The effect of stress on the expression of GnRH and GnRH receptor genes in the discrete regions of the hypothalamus and pituitary of anestrous ewes

Magdalena Łapot\textsuperscript{1,2}, Magdalena Ciechanowska\textsuperscript{3}, Tadeusz Malewski\textsuperscript{2}, Tomasz Misztal\textsuperscript{2}, Krystyna Mateusiak\textsuperscript{2}, Franciszek Przekop\textsuperscript{2}

\textsuperscript{1}The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna, \textsuperscript{2}Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, Poland

Received: 26 August 2006; accepted: 2 March 2007

SUMMARY

Using the reverse transcription-polymerase chain reaction (RT-PCR) technique, the gonadotropin releasing-hormone (GnRH) mRNA and GnRH receptor (GnRH-R) mRNA were analyzed in the preoptic area (POA), anterior (AH) and ventromedial (VM) hypothalamus, stalk/median eminence (SME) and anterior pituitary gland (AP) of anestrous ewes subjected to short or prolonged footshock stimulation. No GnRH gene expression was detected in the SME and AP. The comparable levels of GnRH mRNA were found in the POA, AH and VM in control ewes. Short and prolonged footshock stimulation significantly increased GnRH mRNA in all analyzed tissue. The highest responses in GnRH mRNA to the short stress occurred in the POA whereas to the prolonged stress in the POA and VM. In non-stressed ewes the GnRH-R mRNA were detected in tissue continuum throughout the POA, AH, VM, SME and AP. The highest
concentration of GnRH-R mRNA was detected in the SME. Short as well as prolonged stress stimuli caused an increase in GnRH-R mRNA levels in all analyzed tissue. The highest responses in GnRH-R mRNA expression were found in the VM. In spite of profound up-regulation of GnRH mRNA and GnRH-R mRNA under the short and prolonged stress conditions, the increase of luteinizing hormone (LH) secretion was noted only during acute stress. It is suggested that the increase of expression of GnRH and GnRH-R genes in anestrous ewes are not directly related to GnRH level and GnRH-R activity. Reproductive Biology 2007 7 (1):55–71.

**Key words:** anestrous ewe, stress, GnRH mRNA, GnRH-R mRNA, LH

**INTRODUCTION**

Physiological regulation of gonadotropin releasing-hormone (GnRH) secretion is associated with complex interplay among excitatory and inhibitory neurotransmitter, neuromodulator and neurohormone system activity within the hypothalamus. Changes of activity in these systems under the stress condition alter GnRH/luteinizing hormone (LH) secretion in sheep [43, 45, 46] and disturb physiological ovulatory cycles [11, 29]. It is suggested that the immediate response of GnRH neurons to acute stress depends primarily upon central neural mechanism(s) [33] and, at least in some circumstances, is stimulatory [3, 13, 46]. There is little doubt that prolonged stress stimuli additionally modulate the GnRH secretion by neurotransmitters and hormones released during time-course of stress application and display a rather suppressive effect on this hormone secretion [25, 31, 40]. Despite a number of studies that have been concerned with GnRH/LH release during stress, there is as yet no coherent evidence how stress affects the intraneural events associated with biosynthesis of GnRH and GnRH-R in the preoptic area-hypothalamus and GnRH receptor activity in the anterior pituitary gland. Lack of clarity is exemplified by a debate on the effects of different kinds of stress stimuli on GnRH mRNA levels in the hypothalamus and GnRH-R mRNA in the anterior pituitary gland. Recent studies performed on rats have reported that prolonged as well as acute
stress suppress GnRH and GnRH-R gene expression in the hypothalamus and the anterior pituitary gland, respectively. However, the responses of the expression are highly dependent upon the kind of stress and physiological state of animals [7, 16, 26, 40]. The mode of stress action on the expression of GnRH and GnRH-R genes is still not well recognized.

Currently available data indicate that gonadal hormones [14] and some hypothalamic neurotransmitters [19, 20, 21, 41] which participate in the control of GnRH release may also be involved in the expression of GnRH and GnRH-R genes. Moreover, it is notable that the GnRH-R gene transcription in the pituitary gland is regulated by GnRH, gonadal hormones [22, 37, 48, 51] and GABA [36]. Physiological studies in sheep have implicated catecholaminergic, GABA-ergic and opioidergic systems in the preoptic area-hypothalamus among others, as potential transducers of steroid feedback onto the GnRH system [17]. However in ewes, as a seasonally breeding species, the effect of gonadal hormones [14] and various hypothalamic neural systems, including noradrenergic [10, 15, 32, 44], dopaminergic and β-endorphinergic systems [47], on GnRH release differs during non-breeding and breeding period. Therefore, the expression of GnRH and GnRH-R genes and their responses to stress may be specific for the different stages of reproduction. Thus, the objective of this study was to determine the effect of short and prolonged footshock stimulation on the GnRH gene expression in the hypothalamus and GnRH-R gene expression in the hypothalamus and in the anterior pituitary gland of anestrous ewes.

**MATERIALS AND METHODS**

**Animals**

The studies were performed on three-to-four-year-old Polish Merino ewes during a period of increasing day length, from the end of March to the end of May, corresponding in these ewes to seasonal anestrus. The animals were maintained indoors in individual pens and exposed to natural lighting. Food and water were available *ad libitum*. They were well adapted to the
experimental conditions: they had always visual contact with their neighbours even during blood collection to prevent the stress of the social isolation.

**Stressing procedure**

A state of stress was induced by applying repetitive trains of 3 mA alternative current of 0.5 sec on and 1 sec off arranged in a series of ten during a 20 minute period of every hour. These pulses were delivered to the electrodes on the feet of animals at the level of metacarpus in the programmed schedule: 3 h (from 0800 to 1100) during one day for the short stressed ewes and 5 hours daily (from 0800 to 1300) for the prolonged stressed animals. This procedure was described previously [30]. No stressed ewes served as controls. Six animals were in each group.

To establish the concentration of plasma LH and its profile secretion, the blood samples were collected via indwelling jugular catheters at 10 min intervals during five hours: first, on the day prior to the stressing procedure and second, on the day of the last stimulation. Immediately after the last blood collection, the ewes were euthanized with a barbiturate overdose.

![Fig. 1. Sagittal section through ovine brain. The outlined areas indicate structures taken for analysis of GnRH mRNA and GnRH-R mRNA. AH: anterior hypothalamus, OCH: optic chiasm, POA: preoptic area, SME: stalk/median eminence, MB: mammillary body, TH: thalamus, VM: ventromedial hypothalamus, HD: dorsal hypothalamus.](image-url)
animals were treated in strict compliance with Local Ethical Committee of the Warsaw Agricultural School.

The brains were rapidly removed from the skull and the median eminences were cut out and frozen in liquid nitrogen. The blocks of the brain encompassing the preoptic area-hypothalamus were sectioned sagitally and dissected from both sides on three parts: the preoptic area, anterior hypothalamus and ventromedial hypothalamus, according to the stereotaxic atlas of the ovine brain ([50]; fig.1). The anterior pituitary gland was also taken for GnRH-R gene analysis. All samples were frozen in liquid nitrogen and stored at – 80 °C until assay.

**Measurement of relative genes expression (RT-PCR)**

Total RNA from the frozen tissue was extracted with a GenElute Mammalian Total RNA Kit (Sigma-Aldrich, USA) according to the manufacturer’s instructions. To quantify the amount of total RNA, the optical density was determined with an ultraspectrophotometer DU-68 (Beckmann) and RNA integrity was electrophoretically verified in a 1.5% agarose gel stained with ethidium bromide.

For elimination of probe contamination by genomic DNA, the total RNA was treated with Rnase-free Dnase 1 (Sigma-Aldrich, USA). 1 µg of RNA was treated with 1 U of Dnase 1 for 15 min at room temperature. The reaction was stopped by the addition of stop solution and Dnase was inactivated at 70°C for 10 min. Reverse transcription was carried out using an Enhanced Avian HS RT-PCR Kit (Sigma-Aldrich, USA) according to the manufacturer’s instructions. The cDNA was stored at - 20°C. The primers for the gonadotropin-releasing hormone and gonadotropin-releasing hormone receptor were based on the sheep sequences, whereas primers for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were obtained from bovine sequence. Primer design was performed using Primer 3 software and all sequences with accession numbers are presented in tab. 1.

The PCR amplification produced 152 bp and 200 bp products for GnRH and GnRH-R, respectively and 182 bp for GAPDH. Conditions for PCR were optimized in a gradient cycler (MJ Research Inc.) with regard
to various annealing temperatures, amount of RT product, and a number of cycles. The optimal conditions of amplification for GnRH, GnRH-R and GAPDH were 35 cycles consisting of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and 72°C for 10 min in an MJ DNA engine Tetrad (MJ Research, Inc.). The PCR reaction was performed in a 25 µl reaction mixture using the ReadyMix RedTag PCR Reaction Mix (Sigma, USA) with a forward and reverse primer concentration of 10 pM each and 3 µl of GnRH, 1 µl of GnRH-R and 1 µl of RT product. The expression of GnRH and GnRH-R genes was normalized to the expression of GAPDH. Products were run on 1.5% agarose gel in 1×Tris-Borate-EDTA buffer at 100 V. Bands were visualized by UV after the gel was stained for 20 minutes in a 0.5 µg/ml ethidium bromide solution and the amount of PCR product was measured by densitometry on a Molecular Imager (BioRad, Inc.). The negative controls for GnRH and GnRH-R genes were analyzed in the liver tissue (data not showed). For each pair of primers the non template controls (NTC) were performed.

**LH Radioimmunoassay**

Plasma LH concentration was assayed by a double-antibody radioimmunoassay [39] using anti-ovine LH and rabbit gamma globulin antiserum and ovine LH standard (NIH-LH-S018). The assay sensitivity was 0.6 ng/ml and the intra- and interassay coefficients of variation were 9% to 12%, respectively.

**Statistical analysis**

GnRH mRNA and GnRH-R mRNA concentrations are expressed as a mean±SEM. One-way ANOVA followed by Tukey’s test was used to evaluate differences between the concentrations in control vs. stressed animals. Plasma LH concentrations are expressed as a mean ±SEM. The significance of differences in the LH concentration between control and stressed animals was assessed by one-way ANOVA followed by the least significant difference (LSD) test (STATISTICA TULSA, OK, USA).
The number and amplitude of LH pulses were determined by the PC-PULSAR computer program [24] with G parameters: G1=3.98; G2=2.40; G3=1.68; G4=1.24; G5=0.96. The frequency of LH pulses was defined as the number of identified pulses per collecting period [49]. The amplitude of LH was defined as the difference between peak and nadir values. Differences in LH pulse frequency and amplitude between groups were analyzed by the unilateral Wilcoxon test.

RESULTS

Representative composite diagrams of cytoplasmic GnRH mRNA and GnRH-R mRNA levels in the selected tissues are presented in fig. 2.

Fig. 2. Representative composite diagrams of hypothalamic and pituitary GnRH mRNA, GnRH-R mRNA and a reference gene GAPDH in individual I/ control, II/ short stressed and III/ prolonged stressed ewes; AP: anterior pituitary gland; POA: the preoptic area, AH: anterior hypothalamus, VM: ventromedial hypothalamus, SME: stalk/median eminence.
Stress and GnRH/GnRH-R gene expression

GnRH mRNA
No GnRH gene expression was detected in the SME and AP. Comparable concentrations of GnRH mRNA were found in the brain structures throughout the preoptic area (POA) as well as anterior (AH) and ventromedial (VM) parts of the hypothalamus. In contrast, the amounts of the GnRH mRNA in the POA, AH and VM were significantly higher in ewes subjected to short as well as prolonged stress than those in controls (fig. 3). The highest responses of GnRH mRNA expression to the short stress occurred in the POA and to the prolonged stress stimuli in the POA and VM.

Fig. 3. Effects of short and prolonged stress on GnRH mRNA concentration (mean±SEM) in the preoptic area (POA), anterior hypothalamus (AH) and ventromedial hypothalamus (VM) of ewes (n=6). *p≤0.05, **p≤0.01, ***p≤0.001
In control ewes, the GnRH-R mRNA was found in tissue continuum throughout the POA, AH, VM, stalk/median eminence and in the anterior pituitary gland. The concentration of GnRH-R mRNA in the SME was significantly higher than in any other tissue (fig. 4). Short as well as prolonged stress stimuli caused an increase in GnRH-R mRNA levels in all analyzed tissues and the highest responses of GnRH-R mRNA were found in the VM.

**GnRH-R mRNA**

In control ewes, the GnRH-R mRNA was found in tissue continuum throughout the POA, AH, VM, stalk/median eminence and in the anterior pituitary gland. The concentration of GnRH-R mRNA in the SME was significantly higher than in any other tissue (fig. 4). Short as well as prolonged stress stimuli caused an increase in GnRH-R mRNA levels in all analyzed tissues and the highest responses of GnRH-R mRNA were found in the VM.

**LH secretion**

In ewes subjected to short footshock stimulation, an increase in the LH concentration was observed as compared with controls (fig. 5). The frequency
Stress and GnRH/GnRH-R gene expression

and amplitude of LH pulses showed a tendency to increase, but their value as compared with the control results did not attain statistical significance. In the prolonged-stressed ewes the LH concentration, LH pulse frequency and LH pulse amplitude were in range of control value (data not shown).

DISCUSSION

The results of the present study provide new insight into GnRH mRNA expression in the preoptic area-hypothalamus, and GnRH-R mRNA expression in the above mentioned structures and in the anterior pituitary gland in anestrous ewes subjected to short or prolonged footshock stimulation.

Our study demonstrated that comparable levels of GnRH mRNA were found in structure continuum throughout the preoptic area, and the anterior and ventromedial hypothalamus of control animals. On the basis of available data it has been indicated that most GnRH neurons in sheep (above 50%) are located in the preoptic area [5]. Lack of evident differences in the GnRH mRNA concentration in the selected tissue of hypothalamus shows

Fig. 5. Effect of short stress on plasma LH concentration (mean ± SEM) of ewes (n = 6). *p≤0.05
that cytoplasmic GnRH mRNA is not exclusively coupled with primary transcript levels in particular areas of the preoptic area-hypothalamus in anestrous ewes. Because there is no information about the GnRH mRNA degradation and translation in cellular biosynthetic processes in various structures, it is not possible to establish a clear connection between GnRH mRNA levels and actual transcriptional activity.

The concentration of GnRH mRNA in the preoptic area-hypothalamus in anestrous ewes is substantially lower than in sheep during the follicular phase of the estrous cycle [7]. The differences in the GnRH gene expression in the various physiological states may be related, similarly as GnRH release, to the changes in the steroid-sensitive afferent inputs onto GnRH neurons in the non-breeding and breeding seasons [17]. Available data indicate that estrogens [12, 42] and some neurotransmitters (noradrenaline [20, 21], GABA [19, 23], CRF-like peptide [41]) participating in the regulation of GnRH release in rats are also involved in the control of GnRH gene expression. In sheep, as a seasonally breeding species, the effects of noradrenergic, dopaminergic and β-endorphinergic systems on the hypothalamic GnRH release are different in non-breeding and breeding periods [8, 9, 10, 14, 15, 17, 32, 38, 44, 47]. Thus, their role in the expression of GnRH mRNA in the hypothalamus may also be specific in particular physiological state of animals. Since substantial literature has implicated the noradrenergic [14, 15, 44] and dopaminergic systems [47] in the preoptic area-hypothalamus of anestrous ewes in the suppression of GnRH output, it is reasonable to suggest that these systems may also display an inhibitory influence on the expression of GnRH gene in ewes during the non-breeding period.

The simultaneously performed analysis of GnRH-R mRNA in the preoptic area-hypothalamus shows that the GnRH-R gene is also expressed in these structures. The largest quantities of GnRH-R mRNA were found in the SME. The concentrations of GnRH-R mRNA in the VM, SME and AP of anestrous ewes are lower than in ewes during the follicular phase of the estrous cycle [7]. It has been demonstrated that the expression of GnRH-R gene - at least in the anterior pituitary gland of ewes - is highly dependent upon estradiol [4] and GnRH [1, 6, 18]. This suggests that the decrease in GnRH-R gene
expression in adenohypophysis of anestrous ewes may result from the low levels of circulating estradiol and pulsatile secretion of GnRH [2]. In rats, the GnRH-R mRNA concentration in the hypothalamus and the anterior pituitary gland is highly dependent on estradiol and progesterone concentration, but their action in this aspect is different at the level of the medial basal hypothalamus and pituitary [37]. In the mediobasal hypothalamus of rats the estrogen decreases GnRH-R gene expression but progesterone reinstates the estradiol-induced decrease of GnRH-R mRNA concentration. Other studies have indicated that the stimulatory effect of GnRH on GnRH-R mRNA in the anterior pituitary gland of female rats is markedly enhanced by estradiol [51]. In the pituitary of sheep, the progesterone has a suppressive influence on GnRH-R mRNA [4] thus suggesting that the action of this steroid on GnRH-R gene expression may be species specific. Therefore it is possible that the decrease of GnRH gene expression in the preoptic area-hypothalamus and GnRH-R gene expression in the preoptic area-hypothalamus and AP in anestrous ewes may lead - among other factors - to an anovulatory state during the long photoperiod.

The profound up-regulation of GnRH and GnRH-R transcripts level under the short and prolonged stress stimuli indicates that both kinds of footshock stimulation elicit a cascade of neurochemical processes leading to the activation of transcriptional activity of these genes. Similar kinds of stress applied in animals during the follicular phase of the estrous cycle [7] produced distinct, time-dependent changes in the expression of GnRH and GnRH-R genes: stimulatory under the short stress and suppressive influence under the prolonged procedure. The presented results suggest that different hormones and neurochemical compounds released under stress conditions from the hypothalamic-pituitary-adrenal and hypothalamic-pituitary-gonadal axes may affect differently expression of genes both in anestrous ewes and during the follicular phase of the estrous cycle. The mechanisms by which various hormones and neurotransmitters released under stress conditions modulate GnRH and GnRH-R mRNA expression during different reproductive stages are still not well recognized.

Currently available data indicate that this may be due to seasonal plasticity in the GnRH system expressed in the number of synaptic inputs
onto GnRH neurons [17] as well as changes in functional activity of some neural systems affecting GnRH cells [10, 15, 32, 47]. Analyses of the effects of stress factors on the concentrations of GnRH and GnRH-R mRNAs in the preoptic area-hypothalamus in rats have indicated that the expression of GnRH and GnRH-R genes is highly dependent on the kind of stress, physiological state and gender. For example cold stress suppresses GnRH gene expression in the preoptic area [40] while prolonged food deprivation stress does not affect GnRH gene transcription in the hypothalamus in cycling female rats, but provokes a deep inhibition of GnRH-R mRNA in the pituitary gland [26]. By contrast in male rats a decreased number of neurons expressing GnRH mRNA in the hypothalamus were found after prolonged fasting stress [16]. Acute neurogenic stress caused similar down-regulation of GnRH gene expression in the anterior pituitary gland throughout the entire estrous cycle, but attenuated the GnRH mRNA concentration in the hypothalamus in a relatively short period at proestrus [26]. The differences in the GnRH gene and GnRH-R gene expression in animals under various stress applications cannot be adequately interpreted on the basis of recent literature. The specific stressors may elicit diverse responses in GnRH gene expression during the different reproductive stages and the different stressors may activate or inhibit different neural systems involved in the GnRH gene expression [27].

There is evidence that stress may alter the functional activity of some neural systems involved in the regulation of GnRH release [28, 34, 35]. To what extent the increased levels of GnRH mRNA and GnRH-R mRNA under stress condition are associated with GnRH and GnRH-R biosynthesis waits to be established. It is especially interesting to define a functional relationship between GnRH/GnRH-R mRNA and GnRH/GnRH-R biosynthesis, and the role of GnRH-R in the regulation of hypothalamic GnRH release. In spite of profound up-regulation of GnRH mRNA and GnRH-R mRNA under short and prolonged footshock stimulation, the increase in LH secretion occurs only in acute stressed animals. The increase of transcriptional activity of GnRH and GnRH-R genes is suggested not to be directly related to the GnRH and GnRH-R activity. It seems that the increased secretion of LH in ewes under short stress results from the release of GnRH pooled in the
hypothalamus rather than from the newly synthesized GnRH. Probably
the increase in LH secretion in sheep subjected to short stress is due to an
increase in the interpulse of LH release, because the frequency of LH pulses
as well as amplitude of these pulses did not differ significantly from controls.
Lack of evident changes in LH secretion in prolonged stressed ewes is in
contrast with suppression of GnRH release in animals under similar stress
application [45]. It cannot be excluded that low secretory activity of LH cells
in the anterior pituitary gland of anestrous ewes is due to low GnRH receptor
activity in these cells; hence the suppression of GnRH release has no visible
influence on LH release.

In conclusion, the obtained results indicate, that short and prolonged
footshock stimulation activates both GnRH and its receptor gene expression
in the preoptic area-hypothalamus, and GnRH-R gene in the anterior
pituitary gland of anestrous ewes. In spite of profound up-regulation
of GnRH mRNA and GnRH-R mRNA under the short and prolonged
footshock stimulation, the increase of LH secretion follows only in acute
stressed animals. It is suggested that the increase in the transcriptional
activity of GnRH and GnRH-R genes are not directly related to the GnRH
and GnRH-R activity. The functional link that exists between GnRH
mRNA and GnRH-R mRNA with the neural network regulating GnRH
and GnRH-R synthesis requires further research.

REFERENCES

1. Adams BM, Sakurai H, Adams TE 1996 Concentration of gonadotropin-releasing hormone
(GnRH) receptor messenger ribonucleic acid in pituitary tissue of orchidectomized sheep:
2. Barrell GK, Moenter SM, Caraty A, Karsch FJ 1992 Seasonal changes of gonadotropin-
3. Briski KP, Sylvester PW 1988 Effect of specific acute stressors on luteinizing hormone release
in ovariectomized and ovariectomized-estrogen treated female rats. *Neuroendocrinology* 47
4. Brooks J, McNeilly AS 1994 Regulation of gonadotropin-releasing hormone receptor mRNA
expression in the sheep. *Journal of Endocrinology* 143 175-182.
Reproduction and Fertility* 49 147-162.


38. Skinner DC, Herbison AE 1997 Effect of photoperiod on estrogen receptors, tyrosine
hydroxylase, neuropeptide Y, and β-endorphin immunoreactivity in the ewe hypothalamus. 
*Endocrinology* **138** 2585-2595.

*Endocrinology* **68** 6-13.

interleukin-1β, interleukin-2, and gonadotropin-releasing hormone gene expression in

41. Tellam DJ, Perone MJ, Dunn IC, Radovich S, Brennand J, Rivier JE, Castro MG, Lovejoy DA 
1998 Direct regulation of GnRH transcription by CRF-like peptides in an immortalized neural
cell line. *NeuroReport* **9** 3135-3140.

42. Thomky NR, Slater R, Herbison AE 2003 Sex differences in estrogen-dependent transcription
of gonadotropin-releasing hormone (GnRH) gene revealed in GnRH transgenic mice. 
*Endocrinology* **144** 3351-3358.

43. Tomaszewska D, Przekop F 1999 Catecholaminergic activity in the medial preoptic area and
nucleus infundibularis-median eminence of anestrous ewes in normal physiological state and

44. Tomaszewska-Zaremba D, Przekop F 2005 Effects of GABA<sub>B</sub> receptor modulation on
gonadotropin-releasing hormone and β-endorphin release, and on catecholaminergic activity
in the ventro-medial hypothalamus-infundibular nucleus region of anestrous ewes. 
*Journal of Neuroendocrinology* **17** 49-56.

45. Tomaszewska D, Mateusiak K, Przekop F 1999 Changes in extracellular LHRH and β-endorphin
like immunoreactivity in the nucleus infundibularis-median eminence of anestrous ewes under

46. Tomaszewska D, Przekop F, Mateusiak K 2002 Responses of catecholaminergic, β-endorphinergic
and gonadoliberinergic neuronal systems in the hypothalamus of anestrous ewes to stressful

47. Tortonese DJ 1999 Interaction between hypothalamic dopaminergic and opioidergic systems in
the photoperiodic regulation of pulsatile luteinizing hormone secretion in sheep. *Endocrinology* 
**140** 750-757.

receptor gene expression in sheep: interaction of GnRH and estradiol. *Endocrinology* **139** 4890-
4984.

hormone (LHRR) secretion by melatonin in the ewe. Simultaneous delayed increase in LHRH

50. Welento J, Steyn S, Milard Z 1969 Observation on the stereotaxic configuration of the

hormone (GnRH) pulse pattern regulates GnRH receptor gene expression: augmentation by