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Mini Review Article

Structural and functional diversification of gonadotropin inhibitory hormone (GnIH): A crucial hypothalamic neuropeptide

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Abstract

Prior to the identification of a novel RFamide neuropeptide in birds, pituitary gonadotropins, which are driven by gonadotropin-releasing hormone lacked any recognised inhibitory hormone. RFRP dodecapeptide, which is encoded by the neuropeptide VF (npvf) gene, is often referred to as gonadotropin-inhibitory hormone (GnIH) due to its inhibitory impact on the development and maintenance of gonadal glands by reducing gonadotropin production and release which was initially observed in Japanese quail. This hypothalamo-pituitary-gonadal (HPG) axis down-regulation might be common in all vertebrates. This review aims to provide a comprehensive overview of the neuroanatomical distribution of gonadotropin-inhibitory hormone (GnIH) across vertebrate species. It will trace the discovery of GnIH, which was initially identified as part of the RFamide peptide family and explore the presence of GnIH orthologous peptides in various vertebrate groups. The review will compile and synthesize current data on the localization of GnIH neuronal systems and emphasize their role in regulating reproductive processes, energy balance and other physiological functions.

Keywords: FSH, GnIH, Hypothalamus, Mammals, Reproduction

Highlights

- GnIH found in Japanese quail was one of the prime inhibitory neuropeptides which suppress synthesis of gonadotropin and release from the pituitary
- In mammals, GnIH neuron cell bodies are located in the dorsomedial hypothalamic area (DMH); while in birds, these cell bodies are present in the paraventricular nucleus (PVN). GnIH- and GnRH1- innervating terminals are present in the median eminence and the preoptic area respectively in birds and mammals
- GnIH is referred as LPXRF-amide peptides. The LPXRF-amide peptides' conserved expression in hypothalamic neurons points to a crucial function for these peptides in neuroendocrine function
- By blocking the AC/cAMP/PKA pathway, GnIH selectively suppresses GnRH and prevents the rise in intracellular calcium
- GnIH modifies reproductive behaviors in response to social contextual signals, in addition to its gonadotropin-inhibiting actions

INTRODUCTION

The hypothalamic-pituitary-gonadal axis (HPG) controls reproduction in vertebrates. In order to convey these signals into peripheral organs as reproductive output, the HPG axis receives both internal and external inputs linked to reproductive activities. From agnathans to humans, the hypothalamus and pituitary's coordination via the HPG axis is mostly preserved and it is crucial for controlling reproduction. Essentially, the hypothalamus integrates a variety of sensory inputs from nearby neuronal afferents and peripheral circulatory systems. It serves as a key homeostatic regulator for a variety of physiological processes including the control of reproduction which is one of its most significant roles. An important hypothalamic neuropeptide known as gonadotropin-inhibitory hormone (GnIH) was identified in 2000 from the anterior pituitary gland of cultured quails. This neuropeptide suppressed the production of gonadotropin (Tsutsui *et al*., 2000). Following its initial discovery in birds, GnIH was later discovered in fish, humans and other animals (Tsutsui *et al*., 2013). Although vertebrates have the same basic structure of the HPG axis, the hormones in this axis differ

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structurally and functionally depending on the animal groups and species to support reproduction and sexual activities in their own ways. The current analysis concentrates on the identification and biological significance of one of these neuropeptides, GnIH and addresses future approaches targeted at elucidating its brain targets, mechanism of action, similarities and differences between species.

Discovery of GnIH

In neurological systems, neuropeptides are ancient mediators of neuronal signaling with essential functions in controlling physiological functions and animal behavior. All of them are produced from larger precursor proteins however, they vary greatly in number from 3 to over 40 residues. Simple neuropeptide precursors produce only single neuropeptide, whereas complex precursors produce several related or unrelated neuropeptides (Beets *et al*., 2013). Both in vertebrates and invertebrates, neuropeptides are having Arg-Pheamide motif at their Carboxyl-termini, hence known as RFamide peptides. Family of RFamide peptides in vertebrates includes GnIH, NPFF, PrRP, kisspeptin (kiss1 and kiss2) and QRFP (Tsutsui *et al*., 2018). NPFF, GnIH, kiss (1 and 2) are considered to be paralogous within these groups (Osugi *et al*., 2014). GnIH is present in all avian species and the GnIH precursor encodes two GnIH-related peptides, such as GnIH-RP-1 and GnIH-RP-2 which contain a conserved Leu-Pro-X-Arg-Phe-amide motif at their C-terminal end. From a structural point of view, GnIH and GnIH-related peptides are called LPXRF amide peptides. The LPXRF-amide peptides' conserved expression in hypothalamic neurons particularly in the periventricular areas points to a crucial function for these peptides in neuroendocrine function.

RFRP-1 expression is not uniform throughout the brain regions, with significant expression in the thalamus, midbrain and optic nerve but lower expression in the hippocampus. In mice, RFRP-1 is extremely dense in the hypothalamus, especially in the compact dorsal and ventral parts of the dorsomedial nucleus (Soga *et al*., 2014). Ovine species also show a comparable pattern of distribution with GnIH neurons located in the dorsomedial hypothalamic nucleus, medio-basal hypothalamus and paraventricular nucleus. The dorsomedial hypothalamus (DMH) has a large number of GnIH cell bodies whose projections reach the innermost layer of the medial eminence and other midbrain areas such as the preoptic area (POA), the diagonal band of Broca and the anterior pituitary (Bentley *et al*., 2006). This broad distribution indicates a multifaceted role for RFRP-1 in neuroendocrine control among various species.

GnIH and GnIH receptor orthologs

Mammalian GnIH orthologs seem to function as essential neurohormones that prevent the release of gonadotropin in some mammalian species (Ubuka *et al*., 2012). Gonadotropin release in fish is also inhibited by fish GnIH orthologs, suggesting that GnIH and its orthologs play a conserved role in the regulation of HPG axis in all species (Moussavi *et al*., 2012). Therefore, it appears that GnIH and its orthologs function as a crucial neurohormone regulating vertebrate reproduction.

Three putative peptide sequences (LPXRFa 1, -2, and -3) are encoded by the GnIH gene sequence in the majority of fish species, however only two putative sequences (LPXRFa-1 and -2) are present in some teleosts including the stickleback, tetraodon and takifugu (Zhang *et al*., 2010). Three GnIH orthologs, namely RFRP-1, -2 and -3 were initially identified in bovine and human brain cDNA (Tsutsui *et al*., 2010). In rodents, however only RFRP-1 and RFRP-3 were found. The mammalian GnIH orthologs RFRP-1 and RFRP-3 contain the LPXRFamide (X = Leu or Gln) peptide, which is absent in RFRP-2. Interestingly, the putative horse RFRP-2 exhibits a unique characteristic, possessing an LPLRF-amide motif at its C-terminus (Thorson *et al*., 2014). RFRP-1 and RFRP-3 are therefore considered to be the functioning mammalian GnIH orthologs.This variation in the presence and structure of GnIH orthologs across different species highlights the complexity and diversity of these neuropeptides in mammalian systems.

In vertebrate species, two potential Gonadotropin-Inhibitory Hormone (GnIH) receptors have been identified: GPR74 and GPR147. These receptors play a crucial role in the binding of GnIH and RFRP-3 peptides, particularly within avian species. Research has demonstrated that both GPR74 and GPR147 are present in birds, but further investigation into their characteristics has revealed that GPR147 exhibits a higher affinity for avian GnIH. This finding suggests that GPR147 serves as the primary receptor for GnIH in avian species, highlighting its importance in the regulation of reproductive functions (Ikemoto *et al*., 2005). Although most teleosts have been found to only have one type of GnIH receptor gene, zebrafish have three distinct GnIH receptor types (gnihr1, gnhr2 and gnihr3) (Zhang *et al*., 2010). GnIH exerts its suppressive effects on GnRH (gonadotropin-releasing hormone) through multiple mechanisms. One key pathway involves the adenylyl

cyclase/cyclic adenosine monophosphate (cAMP)/ protein kinase A (PKA) signaling cascade. When GnIH binds to its receptor, it activates the inhibitory G-protein subunit Gαi, which subsequently inhibits adenylyl cyclase activity. This inhibition leads to reduced cAMP production and decreased PKA activation, ultimately suppressing GnRH release. Additionally, GnIH interferes with the extracellular signal-regulated kinase (ERK1/2) signaling pathway, which plays a vital role in gonadotropin transcription. By preventing the activation of ERK1/2, GnIH further inhibits the gonadotropins production. These combined actions of GnIH on multiple signaling pathways contribute to its potent suppressive effects on the reproductive axis.

Functional diversification of GnIH

In addition to innervating the median eminence, GnIH has been shown to interact directly with GnRH neurons in both avian and mammalian brains (Bentley *et al*., 2006). This interaction is facilitated by the expression of G protein-coupled receptor (GPCR) 147 on GnRH neurons (Smith *et al*., 2008). These findings suggest that GnIH can modulate reproductive function through two distinct pathways. First, it can act directly on pituitary gonadotropes via the median eminence influencing the release of gonadotropins. Second, GnIH can indirectly affect the reproductive axis by suppressing GnRH neuron activity (Tsutsui *et al*., 2012). This dual mechanism of action highlights the complex regulatory role of GnIH in the HPG axis and underscores its importance in fine-tuning reproductive processes across various species.

GnIH plays a crucial role in regulating reproductive functions in mammals and birds, acting as an autocrine/ paracrine regulator of steroidogenesis and gametogenesis within the gonads. This hormone exerts its effects through the GnIH receptor, also known as GPR147. The study by Geraghty *et al*. (2016) investigated the involvement of GnIH and its receptor in age-related reproductive decline in female rats. Their research provided insights into how the GnIH system contributes to the gradual decrease in reproductive capacity observed in aging female mammals. Female middle-aged rats showed increased expression of GnIH and GPR147 mRNA in the hypothalamus prior to an irregular estrous cycle. Kisspeptin and GnRH mRNA expression subsequently declined after this transient increase. With advancing age, ovaries also express more GnIH and GPR147. By examining the expression and activity of GnIH and GPR147 across different age groups of female rats, the study elucidated the mechanisms through which this regulatory system influences reproductive senescence, potentially offering new perspectives on reproductive aging in mammals. Photoperiodic mammals regulate their reproductive responses based on the yearly cycle of the variation observed in nocturnal melatonin secretion by the pineal gland. In birds, melatonin is important for the regulation of two seasonal functions: gonadal activity and the secretion of gonadotropin. It has been demonstrated that in quail, melatonin plays a direct role in the stimulation of GnIH neurons via the melatonin receptor subtype Mel1c that leads to the stimulation of its expression and release (Chowdhury *et al*., 2010). In addition, experiments have shown that photoperiods control the expression of GnIH in sheep (Dardente *et al*., 2008) and hamsters (Mason *et al*., 2010) emphasizing the universality of this mechanism across species. These results highlight the key role of melatonin and photoperiodic signals in coordinating reproductive activities in both birds and mammals through the control of GnIH expression and release. Within the paraventricular nucleus (PVN) of the hypothalamus, GnIH neurons directly make synaptic contact with corticotrophin-releasing hormone secreting neurons (Qi *et al*., 2009). Upon experiencing stress, either immunologic or psychological, an organism initiates a cascade of physiological events. These stressors cause a reduction in the secretion of gonadotropins and gonadotropin-releasing hormone and at the same time elevate the expression of GnIH (Iwasa *et al*., 2014). This interaction between stress and reproductive hormone shows the intricate connection between the stress response and reproductive function, how stress can affect fertility and reproductive behavior.

GnIH modifies reproductive behaviors in response to social contextual signals, in addition to its gonadotropin-inhibiting actions. It has been shown to increase neuroestrogen synthesis in the hypothalamus leading to the inhibition of sociosexual behavior in male quail (Ubuka *et al*., 2014). This mechanism demonstrates the complex interplay between neuropeptides and steroid hormones in regulating reproductive behaviors. Furthermore, studies on adult male quail have revealed that peripheral administration of GnIH results in reduced plasma testosterone levels, testicular apoptosis and impaired spermatogenic activity. These effects suggest that GnIH may exert its influence through two potential pathways: direct action on the testes or indirect action via decreased gonadotropin release from the pituitary. The dual mode of action

highlights the multifaceted role of GnIH in modulating both central and peripheral aspects of the reproductive system in avian species. Neuropeptide FF or NPFF supports metabolically advantageous macrophages which are important for preserving healthy adipose tissue (Waqas *et al*., 2017). This function is critical because the quantity and activation state of white adipose tissue macrophages directly influence the development of obesity induced metabolic diseases. The maintenance of these beneficial macrophages by NPFF contributes to overall metabolic health and adipose tissue function. Furthermore, GnIH which is related to NPFF has been demonstrated to impact metabolism and appetite in various animal species.

According to McConn *et al*. (2014), GnIH acts as an endogenous orexigen that promotes feeding in chicks. When GnIH was injected intra-cerebroventricularly (ICV) into Peking ducks, their plasma LH content dropped and their food consumption increased. GnIH's orexigenic impact is mediated by NPY and POMC neuronal activity regulation, with POMC being downregulated when GnIH is present (Jacobi *et al*., 2013). NPY fibres in Indian weaver bird is previously characterized to form a structural connection with GnIH neurons (Rastogi *et al*., 2015), thus any change in NPY could also in turn affect GnIH expression. Intracerebroventricular (ICV) injections of GnIH result in increased neuronal activity in the lateral hypothalamus region in chickens (*Gallus gallus*) as well as a heightened expression of NPY and melanin-concentrating hormone and decreased expression of POMC (McConn *et al*., 2014). In rats and mice, intraperitoneal administration of GnIH increased body mass and elevated insulin resistance, glucose intolerance, hypoinsulinism, hyperphagia, hyper-lipidaemia, and hyperglycemia (Huo *et al*., 2020). Hence, GnIH is a new neuroendocrinological regulator for blood glucose homeostasis that may implicate the pathogenesis of diabetes and obesity. GnIH can suppress reproductive activity and acts as an orexigen to promote fat accumulation in both avian and mammalian species (Anjum *et al*., 2014). Therefore, GnIH neuropeptide may be the endocrine bridge between changes in reproductive status and the dietary changes associated with obesity.

Future way forward: application perspective

The discussion of the structural and functional diversification of GnIH in mammals unveils significant implications for our understanding of reproductive regulation. The identification of GnIH and its widespread presence across various mammalian species underscores its crucial role in the complex neuroendocrine pathways governing fertility. Exploring the multifaceted functions of GnIH, from its inhibitory effects on gonadotropin release to its potential involvement in other physiological processes, provides valuable insights into the intricate mechanisms underlying mammalian reproduction. As research in this field continues to evolve, future studies should explore the potential applications of GnIH-related pathways in areas such as fertility management, reproductive disorders, and the development of novel therapeutic interventions. Continued investigation into the species-specific variations and cross-talk between GnIH and other neuroendocrine systems will further elucidate the broader implications of this critical neuropeptide in mammalian physiology.

Conclusion

GnIH is a neuropeptide that has garnered significant attention in the field of reproductive biology due to its pivotal role in the regulation of reproductive processes in various mammalian species. The discovery of GnIH and its subsequent identification in multiple vertebrates including birds and mammals has unlocked new frontiers in understanding the complex neuroendocrine pathways that govern fertility and reproduction. Its importance in neuroendocrinology is highlighted by its extensive distribution and evolutionary conservation.

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Author's contribution: ND: Conceptualized and wrote the manuscript; JM, DB,PRG,PKD, HKB: Revised the manuscript; KD: revised and approved the manuscript.

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