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Structures, Functions and Expressions of GnRH and GnRH Receptor in Peripheral Reproductive Organs and Their Regulation by Estradiol-17β

Review Article

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ABSTRACT

Studies have shown that estradiol-17β (E_2) regulates gonadotropin-releasing hormone (GnRH) and GnRH receptor expression in hypothalamus and pituitary. Several studies have shown that GnRH and its receptor are also expressed in peripheral reproductive organs and little is known about their regulations. In this study, GnRH and GnRH receptor structures, functions, their peripheral expressions and regulations by E_2 were reviewed. Several *in vivo* and *in vitro* conducted studies indicate that E_2 decreases the expression of GnRH mRNA and regulates GnRH receptor expression in a time dependent manner. Nevertheless, the exact mechanism has not been clearly explained yet.

KEY WORDS estradiol, GnRH, ovary, oviduct, uterus.

INTRODUCTION

Gonadotropin-releasing hormone (GnRH) is a hypothalamic decapeptide regulating gonadotropin biosynthesis and release in the anterior pituitary via specific receptors. GnRH and its receptor are not only expressed in hypothalamus and pituitary. Several previous studies have reported an extra hypothalamic origin of GnRH, as well as an extra pituitary presence of its receptor in the reproductive tract of some of the mammalian species (Bauer-Dantoin and Jameson, 1995; Singh *et al.* 2011). The immuno localization and expression of GnRH and its receptor were shown in ovary of the mice and rat (Bauer-Dantoin and Jameson, 1995; Singh *et al.* 2011). GnRH expression has been shown in oviduct of the rat (Sengupta *et al.* 2007), human (Casañ *et al.* 1999) and porcine (Li *et al.* 1993). The expression of GnRH receptor mRNA and GnRH receptor protein have been shown in the bovine oviduct and uterus (Singh *et al.* 2008) and human (Raga *et al.* 1998). There are also reports regarding the presence of GnRH receptors in the male and female gametes (Dekel *et al.* 1988; Minaretzis *et al.* 1995; Morales *et al.* 1999) and embryos (Casan *et al.* 1999). In the ovary, GnRH is considered to act in an autocrine or a paracrine manner to regulate steroidogenesis by exerting a stimulatory as well as an inhibitory effect on the production of steroid hormones and apoptosis in ovarian follicles and corpora lutea (Dubois *et al.* 2002; Ramakrishnappa *et al.* 2005). The presence of GnRH in gametes indicated that it may have an effect on fertilization. Addition of GnRH to human semen increases the binding ability of sperms to the zona pellucida surrounding oocytes (Morales, 1998). In humans, GnRH and GnRH-receptor mRNA and protein expressions have been shown in endometrium throughout all phases of the menstrual cycle (Raga *et al.* 1998). The stimulatory effects of GnRH on cleavage cell divisions during early embryonic development and implantation have been reported in human (Tesarik *et al.* 2004). The presence of GnRH and the relative expression of its mRNA in the oviduct of pregnant rat (Sengupta *et al.* 2007) have led to the conclusion that it may have a possible

role in post implantation embryonic development and the maintenance of pregnancy (Sengupta *et al.* 2007). GnRH and GnRH receptor mRNA are also expressed in placenta (Lin *et al.* 1995; Raga *et al.* 1998) and might affect the secretion of human chorionic gonadotropin (hCG). Addition of GnRH into the placental explant culture increased the secretion of hCG (Lin *et al.* 1995). The regulation mechanism of GnRH and its receptor in the peripheral reproductive organs is not well known. Estradiol-17β (E_2) is major regulator of GnRH and GnRH receptor expression. Therefore, the aim of this study is to review the structure, function and expression of GnRH and its receptors in the peripheral reproductive organs and their regulation by E_2.

Structure and function of GnRH-1

It was firstly proposed by Geoffrey Harris, John Everett, and Charles Sawyer that the hypothalamus controls reproductive function and a neurochemical signal from the hypothalamus is released into the anterior pituitary gland to stimulate gonadotropin secretion between 1930-1940 (Terasawa *et al.* 2010).

Therefore, there must be a capillary portal vessel network connecting the hypothalamus to the pituitary. After a series of experimentations including the injection of Indian ink into the capillary network, Geoffrey Harris (Harris, 1948) demonstrated the presence of a portal vessel system in 1940 s. The hypothalamic factor controlling the pituitary secretion of gonadotropins was initially named as luteinizing hormone-releasing hormone (LHRH) because of its preferential positive effect on luteinizing hormone (LH) secretion rather than the secretion of follicle stimulating hormone (FSH) (McCann *et al.* 1960). However, injection of a specific LHRH antagonist suppressed both LH and FSH secretion. Therefore, it was named as gonadotropin releasing hormone (GnRH). Following studies focused on the extraction and the purification of GnRH, which was firstly purified from pig, ovine and bovine hypothalamus (Kochman and Domański, 1969; Schally *et al.* 1971).

The molecular structure of GnRH was first explained by Andrew Schally and his team in 1971 (Schally *et al.* 1971). This GnRH is accepted as mammalian GnRH and designated as GnRH-I. The discovery of GnRH led to extensive research in this field and it is still an active field of research.

This is because it is an important peptide for the regulation of the hypothalamic-pituitary-gonadal axis, and because recent advances in molecular biology have enabled the researchers to study its functions at multiple functional levels. The mammalian GnRH (GnRH-I) is consisted of ten amino acids as pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2 respectively. The length of the peptide and the amino acids both at the amino and at the carboxyl-terminal domains, which are important for receptor binding and activation, are highly conserved. In all vertebrate GnRH, glycine at the position 6 is strictly conserved. This amino acid is important for βII-type turn conformation of GnRH to bind its receptor. A three-dimensional structure of mammalian GnRH based on recent nuclear magnetic resonance (NMR) report showing the βII-type turn conformation around the glycine in position 6 (Figure 1).

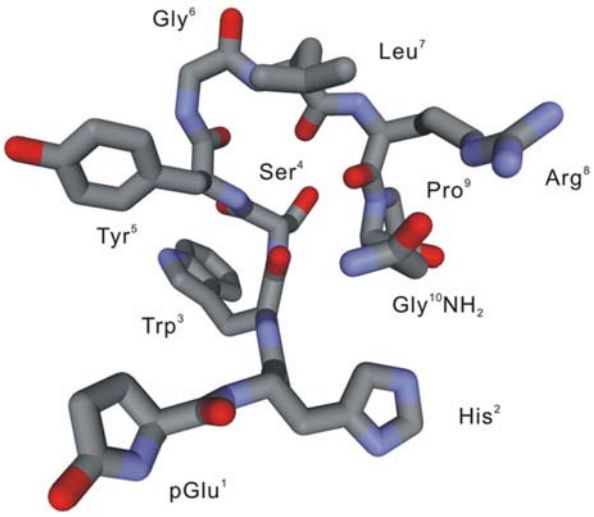

Figure 1 NMR structure of mammalian GnRH showing the βII-type turn conformation around glycine in position 6 (obtained from the Figure 6 of Millar *et al.* 2008)

It is known that GnRH is synthesized from a larger prohormone by an enzymatic process and packaged into storage granules in the neurons in the preoptic area of the hypothalamus, then transported down to the axons located in the external zone of the median eminence (Kaiser *et al.* 1997; Millar *et al.* 2004; Cheng and Leung, 2005). It is released in a pulsatile manner, in synchronized pulses repeating every 30-120 minutes, from the nerve endings of about 1000 neurons into the hypophyseal-portal circulation system to the anterior pituitary, where it binds to its cognate-receptor on pituitary gonadotropin-secreting cells to control the secretion and synthesis of LH and FSH (Conn and Crowley, 1994; Stojilkovic and Catt, 1995; Sealfon *et al.* 1997). The amount of secreted gonadotropin depends on the amount of the GnRH reaching to the pituitary and the response of pituitary to GnRH.

It is well known that the hypothalamus and the pituitary are not only the sites where GnRH and its receptor are expressed. Both GnRH and its receptor expression have been reported in the peripheral reproductive tissues and organs such as testis, ovary, oviducts, endometrium, placenta and in mammary glands of various vertebrate species (Siler-Khodr and Khodr, 1979; Khodr and Siler-Khodr, 1980; Oikawa *et al.* 1990; Li *et al.* 1993; Bahk *et al.* 1995; Raga

et al. 1999; Harrison *et al.* 2004; Ramakrishnappa *et al.* 2005). As mentioned above, GnRH is synthesized from a larger prohormone by an enzymatic process. The gene encoding the preprohormone was first described in humans, which contains four exons and three introns (Figure 2).

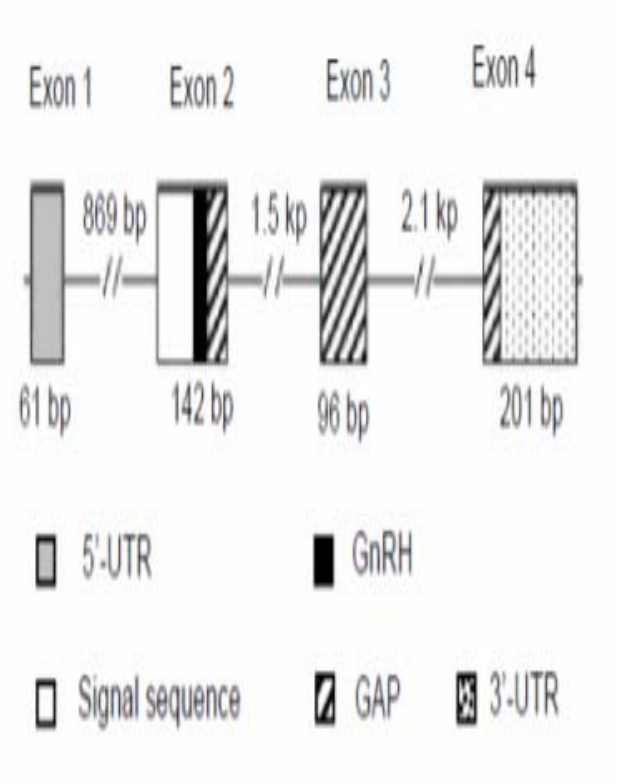

Figure 2 The gene structure of human GnRH-I prohormone. It consists of four exons interrupted by three introns. Exon 1 encodes the 5'-UTR. Exon 2 encodes the signal peptide, GnRH decapeptide and the N-terminus of GAP. Exon 3 encodes the central portion of GAP and exon 4 encodes the C terminus of GAP along with the 3'-UTR (taken from Cheng and Leung, 2005)

The first exon of the gene is untranslated and consists of 61 bp in mRNA expressed in the hypothalamus. The second exon encodes the signal sequence, the GnRH decapeptide and GnRH-associated peptide (GAP) residues. The third exon encodes for the next GAP residues. The fourth exon encodes the remaining GAP residues and contains the translation termination codone and also the entire 3'-UTR (Adelman *et al.* 1986; Radovick *et al.* 1990). According to the pulse frequencies of GnRH, both LH and FSH are secreted by the same cells of pituitary known as gonadotrophs. Higher pulse frequency primes the secretion of LH, while lower frequencies prime the secretion of FSH (Dalkin *et al.* 1989; Haisenleder *et al.* 1993; Burger *et al.* 2002). External administration of GnRH results in LH and FSH secretion within 30 minutes. In peripheral tissues, such as in the ovary, GnRH affects steroid hormone production and in the placenta, it stimulates the secretion of chorionic gonadotropin (Hsueh and Schaeffer, 1985; King and Millar, 1995). A second form of GnRH was discovered in the chicken brain, and named as chicken-GnRH or GnRH-II. It is highly conserved peptide and also expressed in the central nervous system of many mammalian species including monkey (Lescheid *et al.* 1997) and human (White *et al.* 1998). Its amino acid sequence (pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH2) is 70% similar to that of GnRH-I differing in three amino acids (at positions 5, 7 and 8), but is encoded by a different gene (White *et al.* 1998) and is expressed by a distinct population of cells (Latimer *et al.* 2001). Initially, it was thought that GnRH-II promotes LH secretion in birds, but studies have shown that it can also promotes LH secretion in mammalians with a much lower potency than GnRH-I (Millar and King, 1983; Hasegawa *et al.* 1984). The expression and immune reactivity of GnRH-II is not only present in hypothalamus and pituitary gland, but also present in peripheral reproductive tissues of mammals including ovary, oviduct, uterus and placenta (Millar, 2003; Neill *et al.* 2001; Cheon *et al.* 2001; Siler-Khodr and Grayson, 2001; Choi *et al.* 2001; Chen *et al.* 2002).

In addition to GnRH-I and GnRH-II, at least 45 structurally different forms of GnRH have been identified. Fifteen structural variants of the GnRH molecule have been found in vertebrates, and 15, in invertebrates (Millar *et al.* 2004; Roch *et al.* 2011), nine different GnRHs were identified in prochordates, which are vertebrate progenitors (Adams *et al.* 2003; Millar *et al.* 2004). A further six GnRH sequences were determined in other invertebrates.

Gonadotropin-releasing hormone and its analogues have been used for the stimulation of gamete and hormone production, inhibition of ovulation and spermatogenesis, manipulation of puberty, synchronization of estrous in cattle, treatment of cystic ovary problem, treatment of uterine lesions and as well as used for the treatment of cancer.

Structure and function of GnRH receptor

Gonadotropin-releasing hormone (GnRH) acts via G-protein-coupled receptors (GPCRs), which are a family related to the rhodopsin and β-adrenergic receptors. GPCRs are known as the largest family of signaling proteins preferentially coupled to the Gq/11 protein localized in the cytoplasm and associated with the intracellular domains of the receptor (Stojilkovic *et al.* 1994). G-protein-coupled receptors are characterized by a hydrophilic extracellular N-terminal domain followed by hydrophobic seven transmembrane domains linked by a series of hydrophilic intracellular and extracellular loops and finally a hydrophilic intracellular carboxyl-terminal domain. The extracellular N-terminal domain contains ligand-binding and glycosylation sides as well as conserved cysteine residues forming disulfide bridges to stabilize the receptor structure (Figure

3). The seven transmembrane domains are known to be arranged in a tight bundle enclosing a hydrophilic pocket and surrounded by the hydrophobic membrane environment and believed to be involved in conformational change associated with signal propagation (receptor activation), while the intracellular domains are involved in interacting with G-proteins and other proteins for intracellular signal transduction (Sealfon *et al.* 1997; Flanagan *et al.* 1997; Schertler *et al.* 1993; Naor *et al.* 1998; Baldwin, 1993; Donnelly *et al.* 1989; Ballesteros and Weinstein, 1992).

Binding of the mammalian GnRH to its receptor occurs via $pGlu^1$, His^2, Arg^8 $GlyNH_2{}^{10}$ with cognate sites $D^{98}(Asp^{98})$, $K^{121}(Lysine^{121})$, $D^{302}(Asp^{302})$ and N^{102} (Asn^{102}) in the receptor. The $Arginine^8$ in GnRH is essential for high affinity binding and selectivity of the receptor, while its mutation to Glycine leads to very poor binding efficiency (Ballesteros *et al.* 1998; Sealfon *et al.* 1997; Illing *et al.* 1999; Millar *et al.* 1989; Millar and King, 1983; Flanagan *et al.* 1994). Arginine side chain is involved in a triad interaction with $aspargine^{87}$ in the second transmembrane domain (TMD2) and with $aspartate^{318}$ in the seventh transmembrane domain (TMD7) to stabilize the active conformation of the receptor (Ballesteros *et al.* 1998).

In non-mammalian vertebrate species (catfish, frog, chicken, and goldfish) and non-human primates, GnRH receptors have a carboxyl-terminal extension containing potential phosphorylation sites on multiple serine or threonine residues (Neill, 2002; Millar, 2003). When GnRH binds to its receptor, the receptor couple via heterodimeric G protein to phospholipase C (PLC) and adenylyl cyclase (AC) followed by phosphorylation of serine or threonine residues within carboxyl-terminal region. This phosphorylation is typically rapid (seconds to minutes) and mediated by specific G-protein receptor kinases (GRKs), by second messenger-regulated kinases (e.g. protein kinase C (PKC) or PKA), or by casein kinases (Tobin *et al.* 1997; Hanyaloglu *et al.* 2001) leading to β-arrestin binding, which hinders G-protein binding and thereby prevents effector activation (Zhang *et al.* 1997; Ferguson, 2001) and leading to the internalization of GnRH-Rs via clathrin-coated vesicles (Jennes *et al.* 1983; Jennes *et al.* 1986; Conn *et al.* 1987). The formation of these vesicles is typically controlled by a dynamin collar, which separates the vesicle from the plasma membrane by pinching off (or stretching) the neck of the vesicle (Schmid, 1998). After internalization, the receptors are either recycled to the cell surface or proteolytically degraded in lysosomes (Lefkowitz *et al.* 1990; Dohlman *et al.* 1991).

Mammalian GnRH receptors do not desensitized rapidly, and do not undergo agonist-induced phosphorylation or cause β-arrestin translocation. They do show agonist-induced internalization, but this process is much slower and is not influenced by expression of β-arrestin (Heding *et al.* 1998; Heding *et al.* 2000; Vrecl *et al.* 1998; Willars *et al.* 1999). When GnRH binds to its receptor (GnRH-R), the receptor is coupled via Gq/11 to phospholipase C1 (PLC1). PLC1 cleaves phosphatidylinositol 4, 5-bisphosphate (PIP2), producing inositol trisphosphate (IP3), which mobilizes Ca^{2+} and thereby acutely regulates gonadotropin exocytosis. It also yields diacylglycerol (DAG), which activates PKC, feeding in to mitogen-activated protein kinase (MAPK) regulation and consequent regulation of gene expression. The structure of mammalian GnRH receptor, from different animals, shows about 85% homology. The length of the receptor protein varies between the mammalian and the non-mammalian species. Its length is 327-328 amino acids in cow, sheep and in human, while it is 370 amino acid-long in catfish due to the presence of 49 amino acids in the carboxyl-terminal domain, which is not present in mammalian receptors (Tensen *et al.* 1997). The expression both of the GnRH receptor mRNA and GnRH receptor protein are not only localized in pituitary gonadotrophs, but also localized in the peripheral reproductive tissues such as ovary, oviduct, uterus of mammals (Singh *et al.* 2008; Hsueh and Jones, 1981; Imai *et al.* 1994; Minaretzis *et al.* 1995; Borroni *et al.* 2000). GnRH and GnRH receptors are also found in many gonadal steroid-dependent cancers, including those of the breast, endometrium, prostate and ovary (Eidne *et al.* 1985; Miller *et al.* 1985; Emons *et al.* 1998; Limonta *et al.* 1999; Limonta *et al.* 2001; Schally, 1999; Schally and Nagy, 1999; Imai and Tamaya, 2000; Grundker *et al.* 2001).

In humans, the GnRHR gene is located at 4 q 13.2 ± 3 and consists of three exons and two introns encoding a 328 amino acid protein (Figure 4). The proximal 5'-flanking region of the human GnRH receptor gene exhibits a great homology with that of the rodent and ovine sequences (Albarracin *et al.* 1994; Fan *et al.* 1995; Campion *et al.* 1996; Reinhart *et al.* 1997). But, there are certain specific differences in the structure of the gene between the human and rodent, such as the differences in transcription starting side and the differences in presence of multiple TATA and CAAT boxes.

Expression of GnRH and GnRH receptor in the peripheral reproductive organs

The expression of GnRH and GnRH receptor are not peculiar to hypothalamus and pituitary, they are expressed in the peripheral organs and tissues. The determination of peripheral expression of GnRH and GnRH receptor dates back to the late 1970s in rodent species. Specific radioligand binding sites were identified on rodent ovarian ganulosa and luteal cells (Clayton *et al.* 1979; Harwood *et al.* 1980; Reeves *et al.* 1980; Jones *et al.* 1980; Pieper *et al.* 1981).

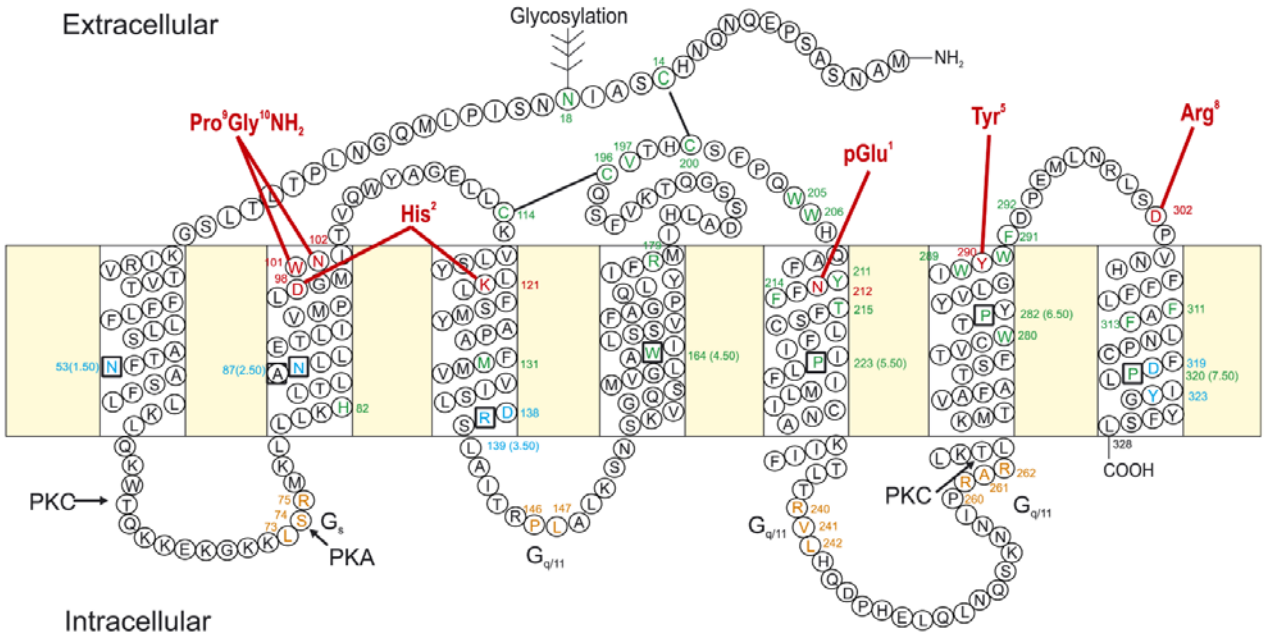

Figure 3 The mammalian GnRH receptor consists of an extracellular N-terminal domain followed by seven transmembrane domains linked by 3 intracellular and 3 extracellular loops. Mammalian GnRH receptor has no intracellular carboxyl-terminal domain. The extracellular N-terminal domain contains ligand binding and glycosylation sides as well as conserved cystein residues forming disulfide bridges to stabilize the receptor structure. GnRH binding sides are indicated in red and the sides are thought to be important in receptor structure or binding pocket configuration are indicated in green, including disulfide bond formation and glycosylation sites. Residues involved in receptor activation are shown in blue. Residues involved in coupling to G proteins are shown in orange. Putative protein kinase C (PKC) and protein kinase A (PKA) phosphorylation sites are indicated (obtained from Millar *et al.* 2004)

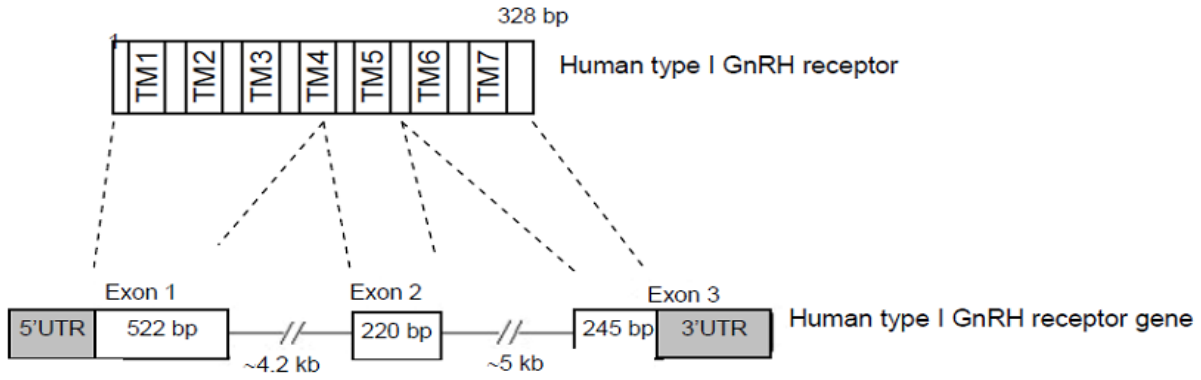

Figure 4 GnRHR gene consists of three exons and two introns. Exon 1 contains the 5'-UTR and encodes the first three transmembrane (TM) domains and a portion of the fourth TM domain. Exon 2 is 220 bp in length and encodes the remainder of the fourth TM domain, the fifth TM domain, and part of the third intracellular loop. Exon 3 encodes the rest of the open reading frame and contains the 3'-UTR (obtained from Cheng and Leung, 2005)

By using *in situ* hybridization and reverse transcription polymerase chain reaction (RT-PCR) techniques, the localization of GnRH mRNA and GnRH receptor mRNA in human ovarian granulosa and luteal cells has been shown (Minaretzis *et al.* 1995; Olofsson *et al.* 1995; Kang *et al.* 2000). Bu using RT-PCR and monoclonal GnRH receptor antibody through immunoblotting (Singh *et al.* 2008), the expressions of GnRH receptor mRNA and GnRH receptor protein have been shown in ovary, oviduct and in the uteri of bovine (Singh *et al.* 2008), porcine (Li *et al.* 1993) and human (Hsueh and Jones, 1981; Imai *et al.* 1994; Minaretzis *et al.* 1995; Ikeda *et al.* 1996; Dong *et al.* 1998; Raga *et al.* 1998; Borroni *et al.* 2000; Casañ *et al.* 2000). The presence of GnRH or a GnRH-like substance has also been reported in the placenta (Khodr and Siler-Khodr, 1978; Tan and Rousseau, 1982) and mammary glands (Amarant *et al.* 1982) of humans. Nucleotide sequence analyses in the rodent, bovine and human have shown that ovarian GnRH and GnRH receptor have sequence identical to those found in the hypothalamus and the pituitary (Kakar *et al.* 1992; Peng *et al.* 1994; Moumni *et al.* 1994; Olofsson *et al.* 1995; Whitelaw *et al.* 1995; Kottler *et al.* 1999).

GnRH is regarded as an important paracrine and autocrine factor in the ovary. It has both inhibitory and

stimulatory effects on ovarian function. GnRH exerts a stimulatory action on preovulatory follicles by inducing oocyte maturation (Hillensjo and LeMaire, 1980) and follicle rupture (Ekholm *et al.* 1981). On the other hand, GnRH has inhibitory effect on steroidogenesis involving the suppression of gonadotropin receptors (Hsueh and Jones, 1981) or the suppression of activity of the intermediary enzymes involved in steroidogenic pathway. It was also suggested that GnRH affects the process of fertilization and the cleavage rate of bovine oocytes *in vitro* (Funston and Seidel, 1995).

Effects of estradiol on peripheral GnRH and GnRH receptor expression

Estrogen is naturally occurring hormone within the body. Animal body naturally produces three main forms of estrogen, which are estradiol-17β (E_2), estrone (E_1) and estriol (E_3). Estrone and estriol were first identified in the urine of pregnant women and this was followed by the identification of E_2 in the follicular fluid of sow by Edward Adelbert Doisy between 1929 and 1936 (Simoni *et al.* 2002). Estradiol-17β is named for its importance in the estrous cycle affecting growth, development, maturation and functioning of reproductive tract, as well as the sexual differentiation and the behavior (Lien *et al.* 1985; Laugier *et al.* 1988; Balthazart *et al.* 2009).

Two receptors, known as ERα (ESR1) and ERβ (ESR2), mediate effects of estrogen (Calatayud *et al.* 2010). Both are members of a large super family of proteins functioning as ligand-activated transcription factors (Katzenellenbogen and Katzenellenbogen, 1996). Their presence have been shown within the hypothalamus, pituitary, ovary, oviduct, uterus, cervix and vagina of mammalians including the human (Brodowska *et al.* 2007), sheep (Juengel *et al.* 2006), cow (Sağsöz, 2011), goat (Cui *et al.* 2009), Porcine (Knapczyk-Stwora *et al.* 2011), rat (Okada *et al.* 2003) and mouse (Hułas-Stasiak and Gawron, 2007).

It is clearly known that estrogen is a major regulator of GnRH neuronal function in the female brain and it has a bimodal effect on the hypothalamic-pituitary axis in females with both an inhibitory (Caraty *et al.* 1989; Sarkar and Fink, 1980; Levine and Ramirez, 1980; Chongthammakun and Terasawa, 1993; Evans *et al.* 1994; Evans *et al.* 1995) and stimulatory effect on GnRH and gonadotropin secretion. The stimulatory effect of estrogen on GnRH secretion is best illustrated at the end of the follicular phase where a gradual and sustained rise in circulating estrogen levels exerts a positive feedback effect on the hypothalamus triggering a preovulatory GnRH surge which, in turn, stimulates the preovulatory LH secretion (Moenter *et al.* 1990; Sarkar *et al.* 1976).

Throughout the remainder of the cycle, estradiol exerts negative feedback actions on the central reproductive axis. GnRH is locally expressed in peripheral reproductive organs (Okrasa *et al.* 2003) and its expression is affected by estradiol. A study in ovariectomized (OVX) gilts showed that intramuscular injection of estradiol benzoate (EB) caused fluctuations in GnRH content according to the part of the reproductive tract. Estradiol benzoate treatment stimulated GnRH content in the ampulla of the oviduct and in the paracervical uterus, while inhibited GnRH content in the middle part of the uterus (Okrasa *et al.* 2003).

The presence of GnRH mRNA have been shown in the human endometrium, while absence of mRNA for GnRH receptors have been shown by using reverse transcriptase-polymerase chain reaction (RT-PCR) and Southern blot analysis (Ikeda *et al.* 1997). Data related to the effect of estradiol on GnRH gene expression is limited. *In vivo* studies conducted in several mammalian species have indicated that estrogen reduces GnRH gene expression (Zoeller and Young, 1988; Petersen *et al.* 1995; Spratt and Herbison, 1997).

Studies in postmenopausal women have shown that they have significant higher levels of GnRH mRNA when compared to premenopausal women (Rance and Uswandi, 1996). This suggests that lack of estrogen in postmenopausal women might have contributed to the high GnRH mRNA levels observed. Another study in adult female macaques indicated that ovariectomy significantly increased the number of GnRH-I expressing cells in the medial basal hypothalamus when compared to ovariectomized-estradiol treated animals (Figure 5, panel A). However, the number of GnRH-II expressing cells in ovariectomized animals was significantly lower than the number of the ovariectomized and estradiol treated group (Figure 5 panel B), Densmore and Urbanski (2004).

The similar results were obtained from *in vitro* studies in human and rat. Treatment of human granulosa luteal cells with estradiol significantly decreased GnRH-I mRNA levels in a time-dependent manner, with maximum inhibition of 77% at 48 h (Khosravi and Leung, 2003). In rat hypothalamic explants, estrogen reduced GnRH mRNA expression (Wray *et al.* 1989).

The studies given above indicate that estrogen negatively regulates GnRH gene expression; however, there is limited amount of information regarding the mechanisms of this negative regulation.

Estradiol-17β (E2) is the major regulator of GnRH receptor gene expression and number during the preovulatory period. Treatment of ewes with estradiol caused significant increase in the concentrations of GnRH receptor mRNA and GnRH receptors on pituitary gonadotrophs (Turzillo *et al.* 1998).

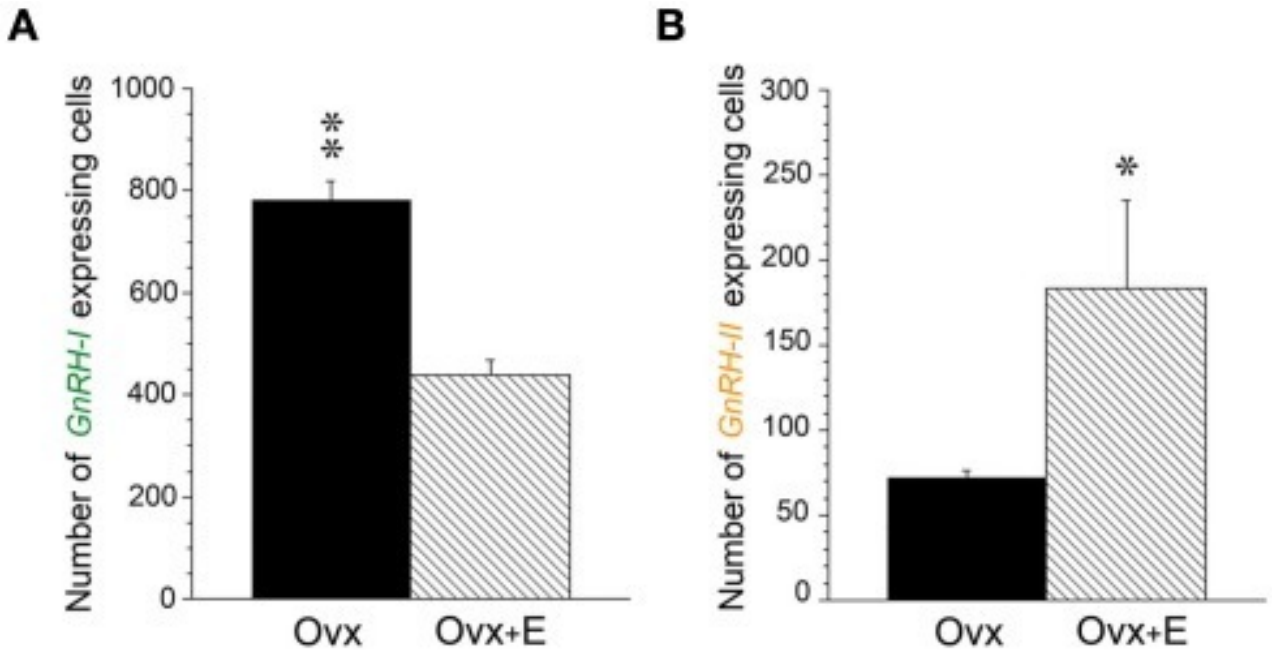

Figure 5 The illustration of the effect of estradiol on GnRH-I and II gene expressions in the medial basal hypothalamus of ovariectomized (Ovx) adult rhesus macaques. Ovariectomy caused a significant increase in the number of cells expressing GnRH-I, while the number of cells expressing GnRH-II decreased. Estradiol treatment of ovariectomized animal (Ovx+E) caused decrease in the number of GnRH-I expressing cells, while the number of GnRH-II expressing cells increased (taken from the figure 2 of Densmore and Urbanski, 2004)

In a previous experimental study (Nathwani *et al.* 2000), human granulosa-luteal cells (hGLCs) were supplemented with different concentrations of estradiol (1-100 nM). It was reported that a short-term treatment (6 h) with E2 significantly increased GnRH receptor mRNA levels by 20%, whereas long-term treatment (48 h) resulted in a 60% decrease in GnRH receptor expression in hGLCs (Nathwani *et al.* 2000).

CONCLUSION

GnRH and GnRH receptor are also expressed in peripheral reproductive organs and estradiol-17β has negative effect on GnRH mRNA expression, but the exact mechanism has not been elucidated yet. The existing data only indicate that estradiol regulate GnRH receptor expression, but it does not clearly indicate that whether estradiol affects the process either positively or negatively.

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