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GONADOTROPIN-RELEASING HORMONE, BRADYKININ AND GONADOTROPIN-INHIBITORY HORMONE AND THEIR POTENTIAL ROLE IN REPRODUCTION

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ABSTRACT: Gonadotropin-releasing hormone (GnRH) is the hypothalamic factor that controls reproductive axis. GnRH, that is synthesized and released in a pulsatile manner from hypothalamic neurosecretory cells, reaches pituitary cells through specialized portal system, and regulates the synthesis and release of pituitary gonadotropins that, in turn, regulate steroidogenesis and gametogenic functions of gonad. Several studies from various laboratories including ours support an emerging concept that an intrinsic GnRH system, complete with ligand, receptor, and biological response exist in the ovary. However, available studies about distribution of GnRH, its receptor proteins, and their physiological roles within ovarian compartments of different vertebrate groups are fragmentary. The factor(s) regulating GnRH synthesis and secretion in the ovary are poorly understood. Bradykinin was shown to bring about GnRH release in the rat hypothalamus. A novel hypothalamic dodecapeptide that directly inhibits Gonadotropin release in quail and termed as gonadotropin-inhibitory hormone (GnIH) was discovered recently in year 2000. The GnIH receptor is found in hypothalamus and may act on the hypothalamus to regulate GnRH release. It would be interesting to investigate the role of GnIH and bradykinin as regulators of ovarian GnRH. Recent studies on rat and human ovaries showed that GnRH function as a local autocrine and/or paracrine factor regulating steroidogenesis, cell proliferation and apoptosis. Whether GnRH I perform similar or additional functions in the ovary of other mammals such as mice etc and/or during pathological conditions, such as in polycystic ovary syndrome (PCOS), are yet to be investigated.

Keywords: GnRH, bradykinin, GnIH, reproduction.

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1. INTRODUCTION

Gonadotropin-releasing hormone (GnRH) was first isolated from mammalian hypothalamus as the decapeptide (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2) [1, 2]. It is one of the earliest hypothalamic releasing hormones to be sequenced and characterized. It is produced by hypothalamic neurosecretory cells and released in a pulsatile manner and reaches pituitary cells by way of specialized hypothalamo-hypophyseal portal circulation. The major function of this decapeptide is to modulate the synthesis and release of gonadotropins from the pituitary. The gonadotropin released, in turn, regulates steroidogenesis and gametogenic functions of the gonads. GnRH was subsequently isolated and characterized from birds [3] and other vertebrates [4]. Thus, GnRH is now established as a key regulator of reproductive axis in all the vertebrate species. Besides the well-established role for GnRH and GnRH receptor in gonadotropins regulation in the pituitary, the detection of both hormone and receptor in multiple mammalians, non-pituitary tissues and cells suggests their numerous and diverse autocrine, paracrine and extra-pituitary roles. The GnRH system has been investigated in many different species with a view towards developing therapies for pathological conditions and for method to assist reproduction. Several studies from various laboratories including our support an emerging concept that an intrinsic GnRH system, complete with ligand, receptor and biological response exists in ovary. GnRH and its receptor mRNA have often been demonstrated in the ovary of several mammalian as well as of several non-mammalian vertebrates using real time polymerase chain reaction (RT-PCR) techniques without functional assays of protein expression and localization. Surprisingly, there is a distinct lack of sufficient information about the distribution and physiological significance of these peptides in the ovaries of vertebrates. The extensive investigation of physiological, cell biological and molecular functions of the GnRH in different animal models are critically required and that will improve our understanding of GnRH role in reproductive processes in various animal groups. Changes in GnRH and its receptor concentration in the ovary during reproductive cycle have not yet been investigated extensively. This study may provide some clue about their control mechanism as well as their physiological significance. The factor(s) regulating GnRH synthesis and secretion in the ovary remains poorly understood. Large number of scientific publications per year indicate that the comprehensive role of GnRH in reproductive biology, although still incompletely understood and remain considerable therapeutic interest. This overview aims to summarize general information as well as recent advances on GnRH and its role in the regulation of reproductive processes.

Taxonomic distribution of GnRHs

GnRH was first isolated in 1971 from the brains of pigs and sheep [5, 6]. Since then, the GnRH family has expanded to include at least 23 molecular isoforms, 13 from various vertebrate species and 10 from invertebrates [7, 4].

Since GnRH was first identified in mammals (pig and sheep), it is now known as mammalian GnRH (mGnRH or GnRH I). To date, six GnRHs are isolated from fish species [8], salmon GnRH (sGnRH) [9], catfish GnRH (cfGnRH) [10], dogfish GnRH (df-GnRH) [11], seabream GnRH (sbGnRH) [12], herring GnRH (hGnRH) [13], and medakaGnRH (mdGnRH) [14]. Primitive species such as the lamprey and the protochordate *Ciona intestinalis* have their own forms of GnRH: lamprey GnRH I and III [15] and tunicate GnRH I and II, respectively [16]. Chicken GnRH I (cGnRH I) [17] and chicken GnRH II (cGnRH II) [18] were both first characterized in chicken. Apart from the common mGnRH, a guinea pig GnRH (gpGnRH) was shown as an alternative form of mammalian GnRH [19]. The most recent finding of a novel GnRH was made in an amphibian, the frog *Ranady bowskii*: ranid GnRH (rGnRH) [20]. In general, all investigated species to date possess two or three different forms of GnRH(Table 1)**.** The most conserved form of GnRH is chicken GnRH II and it coexists in all classes of vertebrates from the *Chondrichtyes* species onward, together with a species-specific GnRH and a possible third form. The two or three forms of GnRH coexisting in one species are transcribed from different genes. The species-specific forms vary, for example, cGnRH I in birds, hGnRH in herring, dfGnRH in sharks, sGnRH in salmonids, and mGnRH in primates. But, if a third form is present, as shown for “modern” fishes, it is always the sGnRH form. The mammalian form of GnRH (mGnRH or GnRH I) found in humans has a wide distribution in vertebrate species. This form of GnRH can be detected in primitive bony fish, but not in species thought to have evolved earlier. Hence, the mammalian form of GnRH appears to have arisen 400 million years ago. High performance liquid chromatography (HPLC) and radioimmunoassay (RIA) studies show that mGnRH is present in the descendants of primitive bony fish that evolved before the teleosts, such as reedfish, sturgeon, alligator gar [21]. However, nucleotide base substitutions may have occurred in the mGnRH gene early in the evolution of the advanced bony fish, the teleosts. Only an early evolving teleost, the eel, appears to retain the mGnRH molecule [22]. Thereafter, the teleosts studied to date do not contain mGnRH. On the contrary, the salmon form of GnRH (sGnRH) is present in a variety of teleosts in addition to members of the salmonid family.

Table 1: Taxonomic distribution of different forms of GnRH and GnRH receptor

GnRH/GnRH R	Type of GnRH	Species	Techniques	References
GnRH	cGnRH II	*R. esculenta*	HPLC, RIA	Battisti *et al* 1994
GnRH-R		Rat	RT-PCR	Olofsson *et al* 1995
GnRH-R		Rat	RT-PCA	Dantoin *et al* 1995
GnRH	sGnRH, cGnRH II	Goldfish	RT-PCR	Lin & Peter 1996
GnRH	GnRH-I, cGnRH-II	Goldfish	RT-PCR	Lin & Peter 1996
GnRH	cGnRH II	Newt	ICC	Batisti *et al* 1997
GnRH		Lamprey	GnRH binding site	Gazourian 1997
GnRH,GnRH-R		Rat	RT-PCR	Botte *et al* 1998
GnRH,GnRH-R		Goldfish	RT-PCR	Yu *et al* 1998
GnRH	sGnRH	Goldfish	HPLC	Pati&Habibi 1998
GnRH-R		monkey	In situ hybridization	Fraser *et al* 1996
GnRH	m, s, c-II &SbGnRH	Rainbow trout	HPLC	Von Schallburg*et al* 1999
GnRH	s-GnRH	Rainbow trout	RT-PCR	Von Schallburg 1999
GnRH-R		Neonatal & adult Rat	In situ hybridization	Kogo*et al* 1999
GnRH,GnRH-R	mGnRH	Human	RT-PCR, Southern Blot	Kang *et al* 2000
GnRH	sGnRH	Rainbow trout	Northern blot	Uzbekova*et al* 2000
GnRH-R		Mouse & Rat	Northern blot	Bull *et al* 2000
GnRH	m & c-I GnRH	*C. intestinalis*	HPLC, RIA, IHC	Fiore *et al* 2000
GnRH	s, c-II, SbGnRH	Seabream	RT-PCR	Nabissi*et al* 2000
GnRH,GnRH-R		Rat	RT-PCR	Park *et al* 2001
GnRH, GnRH-R	sGnRH, cGnRH II	Rainbow trout	RT-PCR	Uzbekova*et al* 2002
GnRH	sGnRH	Rianbow trout	RT-PCR, HPLC	Gray *et al* 2002
GnRH-R	s-I, s-II, c-II, rtGnRH	Rainbow trout	RT-PCR	Uzbekova*et al* 2002
GnRH-R		Walabies	PCR	Chaung *et al* 2003
GnRH	GnRH I, GnRH II	Human	RT-PCR	Khosravi& Leung 2003
GnRH R		Octopus	RT-PCR	Kanda *et al* 2005
GnRH R		Mollusca	RT-PCR	Rodet *et al* 2005
GnRH/GnRH-R	mGnRH/ GnRH-I R	Rat	RT-PCR	Schirman *et al* 2005

Further alterations in GnRH may have led to the catfish form (cfGnRH) as neither mGnRH nor sGnRH peptides are present in three species of catfish [23, 24]. A GnRH gene duplication and

subsequent nucleotide base substitutions may account for a novel GnRH with unknown structure in some advanced fish species that also contain two of the known forms of GnRH. In the line of evolution leading to the land animals, mGnRH was probably inherited by ancestral amphibians [25, 26]. Early in the phylogeny of reptiles, the mGnRH molecule is thought to have had nucleotide base substitutions leading to a single amino acid change in position 8. This resulted in the chicken GnRH I (cGnRH I) form. The evidence for this interpretation is based on the presence of cGnRH I throughout the reptiles in the major groups containing turtles, lizards, snakes, and crocodiles [27]. Similarly, birds, which evolved from the crocodilian line, have the cGnRH I molecule [3, 28, 29]. The mGnRH, meanwhile, was retained in the mammals including the marsupials [30, 31] and placental mammals [32, 33]. The conservation of the GnRH structure reflects the importance of the peptide for reproductive success and, therefore, survival. The presence of mGnRH in members of several vertebrate classes may also explain the effectiveness of mGnRH in nonmammalian species. The GnRH receptors in nonmammmalian species, although evolving, still recognize the mammalian GnRH structure. There is a form of GnRH that appears to have been conserved longer than mGnRH. This form, [His5, Trp7, Tyr'] -GnRH (chicken GnRH II) appears throughout the vertebrates from cartilaginous fish to primitive placental mammals. One key question is why cGnRH II is no longer found in more recently evolved placental mammals. This form is effective for releasing LH and FSH in mammals, but to a lesser extent than mGnRH [18]. There is no question that cGnRH II exists in other vertebrates as the primary structure has been determined in two cartilaginous fishes, ratfish [34] and dogfish shark [11] in a bony fish, catfish [23] and in a reptile, alligator [27]. Additionally, HPLC and RIA studies have detected this GnRH form in a variety of other vertebrates [35]. It is present as a second form of GnRH in all jawed vertebrate classes except for ratfish where it is the sole GnRH in the brain [34]. The long-term conservation of the molecule points to its importance but makes its disappearance in most placental mammals puzzling. The other five GnRH forms are less widely distributed than mGnRH and cGnRH II in vertebrates. Salmon GnRH appears to be limited to the teleosts, whereas cGnRH I is found only in the reptiles and birds to date. Lamprey, catfish, and dogfish GnRHs appear to be confined to species closely related to the one from which the peptide was isolated. All these forms probably evolved independently after the separation of each group from the ancestral vertebrate stem line.

GnRH isoforms

At least two isoforms of GnRH have been identified in the mammalian hypothalamus, GnRH I and GnRH II. GnRH I is a decapeptide responsible for secretion of LH and FSH from pituitary originally isolated and characterized by Guillemin and Schally [2, 36]. GnRH II was initially discovered as chicken GnRH II [37]. In the hypothalamus, GnRH II has been hypothesized to play a role in the behavioural components of reproduction [37]. Both isoforms of GnRH are decapeptides that are characterized by post-translational modification including the pyro-glutamic acid at the amino

terminal and amidated glycine at the carboxyl terminal. GnRH I is conserved throughout evolution and has been identified in both vertebrate and invertebrate [38]. GnRH I share a 60% identity between mammals and tunicate, whereas GnRH II is even more highly conserved with 100% identify between birds and mammals [38]. In addition, in mammals, a third isoform, the salmon GnRH, named GnRH III, was reported [39, 40]. In human genome, only the GnRH I and GnRH II have been found. The expression of GnRH III is reported to be doubtful [41]. All-natural GnRH decapeptides are highly conserved with respect to their length at the sequences of both NH2-terminus (pGlu-His-Trp-Ser), and COOH-terminus (Pro-Gly NH2) [42]. The conservation of these residues, during the evolution of vertebrates, suggests that they are critically important for receptor binding and activation [4].

GnRH Receptors

Mammals, including human, produce two isoforms of GnRH receptors, GnRH I-receptor and GnRH II-receptor [43, 44, 45, 46]. Both isoforms of GnRH receptors belong to the family of rhodopsin-like G protein-coupled receptors (GPCRs), coupled with Gq alpha. These are characterized by seven transmembrane domains connected by alternating intracellular and extracellular loop domains. Peptide ligands appear to bind predominantly to extracellular domains and to the transmembrane domains [47]. A unique feature of the mammalian GnRH receptor (Type I) is the absence of a carboxy terminal tail present in all other GPCRs and in all of the non-mammalian GnRH receptors. This suggests a recently evolved feature, which presumably serve an important role in the functioning of the mammalian GnRH receptor. The absence of the carboxyl terminal tail leads to a slow internalization and failure of a quick desensitization of the receptor [48]. All GnRH receptors that lack carboxy terminal tail are designated type 1 and all GnRH receptors that have carboxy terminal tail are termed as type 2 receptor. The type II, GnRH receptor has been cloned in marmoset as well as in both African green and rhesus monkey [49, 50]. The type II, GnRH receptor has 39% identity (68% conservation) with type I GnRH receptor. The most striking difference between the receptor subtypes is the retention of a 56residues cytoplasmic tail domain at the carboxyl terminus of the type II GnRH receptor, compared with its absence in the type I receptor. It has been presumed that in humans and in the chimpanzee, cow, sheep, horse, rat, and mouse, the type II GnRH receptor is silent [51]. In any case, GnRH I and GnRH II bind in the GnRH I-receptor, and it seems that they have different roles.

Distribution of the GnRH/GnRH receptor system

In addition to the hypothalamus, GnRH I have also been localized to the endometrium, placenta, breast, ovary, testis and prostate [43, 52, 38]. The exact function of GnRH I in these tissues is under active investigation. Several lines of evidence suggest that the GnRH I-receptor is also expressed in the brain in GnRH neurons to contribute to an ultrashort loop feedback mechanism [53].In the ovary, *in situ* studies have shown the presence of the GnRH I mRNA in granulosa cells of primary,

secondary and tertiary follicles [45]. Recently, investigators have shown the presence of GnRH II in human granulosa-luteal cells (hGLCs), immortalized ovarian surface epithelial (OSE) cells and in ovarian cancer cells [45]. Some studies suggest a physiologic role of the GnRH system in the control of atresia [45].GnRH receptor expression changes in the ovary correlate with the degree of follicular development across the estrous cycle [45]. GnRH I induced a biphasic effect on GnRH I and GnRH I-receptor expression in hGLCs and OSE cells. Estrogen treatment resulted in an initial up- and then down-regulation of GnRH I and GnRH I-receptor expression. GnRH agonist administration can also down-regulate estrogen receptors alpha and beta in ovarian cells [45]. Recent studies have suggested that the GnRH receptor promoter is controlled by a unique upstream regulatory sequence in human ovarian granulosa-luteal cells which was not critical in ovarian cancer cells or primary cells [54]. Thus, there may be tissue-specific regulation of GnRH/GnRH receptor pathways.It is now well established that GnRH I and GnRH I-receptor are expressed in many peripheral tissues. The functional physiological role of the ligand and its receptor in these sites is under active investigation.

Regulation of GnRH in the gonads

The factor(s) regulating GnRH I synthesis and secretion in the gonads remains poorly understood. The factors regulating GnRH I, GnRH II and their receptors in the ovary and testis seem to be steroids, gonadotropins, bradykinin, and GnRH itself. In addition, gonadotropin-inhibitory hormone (GnIH) regulates synthesis and biological actions of GnRH too.In human ovarian surface epithelium and granulosa-luteal cells, treatment with GnRH I produces a biphasic response in its own m-RNA level such that high concentration decreases whereas low concentrations increase GnRH I gene expression (55, 56]. This suggests autoregulation of GnRH in the ovary. Both GnRH I and its receptor mRNA level have been shown to be down-regulated by estrogen in ovarian cells of human [56] and this effect can be reversed by co-treatment with an estrogen antagonist, indicating that the estrogen induced down-regulation of gene expressions is mediated via the estrogen receptor. This data indicate that estrogen suppresses GnRH gene expression. In human ovary, the expression of GnRH I and GnRH II mRNAs have been shown to be regulated differentially by FSH and human chorionic gonadotropin (hCG). The gonadotropins increase the mRNA level of GnRH II but decrease that of GnRH I in a dose dependent manner [56]. Several reports demonstrated that the regulation of GnRH I-receptor gene expression by gonadotropins is tissue specific. GnRH I, GnRH I-receptor and RFRP-3 (a mammalian ortholog of GnIH) undergo significant variation during proestrus and are suggested to be responsible for selection of follicle for growth and atresia [57].

Distribution and concentration of neuropeptides, gonadotropin-releasing hormone (GnRH), gonadotropin-inhibitory hormone (GnIH), kisspeptin, and gonadotropin-releasing hormone receptor (GnRH-R) were evaluated in the testis of mice from birth to senescence. GnRH, GnIH, and kisspeptin interact in the testis that causes changes in the levels of GnRH-R and testicular development [58]. Also, testosterone significantly increases GnRH receptor number in testis [59].

Bradykinin as regulator of GnRH release

Bradykinin has been emerged as a potent GnRH stimulator from hypothalamic fragment and bradykinin has been shown to localize in the hypothalamic area [60, 61]. Bradykinin containing neurons are found in the supraoptic nucleus, organum vasculosum of the laministerminalis (OVLT), arcuate nucleus and median eminence of the cycling rat. These reasons are also critical in the control of GnRH secretions. Bradykinin B_2-receptor is also found in the rat pituitary, hypothalamus and the immortalized GnRH neuronal cell line (GT1-7 cells) [60]. The fact that bradykinin neurons in the hypothalamus play a physiological role in the regulation of GnRH and LH release is supported by the finding that central administration of the bradykinin B_2-receptor antagonist into the third cerebral ventricle blocked the steroid-induced LH surge in the ovariectomized adult rat. This suggests that the bradykinin acts directly on GnRH neurons through a mechanism involving mediation by the bradykinin B_2-receptor (Fig. 1). The action of bradykinin on the GnRH release appears to be independent of excitatory amino acids because N-methyl-D-aspartate (NMDA) and non-NMDA antagonist cannot block the bradykinin induced release from hypothalamic fragments [60]. It has already been reported that kinin-producing activity increases during ovulation [62, 63, 64]. In a study, Kihara [65] showed the presence of a component of bradykinin and bradykinin-producing system in the porcine ovarian follicle suggesting its role in early follicular development and ovulation. It has been demonstrated that bradykinin induces ovulation in perfused rabbit ovaries [66, 67] potentiates the action of LH [68] and a physiological role of bradykinin in the LH surge was also implicated [60]. Whether bradykinin is also involved in the regulation of ovarian GnRH I synthesis and secretion require further investigation.

Gonadotropin-Inhibitory Hormone (GnIH)

Two decades ago, a hypothalamic dodecapeptide was identified, which directly inhibits gonadotropin release in the Japanese quail and was termed as “Gonadotropin-inhibitory hormone (GnIH) [69]. This was the first demonstration of a hypothalamic neuropeptide that directly inhibiting gonadotropin release in any vertebrate. A gonadotropin inhibitory system is an intriguing concept and provides an unprecedented opportunity to study the regulation of vertebrate reproduction from an entirely novel standpoint. The GnIH localization was first studied in quail brain (hypothalamus) by immunocytochemistry [69, 70, 71]. Clusters of distinct GnIH immunoreactive neurons were found in the paraventricular nuclei (PVN) in hypothalamus. GnIH-containing fibers were widely distributed in the diencephalic and mesencephalic regions. The

most prominent fibers were seen in the median eminence of the hypothalamus. The presence of GnIH in the PVN was also demonstrated in other birds and thus appears to be a conserved property among several avian species. Interestingly, GnIH-containing fibers were observed in extremely close proximity to GnRH neurons in the preoptic area in birds [72, 71]. It is therefore plausible that GnIH may act at the level of the hypothalamus to regulate gonadotropin through GnRH as well as by directly acting at the pituitary (Fig. 1). A cDNA that encoded the GnIH precursor polypeptide was identified in the quail brain [73]. The deduced GnIH precursor consisted of 173 amino acid residues that encoded one GnIH and two putative GnIH-related peptides (GnIH-RP-1 and GnIH-RP-2) sequences that included-LPXRF at their c-termini. The effect of GnIH on the release of LH, FSH and prolactin was investigated using cultured quail anterior pituitaries [69]. GnIH significantly inhibit the LH and FSH release. There is evidence that GnIH inhibits gonadotropin synthesis *in vitro* [74, 75]. Identification of the receptor for GnIH is crucial to elucidate the mode of action of GnIH. Yin H [76] identified the receptor for GnIH in the quail diencephalons and characterized its expression and binding activity. GnIH receptor possessed seven transmembrane domains, indicating a new member of the G-protein-coupled receptor (GPCR) superfamily [76]. To understand the functional significance of GnIH, effect of GnIH treatment on gonadal development and maintenance in male quail was investigated [75]. In mature birds, chronic treatment with GnIH via osmotic pump decreased gonadotropin synthesis and release in a dose dependent manner [75]. Plasma testosterone concentrations were also decreased dose-dependently. GnIH treatment to mature birds induced testicular apoptosis and decreased spermatogenic activities in the testis. In mammals, GnIH is also expressed in the testis and ovary and suppresses gametogenesis and sex steroid production acting in an autocrine/paracrine manner [57, 77]. GnRH I and GnIH proteins are found in close vicinity suggesting a functional interaction between them. GnIH may also suppress gonadotropin synthesis and release by suppressing gonadotropin-releasing hormone (GnRH) (Fig. 1) [78]. It is, therefore, possible that GnIH may be regulating GnRH release.

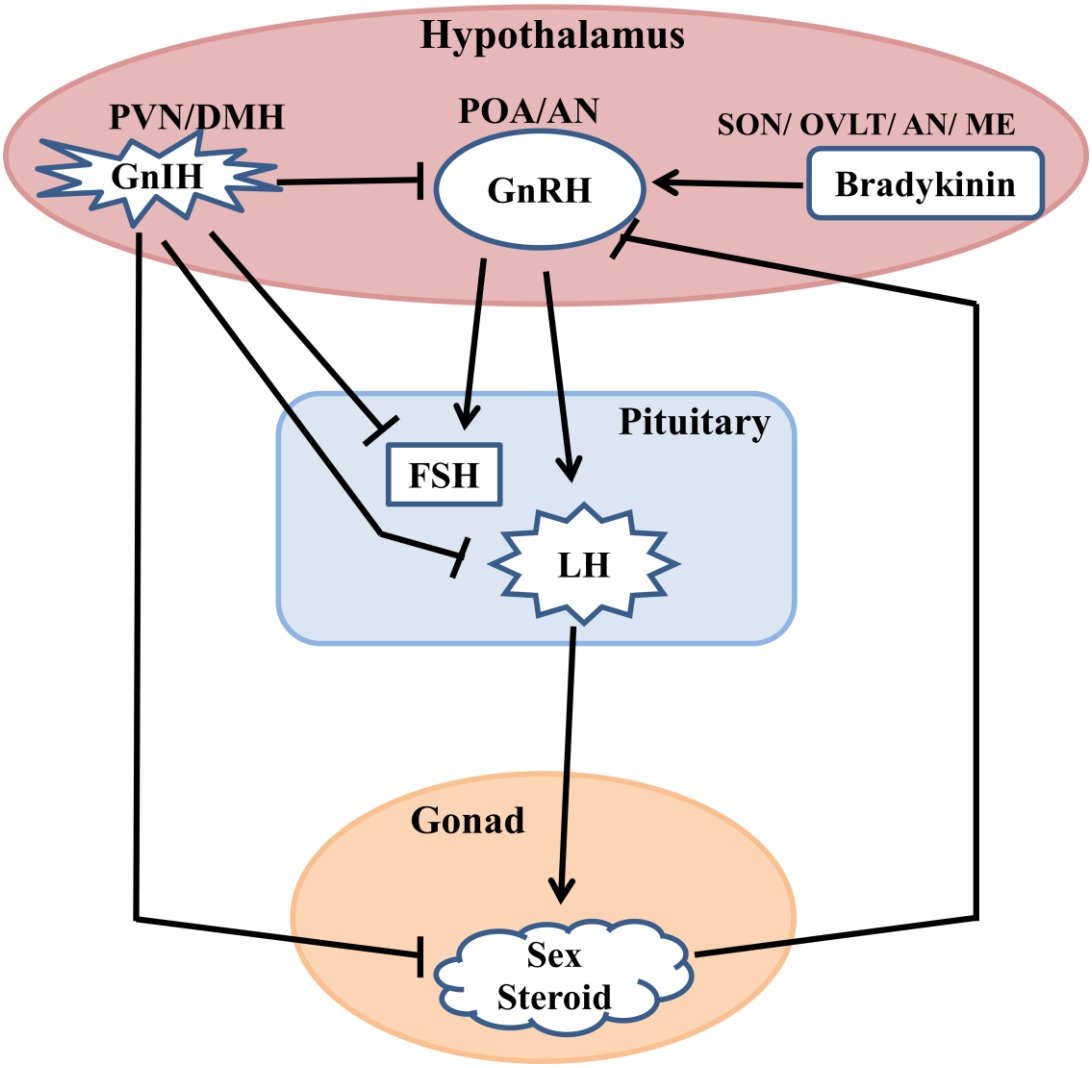

Fig 1. Relationship between GnRH, Bradykinin and GnIH in regulation of Reproduction. Bradykinin in hypothalamus regulate GnRH and LH release through its Bradykinin B_2-receptor. GnIH regulate gonadotropins directly as well as suppress GnRH synthesis and release. GnRH act at the level of hypothalamic-pituitary-gonadal axis to stimulate reproduction.

GnRH Analogs

GnRH analogsare peptides in which primary structure has been altered by the deletion of one or more amino acids and/or substitution of one or more amino acids by other amino acids. Many structural analogs of GnRH including both agonists and antagonists have been synthesized to develop more potent compounds for therapeutic use. The GnRH analogs have a longer half-life and a higher receptor affinity than does the native hormone. A large numbers of GnRH agonists and antagonists with elaborate side chains were synthesized. Since discovery of GnRH some 35 years ago, many GnRH I analogs with enhanced biological potency have been developed and studied extensively [79]. Clinically, some of these synthetic analogs have been used as an effective treatment for a variety of reproductive endocrinopathies, whereas others have been widely adopted

in controlled ovarian hyperstimulation regimens for assisted reproductive technique [80]. They also have potential as novel contraceptives in men and women. The first synthetic GnRH analogs consisted of GnRH agonists, when administered for the prolonged period result in a brief period of increased gonadotropin synthesis and release known as the flare-up effect. This flare-up effect was followed by a more prolonged decrease of pituitary hormone secretion. This paradoxical action, known as desensitization, has turned out to be clinically useful in the treatment of several sex hormone-dependent conditions. Later, GnRH antagonist were introduced, leading to immediate arrest of Gonadotropin secretion, but their clinical use limited by histaminic skin reaction after subcutaneous injection. More advanced compounds include cetrorelix and ganirelix, which showed no significant histamine-releasing effect but a dramatic and rapid suppression of gonadotropin secretion [81].The direct actions of GnRH agonists/antagonists in the human ovary showed some conflicting effects. In granulosa cells from follicles of patients undergoing *in vitro* fertilization, some authors found an increased ovarian steroidogenesis (estrogen/progesterone) induced by GnRH agonists *in vitro* which could not be confirmed by others. With respect to GnRH antagonists, both inhibitory action and no effect on ovarian steroidogenesis have been reported [82]. When GnRH agonist and antagonist was compared for steroidogenesis and ovulation, agonist showed beneficial effect over GnRH antagonist [83].

Reproductive functions of GnRH

The isolation of several forms of GnRH in neural tissue of tunicates and their activation of the gonads suggests that direct regulation of the gonads was an early evolved function and that the neuroendocrine role in regulating the pituitary was a later evolutionary development [84, 85]. The presence of GnRH and GnRH receptors in the gonads of various vertebrate species, including mammals, may reflect this early function. Neurons are probably one of the earliest cells in evolution to elaborate GnRH peptides. Clearly, the basic structure of GnRH peptides was established in primitive fish. In contrast, at least three other identified forms of GnRH have been detected in teleosts or tetrapods: Salmon I, catfish I, and chicken I GnRH. Evidence for the presence of members of the GnRH family and the neurohypophysial hormone family in primitive fishes argues for the importance of neuroendocrine control throughout the history of vertebrates. A reasonable hypothesis based on present evidence is that the mammalian form of GnRH arose in an ancestor of the primitive bony fish during the Silurian Period. This form of GnRH appears to have radiated throughout the primitive bony fish as represented by the 4 orders containing reedfish, sturgeon, alligator gar and bowfin, but there is no clear evidence to date that the mammalian sequence of the peptide is present in teleosts. The mammalian form of GnRH also radiated with the amphibians; the amino acid analysis of GnRH in the bullfrog is the strongest evidence [86], but the immunological and chromatographic evidence of mammalian GnRH in several species of frogs, newts and salamanders supports this concept [25, 26].In addition to its pivotal role in stimulating gonadotropin

secretion, it is well established that GnRH I function as a local autocrine and/or paracrine factor in the mammalian ovary by regulating steroidogenesis, cell proliferation, and apoptosis. So far, *in vitro* and *in vivo* studies in rat and human ovary have indicated that GnRH I is implicated in ovarian steroidogenesis and the transcription of several genes involved in the process of follicular maturation and ovulation [45, 87]. GnRH I also inhibited DNA synthesis *in vitro* and induced apoptosis in rat granulosa cells [88, 87]. It is well documented that GnRH I possess antigonadotropic effect in the rat ovary by downregulating the expressions of FSH and LH-receptors [89] inhibiting gonadotropin-stimulated cAMP production [90] and suppressing steroidogenic enzymes [91]. According to Peng C [92] progesterone secretion decreases from luteal cells was associated with upregulation of mRNA levels for GnRH I and GnRH receptor. The role of GnRH I as a negative autocrine regulator of proliferation in ovary surface epithelium and ovarian cancer cells has been well-documented [93, 94]. Because the growth of these cells can be significantly inhibited at nanomolar concentration of GnRH I agonists, it is believed that the antiproliferative action of the hormone is mediated via GnRH I-receptor. The exact mechanism of the GnRH-growth inhibitory effect in ovarian cancer cell remains to be elucidated. To-date, the role of GnRH I in regulating apoptosis in human ovarian cancer cells remains controversial. GnRH I may function as an autocrine factor to stimulate apoptotic cell death in Fas-positive tumors. The role of GnRH I in regulating apoptosis in rat granulosa cells has been well-established [88]. In rats, treatment with GnRH I agonist *in vivo* produces a time- and dose-dependent increase in DNA fragmentation, a hallmark of apoptotic cell death in granulosa cells of preantral and antral follicles [95]. In addition, GnRH I treatment can partially block the antiapoptotic effect induced by FSH [88]. GnRH I has been suggested as a luteolytic factor, increasing the number of apoptotic luteinized granulosa cells [96]. GnRH I induced an increase in the number of apoptotic human granulosa cells obtained during oocyte retrieval for *in vitro* fertilization [96]. More studies are warranted to dissect definitively the specific functional roles of the GnRH/GnRH receptor in the vertebrate ovary and results from these studies should undoubtedly facilitate our understanding of the biological significance of the hormone in controlling reproductive process.

2. CONCLUSION

It is now well established that the decapeptide, GnRH, is a key molecule of sexual maturation and reproductive functions in vertebrates. In the last several years, there has been increasing evidence that GnRH is an intra-ovarian regulatory factor. However, the detailed information about the distribution, regulation and physiological action of GnRH system (ligand, receptors and its regulators) in different vertebrate species are not fully investigated. Information about the paracrine/autocrine actions of GnRH in the ovary during different reproductive phases may further contribute to the better knowledge of the GnRH in the ovarian functions. Role of GnRH in the ovarian patho-physiology is completely lacking. Recently, GnIH is demonstrated in the ovaries of

birds and mammals and interactions of GnIH and GnRH may also exist in the gonads, as it does in their brains.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The author confirms that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

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