Endocrinology
Series Editors:
Andrea Lenzi · Emmanuele A. Jannini

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Ilpo T. Huhtaniemi *Editors*

Endocrinology of the Testis and Male Reproduction

Springer

Endocrinology

Volume 1

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Within the health sciences, Endocrinology has a unique and pivotal role. This old, but continuously new science is the study of the various hormones and their actions and disorders in the body. The matter of Endocrinology are the glands, i.e., the organs that produce hormones, active on the metabolism, reproduction, food absorption and utilization, growth and development, behavior control, and several other complex functions of the organisms. Since hormones interact, affect, regulate, and control virtually all body functions, Endocrinology not only is a very complex science, multidisciplinary in nature, but is one with the highest scientific turnover. Knowledge in the Endocrinological sciences is continuously changing and growing. In fact, the field of Endocrinology and Metabolism is one where the highest number of scientific publications continuously flourishes. The number of scientific journals dealing with hormones and the regulation of body chemistry is dramatically high. Furthermore, Endocrinology is directly related to genetics, neurology, immunology, rheumatology, gastroenterology, nephrology, orthopedics, cardiology, oncology, gland surgery, psychology, psychiatry, internal medicine, and basic sciences. All these fields are interested in updates in Endocrinology. The aim of the MRW in Endocrinology is to update the Endocrinological matter using the knowledge of the best experts in each section of Endocrinology: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreas with diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenals and endocrine hypertension, sexuality, reproduction, and behavior.

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Endocrinology of the Testis and Male Reproduction

With 127 Figures and 73 Tables

Springer

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Series Preface

Is there an unmet need for a new MRW series in Endocrinology and Metabolism? It might not seem so! The vast number of existing textbooks, monographs, and scientific journals suggest that the field of hormones (from genetic, molecular, biochemical, and translational to physiological, behavioral, and clinical aspects) is one of the largest in biomedicine, producing a simply huge scientific output. However, we are sure that this new series will be of interest for scientists, academics, students, physicians, and specialists alike.

The knowledge in Endocrinology and Metabolism is almost limited to the two main (from an epidemiological perspective) diseases, namely hypo/hyperthyroidism and diabetes mellitus, now seems outdated and closer to the interests of the general practitioner than to those of the specialist. This has led to endocrinology and metabolism being increasingly considered as a subsection of internal medicine rather than an autonomous specialization. But endocrinology is much more than this.

We are proposing this series as the *manifesto* for **Endocrinology 2.0**, embracing the fields of medicine in which hormones play a major part but which, for various historical and cultural reasons, have thus far been "ignored" by endocrinologists. Hence, this MRW comprises "traditional" (but no less important or investigated) topics: from the molecular actions of hormones to the pathophysiology and management of pituitary, thyroid, adrenal, pancreatic, and gonadal diseases, as well as less common arguments. Endocrinology 2.0 is, in fact, the science of hormones, but it is also the medicine of sexuality and reproduction, the medicine of gender differences, and the medicine of well-being. These aspects of Endocrinology have to date been considered of little interest, as they are young and relatively unexplored sciences. But this is no longer the case. The large scientific production in these fields coupled with the impressive social interest of patients in these topics is stimulating a new and fascinating challenge for Endocrinology.

The aim of the **MRW in Endocrinology** is thus to update the subject with the knowledge of the best experts in each field: basic endocrinology, neuroendocrinology, endocrinological oncology, pancreatic disorders, diabetes and other metabolic disorders, thyroid, parathyroid and bone metabolism, adrenal and endocrine

hypertension, sexuality, reproduction, and behavior. We are sure that this ambitious aim, covering for the first time the whole spectrum of Endocrinology 2.0, will be fulfilled in this vast Springer MRW in Endocrinology Series.

Andrea Lenzi M.D.
Emmanuele A. Jannini M.D.

Volume Preface

Generally speaking, the ultimate scope of life is maintenance of the species, which is ensured by reproduction. Reproduction depends on exquisite endocrine signals, which start during fetal life, are dormant during childhood, mature and attain full function at the end of puberty, and variably decline thereafter in a gender-dependent fashion. Therefore, if hormones are essential for life of individuals, reproductive hormones are in addition essential for the maintenance of species. Turned in another way, if some hormonal defects are deadly for the individual, reproductive failure may be deadly for the species. This book illustrates what hormones do for male reproduction, in which way they govern the pathophysiology of the hypothalamo-pituitary-gonadal axis, and, more generally, how they are implicated in the maintenance of the human species.

The book has been compiled by the world experts of the field, both in basic and clinical science. It aims at providing a comprehensive view of the current knowledge ranging from molecular, genetic, and cellular mechanisms to clinical manifestations of male reproductive physiology and its disorders to therapeutic perspectives. Male reproduction is a sensitive issue, with wide socioeconomical and ethical implications, which has been covered as well.

Each chapter of this book is meant to stand on itself as a reference work in its field. It has been written with human male reproduction in mind, but it also covers the animal research relevant to understand the clinical problems andrologists and male reproductive endocrinologists may encounter in their everyday practice.

We are grateful to all distinguished colleagues who generously contributed to this work with their knowledge and experience. We are confident that the readership will find clear answers to their curiosity, to improve their clinical skills and to stimulate them to more creative research in this fascinating field.

Manuela Simoni
Ilpo T. Huhtaniemi

Contents

Volume 1

About the Editors

Manuela Simoni , M.D., Ph.D., trained as a clinical endocrinologist at the Unit of Endocrinology of the University of Modena, Italy, between 1982 and 1990 and, thereafter, as molecular endocrinologist at the Institute of Reproductive Medicine of the University of Münster, Germany, where she was Professor for Endocrinology and Molecular Biology of Reproduction from 1998 to 2008.

Since 2008, she is Full Professor of Endocrinology at the University of Modena and Reggio Emilia, Italy.

Currently, she holds the following positions: Director of the Clinical Unit of Endocrinology at the Ospedale Civile of Modena, Director of the School of Specialization in Endocrinology and Metabolism, Deputy Director of the Department of Biomedical, Metabolic and Neural Sciences, and Director of the Center for Genomic Research of the University of Modena and Reggio Emilia.

Her research interests are gonadotropin and androgen action, testicular function, male infertility, endocrinology, and pathophysiology of reproduction.

She is member of several societies, including the European Academy of Andrology (EAA) and the European Society of Endocrinology (ESE, serving as the Secretary), and is active in the editorial boards of several journals in the fields of endocrinology and reproduction. Since January 2017, she is Co-Editor-in-Chief of ANDROLOGY, the official journal of the European Academy of Andrology and the American Society of Andrology.

Ilpo T. Huhtaniemi received his M.D. and Ph.D. from the University of Helsinki, Finland, did postdoctoral training in USA (UC San Francisco and NIH, Bethesda), and has been on sabbatical leave in Germany, USA, and Scotland. From 1986 to 2002, he held the post of Professor and Chairman of Physiology at University of Turku, Finland. He moved in 2002 to UK as a Chair in Reproductive Endocrinology at Imperial College London, from which post he retired in 2015. He has received several national and international honors, among them a Fellowship of the Academy of Medical Sciences (UK) and a Doctor Honoris Causa of the Medical University Lodz, Poland, and University of Szeged, Hungary. He has been the Chief Managing Editor of *Mol Cell Endocrinol* (1999–2017), has served in the Editorial Board of *Endocrinology* and *Endocr Rev*, and is/has been the Editor or Editorial Board Member of several other scientific journals (e.g., *Eur J Endocrinol*, *Clin Endocrinol*, *Hum Reprod Update*, *J Endocrinol*, *Mol Hum Reprod*, *Reproduction*, *Asian J Androl*, *J Endocr Soc*). He has held positions of trust in many international scientific organizations (e.g., Past President of International Society of Andrology; Executive Committee of European Society of Endocrinology). His research interests include clinical and basic reproductive endocrinology, in particular the function of gonadotrophins and male reproductive endocrinology. He also has long-term interests in male contraception, hormone-dependent cancer, and the endocrinology of aging.

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Part I

Physiology of the Hypothalamus-Pituitary-Gonadal Axis, the Testis, and Testicular Function

Control of GnRH Secretion

1

Marco Bonomi, Valeria Vezzoli, and Anna Cariboni

Abstract

Human reproduction and fertility are completely dependent upon neuroendocrine control of the hypothalamus-pituitary-gonadal (HPG) axis and its hierarchy of secreted hormones. The human reproductive system is controlled by the hypothalamus through the decapeptide gonadotropin-releasing hormone (GnRH), which displays a remarkable conservation over millions of years of evolution in the different species. The neurosecretion of GnRH depends on less than 4,000 GnRH-secreting neurons, which have an extracranial origin and finally migrate into the hypothalamic preoptic area. They secrete GnRH starting from the tenth week of gestation till the first 4–6 months of life, when GnRH secretion is "switched-off" until puberty. At puberty, the GnHR secretion is switched back "on" with a characteristic pulsatile manner that is maintained during adulthood. Regulation of GnRH-secreting neuron activity through the lifespan is not completely understood, but is clearly the result of a sophisticated network of stimulatory and inhibitory inputs, that include centrally different subgroups of neurons afferent to the GnRH-secreting neurons and peripherally the gonadal steroid feedback. The present chapter of the Textbook will focus on the ontogeny

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of the GnRH-secreting neurons and the mechanisms so far known to be implicated in regulating their neurosecretory activity.

Keywords

Gonadotropin-releasing hormone • GnRH receptor • GnRH-secreting neurons • Kisspeptin • KNDy

Contents

Neuroendocrine control of the hypothalamus-pituitary-gonadal (HPG) axis and hormones forms the base of human reproduction and fertility. A small number of neurons, scattered throughout different hypothalamic areas, secrete the neurohormone gonadotropin-releasing hormone (GnRH), which subsequently reaches the adenohypophysis through the pituitary portal vessels. Inside the pituitary, GnRH stimulates synthesis and release of the two gonadotropins, the luteinizing hormone (LH) and the follicle-stimulating hormone (FSH), by interacting with its specific receptor, the GnRHR which is expressed on the membrane of the gonadotrope cells. The two gonadotropins enter the systemic circulation and reach the gonads where they promote steroidogenesis (estrogen, progesterone, and androgens) and gametogenesis (oocytes and spermatozoa). Gonadal steroids, in turn, autoregulate their own secretion through a feedback mechanism, which determines a decrease of GnRH and gonadotropin secretion at the central level of the HPG axis.

The first suggestion of a hypothalamic site of control of the reproductive system came from the original findings of Harris in 1937 and his following "neurohumoral theory" (Harris 1955), which postulated that the secretion of each adenohypophyseal hormone would be controlled by a corresponding hypothalamic neuropeptide. Indeed, only some years later, the first such hypothalamic releasing hormone was identified, thyrotropin-releasing hormone (TRH) (Boler et al. 1969; Burgus et al. 1970), closely followed by the discovery and purification of GnRH (Amoss et al. 1971; Baba et al. 1971; Matsuo et al. 1971; Schally et al. 1971). Following this, the role of GnRH as a crucial regulator of the HPG axis became progressively clearer (Conn and Crowley 1994; Millar et al. 2001).

Comparison of the GnRH sequence from different species reveals a remarkable evolutionary conservation of over millions of years in the peptide length (ten amino acids), the N-terminus (Glu-His-Trp-Ser) and the C-terminus (Pro-Gly-NH_2) (Fig. 1), supporting the crucial role of these sequences in receptor binding and activation. In humans, the gene encoding GnRH consists of four exons and is mapped to 8p.11.2-p.2p21 (Fig. 2) (Yang-Feng et al. 1986; Radovick et al. 1990). GnRH cDNA comprises an open reading frame of 276 base pairs, which encodes a

a

	1	2	3	4	5	6	7	8	9	10	
Mammalian	pGlu-	His-	Trp-	Ser-	Tyr-	Gly-	Leu-	Arg-	Pro-	Gly-	NH_2
Guinea Pig	pGlu-	His-	Tyr-	Ser-	Tyr-	Gly-	Val-	Arg-	Pro-	Gly-	NH_2
Chicken I	pGlu-	His-	Trp-	Ser-	Tyr-	Gly-	Leu-	Gln-	Pro-	Gly-	NH_2
Chicken II	pGlu-	His-	Trp-	Ser-	His-	Gly-	Trp-	Tyr-	Pro-	Gly-	NH_2
Salmon	pGlu-	His-	Trp-	Ser-	Tyr-	Gly-	Trp-	Leu-	Pro-	Gly-	NH_2
Dogfish	pGlu-	His-	Trp-	Ser-	His-	Gly-	Trp-	Leu-	Pro-	Gly-	NH_2
Catfish	pGlu-	His-	Trp-	Ser-	His-	Gly-	Leu-	Gln-	Pro-	Gly-	NH_2
Herring	pGlu-	His-	Trp-	Ser-	His-	Gly-	Leu-	Ser-	Pro-	Gly-	NH_2
Medaka	pGlu-	His-	Trp-	Ser-	His-	Gly-	Leu-	Ser-	Pro-	Gly-	NH_2
Lamprey I	pGlu-	His-	Tyr-	Ser-	Leu-	Glu-	Trp-	Lys-	Pro-	Gly-	NH_2
Lamprey II	pGlu-	His-	Trp-	Ser-	His-	Gly-	Trp-	Phe-	Pro-	Gly-	NH_2
LampreyIII	pGlu-	His-	Trp-	Ser-	His-	Asp-	Trp-	Lys-	Pro-	Gly-	NH_2
Frog	pGlu-	His-	Trp-	Ser-	Tyr-	Gly-	Leu-	Trp-	Pro-	Gly-	NH_2
Seabream	pGlu-	His-	Trp-	Ser-	Tyr-	Gly-	Met-	Ser-	Pro-	Gly-	NH_2
Tunicate I	pGlu-	His-	Trp-	Ser-	Asp-	Tyr-	Phe-	Lys-	Pro-	Gly-	NH_2
Tunicate II	pGlu-	His-	Trp-	Ser-	Leu-	Cys-	His-	Ala-	Pro-	Gly-	NH_2
Whitefish	pGlu-	His-	Trp-	Ser-	Tyr-	Gly-	Met-	Asn-	Pro-	Gly-	NH_2

b

GnRH-I

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2

1 2 3 4 5 6 7 8 9 10

GnRH-II

pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH_2

1 2 3 4 5 6 7 8 9 10

Fig. 1 (**a**) Comparison of GnRH amino acid sequences through evolution of protochordates to mammals. *Grey regions* indicate conserved N- and C-terminal residues throughout the evolution, indicating their important functional role. (**b**) GnRH isoform in humans

precursor protein, subsequently cleaved and processed by a peptidase into secretory granules. The initial 23 amino acids of the prohormone correspond to a signal sequence, which is followed by the mature GnRH, the GKR sequence, and the 56-amino acid GnRH-associated protein, GAP (Fig. 2). The precise role of GAP is not known but is believed to have prolactin release inhibitory activity (Nikolics et al. 1985; Chavali et al. 1997).

Other GnRH isoforms exist and are expressed together with the "classic" type 1 GnRH (GnRH1) (Fig. 1). GnRH2 isoform presents a different amino acid sequence at positions 5, 7, and 8 and is expressed in humans, while GnRH3 has thus far only been found in some classes of fish. In humans, the gene encoding *GnRH2* has been cloned and mapped to chromosome 20p13. It consists of four exons, separated by three introns, encoding a predicted prohormone similarly organized to that of the GnRH1 precursor. However, the human *GnRH1* gene (5 kb) is longer than the *GnHR2* gene (2.1 kb) due to larger introns 2 and 3. The expression of the *GnRH1* and *GnRH2* genes is controlled by different promoters, suggesting different transcriptional regulations (White et al. 1998; Kim 2007). The two GnRH isoforms exhibit an overlapping pattern of tissue expression, which includes the central

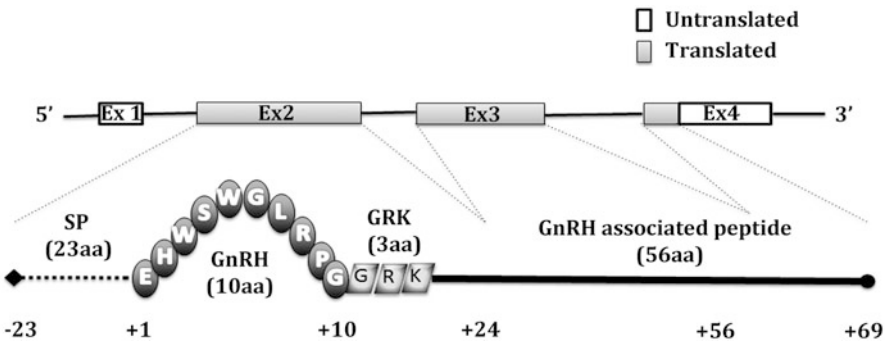

Fig. 2 Human GnRH gene consisting of four exons located on the short arm of chromosome 8. Exon 1 encodes a 5′ untranslated region (5′UTR); Exon 2 encodes the 23 amino acid Signal Peptide (SP), the GnRH decapeptide, the GKR processing sequence, and initial 11 amino acid of the GnRH-associated peptide (GAP); Exon 3 encodes the next 32 amino acid of GAP; Exon 4 encodes the remainder of GAP and an 3′UTR

nervous system and the reproductive organs (ovary, prostate, endometrium, breast, and placenta) (Hong et al. 2008), but GnRH2 is also expressed more widely outside the CNS (Skinner et al. 2009). Indeed, the expression of GnRH2 is primarily detectable in the kidney, prostate, and bone marrow, suggesting both reproductive and nonreproductive roles for this isoform. On the other hand, GnRH1 immunoreactivity is detected not only in the hypothalamus but also in some specific human pituitary cell types, such as thyrotropes and somatotropes, thus indicating a possible supplementary role in the pituitary. Moreover, GnRH2 neurons do not show the same origin of GnRH1 neurons in the olfactory placode and, to a lesser extent, the neural crest (see also below), and the two neuronal populations undergo different regulation by gonadal steroids (Khosravi and Leung 2003).

The short half-life (approximatively few minutes) is due to its rapid cleavage exerted by specific peptidases. Since GnRH is rapidly degraded and largely diluted, it is not possible to precisely measure it in the peripheral bloodstream once it has left the hypophyseoportal circulation. Thus, in humans, the measurement of the two gonadotropins (LH and FSH) is commonly used in the clinical practice as marker of the regular GnRH hypothalamic secretion. Of the two gonadotropins, LH pulses more accurately mirror the GnRH pulses in frequency and amplitude, as also demonstrated in the ewes (Clarke and Cummins 1985), because the longer half-life of FSH can mask FSH secretory troughs between pulses.

Development and Migration of GnRH-Secreting Neurons

Despite their anatomical position within the adult brain, during development GnRH neurons have an extracranial origin. The embryonic development of these cells, which is a conserved process that involves few hundred neurons per hemisphere in

mice (a few thousand in humans), has been extensively studied in mice and other species and is extremely important for the establishment and maintenance of reproduction (Wray 2010). Such studies have highlighted the physical and molecular connection of GnRH neurons with the olfactory system. Thus, GnRH neurons, which can be visualized on sections with *in situ* hybridization and/or immunohistochemistry protocols, are first detected in mice in the nasal placode, a structure which gives rise to the vomeronasal organ (VNO) and the olfactory epithelium at around embryonic day (E) 10.5. Whether GnRH neurons originated entirely within the nasal placode, or were just associated with this region, was for a long time a theme of debate; the current prevailing view is that neural crest-, as well as placodal-derived, cells also contribute to the mature GnRH neuron population.

Following fate specification, GnRH neurons migrate in association with axons of the olfactory/terminal/vomeronasal nerves within the nasal section to reach their definitive position in the forebrain (Hutchins et al. 2013). It is also well established that GnRH neurons co-migrate with other cell populations including other neurons (Fornaro et al. 2003) and neural crest-derived olfactory ensheathing cells (OECs) (Geller et al. 2013; Raucci et al. 2013).

Specifically, GnRH neurons first migrate within the nasal compartment along the intermingled olfactory and terminal-vomeronasal axons, whose cell bodies are located in the olfactory epithelium (OE) and VNO, respectively. Then, once they have reached the nasal-forebrain junction, GnRH neurons make a pause and enter the brain close to the olfactory bulbs. Within the brain, GnRH neurons associate with a transient axonal scaffold, formed by the caudal ramification of the vomeronasal nerve (Fig. 3), which drive the neurons toward the future hypothalamus, where they will set, in mice, at around E18.5. GnRH neuron migration is axophilic, in which the axons of the olfactory and vomeronasal nerves form a scaffold along which GnRH neurons migrate (Marin and Rubenstein 2003). The development of the GnRH-neuroendocrine system is also dependent on the olfactory system. In mammals, olfaction depends on sensory neuronal cells located in the OE and in the VNO, two epithelial structures present in the nose (Mombaerts 2001). The sensory neurons located in the OE are specifically detecting volatile substances and provide information on the external environment. Instead, the neurons placed in the VNO, at least in animals, perceive pheromones, which are not volatile chemicals that mediate reproductive and social behaviors, as well as changes in the neuroendocrine system.

Olfactory neurons send their axons to the principal olfactory bulb, where they are connecting with tufted and mitral cells to form the “glomeruli” (Farbman and Buchholz 1992). Similarly, the vomeronasal neurons are projecting to the accessory olfactory bulb. In humans, it has been recently reported the existence of a potential VNO, which role is still controversial (Dulac and Axel 1995; Stern and McClintock 1998).

Despite the possible functional role of the VNO in humans, it is now established that GnRH neuron maturation and therefore fertility depend on olfactory and vomeronasal neuron development.

In addition to extending olfactory and vomeronasal neuron axons, other cells leave the nasal placode and migrate toward the forebrain. Altogether, the migratory

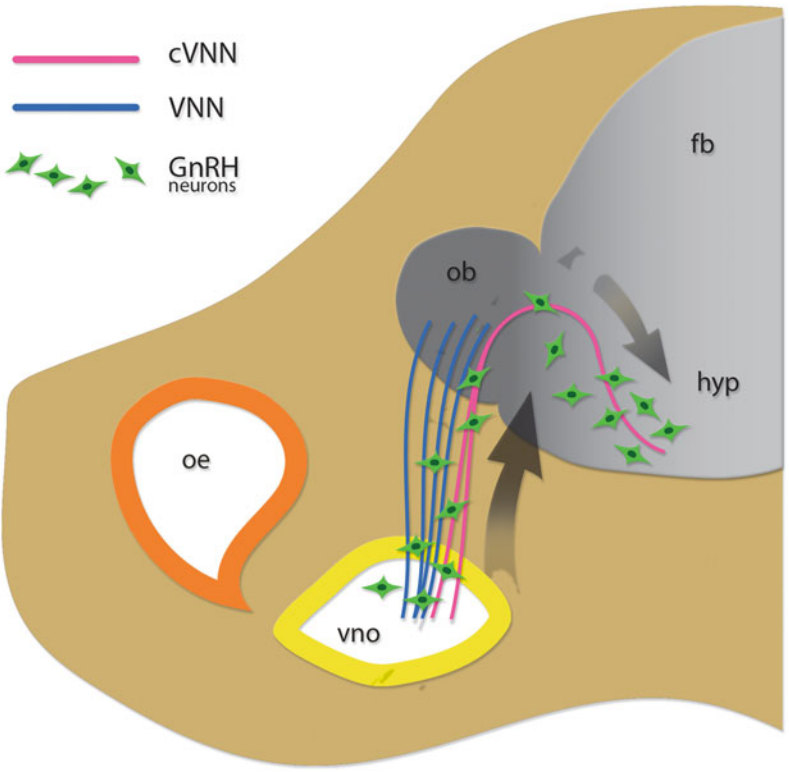

Fig. 3 Schematic drawing of a mouse sagittal section showing the migration of GnRH neurons (*green cells*), olfactory ensheating cells (OECs, *blue cells*), and the patterning of olfactory and vomeronasal axons (*orange, pink, and blue lines*), emerging from the olfactory epithelium (oe) and the vomeronasal organ (vno). To enter the forebrain (fb) and position in the hypothalamus (hyp), GnRH neurons follow the caudal branch of the vomeronasal nerve (cVNN)

cells and the extending axons form the so-called migratory mass. A first population of GnRH-negative neurons emerges from the nasal placode before the extension of olfactory axons; the role of these early migratory cells is to establish a scaffold used by the extending olfactory axons later on (Croucher and Tickle 1989; De Carlos et al. 1995; Maier and Gunhaga 2009). Cell populations that migrate later include GnRH neurons, OMP-positive and acetylcholine esterase-positive cells, and glial OECs (De Carlos et al. 1995; Miller et al. 2010).

OECs are the glial cell component of the olfactory system and derive from neural crest. Recent studies (Barraud et al. 2013; Geller et al. 2013) have highlighted that OECs form a microenvironment suitable for GnRH neuron migration, by secreting trophic factors; these cells also ensheathe olfactory neurons and regulate their fasciculation/defasciculation and subsequently the correct formation of the scaffold along which GnRH neurons migrate.

Interestingly, the discovery of OECs as neural crest derivatives is offering novel insights into the etiopathogenesis of human diseases such as Kallmann syndrome (KS) that, besides GnRH deficiency, often displays several neural crest defects.

In humans, the time of GnRH neuron appearance and their pattern of migration have been determined by performing immunolocalization studies on human embryos (Schwanzel-Fukuda et al. 1996). In embryos of 42 days of development, GnRH immunoreactivity is revealed in epithelial cells of the medial nasal placode, in cells along the terminal nerve in the nasal septum with a similar trajectory observed in mouse embryos toward the forebrain. Concomitant to the migrating GnRH neurons, bundles of fibers expressing the adhesion molecule N-CAM and serving as guides for migrating GnRH neurons are found to elongate form the olfactory pit into the forebrain.

Further evidence of the origin and initial migration of GnRH neurons arises from the analysis of a human fetus, the single so far analyzed, carrying a mutation in the KAL1/ANOS1 gene, which causes the X-linked form of KS; in this fetus, GnRH neurons did not enter the forebrain, and they gathered together in the cribriform plate in a tangle of neurons and olfactory/vomeronasal nerves (Schwanzel-Fukuda et al. 1989).

Besides KAL1/ANOS1 other causal genes have been discovered so far in patients with GnRH deficiency (Vezzoli et al. 2016), and they account, altogether, for only 35–45% of the cases. This is because genetic linkage studies have proven difficult to identify further causative genes, as most pedigrees are small due to infertility, and because sporadic mutations cannot be identified with this technique. Thus, researchers in the field have adopted different experimental paradigms, including immortalized GnRH neuron cell lines (Cariboni et al. 2004), nasal explants (Fueshko and Wray 1994; Tobet et al. 1996) and genetically modified mouse models, to study the molecular mechanisms of GnRH neuron development and, ultimately, to predict new candidate causative genes underlying the etiopathogenesis of KS and hypogonadotropic hypogonadism (HH). These studies have identified some of the molecular mechanisms that directly or indirectly regulate GnRH neuron development: these include transcription factors, i.e., Ebf2 (Corradi et al. 2003), neurotransmitters, i.e., GABA (Wray et al. 1996), adhesion molecules, i.e., N-CAM (Yoshida et al. 1999), and classical secreted cues such as semaphorins (Giacobini et al. 2008; Cariboni et al. 2011, 2015; Messina et al. 2011), Slits (Cariboni et al. 2012), ephrins (Gamble et al. 2005), and SDF-1(Schwarting et al. 2006).

For example, by applying mouse models and cell lines, it has been recently proved that the semaphorin SEMA3A is playing a key role in axon guidance in mice during development of the GnRH neuron (Cariboni et al. 2011), and subsequently genetic variations in SEMA3A in patients with KS have been identified (Hanchate et al. 2012; Young et al. 2012). These and other studies (Pitteloud et al. 2010) show that genetic mouse models are esteemed tools to uncover new causal genes for HH/KS and, when combined to next-generation sequencing (NGS) techniques, will help to validate the functional relevance of the novel genes in the GnRH system.

GnRH-Secreting Neuron Function and GnRH Secretion

Pulsatile GnRH Secretion Throughout Lifespan

The activity of human GnRH-secreting neurons is detectable in the hypothalamus by the tenth week of gestation followed by secretion of the two gonadotropins, which are present by the 10th–13th week of gestation when the hypophyseal portal system has developed (Fig. 4). Their secretion continues until the mid-gestation period when the typical surge of the placental steroids, via a negative feedback mechanism, causes a decrease, which is maintained until delivery. Indeed, after birth, the lack of this inhibition gives rise to a new surge in GnRH secretion. This central stimulation of the HPG axis is typical of the first 12–24 or 6 months of life in girls and boys, respectively, and it is so-called minipuberty. Subsequently and due to inhibitory mechanisms that are not fully understood and might involve the neurotransmitters γ-aminobutyric acid (GABA) and the neuropeptide Y (NPY), GnRH, LH, and FSH levels decrease and remain suppressed until puberty (Fig. 4) (Waldhauser et al. 1981; Blogowska et al. 2003). At puberty, GnRH and gonadotropin secretion resumes with a typical pulsatile manner, which is controlled by the GnRH pulse generator. The neurobiological origins and the precise location of this pulse generation are not yet fully elucidated. Recent work supports two possible hypotheses: (i) the GnRH-secreting neurons are able to generate autonomously the secreting pulses; (ii) the pulse generator is due to the influence of peptidergic neurons usually positioned in the infundibular region (INF) and in the hypothalamic arcuate nucleus (ARC) (Piet et al. 2015; Plant 2015). The pulsatile secretion of GnRH at puberty begins first at night with a low amplitude and slow frequency, and then both amplitude and frequency increase during pubertal development to achieve the normal pattern in adulthood (Fig. 4) (McCartney 2010). In adult men, GnRH secretion is characterized by pulses occurring approximately every 2 h, whereas in the fertile female, the frequency of GnRH pulse is more complicated and is intrinsically dependent on the timing of the ovulatory cycle. GnRH pulsatility is crucial in regulating the synthesis, secretion, and ratio release of the two gonadotropins from

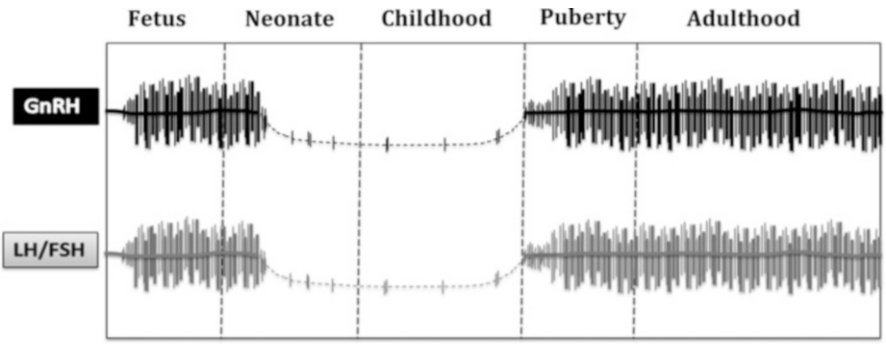

Fig. 4 Representation of the GnRH pulsatile secretion variation during the male lifespan

the pituitary (Fig. 4) (Reame et al. 1984; Nippoldt et al. 1989; Hall et al. 1992), and it is dependent on the fine-tuned modulation of the GnRH-secreting neurons as firstly demonstrated by Knobil and colleagues (Knobil 1992). Indeed, in patients affected by isolated GnRH deficiency, the substitutive use of pulsatile exogenous GnRH allows the pubertal development to occur (Marshall and Kelch 1979; Hoffman and Crowley 1982) and reproduces the hormonal changes normally seen in the menstrual cycle, thus stimulating the ovulation (Crowley and McArthur 1980). On the contrary, when GnRH is infused continuously, it is inhibiting the gonadotropin secretion, while the return to a pulsing stimulation is able to revert this negative effect. The molecular explanation for this phenomenon resides in the downregulation of the GnRHR (Loumaye and Catt 1982; Cheng et al. 2000; McArdle 2012), and this characteristic is currently used in the clinic to temporarily block the HPG axis through the administration of long-acting GnRH agonists. This downregulation of the GnRHR on the gonadotrope cells, when continuously stimulated, gives reason of the importance of the normal pulsatile GnRH secretion in order to induce the synthesis and release of the two gonadotropins from the pituitary.

Regulation of GnRH Secretion

The mechanisms regulating GnRH secretion are extremely complex. Studies on immortalized GnRH-secreting neurons (GT1) (Mellon et al. 1990), on primary GnRH neurons (Tobet et al. 1996; Maurer and Wray 1997), and in animal models (Negro-Vilar et al. 1982; Gore and Terasawa 1991; Levine et al. 1995) show that GnRH secretion is modulated by a network of excitatory and inhibitory inputs that include either a central control exerted by distinct subgroups of neurons afferent to the GnRH-secreting neurons or the peripheral gonadal steroid feedback (Fig. 5) (Ojeda et al. 2006; Christian and Moenter 2010; Herbison 2016).

Central Control by Kisspeptin Neuronal System

The identification of the hypothalamic kisspeptin neuronal network has deeply changed our perceptions of the control and activation of GnRH-secreting neurons at puberty. Indeed, kisspeptin (formerly known as metastin) is a strong activator of the hypothalamic-pituitary-gonadal axis in humans and animal models. It is encoded by the *KISS1* gene (chromosome 1q32) which consists of two untranslated and two coding exons. *KISS1* gene encodes a precursor of 145 amino acid, which cleavage generates a 54 amino acid peptide (West et al. 1998), subsequently processed in two smaller fragments, kisspeptin-13 and kisspeptin-14. Kisspeptin binds to GPR54 (now termed KISS1R) (Gottsch et al. 2004), described both in the rat and in human brain (Lee et al. 1999; Muir et al. 2001; Ohtaki et al. 2001). The five exons of the *KISS1 receptor* gene (chromosome 19p13.3) encode for a 398 amino acid G-protein-coupled receptor (Muir et al. 2001). Kisspeptin-mediated KISS1R activation (Muir et al. 2001; Liu et al. 2008; Constantin et al. 2009) determines a biphasic surge of cytosolic Ca^{2+} concentration with a more persistent second phase (Min et al. 2014). In order to support this second phase and to prevent receptor desensitization

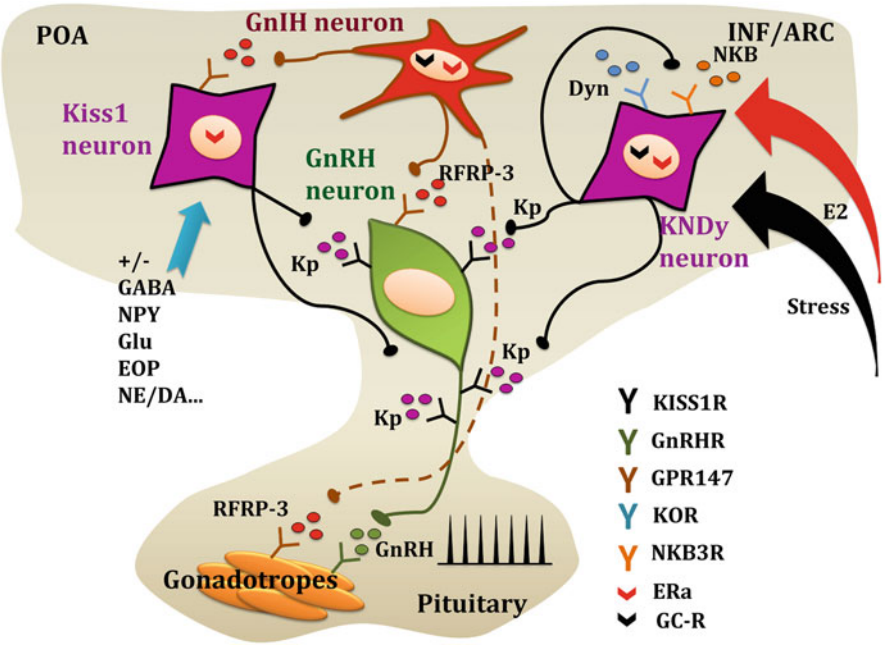

Fig. 5 Summary of the physiological mechanisms possibly involved in the control of GnRH secretion and its action on gonadotrope cells. *POA* preoptic area, *INF* infundibular region, *ARC* arcuate nucleus, *Kp* Kisspeptin, *NKB* neurokinin B, *DYN* dynorphin, *RFRP-3* RFamide-related peptide, *E2* estradiol, *GABA* gamma aminobutirric acids, *NPY* neuropeptide Y, *NA* noradrenaline, *EOP* endogenous opioid peptides, *NE/DA* Norepinephrine/Dopamine, *KISS1R* Kiss1 receptor, *GnRHR* GnRH receptor, *GPR147* RFP-3 receptor, *KOR* kappa-opioid receptor, *NKB3R* neurokinin B receptor, *ERa* estrogen receptor alpha, *GC-R* Glucocorticoid receptor.

following the first activation, an intense KISS1R trafficking is needed (Min et al. 2014).

The key role played by kisspeptin system became evident from studies performed in a model of human disease which is represented by patients with congenital GnRH deficiency. Indeed, patients with inactivating allelic variants of either *KISS1R* or *KISS1* resulted in idiopathic hypogonadotropic hypogonadism (de Roux et al. 2003; Seminara et al. 2003; Topaloglu et al. 2012). This evidence was supported by studies in knockout mouse models for either the *Kiss1r* or the *Kiss1* genes, which phenocopy the human GnRH congenital deficiency (d'Anglemont de Tassigny et al. 2007; Lapatto et al. 2007; Chan et al. 2009). Thus, the crucial importance of the kisspeptin effect on the GnRH secretion became evident across mammalian species. Moreover, clinical studies involving the administration of kisspeptin to both healthy controls and patients with idiopathic hypogonadotropic hypogonadism further confirmed the importance of the kisspeptin system in the control of the GnRH neuron activity (Dhillo et al. 2005; Jayasena et al. 2009, 2010, 2011; George et al. 2011; Chan et al. 2012; Young et al. 2013). Indeed, the acute injection of exogenous kisspeptin is able

to induce the rise of LH and FSH in male healthy volunteers, while its action in female is variably dependent on the period of the menstrual cycle. Furthermore, non-chronical administration of kisspeptin (in order to avoid the receptor desensitization) is also effective in stimulating GnRH secretion in men with acquired hypogonadotropic hypogonadism associated with obesity and type 2 diabetes (George et al. 2010) and in female with hypothalamic amenorrhea (Jayasena et al. 2010).

Kisspeptin Neurons

The majority of the studies on the Kisspeptin system have been performed in rodents, but many correlations with higher mammals have been found. Kisspeptin neurons are positioned in the infundibular (INF)/arcuate (ARC) nucleus in all species and in the rostral preoptic area (POA) with a species-specific distribution (Clarkson and Herbison 2006; Pompolo et al. 2006; Ramaswamy et al. 2008; Clarkson et al. 2009; Hrabovszky et al. 2010): in rodents, they are positioned in the periventricular nucleus (PeN) and the anteroventral periventricular nucleus (AVPV) (Clarkson and Herbison 2006; Clarkson et al. 2009), while in humans and ruminants, their cell bodies are more sprinkled within the POA (Pompolo et al. 2006; Rometo et al. 2007; Oakley et al. 2009; Hrabovszky et al. 2010). Moreover, in humans there is also a sexual dimorphism in respect to the kisspeptin neuron distribution and numerosity. Female hypothalamus have considerably more kisspeptin fibers and cell bodies in the INF nucleus compared to men hypothalamus (Hrabovszky et al. 2010). Furthermore, kisspeptin cells are detected in the rostral periventricular area only in female (Hrabovszky et al. 2010). This different hypothalamic architecture of the kisspeptin system in male and female, as discussed in the next section of the chapter, has been linked to the different effect of the sex steroids on this cell population.

Kisspeptin neurons may act both directly or transsynaptically through neurotransmitters (Skorupskaite et al. 2014). The close proximity between the kisspeptin- and GnRH-secreting neurons seen in rodents, sheep, and monkeys was also seen in humans, where kisspeptin axons form dense pericapillary plexus in the pituitary stalk, engaging contacts with the GnRH neuron cell body, axons, and dendritic spine (Hrabovszky et al. 2010) (Fig. 5). However, in humans the occurrence of these connections seems lower, and not all GnRH neurons receive kisspeptin neuronal contacts (Clarkson and Herbison 2006; Ramaswamy et al. 2008; Smith et al. 2008a; Hrabovszky et al. 2010). This indicates a fine modulation of GnRH release by kisspeptin and other neuropeptides. Indeed, in the hypothalamus an interconnected and composite system of modulators of kisspeptin neurons has been identified, including neuroendocrine factors and sex steroids (see below), that guarantees the correct gonadotropic function (Fig. 5).

KNDy Neurons

Inside the hypothalamus, two kisspeptin neuron populations are present with a differential expression of neuropeptides (Fig. 5) and distinct functions (Ojeda et al. 2010). In addition to neurons that exclusively express and secrete kisspeptin, there

are other neurons, designated as KNDy neurons (Cheng et al. 2010), which are co-expressing kisspeptin (KP), neurokinin B (NKB), and dynorphin (Dyn) (Lehman et al. 2010; Hrabovszky et al. 2012; Navarro 2012; Skrapits et al. 2015). KNDy neurons are preserved among species and are localized in the ARC nucleus of sheep and rodents and in the corresponding INF region of humans (Burke et al. 2006; Goodman et al. 2007; Navarro et al. 2009). The three secreted KNDy neuropeptides shape kisspeptin secretion through paracrine/autocrine action, operating in a coordinated fashion (Fig. 5). KNDy neurons in the ARC form a complex system where individual neurons are interconnected to each other and project the median eminence (ME) (Lehman et al. 2010). Moreover in humans it was observed that KNDy neurons are in straight relationship with GnRH neuron cell body and dendrites (Ciofi et al. 1994; Krajewski et al. 2005; Clarkson and Herbison 2006; Ramaswamy et al. 2008; Dahl et al. 2009). This distribution suggests that KNDy neurons located in ARC/INF act as a central hub for the regulation of GnRH release. They stimulated GnRH neurons via the release of kisspeptins, but they also participate in the mutual (auto)-regulation of kisspeptin system through the secretion of NKB and Dyn (Navarro et al. 2009; Wakabayashi et al. 2010). NKB pathway is able to stimulate LH release, which mirrors GnRH release (Billings et al. 2010; Navarro et al. 2011). These stimulatory activities are in accordance with the altered reproductive defects observed in TAC3- and TACR3-mutated patients (Topaloglu et al. 2009; Gianetti et al. 2010; Young et al. 2010). In contrast, dynorphin, working via K-opioid receptors (KOR), exerts its inhibitory control on pulsatile GnRH release (Wakabayashi et al. 2010) by mediating the negative feedback of progesterone, as discussed in the next section (Goodman et al. 2004; Foradori et al. 2005). Additionally, although the principal target of kisspeptin stimulation pathway is the control of GnRH secretion (Gottsch et al. 2004; Irwig et al. 2004; Smith et al. 2008b), the expression of *KISS1* and *KISS1R* genes in the gonadotropes suggests a possible direct effect of kisspeptin on gonadotrope functionality. Indeed some studies have demonstrated that pituitary explants stimulated with kisspeptin secrete gonadotropins (Kotani et al. 2001; Navarro et al. 2005; Gutierrez-Pascual et al. 2007; Richard et al. 2008) and that in the sheep low amount of kisspeptin is present in the pituitary portal vessels (Smith et al. 2008b).

Gonadal Steroid Regulation

Another important mechanism that controls dynamically the GnRH synthesis and release from the GnRH-secreting neurons is represented by the gonadal steroid feedback. Indeed, in both sexes the severe deprivation of estrogens (E), androgens (A), and progesterone (P), such as in human females menopause, after castration or due to gonadal dysgenesis, triggers an increase in the secretion of GnRH and of gonadotropins, which, in turn, is controverted by the substitutive therapy (Kalra and Kalra 1989; Herbison 2016). The idea that sex steroids modulate the release of GnRH and gonadotropins was postulated for the first time in the 1930s, although several aspects concerning their precise molecular mechanisms of action are still not fully understood. Whether the sex steroids exert their regulatory functions on the GnRH neurons directly or through intermediate connected pathways or neurons is

still debated. Direct action of gonadal steroids on the GnRH cells would imply the expression of specific receptors. Indeed, estrogens bind to two specific nuclear receptors isoforms: the estrogen receptor alfa (ERα) and the estrogen receptor beta (ERβ), but the presence of the ERα on the GnRH-secreting neurons is still debated, and only recently some studies were detecting the ERα mRNA in these cells (Herbison and Pape 2001; Hu et al. 2008). In addition, after the discovery of a second ER isoform, the ERβ (Kuiper et al. 1996; Mosselman et al. 1996), a small subgroup of GnRH neuron, was found to express this isoform (Butler et al. 1999; Skynner et al. 1999; Hrabovszky et al. 2000; Kallo et al. 2001). Moreover, in vitro studies have demonstrated the presence of ERs in the immortalized GT1 cell line. Therefore, it is also possible that GnRH neurons are temporally expressing the ERs, and the direct action of E on these neurons might exist only during embryogenesis and/or during the early stages of postnatal life. This possibility could be also extended to the A receptor, AR, which is not expressed in vivo in GnRH neurons (Huang and Harlan 1993), while detectable in vitro in the GT1 cells (Poletti et al. 1994; Belsham et al. 1998). Nevertheless, regarding the effects of A on GnRH secretion, we have to consider that testosterone is enzymatically converted through a specific aromatase into E, which is mediating its principal negative effect on the HPG axis. Lastly, even the expression of the P receptor, PR, on the GnRH neurons is still controversial, thus keeping open the question whether the P regulatory effect is directly exerted on GnRH neurons or indirectly through neuronal intermediates. P is important in the regulation of the GnRH secretion at the hypothalamic level (Ramirez et al. 1980; Kim et al. 1989), although its action occurs mainly at the pituitary level where, acting in synergy with E, it induces a full gonadotrope response to GnRH (Nippoldt et al. 1987; Mahesh and Brann 1998).

Following the characterization of the kisspeptin system, further important improvements in our comprehension of the central control of reproduction became possible, including the elucidation of the molecular mechanisms underlying the sex steroid feedback on GnRH neuronal activity. Indeed, kisspeptin system is implicated in the transmission of both negative and positive feedback of sex steroids on GnRH neurons. Accordingly the majority of the hypothalamic kisspeptin-secreting cells, including KNDy neurons, express ERα, AR, PR, and, in a small proportion, ERβ, while GnRH neurons do not (Smith et al. 2005a, b, 2006, 2007, Franceschini et al. 2006; Adachi et al. 2007; Clarkson et al. 2008, 2012). Moreover, *Kiss1* mRNA expression level in the ARC/INF is upregulated following gonadectomy, in accordance with the rise of the gonadotropins levels, while this effect is prevented by the estradiol replacement (Smith et al. 2006; Adachi et al. 2007; Clarkson et al. 2008; Oakley et al. 2009; Lehman et al. 2010). Additionally, KissR inactivation, either as in the KO animal model (Dungan et al. 2007) or in the presence of a specific Kiss1 antagonist (Roseweir et al. 2009), mitigates the rise in circulating LH after gonadectomy. The E negative feedback on the kisspeptin system is mainly dependent upon ERα. Indeed, the administration of an ERα-selective agonist suppresses Kiss1 mRNA expression and LH blood concentrations in castrated animals (Navarro et al. 2004), while the LH increase after castration is absent only in the ERα KO mice and still present in the ERβ null mice (Dungan et al. 2007). Thus, E exerts its

negative feedback on the kisspeptin system, mainly through the ERα, by inhibiting kisspeptin and neurokinin B secretion, which, in turn, lowers their synergic stimulation on the GnRH neurons (Fig. 5). Moreover, dynorphin, which is normally co-secreted with kisspeptin and neurokinin B from the KNDy neurons, is able to inhibit GnRH pulsatility following progesterone administration, while the progesterone receptor expression is upregulated from the E (Nippoldt et al. 1989; Soules et al. 1984). Thus, E also mediates the inhibiting effect on the GnRH secretion by enhancing the dynorphin secretion and action.

The E negative feedback effect so far described is dependent on constant low levels of the hormone, since it is well known that a rapid increase in estradiol levels, such as at the end of the follicular phase in the female menstrual cycle, accompanied by the upregulation of the PR, characteristically switches the feedback from inhibitory (negative feedback) to stimulatory (positive feedback). This E positive feedback is a key point in the determination of the GnRH preovulatory surge which allows the LH peak and the following ovulation. The neuroendocrine mechanisms based on such positive feedback of E are less well characterized, compared to the negative ones, and appear to be more site- and species-specific. Experimental data in humans and animal models indicate that at least a subgroup of kisspeptin-secreting neurons plays a key role in mediating the E positive feedback. Indeed, in humans, the administration of kisspeptin, instead of hCG, was effective in generating the ovulatory LH surge and in triggering the oocyte maturation (Jayasena et al. 2010). A more precise characterization of this positive feedback comes from experimental data on female rodents (Roa et al. 2009). In these animal model, estradiol is able to enhance the Kiss1 mRNA level in the AVPV/RPV3 nucleus (Smith et al. 2005b), and this is associated with the observation that AVPV kisspeptin neurons are stimulated during the preovulatory sex steroid-induced LH surge (Smith et al. 2006). Further, these kisspeptin neurons in the AVPV are connected with the GnRH neurons (Clarkson and Herbison 2006), and the offsetting of kisspeptin in the POA eliminates the preovulatory LH peak (Kinoshita et al. 2005). Furthermore, the KO animal models for *Kiss1* and *Kiss1R* genes are characterized by an anovulatory state, and specific kisspeptin antagonists are able to markedly inhibit the gonadotropin preovulatory surge (Pineda et al. 2010a). Altogether these data indicate a key role of the kisspeptin neurons of the AVPV hypothalamic nucleus in mediating the positive feedback control of the GnRH secretion in rodents. In humans, the homologous of the AVPV kisspeptin neurons has so far not been identified (Rometo et al. 2007; Oakley et al. 2009). Nevertheless, this sexual dimorphism, observed in the kisspeptin pathway in the INF region, might be related to E positive feedback. Indeed, E positive feedback occurs only in females, where the system appears more represented and might be constituted by distinct kisspeptin neurons mediating the negative or the positive E feedback on the GnRH secretion. As for the positive feedback, the effect seems to be mediated by the ERα since the selective elimination of this signaling in the kisspeptin cells in vivo is associated with a lack of the E action on the AVPV neurons and subsequently the absence of ovulation (Mayer et al. 2010).

To summarize the data so far accumulated regarding the sexual steroid action on the GnRH secretion, we can conclude that the negative and positive feedbacks are

surely mediated by the kisspeptin system, although a certain direct effect through specific receptors has to be considered and might be better characterized in the future.

Neurotransmitters and Neuropeptide Regulation

As demonstrated either in vivo or in vitro, the GnRH secretion and pulsatility is also modulated by local release of different neurotransmitters and neuropeptides, which are also interrelated with other control systems such as the KNDy neurons and the gonadal steroids.

Two of the first neurotransmitters able to stimulate the GnRH secretion are represented by norepinephrine and dopamine (Herbison 1997). The initial evidence came from in vitro studies of the GT1 GnRH neuron cell line that expresses β1-adrenergic and D1-dopaminergic receptors and whose activity is pharmacologically interfered by the treatment with specific agonists and antagonists of the so far mentioned receptors (Martinez de la Escalera et al. 1992; Findell et al. 1993; Uemura et al. 1997). Subsequently, experimental data in humans and primates have confirmed the modulation of the GnRH pulsatile secretion by norepinephrine and dopamine also in vivo. Indeed, the α-adrenergic receptor blocking agents (i.e., phentolamine or prazosin) and the dopamine antagonist (i.e., metoclopramide) are able to arrest or at least inhibit the GnRH pulse generator in ovariectomized rhesus monkey (Kaufman et al. 1985; Gearing and Terasawa 1991). Furthermore, in humans, while the administration of dopamine and its agonists has been shown to be able to decrease the mean LH circulating levels, the α-adrenergic receptor blocking agents do not alter the LH pulsatile frequency (Leblanc et al. 1976; Lachelin et al. 1977; Pehrson et al. 1983).

Glutamate represents another important excitatory neurotransmitter in the hypothalamus, and its role in the stimulation of the GnRH secretion has been demonstrated in several species (Goldsmith et al. 1994; Dhandapani and Brann 2000; Gore 2001; Ottem et al. 2002; Lin et al. 2003; Pompolo et al. 2003). Glutamate mediates its role by binding to the N-methyl-D-aspartate (NMDA) receptors which are expressed on GnRH neurons (Gore 2001). Additionally, since the GABA agonists and the opioids agents are able to act at presynaptic NMDA receptor to inhibit the glutamate exocytosis (Potashner 1979; Weisskopf et al. 1993), an interaction between the glutamate and the opioid neurons may occur in the regulation of the GnRH control (Brann and Mahesh 1997). Indeed, in GT1 cells, $GABA_A$ and $GABA_B$ NMDA receptors have been identified (Stojilkovic et al. 1994).

GABA, in contrast to the previously described stimulatory neurotransmitters, inhibits GnRH secretion through the binding of the $GABA_A$ receptor (Urbanski and Ojeda 1987; Herbison 1998). This was demonstrated both in in vivo and in vitro experimental models following the treatment with specific agonist and antagonist of the $GABA_A$ receptor (Li and Pelletier 1993; Leonhardt et al. 1995; Han et al. 2004).

Among the different neuropeptides, an important inhibitory role on the GnRH secretion is surely played by the opioids (Kalra and Kalra 1984; Grosser et al. 1993). Indeed, in vitro experimental evidences revealed that opioids weaken the adrenergic stimulation on GT1 cell line (Nazian et al. 1994), and this inhibitory effect can be solved by the use of the opiate antagonist, naloxone (Ferin et al. 1982; Van Vugt

et al. 1989; Williams et al. 1990). Moreover, the in vivo data demonstrate that the activation of endogenous opioids mediate the suppression of the GnRH secretion following the treatment with corticotropin-releasing hormone (CRH) (Knobil 1989; Williams et al. 1990), although this effect was not observed in humans (Fischer et al. 1992).

Neuropeptide Y (NPY), galanin, and aspartate represent other putative neuromodulators of the GnRH system, although only the last one stimulates directly the GnRH pulse generator, whereas the first two are gonadal steroid dependent for their action (Woller and Terasawa 1992; Brann et al. 1993). Indeed, studies on the effect of NPY on the GnRH secretion reveal a complex picture. Central injection of NPY in intact animal (or castrated animal substituted with sex steroids) leads to the stimulation, while in castrated animal to the inhibition of the GnRH secretion. This bimodal action of NYP on the GnRH system is then sex steroid dependent. Nevertheless, the *NPY* null animal models present a relatively normal reproductive function, indicating that the role played by NPY in reproduction is only one of several inputs and it is part of a highly redundant network.

Another important peptide in regulating the GnRH secretion is leptin, a peptide hormone secreted by the adipose tissue that helps to regulate the energy balance and mirrors the amount of energy reserve. Thus, leptin might play a key role either by signaling to the central nervous system the information regarding the amount of fat stores that are present or by enabling the activation of the HPG axis through the GnRH secretion when convenient. Specifically, it has been demonstrated that leptin is able to stimulate the GnRH secretion either increasing the release of aspartate or reducing the release of GABA in peri-pubertal rats, whereas in the prepubertal rats, it increases the release of GABA (Reynoso et al. 2003). Furthermore, the exogenous replacement therapy with leptin in leptin-deficient prepubertal girls results in a LH/FSH secretory pattern consistent with an early puberty, thus confirming the stimulation of the GnRH secretion (Farooqi et al. 1999).

GnIH

The neuronal network that regulates the HPG axis is additionally complicated by gonadotropin inhibitory hormone (GnIH), a novel neuropeptide capable of inhibiting gonadotropin synthesis and secretion, which was first identified in birds (Tsutsui et al. 2000). GnIH is synthetized as a 173 amino acid precursor that is proteolytically processed into three peptides, respectively, named GnIH, GnIH-1, and GnIH-2 (Satake et al. 2001). These peptides share a carboxyl-terminal LPXRF-amide structure, in which X might be replaced by L or Q. Two perfectly conserved peptides of the RF-amide family, RFRP-1 and RFRP-3, were also reported in mammals. Intriguingly, even if RFRP-1 peptide exhibits greater structural homology with GnIH (Kriegsfeld et al. 2006), the RFRP-3 appears as the mammalian functional ortholog of avian GnIH (Pineda et al. 2010b; Smith and Clarke 2010). Both RFRP-1 and RFRP-3 act primarily by binding NPFF1R (also termed Gpr147), a G-protein-coupled receptor. Moreover the two peptides are able to signal binding the NPFF2R receptor (also named Gpr74) (Clarke et al. 2009). In mammals RFRP-3 neurons are principally identified in the hypothalamic dorsomedial nucleus (DMN)

or adjacent areas, where they project to different districts of the hypothalamus, such as the ARC, the ventromedial nucleus (VMN), the lateral hypothalamus, and the paraventricular nucleus (PVN). It is well known that these regions play crucial functions in the regulation of fertility and energy equilibrium (Qi et al. 2009). In this scenario, recent experimental data suggest a possible function of GnIH/RFRP peptide as a connecting hub, together with leptin, between reproductive and metabolic homeostasis. Overall the pharmacological data so far available are in favor of an orexigenic role for RFRP-3. Considering that orexigenic mediators are turned on in negative energy balance conditions (e.g., to promote food intake), it is reasonable to suppose that RFRP mediates the suppression of reproductive function during an energy absence state (Clarke et al. 2012).

Even though the link between the GnIH/RFRP and the physiology of reproduction was described in animal models (Tsutsumi et al. 2010), a possible regulation of human pubertal development by the RFRP-3/GPR147 system was only recently suggested (Maggi et al. 2016). While the experimental evidences collected until now suggest a main inhibitory effect exerted by GnIH/RFRP on LH/FSH release among mammals, several arguments persist concerning the nature (stimulatory in some instance), preferential area of action (pituitary vs. hypothalamic), and significance (in relation with different neuropeptides) of the GnIH/RFRP network in the control of the hypothalamic-pituitary-gonadal axis (Fig. 5). Equally, the relationship between the RFRP and the other mediators with crucial roles in the physiology of reproduction, including kisspeptins (Fig. 5), was proposed, but additional evidence is required in order to have a complete picture of the main interaction and connections between GnIH/RFRP peptides and central/peripheral actors involved in the regulation of the hypothalamic-pituitary-gonadal axis.

Other Factors Influence on GnRH Regulation

Other factors may influence the GnRH secretion including various stressors as infection, malnutrition, anxiety, depression, and chronic illness. Indeed, in humans and in animal models, acute fasting is able to induce infertility throughout the inhibition of the GnRH secretion (Bergendahl et al. 1998). Similar effect was demonstrated in animal models treated with intravenous lipopolysaccharide, a bacterial endotoxin that mimics an infectious stress (Takeuchi et al. 1997; Yoo et al. 1997; Refojo et al. 1998). Finally, men affected with prolonged critical illness present a decrease and a blunted 24 h pulsatile profile of the LH secretion, which causes a reduced androgen circulating levels with the settle of an acquired central hypogonadism (Van den Berghe et al. 1994).

All these chronic stressors trigger a rise in glucocorticoids, which are the classic endocrine response to stress. GC suppresses reproductive function at different levels of the HPG axis (Collu et al. 1984; Rabin et al. 1988) mainly through the inhibition of the GnRH secretion (Dubey and Plant 1985; Kamel and Kubajak 1987), which, in turn, leads to a reduced LH release (Briski and Sylvester 1991). This is consistent with the observation that in Cushing's disease, a disorder characterized by hypercortisolemia, the presence of an associated hypogonadism is a common aspect. Moreover, hypothalamic neurons are known to express the glucocorticoid receptor

(GC-R) (Chandran et al. 1994), and the treatment of the GT1 cell line with GC results in the inhibition of both the GnRH-mRNA levels and the transcriptional activity of transfected GnRH promoter-reporter gene vectors (Chandran et al. 1996). Additionally, the downregulation of the GnRH secretion exerted by the GC is mediated through the interaction with the GnIH/RFPR system (Fig. 5). Indeed, acute and chronic stress stimulates expression of RFRP in the adult male rat hypothalamus, where expression of RFRP and GC-R overlap (Kirby et al. 2009), and systemic administration of RFRP combined to stress suppresses the LH release and the sexual behavior. On the other hand, adrenalectomy prevents the stress-induced increase of the RFPR expression in the hypothalamus and in turn also the suppression of LH secretion.

On the opposite, in response to acute stress, endogenous GC may protect gonadotropin secretion (Matsuwaki et al. 2006). This is related to the secretion of prostaglandins (PGs) in the central nervous system, which inhibit the LH pulses, as reported in the case of several stress factors such as infections, hypoglycemia, and restriction (Konsman et al. 2004). The increased secretion of GC may balance the effects of stress-induced PG synthesis in order to support, instead to inhibit, the reproductive function in response to acute stress circumstances.

Summary

Reproduction is crucial for species survival and is fully dependent on a complex axis involving different organs such as hypothalamus, pituitary, gonads, and genitalia. This network is controlled by neuroendocrine mechanisms which are not fully characterized but surely interacting at the hypothalamic level where relatively few neurons secrete the neurohormone GnRH. In the last decades, advances have been made to better understand the action of GnRH either in physiologic or pathologic conditions. Researchers are progressively starting to better understand which factors are mandatory for GnRH neuronal migration, the mechanisms involved in the starting of the pulsatile GnRH secretion at puberty, and the maintenance of the normal adult reproductive function.

Cross-References

- ▶ Anabolic and Metabolic Effects of Testosterone and Other Androgens: Direct Effects and Role of Testosterone Metabolic Products
- ▶ Androgen Receptor
- ▶ Androgen Resistance
- ▶ Classification and Epidemiology of Hypogonadism
- ▶ Clinical Manifestation and Diagnosis of Androgen Deficiency
- ▶ Delayed Puberty
- ▶ Estrogen Deficiency in Men
- ▶ Genetic Analysis in Male Infertility

- ▶ GnRH Action
- ▶ Gonadotropins
- ▶ Hormonal Laboratory Examination
- ▶ Primary and Secondary Hypogonadism
- ▶ Testicular Steroidogenesis

References

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. J Reprod Dev. 2007;53(2):367–78.

Amoss M, Burgus R, Blackwell R, Vale W, Fellows R, Guillemin R. Purification, amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. Biochem Biophys Res Commun. 1971;44(1):205–10.

Baba Y, Matsuo H, Schally AV. Structure of the porcine LH- and FSH-releasing hormone. II. Confirmation of the proposed structure by conventional sequential analyses. Biochem Biophys Res Commun. 1971;44(2):459–63.

Barraud P, John JAS, Stolt CC, Wegner M, Baker CV. Olfactory ensheathing glia are required for embryonic olfactory axon targeting and the migration of gonadotropin-releasing hormone neurons. Biol Open. 2013;2(7):750–9.

Belsham DD, Evangelou A, Roy D, Duc VL, Brown TJ. Regulation of gonadotropin-releasing hormone (GnRH) gene expression by 5alpha-dihydrotestosterone in GnRH-secreting GT1-7 hypothalamic neurons. Endocrinology. 1998;139(3):1108–14.

Bergendahl M, Aloi JA, Iranmanesh A, Mulligan TM, Veldhuis JD. Fasting suppresses pulsatile luteinizing hormone (LH) secretion and enhances orderliness of LH release in young but not older men. J Clin Endocrinol Metab. 1998;83(6):1967–75.

Billings HJ, Connors JM, Altman SN, Hileman SM, Holaskova I, Lehman MN, McManus CJ, Nestor CC, Jacobs BH, Goodman RL. Neurokinin B acts via the neurokinin-3 receptor in the retrochiasmatic area to stimulate luteinizing hormone secretion in sheep. Endocrinology. 2010;151(8):3836–46.

Blogowska A, Rzepka-Gorska I, Krzyzanowska-Swiniarska B, Zoltowski S, Kosmowska B. Leptin, neuropeptide Y, beta-endorphin, gonadotropin, and estradiol levels in girls before menarche. Gynecol Endocrinol. 2003;17(1):7–12.

Boler J, Enzmann F, Folkers K, Bowers CY, Schally AV. The identity of chemical and hormonal properties of the thyrotropin releasing hormone and pyroglutamyl-histidyl-proline amide. Biochem Biophys Res Commun. 1969;37(4):705–10.

Brann DW, Mahesh VB. Excitatory amino acids: evidence for a role in the control of reproduction and anterior pituitary hormone secretion. Endocr Rev. 1997;18(5):678–700.

Brann DW, Chorich LP, Mahesh VB. Effect of progesterone on galanin mRNA levels in the hypothalamus and the pituitary: correlation with the gonadotropin surge. Neuroendocrinology. 1993;58(5):531–8.

Briski KP, Sylvester PW. Acute inhibition of pituitary LH release in the male rat by the glucocorticoid agonist decadron phosphate. Neuroendocrinology. 1991;54(4):313–20.

Burgus R, Dunn TF, Desiderio D, Ward DN, Vale W, Guillemin R. Characterization of ovine hypothalamic hypophysiotropic TSH-releasing factor. Nature. 1970;226(5243):321–5.

Burke MC, Letts PA, Krajewski SJ, Rance NE. Coexpression of dynorphin and neurokinin B immunoreactivity in the rat hypothalamus: morphologic evidence of interrelated function within the arcuate nucleus. J Comp Neurol. 2006;498(5):712–26.

Butler JA, Sjoberg M, Coen CW. Evidence for oestrogen receptor alpha-immunoreactivity in gonadotrophin-releasing hormone-expressing neurones. J Neuroendocrinol. 1999;11(5):331–5.

Cariboni A, Pimpinelli F, Colamarino S, Zaninetti R, Piccolella M, Rumio C, Piva F, Rugarli EI, Maggi R. The product of X-linked Kallmann's syndrome gene (KAL1) affects the migratory activity of gonadotropin-releasing hormone (GnRH)-producing neurons. Hum Mol Genet. 2004;13(22):2781–91.

Cariboni A, Davidson K, Rakic S, Maggi R, Parnavelas JG, Ruhrberg C. Defective gonadotropin-releasing hormone neuron migration in mice lacking SEMA3A signalling through NRP1 and NRP2: implications for the aetiology of hypogonadotropic hypogonadism. Hum Mol Genet. 2011;20(2):336–44.

Cariboni A, Andrews WD, Memi F, Ypsilanti AR, Zelina P, Chedotal A, Parnavelas JG. Slit2 and Robo3 modulate the migration of GnRH-secreting neurons. Development. 2012;139 (18):3326–31.

Cariboni A, Andre V, Chauvet S, Cassatella D, Davidson K, Caramello A, Fantin A, Bouloux P, Mann F, Ruhrberg C. Dysfunctional SEMA3E signaling underlies gonadotropin-releasing hormone neuron deficiency in Kallmann syndrome. J Clin Invest. 2015;125(6):2413–28.

Chan YM, Broder-Fingert S, Wong KM, Seminara SB. Kisspeptin/Gpr54-independent gonadotrophin-releasing hormone activity in Kiss1 and Gpr54 mutant mice. J Neuroendocrinol. 2009;21 (12):1015–23.

Chan YM, Butler JP, Sidhoum VF, Pinnell NE, Seminara SB. Kisspeptin administration to women: a window into endogenous kisspeptin secretion and GnRH responsiveness across the menstrual cycle. J Clin Endocrinol Metab. 2012;97(8):E1458–67.

Chandran UR, Attardi B, Friedman R, Dong KW, Roberts JL, DeFranco DB. Glucocorticoid receptor-mediated repression of gonadotropin-releasing hormone promoter activity in GT1 hypothalamic cell lines. Endocrinology. 1994;134(3):1467–74.

Chandran UR, Attardi B, Friedman R, Zheng Z, Roberts JL, DeFranco DB. Glucocorticoid repression of the mouse gonadotropin-releasing hormone gene is mediated by promoter elements that are recognized by heteromeric complexes containing glucocorticoid receptor. J Biol Chem. 1996;271(34):20412–20.

Chavali GB, Nagpal S, Majumdar SS, Singh O, Salunke DM. Helix-loop-helix motif in GnRH associated peptide is critical for negative regulation of prolactin secretion. J Mol Biol. 1997;272 (5):731–40.

Cheng KW, Nathwani PS, Leung PC. Regulation of human gonadotropin-releasing hormone receptor gene expression in placental cells. Endocrinology. 2000;141(7):2340–9.

Cheng G, Coolen LM, Padmanabhan V, Goodman RL, Lehman MN. The kisspeptin/neurokinin B/dynorphin (KNDy) cell population of the arcuate nucleus: sex differences and effects of prenatal testosterone in sheep. Endocrinology. 2010;151(1):301–11.

Christian CA, Moenter SM. The neurobiology of preovulatory and estradiol-induced gonadotropin-releasing hormone surges. Endocr Rev. 2010;31(4):544–77.

Ciofi P, Krause JE, Prins GS, Mazzuca M. Presence of nuclear androgen receptor-like immunoreactivity in neurokinin B-containing neurons of the hypothalamic arcuate nucleus of the adult male rat. Neurosci Lett. 1994;182(2):193–6.

Clarke IJ, Cummins JT. GnRH pulse frequency determines LH pulse amplitude by altering the amount of releasable LH in the pituitary glands of ewes. J Reprod Fertil. 1985;73 (2):425–31.

Clarke IJ, Qi Y, Puspita Sari I, Smith JT. Evidence that RF-amide related peptides are inhibitors of reproduction in mammals. Front Neuroendocrinol. 2009;30(3):371–8.

Clarke IJ, Smith JT, Henry BA, Oldfield BJ, Stefanidis A, Millar RP, Sari IP, Chng K, Fabre-Nys C, Caraty A, Ang BT, Chan L, Fraley GS. Gonadotropin-inhibitory hormone is a hypothalamic peptide that provides a molecular switch between reproduction and feeding. Neuroendocrinology. 2012;95(4):305–16.

Clarkson J, Herbison AE. Postnatal development of kisspeptin neurons in mouse hypothalamus; sexual dimorphism and projections to gonadotropin-releasing hormone neurons. Endocrinology. 2006;147(12):5817–25.

Clarkson J, d'Anglemont de Tassigny X, Moreno AS, Colledge WH, Herbison AE. Kisspeptin-GPR54 signaling is essential for preovulatory gonadotropin-releasing hormone neuron activation and the luteinizing hormone surge. J Neurosci. 2008;28(35):8691–7.

Clarkson J, d'Anglemont de Tassigny X, Colledge WH, Caraty A, Herbison AE. Distribution of kisspeptin neurones in the adult female mouse brain. J Neuroendocrinol. 2009;21 (8):673–82.

Clarkson J, Shamas S, Mallinson S, Herbison AE. Gonadal steroid induction of kisspeptin peptide expression in the rostral periventricular area of the third ventricle during postnatal development in the male mouse. J Neuroendocrinol. 2012;24(6):907–15.

Collu R, Gibb W, Ducharme JR. Effects of stress on the gonadal function. J Endocrinol Investig. 1984;7(5):529–37.

Conn PM, Crowley Jr WF. Gonadotropin-releasing hormone and its analogs. Annu Rev Med. 1994;45:391–405.

Constantin S, Caligioni CS, Stojilkovic S, Wray S. Kisspeptin-10 facilitates a plasma membrane-driven calcium oscillator in gonadotropin-releasing hormone-1 neurons. Endocrinology. 2009;150(3):1400–12.

Corradi A, Croci L, Broccoli V, Zecchini S, Previtali S, Wurst W, Amadio S, Maggi R, Quattrini A, Consalez GG. Hypogonadotropic hypogonadism and peripheral neuropathy in Ebf2-null mice. Development. 2003;130(2):401–10.

Croucher SJ, Tickle C. Characterization of epithelial domains in the nasal passages of chick embryos: spatial and temporal mapping of a range of extracellular matrix and cell surface molecules during development of the nasal placode. Development. 1989;106(3):493–509.

Crowley Jr WF, McArthur JW. Simulation of the normal menstrual cycle in Kallman's syndrome by pulsatile administration of luteinizing hormone-releasing hormone (LHRH). J Clin Endocrinol Metab. 1980;51(1):173–5.

d'Anglemont de Tassigny X, Fagg LA, Dixon JP, Day K, Leitch HG, Hendrick AG, Zahn D, Franceschini I, Caraty A, Carlton MB, Aparicio SA, Colledge WH. H\ypogonadotropic hypogonadism in mice lacking a functional Kiss1 gene. Proc Natl Acad Sci U S A. 2007;104 (25):10714–9.

Dahl SK, Amstalden M, Coolen L, Fitzgerald M, Lehman M. Dynorphin immunoreactive fibers contact GnRH neurons in the human hypothalamus. Reprod Sci. 2009;16(8):781–7.

De Carlos JA, Schlaggar BL, O'Leary DD. Development of acetylcholinesterase-positive thalamic and basal forebrain afferents to embryonic rat neocortex. Exp Brain Res. 1995;104 (3):385–401.

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. Proc Natl Acad Sci U S A. 2003;100(19):10972–6.

Dhandapani KM, Brann DW. The role of glutamate and nitric oxide in the reproductive neuroendocrine system. Biochem Cell Biol. 2000;78(3):165–79.

Dhillo WS, Chaudhri OB, Patterson M, Thompson EL, Murphy KG, Badman MK, McGowan BM, Amber V, Patel S, Ghatei MA, Bloom SR. Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. J Clin Endocrinol Metab. 2005;90(12):6609–15.

Dubey AK, Plant TM. A suppression of gonadotropin secretion by cortisol in castrated male rhesus monkeys (*Macaca mulatta*) mediated by the interruption of hypothalamic gonadotropin-releasing hormone release. Biol Reprod. 1985;33(2):423–31.

Dulac C, Axel R. A novel family of genes encoding putative pheromone receptors in mammals. Cell. 1995;83(2):195–206.

Dungan HM, Gottsch ML, Zeng H, Gragerov A, Bergmann JE, Vassilatis DK, Clifton DK, Steiner RA. The role of kisspeptin-GPR54 signaling in the tonic regulation and surge release of gonadotropin-releasing hormone/luteinizing hormone. J Neurosci. 2007;27(44):12088–95.

Farbman AI, Buchholz JA. Growth of olfactory epithelial tissue in vitro: lectin staining of axons. Microsc Res Tech. 1992;23(2):173–80.

Farooqi IS, Jebb SA, Langmack G, Lawrence E, Cheetham CH, Prentice AM, Hughes IA, McCamish MA, O'Rahilly S. Effects of recombinant leptin therapy in a child with congenital leptin deficiency. N Engl J Med. 1999;341(12):879–84.

Ferin M, Wehrenberg WB, Lam NY, Alston EJ, Vande Wiele RL. Effects and site of action of morphine on gonadotropin secretion in the female rhesus monkey. Endocrinology. 1982;111 (5):1652–6.

Findell PR, Wong KH, Jackman JK, Daniels DV. Beta 1-adrenergic and dopamine (D1)-receptors coupled to adenylyl cyclase activation in GT1 gonadotropin-releasing hormone neurosecretory cells. Endocrinology. 1993;132(2):682–8.

Fischer UG, Wood SH, Bruhn J, Roseff SJ, Mortola J, Rivier JE, Yen SS. Effect of human corticotropin-releasing hormone on gonadotropin secretion in cycling and postmenopausal women. Fertil Steril. 1992;58(6):1108–12.

Foradori CD, Goodman RL, Adams VL, Valent M, Lehman MN. Progesterone increases dynorphin a concentrations in cerebrospinal fluid and preprodynorphin messenger ribonucleic Acid levels in a subset of dynorphin neurons in the sheep. Endocrinology. 2005;146(4):1835–42.

Fornaro M, Geuna S, Fasolo A, Giacobini-Robecchi MG. HuC/D confocal imaging points to olfactory migratory cells as the first cell population that expresses a post-mitotic neuronal phenotype in the chick embryo. Neuroscience. 2003;122(1):123–8.

Franceschini I, Lomet D, Cateau M, Delsol G, Tillet Y, Caraty A. Kisspeptin immunoreactive cells of the ovine preoptic area and arcuate nucleus co-express estrogen receptor alpha. Neurosci Lett. 2006;401(3):225–30.

Fueshko S, Wray S. LHRH cells migrate on peripherin fibers in embryonic olfactory explant cultures: an in vitro model for neurophilic neuronal migration. Dev Biol. 1994;166(1):331–48.

Harris GW. The induction of ovulation in the rabbit, by electrical stimulation of the hypothalamo-hypophysial mechanism. Proc Roy Soc Ser B. 1937;122:374–94.

Gamble JA, Karunadasa DK, Pape JR, Skynner MJ, Todman MG, Bicknell RJ, Allen JP, Herbison AE. Disruption of ephrin signaling associates with disordered axophilic migration of the gonadotropin-releasing hormone neurons. J Neurosci. 2005;25(12):3142–50.

Gearing M, Terasawa E. The alpha-1-adrenergic neuronal system is involved in the pulsatile release of luteinizing hormone-releasing hormone in the ovariectomized female rhesus monkey. Neuroendocrinology. 1991;53(4):373–81.

Geller S, Kolasa E, Tillet Y, Duittoz A, Vaudin P. Olfactory ensheathing cells form the microenvironment of migrating GnRH-1 neurons during mouse development. Glia. 2013;61(4): 550–66.

George JT, Millar RP, Anderson RA. Hypothesis: kisspeptin mediates male hypogonadism in obesity and type 2 diabetes. Neuroendocrinology. 2010;91(4):302–7.

George JT, Veldhuis JD, Roseweir AK, Newton CL, Faccenda E, Millar RP, Anderson RA. Kisspeptin-10 is a potent stimulator of LH and increases pulse frequency in men. J Clin Endocrinol Metab. 2011;96(8):E1228–36.

Giacobini P, Messina A, Morello F, Ferraris N, Corso S, Penachioni J, Giordano S, Tamagnone L, Fasolo A. Semaphorin 4D regulates gonadotropin hormone-releasing hormone-1 neuronal migration through PlexinB1-Met complex. J Cell Biol. 2008;183(3):555–66.

Gianetti E, Tusset C, Noel SD, Au MG, Dwyer AA, Hughes VA, Abreu AP, Carroll J, Trarbach E, Silveira LF, Costa EM, de Mendonca BB, de Castro M, Lofrano A, Hall JE, Bolu E, Ozata M, Quinton R, Amory JK, Stewart SE, Arlt W, Cole TR, Crowley WF, Kaiser UB, Latronico AC, Seminara SB. TAC3/TACR3 mutations reveal preferential activation of gonadotropin-releasing hormone release by neurokinin B in neonatal life followed by reversal in adulthood. J Clin Endocrinol Metab. 2010;95(6):2857–67.

Goldsmith PC, Thind KK, Perera AD, Plant TM. Glutamate-immunoreactive neurons and their gonadotropin-releasing hormone-neuronal interactions in the monkey hypothalamus. Endocrinology. 1994;134(2):858–68.

Goodman RL, Coolen LM, Anderson GM, Hardy SL, Valent M, Connors JM, Fitzgerald ME, Lehman MN. Evidence that dynorphin plays a major role in mediating progesterone negative