBU)^{6}-EA^{10}-LRH on the gonadotropin and gonadal steroid secretion in women with amenorrhea. *Ups. J. Med. Sci* **85** (1980) 103–112.
29. Nillius S. J., Bergquist C. and Wide L.: Inhibition of ovulation in women by chronic treatment with a stimulatory LRH analog- a new approach to birth control? *Contraception* **17** (1978) 537–545.
30. Bergquist C., Nillius S. J. and Wide L.: Inhibition of ovulation in women by intranasal treatment with a luteinizing hormone releasing hormone agonist. *Contraception* **19** (1979) 497–506.
31. Lemay A., Labrie F., Ferland L. and Raynaud J. P.: Possible luteolytic effects of luteinizing hormone-releasing hormone in normal woman. *Fert. Steril.* **31** (1979) 29–34.
32. Lemay A., Labrie F., Belanger A. and Raynaud J. P.: Luteolytic effect of intranasal administration of (D-Ser (TBU)^{6}, des Gly-NH$_2^{10}$) luteinizing hormone-releasing hormone ethylamide in normal woman. *Fert. Steril.* **32** (1979) 646–651.
33. Bergquist C., Nillius S. J. and Wide L.: Luteolysis induced by a luteinizing hormone releasing hormone agonist is prevented by human chorionic gonadotropin. *Contraception* **22** (1980) 341–347.
34. Casper R. F., Shechan K. L. and Yen S. S. C.: Chorionic gonadotropin prevents LRH-agonist induced luteolysis in the human. *Contraception* **21** (1980) 471–478.
35. Gudmundsson J. A., Nillius S. J. and Bergquist C.: Inhibition of ovulation by intranasal Nafarelin, a new superactive agonist of GnRH. *Contraception* **30** (1984) 107–114.