

MINIREVIEW

Luteinizing Hormone-Releasing Hormone I (LHRH-I) and Its Metabolite in Peripheral Tissues

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Luteinizing hormone-releasing hormone (LHRH) was first isolated in the mammalian hypothalamus and shown to be the primary regulator of the reproductive system through its initiation of pituitary gonadotropin release. Since its discovery, this form of LHRH (LHRH-I) has been shown to be one of many structural variants with a variety of roles in both the brain and peripheral tissues. Enormous interest has been focused on LHRH-I and LHRH-II and their cognate receptors as targets for designing therapies to treat cancers of the reproductive system. LHRH-I is processed by a zinc metalloendopeptidase EC 3.4.24.15 (EP24.15) that cleaves the hormone at the fifth and sixth bond of the decapeptide (Tyr⁵-Gly⁶) to form LHRH-(1–5). We have previously reported that the autoregulation of LHRH gene expression can also be mediated by its processed peptide, LHRH-(1–5). Furthermore, LHRH-(1–5) has also been shown to be involved in cell proliferation. This review will focus on the possible roles of LHRH and its processed peptide, LHRH-(1–5), in non-hypothalamic tissues. *Exp Biol Med* 233:123–130, 2008

Key words: luteinizing hormone-releasing hormone; gonadotropin-releasing hormone; metabolism; endopeptidase; proliferation

Introduction

Considerable evidence has accrued to suggest that luteinizing hormone-releasing hormone (LHRH; also referred to as gonadotropin-releasing hormone, or GnRH) may be expressed in both neural and non-neural tissues. The complexity of the anatomical distribution and functional effects of LHRH is suggested by the variety and number of studies to date. While many studies focus on the hypothalamic–pituitary axis, a significant number of studies also focus on its peripheral and non-pituitary neural effects. Historically, this neuropeptide was discovered for its role as the central regulator of vertebrate reproduction. LHRH-I acts via a specific G-protein–coupled receptor, which is located within pituitary gonadotrophs, the LHRH Receptor (LHRH-R), to control the synthesis and release of the gonadotropins, luteinizing hormone, and follicle stimulating hormone (30). Many laboratories, including those of Drs. Andrew Schally, Roger Guillemin, and Samuel McCann, were involved in the characterization of this neurohormone. The studies from these laboratories eventually led to the elucidation of its structure and functions (30, 66).

The hypophysiotropic form of the peptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂) is part of a larger family of decapeptides and was the first to be discovered and characterized in mammals. The discovery of multiple LHRHs in mammalian and non-mammalian species across a wide evolutionary taxa [over 20 LHRHs identified to date; (69)] has resulted in a nomenclature based on the species each of the LHRHs was first discovered. For example, LHRH was first discovered in mammals and thus named mammalian LHRH. The second form of LHRH was discovered and sequenced from the chicken (pGlu-His-

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Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH₂). In this review, the mammalian LHRH is designated LHRH-I as recommended by others (94, 116).

Those LHRH-I neurons that comprise the final common pathway for the regulation of reproductive function via gonadotropin secretion differentiate within the epithelium of the olfactory placode during embryogenesis, and then migrate into the ventral forebrain through the cribriform plate via the central roots of the terminal nerve and olfactory and vomeronasal nerves (95, 116, 119). In the adult, LHRH-I neurons are widely distributed along the rostro-caudal axis in the basal forebrain region around the preoptic area. The brain and pituitary localization of the LHRHs has been extensively reviewed elsewhere (30, 117).

Even though the LHRH-I peptide was first isolated and sequenced in mammalian brain (porcine and ovine), this peptide has been shown in a growing number of studies also to be present in non-neural tissue. These studies, including some dating back to the 1970s that use radioligand binding assays and molecular techniques, show that LHRH-I and its binding site/receptor are present in numerous peripheral tissues (63, 91). The short half-life of LHRH-I [less than 4 min; (24, 26, 42)] has led many to suggest that peripheral LHRH-I likely acts locally in an autocrine and/or paracrine manner. What began as studies to understand how a simple neuroendocrine hormone is thought to primarily control reproductive function by acting at the anterior pituitary to regulate the release of gonadotropins now has been shown to have a diversity of reproductive and other non-reproductive functions. In this minireview, we will focus on two aspects of LHRH-I: 1) the potential functional role of LHRH-I and its cognate receptor, LHRH-RI, in peripheral reproductive tissues, and 2) the conversion of the decapeptide, LHRH-I, via its metabolism to produce a bioactive pentapeptide, LHRH-(1–5).

Peripheral Localization of LHRH-I and LHRH-RI

It is now well-established that LHRH-I and its cognate receptor, LHRH-RI, are expressed in many non-hypothalamic tissues, including the ovary, uterus, placenta, testes, breast, immune system, pancreas, adrenals, tooth, and prostate, as well as in numerous cell lines and tumors (see Table 1). The ubiquitous nature of LHRH-I and its cognate receptor, LHRH-RI, expression in numerous peripheral tissues further underscores the complexity of this LHRH-I system. While the developmental origins of these LHRH-I-producing cells in the periphery remain to be determined, many of its physiological functions in normal and cancer cells have been identified. A number of studies suggest that LHRH-I might have a diversity of actions or may be associated with a number of functions to include the facilitation of steroidogenesis, cell proliferation, apoptosis, fertilization, embryonic implantation, cell-matrix adhesion, and cell migration (36, 39, 66, 90, 106, 110). A role for LHRH-I and LHRH-RI in regulating tumor growth has been

also identified in human ovarian cancer cells, breast tumor tissues, endometrial carcinoma, and in prostate cancer (93) via an autocrine/paracrine role (52).

As indicated above, LHRH-I has been shown to exert several effects that may have physiological significance (12, 52). Within its larger context in the regulation of the hypothalamic-pituitary-gonadal axis, LHRH-I also have local reproductive roles. For example, LHRH-I can inhibit progesterone secretion (79, 82), steroidogenesis (52), regulate gonadotropin receptors (51), and facilitate fertilization and subsequent implantation (9, 74, 75). Not only does LHRH-I increase transcriptional activity of genes known to be involved in follicular rupture (81) and follicular atresia via apoptosis (109), the expression of LHRH-I and LHRH-RI is greatest in the granulosa cells of preovulatory follicles and also the granulosa luteal cells, but not the theca luteal cells (13). Furthermore, its ability to regulate the extracellular matrix also suggests its possible role in luteinization/luteolysis (12, 31). In this latter role, LHRH-I was shown to induce structural luteolysis in corpora lutea by the upregulation of matrix metalloproteinase (MMP)-2 and membrane type 1-MMP expression (12, 31). This ability of LHRH-I to also remodel the extracellular matrix may also be true in other respects such as during development (102, 106) and in disease (13, 33, 37, 91).

There is also increasing data to suggest that there may be direct LHRH-I effect regulating cancer growth locally (93). The majority of reproductive cancers express LHRH-RI through which LHRH-I have anti-proliferative and apoptotic effects (33, 34, 52, 66). The precise role of LHRH-I and its receptor in this regard remains unclear as the effects of LHRH-I agonists and antagonists can both induce growth inhibition [reviewed in (33, 52)]. Nevertheless, it has been shown that the activation of phosphotyrosine phosphatase (PTP) through the LHRH-RI in tumors has a necessary counteracting role against growth factors (34, 46). One pathway that has been described suggests that the activation of the LHRH-RI leads to a sequelae of events that include the coupling of the LHRH-RI to G_{αi} protein, activation of PTP, dephosphorylation of EGF receptors (34, 46), decrease of mitogen-activated protein kinase activity (22), c-fos expression (35), and subsequent suppression of proliferation (34–36, 46). Whether LHRH-I regulates cellular growth via the apoptotic pathway (33, 37, 52, 112) or the cessation of cellular growth via the regulation of JunD and the activator protein-1 (38) remains to be determined.

The diversity of activities of LHRH-I alone is consistent with the view suggesting that the LHRH family of peptides evolved from its original function in cellular communication within simple organisms (1, 87, 107). Subsequently, they were recruited by the nervous system, where this decapeptide transduces external and internal cues; their relative specialization in reproduction may have occurred concomitantly with the development of the hypothalamo-pituitary-gonadal (HPG) axis in vertebrates

Table 1. LHRH-I and LHRH-RI Localization in Peripheral Tissues

	LHRH-I	LHRH-RI
Breast	(27, 40, 66)	(40, 66)
Immune system	(10, 47, 103, 104)	(10, 103, 104)
Ovary	(13, 16, 45, 54)	(6, 13, 16)
Pancreas	(29)	(29)
Placenta	(14, 21, 45, 53)	(15, 89, 114)
Prostate	(3, 65, 105)	(105)
Sperm	(7)	(7, 62, 75)
Testes	(7, 45)	(7)
Tooth	(106)	—
Uterus	(13, 45, 99)	(9, 22, 77, 88)

(69, 107). In support of this, comparative studies indicated diverse distribution and essential biological activities of LHRH in central and peripheral tissues of vertebrates (107). The LHRH system may have originated as a general neuropeptide with a neurotransmitter or neuromodulator role in cellular communication of organisms lacking the HPG axis, and eventually recruited to regulate reproduction in chordates (44, 107). While the LHRH system appears to have been recruited to regulate vertebrate reproduction centrally through the hypothalamic-pituitary-gonadal axis and locally in reproductive tissues such as the breast, ovary, the testes, and the uterus (69), LHRH can also regulate non-reproductive tissues such as the immune system, olfactory system, and the tooth (43, 48, 55, 70–72). To our knowledge, the central regulation of reproductive function remains to be the most (and perhaps the only) indispensable function of LHRH-I in vertebrates. That LHRH-I may be involved with the regulation of diverse neural and peripheral tissues in many species studied to date suggests that it may equally have reproductive and non-reproductive roles in vertebrates. This will remain a subject of future studies and speculation.

Processing of LHRH-I

The ability of a peptide to exert a biological action is dependent on its availability at the receptor. This availability, in turn, is dependent on the balance between production and degradation. Bioactive peptides are produced from larger precursor peptides by selective processing by peptidases, and are then broken down by further cleavages. Likewise, LHRH-I is translated from the mRNA as a pro-hormone, which is subsequently converted to the mature decapeptide in secretory vesicles prior to its release (68, 113). Subsequent to its secretion, LHRH-I may be further cleaved by soluble peptidases for the purposes of degrading, converting, or transforming (2, 60, 61, 78, 80, 117, 120).

One identified peptidase important for the metabolism of LHRH-I is the metalloendopeptidase EC 3.4.24.15 (EP24.15; also known as thimet oligopeptidase), a 75-kDa neutral metalloendopeptidase (85). EP24.15 and its enzymatic activity have been shown to be widely distributed in cells and tissues throughout the body and exist primarily in a soluble form (28, 61, 73, 84, 85). The enzyme is zinc-dependent (19), and its activity is dependent on phosphorylation by protein kinase A (108). This enzyme prefers peptide substrates containing a hydrophobic amino-acid residue in the P1 and P2 positions and a bulky hydrophobic residue in the P3' position, relative to the scissile bond (80). EP24.15 cleaves LHRH-I at the Tyr⁵-Gly⁶ bond and is known to have an important role for the modulation of the LHRH-I signal to the pituitary (25, 32, 97, 98, 101). Interestingly, a common structural feature of LHRH-I “super-agonists” is modification at the Tyr⁵-Gly⁶ residues (26, 57–59), which confers resistance to cleavage to EP24.15 (Table 2).

The transition-state analog inhibitor, N-(1-(R,S)-carboxy-3-phenylpropyl)-Ala-Ala-Phe-pAB (cFP-AAF-pAB), specific for EP24.15, prevents the cleavage of LHRH-I at

Table 2. Amino-Acid Sequences of LHRHs and Agonists with Their Half-life. A color version of the table is available online.

	Amino Acid Sequence	Half-Life	References
LHRH-I	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH ₂	< 4 min	[8, 24, 42, 83]
Buserelin	pGlu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-NHC ₂ H ₅	72-80 min	[92]
Goserelin	pGlu-His-Trp-Ser-Tyr-D-Ser(tBu)-Leu-Arg-Pro-AzGly-NH ₂	2.3-4.2 h	[17, 56]
Leuprolide	pGlu-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-Gly-NHC ₂ H ₅	3 h	[86]
Nafarelin	pGlu-His-Trp-Ser-Tyr-D-Nal(2)-Leu-Arg-Pro-Gly-NHC ₂ H ₅	2.6-4 h	[15]
Triptorelin	pGlu-His-Trp-Ser-Tyr-D-Trp-Leu-Arg-Pro-Gly-NH ₂	2.8 h	[76]

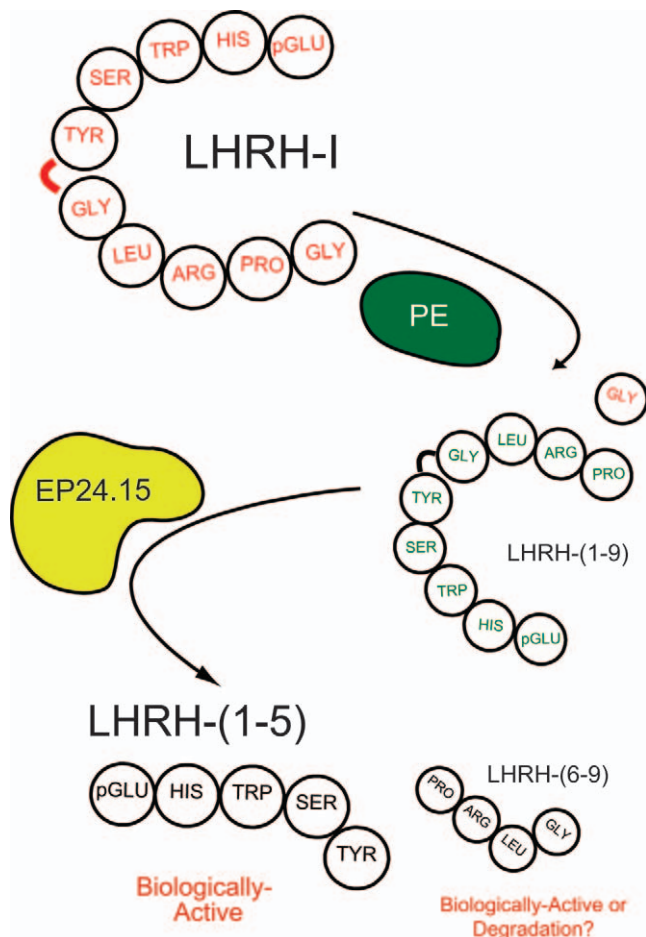


Figure 1. A diagram showing LHRH-I in the folded conformation with the Tyr⁵-Gly⁶ bond available for hydrolysis by EP24.15. Substitutions with D-amino acids in the sixth position (Gly) increases binding affinity to the LHRH-R but prevents hydrolysis [see (55) and Table 2]. EP24.15 plays a crucial role *in vivo* in a two-step mechanism with another enzyme, prolyl endopeptidase (PE), in the degradation of LHRH-I producing the LHRH-(1-5). LHRH-I is initially processed by PE, which cleaves the 10th amino acid from the decapeptide to produce LHRH-(1-9). This 9 amino acid peptide has a greater affinity for EP24.15 and is processed/converted to produce two sub-products, LHRH-(1-5) and LHRH-(6-9), of which LHRH-(1-5) is known to have biological activity. A color version of this figure is available in the online version of the journal.

this bond by isolated membranes and the recombinant enzyme (19, 64, 108). Interestingly, in an *in vitro* study, it was shown that there is a 10–15-fold improvement in the K_m for the cleavage of LHRH-(1-9) compared with LHRH-I (64). This observation suggests that the initial cleavage of LHRH-I may occur via a two-step mechanism, involving the initial removal of the COOH-terminal glycnamide by a prolyl endopeptidase-like enzyme, followed by hydrolysis by EP24.15 (64) (Fig. 1). Interestingly, phosphorylation of EP24.15 by protein kinase A on serine 644 results in an alteration of LHRH-I hydrolyzing activity, suggesting that phosphorylation may also be a physiological regulator of EP24.15 activity (108). Furthermore, EP24.15 is also localized to the extracellular surface of the plasma

membrane (18) as well as the exofacial leaflet of the lipid raft microenvironment (49, 50) to provide more evidence that LHRH-I may be further processed to produce a biologically active LHRH-(1-5), resulting in a divergence of effect. On the other hand, EP24.15 is locally regulated by steroid hormones (101, 117). Interestingly, agonists of LHRH-I, those with a hydrophobic residue at the sixth position, have a direct inhibitory effect on EP24.15 (101). Collectively, these data suggest that EP24.15 is more than a degradation enzyme, serving but serves as a converting enzyme that processes the bioactive LHRH-I to another biologically active peptide, LHRH-(1-5), which has a different activity. Thus, EP24.15 may, in turn, indirectly influence signal transduction pathways through its processing properties.

Possible Role for a Metabolite of LHRH, LHRH-(1-5)

Others and our laboratory have hypothesized and shown that LHRH-(1-5), a specific metabolite of LHRH-I composed of the first 5 amino acids, may be bioactive (4, 5, 20, 112, 118, 120). Previous studies in our laboratory showed that this metabolite stimulates LHRH-I mRNA expression in the neuronal GT₁₋₇ cell line (120). LHRH-I treatment, on the other hand, had a negative regulatory effect on its own expression in the same cells, indicating that LHRH-(1-5) operates through a distinct signaling mechanism when compared with its parent molecule. More recent data indicate that LHRH-(1-5) functions differently in an endometrial cell line than in neuronal GT₁₋₇ cells, where LHRH-I stimulate their own gene expression (4). Specifically, the metabolite regulates LHRH-II mRNA expression in the endometrial cell line as opposed to LHRH-I expression (4). LHRH-I, on the other hand, induces its own expression as well as its cognate receptor, as opposed to the inhibitory effect observed in GT₁₋₇ cells. This divergence between the cell lines is not at all surprising, considering the numerous reports of differences between LHRH-I effects and signaling pathways between pituitary and non-pituitary cells and reports of differences between the LHRH-I and the LHRH-II systems.

In pituitary gonadotrophs, for example, LHRH-I is coupled to G protein q/11, thereby activating phospholipase C and protein kinase C. LHRH-I in reproductive cancers, on the other hand, couple to G_i proteins, subsequently activating a phosphotyrosine phosphatase (36, 41, 67, 96, 100). The dichotomy in LHRH-(1-5) functioning between GT₁₋₇ cells and endometrial cells (4, 112), therefore, is in keeping with previous findings. In contrast, the difference between LHRH-I and LHRH-II is more complicated. While there are similar functions of both neuropeptides such as their effect in cellular proliferation, there are also differences in function. For example, LHRH-I has been shown to upregulate, whereas LHRH-II has been shown to down-regulate both LH and FSH receptors in ovarian steroido-

genic cells. The contrasting role of LHRH-I and LHRH-II has been recently reviewed (11, 52, 65).

It is interesting to note also that LHRH-(1–5) has proliferative effects in the Ishikawa endometrial cell line (112) and a number of ovarian surface epithelial cell lines such as those previously shown to be responsive to direct LHRH-I effect (Walters and Wu, unpublished observations). This is in contrast to LHRH-I agonists, which have anti-proliferative effects, while LHRH-(1–5) may directly effect cell growth. The ability of LHRH-(1–5) to stimulate proliferation appears to be linked to its ability to suppress caspase-3/7 expression as well as ERK-1/2 expression (112). It is possible that EP24.15 expression may be altered in cancer that results in an increase in the conversion of LHRH-(1–5). This altered LHRH-(1–5) production, in turn, may contribute to greater growth and the diminishing role of LHRH-I in regulating the extracellular matrix and activating the apoptosis pathway (11, 12, 91, 112, 115).

Summary

While it has long been presumed that LHRH-I agonists and antagonists have been used clinically to depress pituitary gonadotropins release and its subsequent suppression of steroid hormones from the gonads, a number of studies, including ours, also suggest that they might have direct local effects in tissues. Thus, understanding the role(s) of LHRH-I in peripheral systems is clinically relevant; the potential local tissue effects are not typically considered nor are the potential local activities well understood.

Superimposed on this, is the discovery of multiple forms of LHRH and their cognate receptors (1, 55, 69). In humans and most vertebrates, there are two forms of LHRHs, LHRH-I and LHRH-II, that exist in the brain and in peripheral tissues. Furthermore, the processed peptide of LHRH-I, LHRH-(1–5), appears also to have biological activities that are in contrast to its parent activities. Data from our laboratory suggest that LHRH-(1–5) is not likely to act via the LHRH-RI, but acts via an alternate path. It is possible that LHRH-(1–5) may act through one of the alternative LHRH receptors, such as the LHRH-RII. This is plausible since LHRH-(1–5) share the first 4 amino acids with at least 9 LHRH-I forms. Our finding showing that LHRH-(1–5) may have contrasting effects from its parent peptide, LHRH-I, could help explain the lack of correlation between the action of LHRH-I analogs that behave as antagonists at the pituitary level but result in agonist-like anti-proliferative effects in many cancers (36, 40, 66, 91).

The identification of alternative LHRH-I receptors or a unique LHRH-(1–5) receptor will lead to better agonist/antagonist design as well as better and more biologically relevant *in vitro* and *in vivo* assays to screen for future drug designs. Furthermore, the identification of LHRH-(1–5) binding sites and its processing will provide clearer understanding of this LHRH-I system. These studies

suggest a rich and highly complex neuropeptide system with a diverse mode of action that extends beyond its name.

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