Chapter 2

Studies on the Character of Hypothalamic GnRH Neurons and Kisspeptin Neurons Using Hypothalamic Cell Models

Haruhiko Kanasaki, Aki Oride,
Tuvshintugs Tumurbaatar and Satoru Kyo

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.73128

Abstract

The hypothalamic-pituitary-gonadal (HPG) axis controls the hormonal network responsible for reproductive functions. In the past, hypothalamic gonadotropin-releasing hormone (GnRH) neurons have been positioned at the highest level in the HPG axis. After the discovery of the indispensable roles of hypothalamic kisspeptin in GnRH neurons, our understanding of the neuroendocrine regulation of the HPG axis was reconfirmed, and it is now recognized that hypothalamic kisspeptin neurons are positioned at the summit of the HPG axis. Accumulating evidence shows that kisspeptin neurons are responsible for the onset of puberty and sex steroid feedback mechanisms by modulating the activity of GnRH neurons. Furthermore, the identification of kisspeptin in the hypophyseal portal circulation suggests that this peptide has some direct roles in the pituitary gland. The detailed mechanisms underlying the regulation of GnRH by kisspeptin and the regulatory control of kisspeptin neurons are still largely unknown because of the limitations of the experimental models. The establishment of GnRH-expressing and kisspeptin-expressing cell models has enabled us to examine the character of these neuronal cells. In this chapter, we describe our in vivo studies examining the character of GnRH neurons and kisspeptin neurons in the hypothalamus using hypothalamic GnRH- and/or kisspeptin-expressing cell models.

Keywords: kisspeptin, GnRH, gonadotropin, HPG axis

1. Introduction

The hypothalamus maintains the homeostasis within the body and controls endocrine functions. The hypothalamic-pituitary-gonadal (HPG) axis is a hormonal network responsible

for female reproductive function. After the discovery of inactivating mutations in the gene encoding the kisspeptin receptor (Kiss1R) in patients with idiopathic hypogonadotropic hypogonadism [1, 2], a new concept of the HPG axis was established. Now, it is generally agreed that kisspeptin produced from kisspeptin neurons, which are located in different regions of hypothalamus, stimulates gonadotropin-releasing hormone (GnRH) synthesis and release through Kiss1R within the GnRH neurons.

2. Kisspeptin as a regulator of the HPG axis

Kisspeptin is positioned upstream of GnRH in the HPG axis. Kisspeptin, which is encoded by the Kiss1 gene, was first discovered as a peptide that has potency to suppress metastasis of malignant melanoma and was initially named metastin [3]. The Kiss1 gene product is translated into a 145-amino acid precursor protein and further cleaved into a 54-residue peptide (kisspeptin 54), which can be further cleaved into 14-, 13-, and 10-amino acid peptides [4]. Kiss1R was discovered 4 years after kisspeptin, and it was found that Kiss1R is a member of the G protein-coupled receptor superfamily and is structurally similar to the galanin receptor [4, 5]. Discovery of loss-of-function mutations in Kiss1R in the family of a hypogonadotropic hypogonadism patient [1, 2] clearly linked kisspeptin and reproduction, and these observations indicated that kisspeptin acts as a major stimulator of the HPG axis.

Two different populations of kisspeptin-expressing neurons have been identified in rodents. The most predominant population is located in the arcuate nucleus (ARC) of the hypothalamus, where kisspeptin-expressing neurons co-express dynorphin and neurokinin B (NKB). Another kisspeptin-expressing cell population is located in the anteroventral periventricular nucleus (AVPV) of the hypothalamus. In humans and primates, kisspeptin is expressed predominantly within the infundibular nucleus, which is equivalent to the ARC in rodent [6]. Kisspeptin neurons in both populations make direct synaptic contacts with GnRH neurons and their terminals in the median eminence [7].

3. GnRH release is influenced by kisspeptin

In rodents, GnRH is released into the portal circulation by GnRH neurons located in the preoptic area and eventually reaches the anterior pituitary. GnRH is released in a pulsatile manner, and the pulse frequency and amplitude of GnRH release vary physiologically during reproductive cycles in females. Secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) is maintained by pulsatile, not continuous, release of GnRH [8]. Moreover, the frequency of the GnRH pulse differentially regulates the production and release of FSH and LH from the anterior pituitary [9]. Administration of high-frequency GnRH pulses increases the secretion of LH, whereas a lower frequency of GnRH pulses shifts the gonadotropin secretion to more FSH dominant [10]. Taken together, these

observations show that the secretory mode of pituitary gonadotropins is controlled by the so-called GnRH pulse generator.

The neuronal mechanisms underlying the pulsatile release of GnRH are still not fully understood, but at present it is agreed that kisspeptin neurons located in the ARC of the hypothalamus may be involved. Previous studies recorded the electrical activity of neurons, measured as multiunit activity (MUA), within the ARC region of the hypothalamus and reported that MUA correlates with pulsatile secretion of LH in several animal models [11, 12]. The neuronal population of kisspeptin-expressing cells is called KNDy neurons because kisspeptin-expressing cells located in the ARC region of the hypothalamus co-express kisspeptin, neurokinin B, and dynorphin. KNDy neurons generate synchronized oscillatory patterns of neuronal activity by sharing excitatory and inhibitory input from NKB and dynorphin produced within themselves [13, 14]. Selective and synchronized activation of KNDy neurons induces pulsatile release of LH in a mouse model [15]. Furthermore, exogenous kisspeptin administration can increase LH pulse frequency and amplitude in healthy women [16]. Because kisspeptin antagonism suppresses both mean GnRH and GnRH pulses [17], it is natural to think that kisspeptin neurons in the ARC of the hypothalamus (KNDy neurons) comprise the GnRH pulse generator.

The pattern of GnRH release and subsequent LH release across the reproductive cycle is modulated by gonadal steroid feedback. In rodents, the estrogen-induced GnRH/LH surge is mediated by kisspeptin neurons in the AVPV region (positive feedback). However, KNDy neurons in the ARC region of the hypothalamus are sensitive to estradiol (E2) and reduce the GnRH/LH secretion (negative feedback). This concept is based on the observations that kisspeptin expression was increased in the AVPV region at the time of the estrogen- and progesterone-induced LH surge in ovariectomized rats, whereas kisspeptin expression levels in the ARC were lowest during this time [18]. Another experiment showing that Kiss1 gene expression in the kisspeptin neurons in the AVPV is upregulated by E2, whereas those in the ARC are inhibited by E2 [19], also supports the current understanding that kisspeptin neurons in the AVPV play a role in the GnRH/LH surge, and kisspeptin neurons in the ARC region maintain the pulsatile release of GnRH and also play a pivotal role in the negative feedback control by E2.

4. Hypothalamic cell models, GT1-7 and rHypoE8, for investigating neuroendocrine mechanisms of the HPG axis

The hypothalamus is the control center for the HPG axis; however, it has been difficult to study in detail the GnRH neurons as well as the kisspeptin neurons because of the inherent heterogeneity of this brain region. The hypothalamus is composed of a complex network of neurons, and there are different neuronal phenotypes that express a specific complement of neuropeptides, neurotransmitters, and receptors [20]. Immortalized, clonal cell lines represent a relatively homogeneous population of specific neuronal cells and can be used as

experimental models. For the study of the character or the functions of hypothalamic GnRH neurons, we are using GT1-7 cell lines, which have proven to be a valuable GnRH-expressing cell model for GnRH neurons. These cells were created from the hypothalamic tumor cells in a transgenic female mouse that expressed the SV40 T-antigen under the control of the GnRH promoter [21]. GT1-7 cells display neuronal morphology and secrete GnRH; therefore, these cells have become one of the most highly utilized neuronal cell models for studies related to GnRH neurons.

The embryonic rat hypothalamic cell line R8 (rHypoE8) consists of hypothalamic neurons from rat embryonic day 18 hypothalamic primary cultures immortalized by retroviral transfer of SV40 T-antigen. These cells express neuroendocrine markers such as kisspeptin, GnRH, and RF-amide-related peptide-3 (RFRP-3, the mammalian ortholog of the avian gonadotropin-inhibiting hormone, GnIH). Because the expression of kisspeptin or RFRP-3 is functionally altered by physiological neuropeptides, these cells serve as tools for the analysis of the cellular and molecular mechanisms involved in the hypothalamic control of a number of physiological processes [22].

5. Effect of kisspeptin on hypothalamic GT1-7 cells

It is generally agreed that hypothalamic kisspeptin regulates GnRH release from GnRH neurons by kisspeptin binding the Kiss1R that is expressed by GnRH neurons [23]. A previous study by Novaira et al. demonstrated a functional role for kisspeptin in GT1-7 cells, in which they showed that kisspeptin stimulates the expression and secretion of GnRH [24]. Similarly, Terasaka et al. demonstrated the stimulatory effect of kisspeptin on GnRH gene expression, and they also found that this stimulatory effect was antagonized in the presence of bone morphogenetic protein in these cells [25]. In our study using GT1-7 cells, we did not observe any effect of kisspeptin on GnRH expression [26]. Because GT1-7 cells express Kiss1R, we suspected that Kiss1R function was lost or diminished in our GT1-7 cells, probably because of a change in cell character due to cell immortalization or multiple passages. On the other hand, when GT1-7 cells overexpressed Kiss1R after transfection with a Kiss1R expression vector, exogenous Kiss1R was absolutely functional. Furthermore, both extracellular signal-regulated kinase (ERK) and cAMP/protein kinase A (PKA) pathways were activated by kisspeptin in Kiss1R-overexpressing GT1-7 cells. These observations suggested that overexpression of exogenous Kiss1R could lead to activation of the intracellular signaling pathways mediated by kisspeptin stimulation in these cells. It is also noteworthy that, even when GT1-7 cells overexpressed Kiss1R, GnRH expression was not stimulated by kisspeptin [26]. It is still unclear why kisspeptin did not increase GnRH expression in our GT1-7 cells even when Kiss1R was overexpressed; instead, we clearly observed that kisspeptin could stimulate the expression of the GnRH receptor (GnRHR) in GT1-7 cells overexpressing Kiss1R [26] (**Figure 1**). GnRH-producing cells have been reported to respond to GnRH and modify their GnRH expression levels [27]. Furthermore, GnRHRs within GnRH neurons were reported to be involved in the pulsatile secretion of GnRH by an autocrine or paracrine interaction between GnRH and GnRHR [28, 29]. These

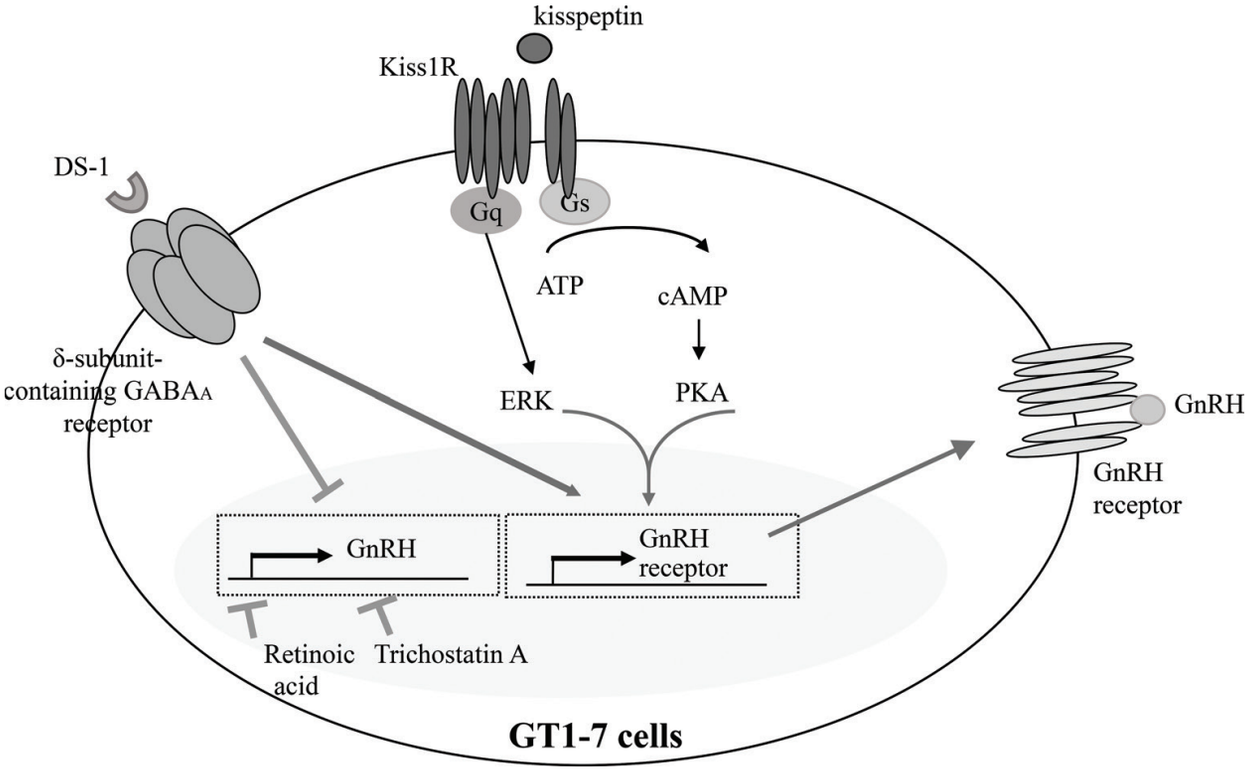

Figure 1. Schematic summary of the regulation of GnRH in GT1-7 cells. GT1-7 cells express Kiss1R, but endogenous Kiss1R does not respond to kisspeptin. Therefore, we used GT1-7 cells overexpressing Kiss1R. GT1-7 cells overexpressing Kiss1R did not show an increase in GnRH mRNA expression upon kisspeptin treatment. However, kisspeptin increased GnRH receptor expression in these cells. We also found that a γ-subunit-containing $GABA_A$ receptor agonist, DS1, as well as histone deacetylase inhibitor trichostatin A, reduces GnRH mRNA expression. Retinoic acid also has an inhibitory effect on GnRH expression.

observations implied that kisspeptin could affect the function of GnRH neurons by changing their expression levels of GnRHR.

6. Effect of kisspeptin on primary cultures of fetal rat brain

Although GT1-7 cells endogenously express Kiss1R, kisspeptin does not affect these cells. When GT1-7 cells overexpress Kiss1R, kisspeptin stimulates intracellular signaling pathways and increases GnRHR, but not GnRH expression. To determine the character of kisspeptin neurons in their original, non-transformed state, we used primary cultures of fetal rat brain that contain both GnRH and kisspeptin neurons. GnRH neurons in these cells did not respond to E2, which failed to stimulate GnRH mRNA expression. This observation was consistent with a previous study that revealed a lack of estrogen receptor immunoreactivity in GnRH neurons, raising doubts about the role of E2 in GnRH neuronal function [30]. In contrast, kisspeptin neurons in these primary cultures responded to E2, and Kiss1 mRNA expression was upregulated by E2 [31], suggesting that kisspeptin neurons, but not GnRH neurons, could be a target of E2 in neuronal cells in the fetal brain. GnRH mRNA expression within these primary cultures of fetal rat brain containing GnRH-producing neurons was

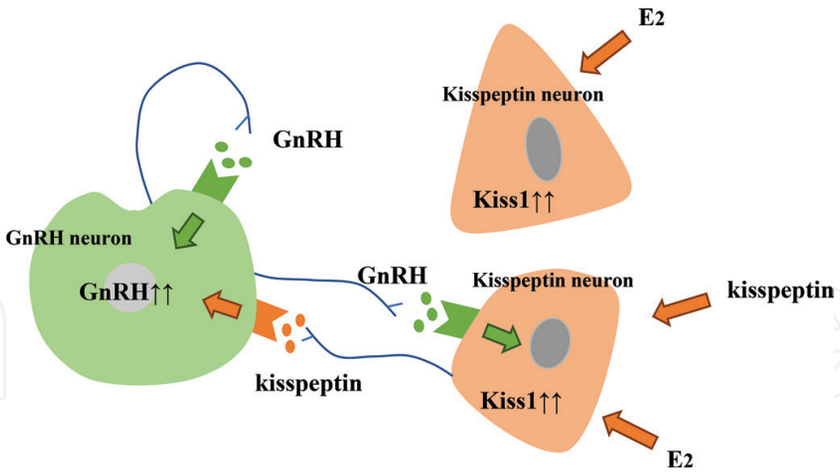

Figure 2. Schematic summary of the regulation of Kiss1 mRNA and GnRH mRNA expression in primary cultures of fetal rat brain and the proposed interaction between kisspeptin neurons and GnRH neurons. In experiments using primary cultures of fetal rat neuronal cells, Kiss1 mRNA, but not GnRH mRNA expression, was upregulated by estradiol (E2). GnRH mRNA expression was clearly increased by treatment with kisspeptin. GnRH stimulation increased the expression of both Kiss1 and GnRH mRNAs, and kisspeptin itself was found to increase the expression of the Kiss1 gene. We postulate that GnRH neurons reversibly interact with kisspeptin neurons and also form an autocrine interaction with kisspeptin neurons.

clearly increased by treatment with kisspeptin. Therefore, we could conclude that kisspeptin can stimulate GnRH synthesis in GnRH-expressing neurons in vivo. However, kisspeptin increased GnRH mRNA expression only up to about 1.5-fold [31]. In addition, we have found that GnRH stimulation increased the expression of the Kiss1 gene as well as that of the GnRH gene and also found that kisspeptin itself increased the expression of the Kiss1 gene. We postulate that GnRH neurons reversibly interact with kisspeptin neurons and also form an autocrine interaction with kisspeptin neurons (**Figure 2**).

7. Trichostatin A, a selective inhibitor of mammalian histone deacetylase, reduces GnRH expression in GT1-7 cells

Observations from the studies using GT1-7 cells and primary cultures of fetal rat brain imply that kisspeptin could affect GnRH neurons and increase GnRH expression. In addition, kisspeptin may change the GT1-7 cells' expression levels of Kiss1R. GnRH synthesis is not only regulated by kisspeptin, but several experimental reagents can modify the GnRH synthesis in GnRH-producing cells. Trichostatin A (TSA), a selective inhibitor of histone deacetylase, is an experimental reagent that modifies gene expression by opening chromatin structure through hyperacetylation of histones [32]. The structural change in chromatin allows transcription factors to bind DNA to modify gene expression. In GnRH-producing GT1-7 cells, TSA significantly reduced GnRH expression, with a concomitant increase in the gene encoding retinaldehyde dehydrogenase, which catalyzes the oxidation of retinol to retinoic acid [33]. Because retinoic acid also reduces GnRH expression in these cells, epigenetic mechanisms modified through retinaldehyde dehydrogenase, and retinoic acid might have an inhibitory effect on GnRH production (**Figure 1**).

8. DS1, a δ-subunit-containing $GABA_A$ receptor agonist, reduces GnRH mRNA expression and increases that of GnRHR in GT1-7 cells

It is well documented that the neurotransmitter γ-aminobutyric acid (GABA) can modulate the activity of GnRH neurons. GnRH neurons possess functional $GABA_A$ receptors [34], and GABAergic neurons establish synapses with GnRH neurons [35]. GABA neurons predominantly exert their inhibitory effect on GnRH neurons in rodents and sheep. GT1-7 cells also express functional $GABA_A$ receptors [36]. $GABA_A$ receptors are multimeric proteins that are composed of five subunits drawn from a repertoire of several homologous protein groups (α1-6, β1-3, γ1-3, δ, ε, θ, and π); the majority of $GABA_A$ receptors in the central nervous system are composed of α, β, and γ subunits, and less abundant populations of $GABA_A$ receptor contain the δ subunit [37]. DS1, an α4β3δ $GABA_A$ receptor agonist, reduces GnRH mRNA expression in GT1-7 cells, although DS1 can exert a stimulatory effect on signal transduction systems, such as ERK and cAMP/PKA [38]. The δ-subunit-containing α4β3δ $GABA_A$ receptor was found in extra-synaptic sites and is known to control neuronal excitability [39]. Interestingly, although GnRH mRNA expression was decreased, GnRHR expression within GT1-7 cells was significantly increased by DS1 stimulation [38] (**Figure 1**). At present, it is still unknown why δ-subunit-containing $GABA_A$ receptor agonism decreases the production of GnRH in spite of increasing GnRHR expression. We currently speculate that GABA could modulate GnRH-producing neurons through δ-containing $GABA_A$ receptors and deplete their GnRH content by modulating gene expression and secretory function in association with the expression of their GnRHR within the cell (**Figure 1**).

9. Kisspeptin expression is induced by glucagon-like peptide-1 in rHypoE8 cells and GT1-7 cells

As described above, we used GT1-7 cells as a model for GnRH-producing neurons; however, GT1-7 cells also express the Kiss1 gene, which encodes kisspeptin [40]. rHypoE8 cells, another hypothalamic model that was developed from rat embryonic hypothalamic primary cultures, express the Kiss1 gene, and they also express the GnRH gene [22]. Because both rHypoE8 and GT1-7 are immortalized cell lines derived from heterogeneous hypothalamic cell populations, they express several types of neuropeptides. Using these hypothalamic cell models, we found that Kiss1 mRNA was regulated by several metabolic factors. Glucagon-like peptide-1 (GLP-1) is a gastrointestinal hormone produced by the small intestine and colon in response to food intake [41]. GLP-1 is also expressed in the central nervous system, and its expression in the brain is altered during fasting or feeding [42], suggesting that GLP-1 plays a role as a satiety factor. We found that GLP-1 increased the expression of Kiss1 mRNA in rHypoE8 cells as well as GT1-7 cells [43]. Moreover, leptin, which is an anorexigenic factor that is released from adipocytes, can also stimulate Kiss1 mRNA expression in these cells (**Figure 3**). These observations suggest that the levels of metabolic factors such as GLP-1 or leptin, which change during a state of starvation or negative energy balance, can critically influence the HPG axis by changing kisspeptin expression.

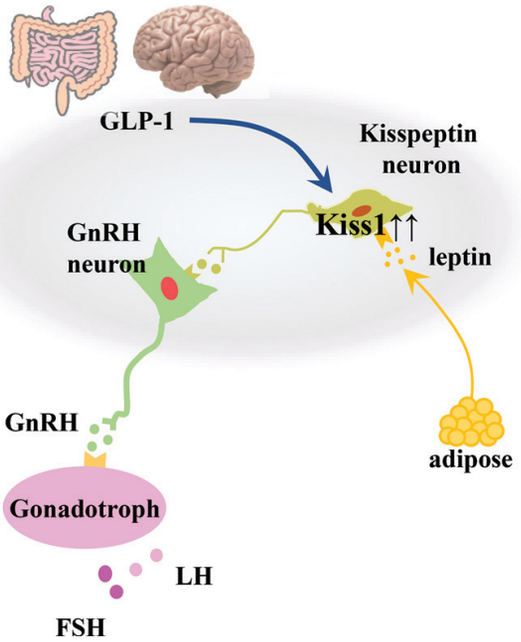

Figure 3. The metabolic factors glucagon-like peptide-1 (GLP-1) and leptin can affect the HPG axis via kisspeptin neurons. From experiments using the hypothalamic cell models rHypoE8 and GT1-7 cells, we found that metabolic factors such as GLP-1 and leptin had the ability to stimulate Kiss1 gene expression. These metabolic factors may affect the HPG axis by modulating the synthesis and release of kisspeptin.

10. Conclusion

Within the past decade, our understanding of the hypothalamic control of female reproductive function has matured considerably. The identification of hypothalamic kisspeptin, a regulator of GnRH, has provided us decisive insight into previously unanswerable questions. Kisspeptin neurons within the hypothalamus play a pivotal role in the control of the HPG axis, but it is still not entirely clear how kisspeptin release and expression are regulated in the brain during the reproductive cycle. Furthermore, the precise biology of kisspeptin and GnRH neurons remains unknown because of the difficulty of isolation of these neurons from heterogeneous neuronal populations of the hypothalamus.

In this review, we described our observations concerning the regulation of kisspeptin and GnRH neurons using hypothalamic cell models. Because we believe these cell models may reflect the original character of genuine kisspeptin and GnRH neurons, future studies using these cells are likely to contribute to our understanding of the mechanisms of regulation of the HPG axis.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (grant number 20791144).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review reported.

Author details

Haruhiko Kanasaki*, Aki Oride, Tuvshintugs Tumurbaatar and Satoru Kyo

*Address all correspondence to: kanasaki@med.shimane-u.ac.jp

Department of Obstetrics and Gynecology, Shimane University Faculty of Medicine, Izumo, Japan

References

[1] de Roux N, Genin E, Carel JC, Matsuda F, Chaussain JL, Milgrom E. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(19):10972-10976. DOI: 10.1073/pnas.1834399100

[2] Seminara SB, Messager S, Chatzidaki EE, Thresher RR, Acierno JS Jr, Shagoury JK, Bo-Abbas Y, Kuohung W, Schwinof KM, Hendrick AG, Zahn D, Dixon J, Kaiser UB, Slaugenhaupt SA, Gusella JF, O'Rahilly S, Carlton MB, Crowley WF Jr, Aparicio SA, Colledge WH. The GPR54 gene as a regulator of puberty. The New England Journal of Medicine. 2003;**349**(17):1614-1627. DOI: 10.1056/NEJMoa035322

[3] Lee JH, Miele ME, Hicks DJ, Phillips KK, Trent JM, Weissman BE, Welch DR. KiSS-1, a novel human malignant melanoma metastasis-suppressor gene. Journal of the National Cancer Institute. 1996;**88**(23):1731-1737

[4] Kotani M, Detheux M, Vandenbogaerde A, Communi D, Vanderwinden JM, Le Poul E, Brezillon S, Tyldesley R, Suarez-Huerta N, Vandeput F, Blanpain C, Schiffmann SN, Vassart G, Parmentier M. The metastasis suppressor gene KiSS-1 encodes kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. The Journal of Biological Chemistry. 2001;**276**(37):34631-34636. DOI: 10.1074/jbc.M104847200

[5] Ohtaki T, Shintani Y, Honda S, Matsumoto H, Hori A, Kanehashi K, Terao Y, Kumano S, Takatsu Y, Masuda Y, Ishibashi Y, Watanabe T, Asada M, Yamada T, Suenaga M, Kitada C, Usuki S, Kurokawa T, Onda H, Nishimura O, Fujino M. Metastasis suppressor gene KiSS-1 encodes peptide ligand of a G-protein-coupled receptor. Nature. 2001;**411**(6837):613-617. DOI: 10.1038/35079135

[6] Skorupskaite K, George JT, Anderson RA. The kisspeptin-GnRH pathway in human reproductive health and disease. Human Reproduction Update. 2014;**20**(4):485-500. DOI: 10.1093/humupd/dmu009

[7] Colledge WH. Kisspeptins and GnRH neuronal signalling. Trends in Endocrinology and Metabolism. 2009;**20**(3):115-121. DOI: 10.1016/j.tem.2008.10.005

[8] Knobil E. The neuroendocrine control of the menstrual cycle. Recent Progress in Hormone Research. 1980;**36**:53-88

[9] Crowley WF Jr, Filicori M, Spratt DI, Santoro NF. The physiology of gonadotropin-releasing hormone (GnRH) secretion in men and women. Recent Progress in Hormone Research. 1985;**41**:473-531

[10] Wildt L, Hausler A, Marshall G, Hutchison JS, Plant TM, Belchetz PE, Knobil E. Frequency and amplitude of gonadotropin-releasing hormone stimulation and gonadotropin secretion in the rhesus monkey. Endocrinology. 1981;**109**(2):376-385. DOI: 10.1210/endo-109-2-376

[11] Wilson RC, Kesner JS, Kaufman JM, Uemura T, Akema T, Knobil E. Central electrophysiologic correlates of pulsatile luteinizing hormone secretion in the rhesus monkey. Neuroendocrinology. 1984;**39**(3):256-260

[12] Mori Y, Nishihara M, Tanaka T, Shimizu T, Yamaguchi M, Takeuchi Y, Hoshino K. Chronic recording of electrophysiological manifestation of the hypothalamic gonadotropin-releasing hormone pulse generator activity in the goat. Neuroendocrinology. 1991;**53**(4):392-395

[13] Wakabayashi Y, Nakada T, Murata K, Ohkura S, Mogi K, Navarro VM, Clifton DK, Mori Y, Tsukamura H, Maeda K, Steiner RA, Okamura H, Neurokinin B. Dynorphin A in kisspeptin neurons of the arcuate nucleus participate in generation of periodic oscillation of neural activity driving pulsatile gonadotropin-releasing hormone secretion in the goat. The Journal of Neuroscience. 2010;**30**(8):3124-3132. DOI: 10.1523/JNEUROSCI.5848-09.2010

[14] Lehman MN, Coolen LM, Goodman RL. Minireview: Kisspeptin/neurokinin B/dynorphin (KNDy) cells of the arcuate nucleus: A central node in the control of gonadotropin-releasing hormone secretion. Endocrinology. 2010;**151**(8):3479-3489. DOI: 10.1210/en.2010-0022

[15] Han SY, McLennan T, Czieselsky K, Herbison AE. Selective optogenetic activation of arcuate kisspeptin neurons generates pulsatile luteinizing hormone secretion. Proceedings of the National Academy of Sciences of the United States of America. 2015;**112**(42):13109-13114. DOI: 10.1073/pnas.1512243112

[16] Jayasena CN, Comninos AN, Veldhuis JD, Misra S, Abbara A, Izzi-Engbeaya C, Donaldson M, Ghatei MA, Bloom SR, Dhillo WS. A single injection of kisspeptin-54 temporarily increases luteinizing hormone pulsatility in healthy women. Clinical Endocrinology. 2013;**79**(4):558-563. DOI: 10.1111/cen.12179

[17] Roseweir AK, Kauffman AS, Smith JT, Guerriero KA, Morgan K, Pielecka-Fortuna J, Pineda R, Gottsch ML, Tena-Sempere M, Moenter SM, Terasawa E, Clarke IJ, Steiner RA, Millar RP. Discovery of potent kisspeptin antagonists delineate physiological mechanisms of gonadotropin regulation. The Journal of Neuroscience. 2009;**29**(12):3920-3929. DOI: 10.1523/JNEUROSCI.5740-08.2009

[18] Smith JT, Popa SM, Clifton DK, Hoffman GE, Steiner RA. Kiss1 neurons in the forebrain as central processors for generating the preovulatory luteinizing hormone surge. The Journal of Neuroscience. 2006;**26**(25):6687-6694. DOI: 10.1523/JNEUROSCI.1618-06.2006

[19] Adachi S, Yamada S, Takatsu Y, Matsui H, Kinoshita M, Takase K, Sugiura H, Ohtaki T, Matsumoto H, Uenoyama Y, Tsukamura H, Inoue K, Maeda K. Involvement of anteroventral periventricular metastin/kisspeptin neurons in estrogen positive feedback action on luteinizing hormone release in female rats. The Journal of Reproduction and Development. 2007;**53**(2):367-378

[20] Everitt BJ, Hokfelt T. Neuroendocrine anatomy of the hypothalamus. Acta Neurochirurgica. Supplementum (Wien). 1990;**47**:1-15

[21] Mellon PL, Windle JJ, Goldsmith PC, Padula CA, Roberts JL, Weiner RI. Immortalization of hypothalamic GnRH neurons by genetically targeted tumorigenesis. Neuron. 1990; **5**(1):1-10

[22] Gingerich S, Wang X, Lee PK, Dhillon SS, Chalmers JA, Koletar MM, Belsham DD. The generation of an array of clonal, immortalized cell models from the rat hypothalamus: Analysis of melatonin effects on kisspeptin and gonadotropin-inhibitory hormone neurons. Neuroscience. 2009;**162**(4):1134-1140. DOI: 10.1016/j.neuroscience.2009.05.026

[23] Maggi R, Cariboni AM, Marelli MM, Moretti RM, Andre V, Marzagalli M, Limonta P. GnRH and GnRH receptors in the pathophysiology of the human female reproductive system. Human Reproduction Update. 2016;**22**(3). DOI: 10.1093/humupd/dmv059

[24] Novaira HJ, Fadoju D, Diaczok D, Radovick S. Genetic mechanisms mediating kisspeptin regulation of GnRH gene expression. The Journal of Neuroscience. 2012;**32**(48):17391-17400. DOI: 10.1523/JNEUROSCI.2438-12.2012

[25] Terasaka T, Otsuka F, Tsukamoto N, Nakamura E, Inagaki K, Toma K, Ogura-Ochi K, Glidewell-Kenney C, Lawson MA, Makino H. Mutual interaction of kisspeptin, estrogen and bone morphogenetic protein-4 activity in GnRH regulation by GT1-7 cells. Molecular and Cellular Endocrinology. 2013;**381**(1-2):8-15. DOI: 10.1016/j.mce.2013.07.009

[26] Sukhbaatar U, Kanasaki H, Mijiddorj T, Oride A, Miyazaki K. Kisspeptin induces expression of gonadotropin-releasing hormone receptor in GnRH-producing GT1-7 cells overexpressing G protein-coupled receptor 54. General and Comparative Endocrinology. 2013;**194**:94-101. DOI: 10.1016/j.ygcen.2013.09.002

[27] Neithardt A, Farshori MP, Shah FB, Catt KJ, Shah BH. Dependence of GnRH-induced phosphorylation of CREB and BAD on EGF receptor transactivation in GT1-7 neuronal cells. Journal of Cellular Physiology. 2006;**208**(3):586-593. DOI: 10.1002/jcp.20697

[28] Krsmanovic LZ, Martinez-Fuentes AJ, Arora KK, Mores N, Navarro CE, Chen HC, Stojilkovic SS, Catt KJ. Autocrine regulation of gonadotropin-releasing hormone secretion in cultured hypothalamic neurons. Endocrinology. 1999;**140**(3):1423-1431. DOI: 10.1210/endo.140.3.6588

[29] Martinez-Fuentes AJ, Hu L, Krsmanovic LZ, Catt KJ. Gonadotropin-releasing hormone (GnRH) receptor expression and membrane signaling in early embryonic GnRH neurons: Role in pulsatile neurosecretion. Molecular Endocrinology. 2004;**18**(7):1808-1817. DOI: 10.1210/me.2003-0321

[30] Shivers BD, Harlan RE, Morrell JI, Pfaff DW. Absence of oestradiol concentration in cell nuclei of LHRH-immunoreactive neurones. Nature. 1983;**304**(5924):345-347

[31] Sukhbaatar U, Kanasaki H, Mijiddorj T, Oride A, Hara T, Yamada T, Kyo S. Expression of GnRH and Kisspeptin in primary cultures of fetal rat brain. Reproductive Sciences. 2017;**24**(2):227-233. DOI: 10.1177/1933719116653679

[32] Tsuji N, Kobayashi M, Nagashima K, Wakisaka Y, Koizumi K. A new antifungal antibiotic, trichostatin. Journal of Antibiotics (Tokyo). 1976;**29**(1):1-6

[33] Kanasaki H, Mijiddorj T, Sukhbaatar U, Oride A, Ishihara T, Yamagami I, Kyo S, Trichostatin A. Reduces GnRH mRNA expression with a concomitant increase in retinaldehyde dehydrogenase in GnRH-producing neurons. Molecular and Cellular Endocrinology. 2015;**413**:113-119. DOI: 10.1016/j.mce.2015.06.017

[34] Pape JR, Skynner MJ, Sim JA, Herbison AE. Profiling gamma-aminobutyric acid (GABA(A)) receptor subunit mRNA expression in postnatal gonadotropin-releasing hormone (GnRH) neurons of the male mouse with single cell RT-PCR. Neuroendocrinology. 2001;**74**(5):300-308. DOI: 10.1159/000054697

[35] Leranth C, MacLusky NJ, Sakamoto H, Shanabrough M, Naftolin F. Glutamic acid decarboxylase-containing axons synapse on LHRH neurons in the rat medial preoptic area. Neuroendocrinology. 1985;**40**(6):536-539

[36] Garyfallou VT, Lemos D, Urbanski HF. Expression profiling of genes encoding glutamate and GABA receptor subunits in three immortalized GnRH cell lines. Brain Research. 2006;**1086**(1):50-54. DOI: 10.1016/j.brainres.2006.02.105

[37] Brown N, Kerby J, Bonnert TP, Whiting PJ, Wafford KA. Pharmacological characterization of a novel cell line expressing human alpha(4)beta(3)delta GABA(A) receptors. British Journal of Pharmacology. 2002;**136**(7):965-974. DOI: 10.1038/sj.bjp.0704795

[38] Sukhbaatar U, Mijiddorj T, Oride A, Kanasaki H. Stimulation of delta subunit-containing GABAA receptor by DS1 increases GnRH receptor expression but reduces GnRH mRNA expression in GnRH-producing GT1-7 cells. Endocrine. 2015;**49**(1):222-230. DOI: 10.1007/s12020-014-0464-y

[39] Bhattarai JP, Park SA, Park JB, Lee SY, Herbison AE, Ryu PD, Han SK. Tonic extrasynaptic GABA(A) receptor currents control gonadotropin-releasing hormone neuron excitability in the mouse. Endocrinology. 2011;**152**(4):1551-1561. DOI: 10.1210/en.2010-1191

[40] Li D, Mitchell D, Luo J, Yi Z, Cho SG, Guo J, Li X, Ning G, Wu X, Liu M. Estrogen regulates KiSS1 gene expression through estrogen receptor alpha and SP protein complexes. Endocrinology. 2007;**148**(10):4821-4828. DOI: 10.1210/en.2007-0154

[41] Herrmann C, Goke R, Richter G, Fehmann HC, Arnold R, Goke B. Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. Digestion. 1995;**56**(2):117-126

[42] Knauf C, Cani PD, Ait-Belgnaoui A, Benani A, Dray C, Cabou C, Colom A, Uldry M, Rastrelli S, Sabatier E, Godet N, Waget A, Penicaud L, Valet P, Burcelin R. Brain glucagon-like peptide 1 signaling controls the onset of high-fat diet-induced insulin resistance and reduces energy expenditure. Endocrinology. 2008;**149**(10):4768-4777. DOI: 10.1210/en.2008-0180

[43] Oride A, Kanasaki H, Mijiddorj T, Sukhbaatar U, Hara T, Tumurbaatar T, Kyo S. GLP-1 increases Kiss-1 mRNA expression in kisspeptin-expressing neuronal cells. Biology of Reproduction. 2017;**97**(2):240-248. DOI: 10.1093/biolre/iox087

IntechOpen