

## REVIEW ARTICLE

# The neuroendocrine pathways and mechanisms for the control of the reproduction in female pigs

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## Abstract

Within the hypothalamic-pituitary-gonad (HPG) axis, the major hierarchical component is gonadotropin-releasing hormone (GnRH) neurons, which directly or indirectly receive regulatory inputs from a wide array of regulatory signals and pathways, involving numerous circulating hormones, neuropeptides, and neurotransmitters, and which operate as a final output for the brain control of reproduction. In recent years, there has been an increasing interest in neuropeptides that have the potential to stimulate or inhibit GnRH in the hypothalamus of pigs. Among them, Kisspeptin is a key component in the precise regulation of GnRH neuron secretion activity. Besides, other neuropeptides, including neurokinin B (NKB), neuromedin B (NMB), neuromedin S (NMS),  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ -MSH), Phoenixin (PNX), show potential for having a stimulating effect on GnRH neurons. On the contrary, RFamide-related peptide-3 (RFRP-3), endogenous opioid peptides (EOP), neuropeptide Y (NPY), and Galanin (GAL) may play an inhibitory role in the regulation of porcine reproductive nerves and may directly or indirectly regulate GnRH neurons. By combining data from suitable model species and pigs, we aim to provide a comprehensive summary of our current understanding of the neuropeptides acting on GnRH neurons, with a particular focus on their central regulatory pathways and underlying molecular basis.

**Keywords:** pig; reproduction; neuroendocrine; GnRH neuron; RFamide-related peptide-3.

## Introduction

The reproductive function of pigs is controlled by complex regulatory networks, which integrate peripheral and internal cues and impinge at the brain centers driving the reproductive axis. GnRH is synthesized in a small subset of hypothalamic neurons, which form the final common pathway for the central control of reproduction (Herbison 2016). They integrate steroidal, lactational, hunger, stress, satiety, circadian, odorant, and pheromone signals (Spergel 2019). These signals are conveyed to a large extent by neuropeptides directly and/or indirectly, as well as by conventional neurotransmitters, gaseous transmitters, gliotransmitters, and other factors. GnRH neurons synthesize and secrete GnRH in a pulsatile manner from axon terminals in the median eminence (ME) into the hypothalamo-hypophyseal circulation through which it is transported to the anterior pituitary gland. By binding to specific receptors (gonadotropin-releasing hormone receptors, GnRHRs) on pituitary gonadotropin cells, GnRH stimulates the biosynthesis and the release of two gonadotropins (luteinizing hormone, LH; follicle-stimulating hormone, FSH). LH and FSH, which are required for the development and maintenance of the gonads and thus for fertility, bind to receptors on the

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gonads to regulate gametogenesis and gonadal steroidogenesis in both sexes (Millar 2005; Muro et al., 2021).

Extensive research has shown that several neuropeptides have been viewed as modulators or regulators of GnRH neurons in the porcine hypothalamus, including Kisspeptins, which have a stimulating effect on the activity and synthesis of GnRH neurons, and RFamide-related peptide-3 (RFRP-3; gonadotropin inhibitory hormone, GnIH), which has an inhibitory effect on the activity and synthesis of GnRH neurons (Herbison 2016). It is now well established from a variety of studies, that the coordination effect of excitatory neuronal signaling coupled with inhibitor neuronal input to the GnRH pulse generator controls the function of the HPG axis, thereby driving and maintaining the reproductive ability of pigs (Spergel 2019). Although recent advancements in neuropeptides that regulate porcine GnRH have been made, our understanding of the central nervous system network of porcine reproduction remains incomplete. This review focuses on the neuropeptides which have been viewed as regulators of GnRH neuronal activity and/ or reproductive function and whether they act directly on GnRH neurons in pigs. The complete elucidation of the novel neuropeptidergic and molecular mechanisms summarized in this review will not only expand our knowledge of the intimate mechanisms responsible for the reproductive in pigs but might also provide new tools and targets for better prevention and management of pig reproduction in practice (Peltoniemi et al., 2019).

### **The important role of gonadotropin-releasing hormone (GnRH) neurons in pig reproduction**

The activation of the hypothalamic-pituitary axis is critical for the initiation and maintenance of reproductive cycles in pigs and is influenced by a number of factors, such as nutrition, metabolism, and gonadal steroids (Garcia et al., 2020; Marín-García and Llobat 2021). At present, it is universally admitted that GnRH neurons in pigs function as brain sensors and main effectors for the modulation of the hypothalamus level. The cell bodies of GnRH neurons, which receive neuropeptidergic inputs from neurons in the hypothalamus and other brain areas, are distributed in the preoptic area (POA) at the organum vasculosum of the lamina terminalis (OVLT) level, medial basal hypothalamus (MBH) including the arcuate nucleus (ARC), and in the anterior hypothalamic area (AHA) (Lents et al., 2020).

In pigs, GnRH pulsation is essential for maintaining gonadotropin gene expression and the physiological pattern of gonadotropin secretion. The pulse frequencies of GnRH and LH are known to change throughout the estrous cycle and postpartum period. In the luteal phase of gilts, a pattern of LH secretion characterized by high-amplitude, low-frequency pulses, and reduced serum concentrations of LH, as well as increased serum concentrations of FSH were associated with the low-frequency GnRH pulse. In the follicular phase of gilts, the transition of the GnRH pulse mode to a high frequency resulting in the pattern of LH secretion changing to high-frequency, low-amplitude pulses, and a decrease in FSH synthesis and release. The interaction between GnRH and LH/FSH found that the synthesis and pulsatile secretion of GnRH from neurons in the hypothalamus drives pulsatile secretion of LH, and to a lesser extent, FSH in gilts (Tsutsumi and Webster 2009).

### **Neuropeptide modulators of GnRH neuronal activity, GnRH secretion and reproduction that act directly on GnRH neurons**

#### **Kisspeptin**

Kisspeptin is the peptide encoded by the KISS1 gene, and the Kisspeptin receptor is a G-protein-coupled receptor, GPR54 (Ohtaki et al., 2001; Seminara et al., 2003). Kisspeptin has emerged as a key regulator of reproductive function in pigs when it was discovered that boars in which a functional Kisspeptin receptor was knocked out with gene-editing technology presented a condition of hypogonadotropic hypogonadism (Sonstegard et al., 2017). The boars

are characterized by a lack of gonadal development and low levels of gonadotropin secretion from the anterior pituitary gland that failed to transition through puberty (de Roux et al., 2003; Semple et al., 2005). A substantial body of evidence indicates that Kisspeptin has potent stimulatory action on the secretion of gonadotropin hormones in gilts (Lents et al., 2008; Ralph et al., 2017). Likewise, accumulating evidence showed that central and peripheral treatment with Kisspeptin stimulated gonadotropin secretion, particularly LH secretion, in various mammalian species, including rodents, sheep, goats, cattle, and horses (Thompson et al., 2004; Messenger et al., 2005; Shahab et al., 2005; Caraty et al., 2007; Kadokawa et al., 2008; Magee et al., 2009; Hashizume et al., 2010). In sheep, Kisspeptin receptor is expressed in the GnRH neurons of the hypothalamus, and intracerebroventricular infusion of Kisspeptin caused a dramatic increase in serum LH and FSH, accompanied by a concomitant release of the cerebrospinal fluid GnRH content (Messenger et al., 2005; Caraty et al., 2007; Smith et al., 2011). Moreover, Kisspeptin-induced LH secretion was abolished in ewes treated with neutralizing antibodies to GnRH, and in ewes in which the hypothalamus had been disconnected from the pituitary to eliminate GnRH input to gonadotroph cells, indicating that Kisspeptin stimulates LH secretion in a GnRH-dependent manner (Arreguin-Arevalo et al., 2007; Smith et al., 2008b). Although there are no similar studies in pigs, the direct action of Kisspeptin on GnRH neurons is also inferred from the spatial distribution of the Kisspeptin expression within the porcine hypothalamus.

The localization of the Kisspeptin expression within the porcine hypothalamus has not been fully characterized (Lents 2019). In the central nervous system of pigs, Kisspeptin cells are localized primarily in two discrete regions involved in the regulation of gonadotropin secretion, including the MBH within the ARC and the periventricular (PeV) nucleus (Tomikawa et al., 2010; Ieda et al., 2014; Thorson et al., 2017). Within the ARC of the pig, a spatially distinct pattern of KISS1 is seen, with the greatest expression occurring in the medio-caudal sections, similar to the ARC distribution of Kisspeptin observed in sheep and cattle (Redmond et al., 2011; Cardoso et al., 2015; Lents et al., 2020). Preliminary immunocytochemistry data illustrate that neuronal cell bodies as well as nerve fibers for Kisspeptin are evident in the porcine ARC. Thus, it is anticipated that the neuroanatomical distribution of Kisspeptin neurons in the porcine ARC is like that of other species. Specifically, Kisspeptin neurons in the POA regulate GnRH cell bodies, whereas Kisspeptin neurons in the ARC act on GnRH terminal axons in the median eminence (Lents et al., 2020).

Estradiol has a biphasic effect in pigs, inhibiting basal LH pulses via negative feedback then stimulating an ovulatory surge of LH through positive feedback (Ieda et al., 2014; Thorson et al., 2017). When sexually mature OVX gilts were given a dose of estradiol sufficient to stimulate an ovulatory surge of LH, the expression of Kisspeptin in the PeV was upregulated compared with control OVX gilts (Tomikawa et al., 2010; Silva et al., 2021). It is inferred that separate populations of Kisspeptin neurons in the ARC and the PeV of gilts mediate negative and positive estrogen feedback for the control of tonic and surge LH secretion, respectively. Moreover, previous research has established that the initiation of puberty and postpartum reproductive cycles in gilts are metabolically gated. Recent work by Thorson et al. (2018) has established that short-term (10 days) negative energy balance induced reduced frequency and increased amplitude of LH pulses, but no differences in ARC the transcription of Kisspeptin between feed-restricted and full-fed gilts were observed (Thorson et al., 2018). Surveys such as that conducted by Zhou et al. (2014) have shown that feed restriction to cyclic gilts for a prolonged period (100 days) resulted in the point that they ceased cycling, and mRNA expressions for Kisspeptin, Kisspeptin receptor, and GnRH were all downregulated in the MBH; on the contrary, Kisspeptin and its receptor mRNA expression were upregulated in the hypothalamic tissue containing the caudal POA and PeV of pigs fed a higher-energy diet (Zhou et al., 2014). This implies that nutrition-induced changes in LH pulse patterns of pigs may depend on hypothalamic subpopulations of Kisspeptin neurons that respond differently to nutritional signals in mediating the GnRH pulse generator.

### **$\alpha$ -Melanocyte-stimulating hormone ( $\alpha$ -MSH)**

The anorexigenic neuropeptide  $\alpha$ -MSH is synthesized and released by pro-opiomelanocortin (POMC) neurons and the  $\alpha$ -MSH analogue melanotan II, which act directly via MC3Rs and MC4Rs on most (70%) GnRH neurons to increase their firing rate and induce the expression of c-Fos in rats (Israel et al., 2012; Roa and Herbison 2012; Lane and Whitaker 2018; Spergel 2019; Xu et al., 2021). In pigs, the distribution and location of POMC perikarya are identified specifically within and around the immediate location of the ARC. Fibers for POMC were noted as projecting rostrally from the ARC to the SCN, SOP, LHA, medial POA, and dbB (Lents et al., 2020). GnRH neurons in the hypothalamus of gilts showed numerous close contacts with POMC-containing varicosities, and both GnRH and POMC fibers have extensive overlap within the ME, supporting the expectation that  $\alpha$ -MSH may control the release of GnRH and(or) Kisspeptin for increased LH pulses. Neither ICV treatment of prepubertal OVX gilts with a melanocortin agonist (NDP-MSH) or SHU9119 affected LH secretion, offering contradictory findings (Barb et al., 2004). Moreover, NPY expression increased, while promelanin-concentrating hormone expression decreased with no change in AGRP and POMC expression in NDP-MSH-treated pigs, suggesting  $\alpha$ -MSH may be involved in regulating energy homeostasis to reproduction in pigs (Barb et al., 2010).

### **RFamide-related peptide-3 (RFRP-3)**

The RFamide-related peptide (RFRP) gene, an ortholog to the gonadotropin-inhibiting hormone (GnIH) gene that was first detected in the hypothalamic-hypophysial system of avian species and regulates avian reproduction by decreasing gonadotropin release and synthesis by acting on the GnRH system and the anterior pituitary gland, was found to encode 3 biological peptides, RFRP-1, RFRP-2, and RFRP-3 (Hinuma et al., 2000; Legagneux et al., 2009). The receptors of RFRP widely distributed in the central nervous system, pituitary, and gonads in pigs include GPR147 or NPFF1R, the canonical receptor for RFRP, and GPR74 or NPFF2R albeit with much less potency (Yoshida et al., 2003; Bentley et al., 2010). Compelling evidence has documented a role of RFRP-3 in regulating GnRH and GHRH neuronal expression and function as well as secretion of gonadotrophin and steroid hormones and impacting the expression of Kisspeptin in mammals (Johnson et al., 2007; Ducret et al., 2009; Kadokawa et al., 2009; Ancel et al., 2012; Li et al., 2013). The pig RFRP gene was found to be abundantly expressed in the nervous system (cerebellum, cerebrum, hypothalamus, and pituitary) and reproduction system (ovary, oviduct, uterus, and testis), and is thought to be a candidate gene for porcine reproductive traits (Fang et al., 2014).

Using in situ hybridization and immunohistochemistry, the spatial distribution of RFRP has been investigated in the porcine hypothalamus. RFRP-ir neuronal cell bodies and nerve fibers were located in the posterior hypothalamus, dorsomedial hypothalamic nucleus (DMH), and ventromedial hypothalamic nucleus (VMH), with a similar distribution as that in the brain of rodents, sheep, and mares (Yano et al., 2003; Kriegsfeld et al., 2006; Gibson et al., 2008; Smith et al., 2008a; Qi et al., 2009; Thorson et al., 2014). The most abundant population of porcine RFRP-ir was observed in the paraventricular nucleus of the hypothalamus (PVN), agreeing with observations in sheep and nonhuman primates (Yano et al., 2003; Kriegsfeld 2006; Smith et al., 2012). In sheep, RFRP fibers project from the PVN to the lateral hypothalamic area, the ARC, and the external zone of the ME, as well as being closely associated with many other neurons, such as GnRH, POMC, NPY, orexin, and Kisspeptin neurons (Smith et al., 2008a, 2012). Moderately dense RFRP fibers were observed in the lateral hypothalamic area of the porcine hypothalamus, but no RFRP fibers were observed in the external zone of the ME of gilts, which would suggest that RFRP is not released into the hypophyseal portal vasculature of the pig as has been observed in sheep (Li et al., 2013).

Early reports indicated that RFRP-3 attenuated GnRH-stimulated gonadotropin synthesis and the release from primary cultures of porcine anterior pituitary cells (Zmijewska et al., 2020). Furthermore, GnRH secretion from porcine hypothalamic explants was suppressed upon treatment with RFRP-3 (Li et al., 2013). Additionally, there was a study demonstrated that RFRP-3 inhibits the Kisspeptin-activated GnRH firing rate in vitro, but the addition of RFRP-3 to porcine cultured pituitary cells had no antagonistic effect on the Kisspeptin-induced stimulation of LH secretion (Wu et al., 2009;

Zmijewska et al., 2020). These results indicate that RFRP-3 may be a negative regulator of pituitary gonadotropin synthesis and release via GnRH neurons. On the contrary, accumulating evidence further showed that central administration of RFRP-3 into the lateral ventricles of the brain within OVX gilts had no effect on the secretory patterns of LH (Thorson et al., 2017), which is consistent with the effects of RFRP-3 injected into the third ventricle of OVX ewes (Caraty et al., 2012; Decourt et al., 2016). When RFRP-3 was largely infused into the peripheral circulation of OVX gilts, LH secretion remained unchanged, unless very high doses were given. It also took large pharmacological doses of RFRP-3 to impact the pulsatile secretion of LH in intact mature boars (Thorson et al., 2015). As noted, the porcine RFRP preproprotein can yield an RFRP-2 peptide that ruminants and rodents do not produce (Yoshida et al., 2003). RFRP-2 is in the same position within the preproprotein as is avian GnIH and the amino acid sequence of porcine RFRP-2 has greater sequence homology with avian GnIH than RFRP-3, so it was speculated that RFRP-2 was a porcine-specific GnIH. However, when OVX gilts received infusions of RFRP-2 into the peripheral circulation, LH pulses were unaffected (Thorson et al., 2017). Because RFRP does not appear to have a potent suppressive effect on *in vivo* LH secretion in gilts, either centrally or peripherally, some authors have suggested that RFRP does not appear to act as a hypophysiotropic GnIH in pigs. Interestingly, the influence of RFRP on LH within mammals has been highly inconsistent; for instance, several studies have shown that RFRP-3 can inhibit, have no effect on, or even stimulate LH secretion (Yano et al., 2003; Murakami et al., 2008; Anderson et al., 2009; Rizwan et al., 2009; Pineda et al., 2010). It therefore remains unclear what role RFRP plays in porcine reproduction, requiring further research and analysis for pigs.

### Endogenous opioid peptides (EOP)

Endogenous opioid peptides (EOP) consist collectively of endorphins, enkephalins, and dynorphins, playing important roles in suppressing LH secretion in luteal phase gilts and lactating sows. Immunocytochemical studies in gilts demonstrated the existence of pro-opiomelanocortin (POMC) perikarya in the ARC with fibers projecting to the MBH, PeV, ME, and preoptic area (Weems et al., 2018). Following intracerebroventricular (ICV) treatment with morphine, an EOP agonist, decreased secretion of LH, and FSH in prepubertal OVX gilts. In contrast, the general opioid receptor antagonist, naloxone, increased LH release in prepubertal gilts. Naloxone stimulated GnRH secretion from the hypothalamic-preoptic area collected from gilts, indicating that the EOP may inhibit LH secretion by action at the central nervous system. Moreover, naloxone failed to increase LH release in boars given antisera against GnRH. An adeno-hypophysial site of action in modulating LH release is supported by *in vitro* studies in which  $\beta$ -endorphin decreased basal and GnRH-induced LH secretion by porcine pituitary cells. In contrast, exposure to naloxone increased basal secretion of LH and enhanced pituitary responsiveness to GnRH (Wylot et al., 2013).

### Neuropeptide Y (NPY)

Neuropeptide Y (NPY) is a 36-amino-acid tyrosine-rich peptide that was first isolated from porcine brain extracts in 1982 and belongs to the "NPY family" of biologically active peptides. It includes two gut hormones: peptide YY (PYY) and pancreatic polypeptide (PP). NPY affects target cells by activating various G-coupled receptors belonging to the rhodopsin-like superfamily of receptors (Siawrys and Buchowski, 2018). Additionally, NPY as an orexigenic peptide is simultaneously involved in the modulation of the GnRH/LH system and could provide a link between nutrition and reproduction. In the pig, NPY receptors are widely distributed in the structures of the central nervous system and innervating peripheral organs, with a predominant localization in stalk-median eminence (SME), POA, MBH, and the pituitary (Óvilo et al., 2010). NPY is widespread in the porcine CNS, including the limbic system, olfactory system, hypothalamoneurohypophysial tract, corpus striatum, and cerebral cortex. Barb revealed that the central administration of NPY suppresses serum LH concentrations and LH pulse frequency in OVX prepubertal gilts (Barb et al., 2006). Generally, based on studies performed with different species, there are many reports indicating that NPY may regulate LH secretion at the hypothalamic level by directly and/or indirectly modulating the activity of the GnRH neuronal system (Wójcik-Gładysz and Polkowska 2006; Dhillon et al., 2009; Roa and Herbison 2012; Amstalden et al., 2014). Moreover, morphological studies have proved that NPY neurons come in close contact with

GnRH neurons in POA, ARC, and ME (Klenke et al., 2010). It is expected that NPY suppresses LH pulse frequency by inhibiting both GnRH and Kisspeptin cells in the gilt hypothalamus, but this problem requires further and more detailed investigation, including on the use of specific agonists and/or antagonists of all NPY receptor subtypes.

### **Galanin (GAL)**

Galanin (GAL) is a brain-gut neuropeptide widely distributed in the POA, MBH, and pituitary stalk-median eminence of the cyclic gilt (Czujkowska and Arciszewski 2016). The anorexigenic neuropeptide GAL expression and immunoreactivity are regulated by Estrogen (Spiegel 2019). GAL is likely co-released with Kisspeptin from subsets of Kisspeptin neuron axons onto GnRH neurons and both the GALR1 and GALR2 subtypes of GAL receptor appear to be expressed in GnRH neurons (Constantin and Wray 2016). GAL stimulated basal but not GnRH-induced LH secretion from porcine pituitary glands in vitro and antiserum to galanin suppressed GnRH-induced LH release (Elsaesser 2001).

### **Neuropeptides that may or may not act directly on GnRH neurons**

#### **Neurokinin B (NKB)**

Inactivating mutations in genes encoding neurokinin B (NKB) or its receptor NK3R results in hypogonadotropic hypogonadism and failure to attain puberty in rodents and humans, a phenotype reminiscent of that of patients with mutations of Kiss1 or GPR54 (de Roux et al., 2003; Topaloglu et al., 2009). In addition, initial studies in mice and sheep, later confirmed in other species, found that the Kisspeptin neurons in the ARC co-express neurokinin B (NKB), also known as tachykinin 3 (TAC3), which acts through its homologous receptor (TAC3R) (Burke et al., 2006; Goodman et al., 2007; Navarro et al., 2009). Similarly, studies in pig ARC have shown that virtually all Kisspeptin neurons in co-express NKB (Lents et al., 2020). Because the roles of NKB and its receptor in regulating GnRH of the pig are not presently understood, the expression of TAC3R has recently been localized throughout several areas of the hypothalamus in ovariectomized (OVX) gilts (Lindo 2018). Many of the hypothalamic regions that display TAC3R immunostaining in the pig also contain GnRH neurons. Furthermore, the greatest number of TAC3R expressing cells are the dbB and the PVN, followed by the POA and SCN. The PeV and RCh have evidence of TAC3R immunostaining (Lindo 2018). Although about 40% of GnRH neurons occurred in close apposition to TAC3R containing cells, close contacts were few, suggesting that the TAC3R containing cells in the porcine hypothalamus do not directly regulate GnRH neurons in pigs.

It is postulated from molecular, anatomical, and physiological data that NKB acts as an autoregulatory signal for Kisspeptin-neurokinin B-dynorphin (KNDy) neurons, stimulating Kisspeptin output to GnRH neurons. The fact that NKB operates via Kisspeptin signaling to modulate GnRH neurons is supported by a wealth of data, including the demonstration that within monkeys the desensitization of TAC3R blocks the effect of senktide (an NKB agonist) on gonadotropin release, and the fact that ICV injection of senktide into rodents induces c-Fos in ARC Kiss1 neurons, which express NK3R and are excited by NKB (Navarro et al., 2009, 2011; Ramaswamy et al., 2011). Moreover, the effect of senktide is absent in GPR54 null mice but is preserved in mice engineered to maintain Kisspeptin actions only in GnRH neurons, attesting that NKB signaling is upstream of Kisspeptin in the control of GnRH neurons (de Croft et al., 2013). There is little published data on NKB in pigs, but the spatial distribution of NKB expression within the porcine hypothalamus has inferred the function of NKB on GnRH neurons as well. Furthermore, some studies in the gilt suggested that the amplitude of LH pulses may be regulated by NKB, and the pubertal decrease in sensitivity to oestrogen negative feedback in the gilt involves the gene expression for TAC3 and the TAC3R gene in the medial basal hypothalamus (MBH) (Thorson et al., 2018). Thus, it is likely that NKB in pigs acts through an autoregulatory mechanism involving TAC3R to induce the release of Kisspeptin, which acts directly on the GnRH neuronal network to stimulate a pulsatile release of GnRH and subsequently LH.

### **Neuromedin B (NMB)**

Neuromedin B (NMB) is a member of a family of bombesin-like peptides in mammals, which are decapeptides originally identified in porcine spinal cords (Jensen et al., 2008). Amino acid sequences from sequenced cDNA show that the Neuromedin B receptor (NMBR) in pigs is typical of the G protein-coupled receptor (GPCR) family, serving as a 390-amino acid protein with seven membrane spanning domains (Ohki-Hamazaki et al., 2005). NMB/NMBR is an important physiological regulator of smooth muscle contraction, via activation of intracellular signaling pathways. The expression of NMB mRNA in the central nervous system (CNS) as well the presence of NMBR mRNA and protein in the pituitary, testis, ovaries, and uterus, suggest the potential physiological functions of the NMB/NMBR system during reproduction in pigs (Ma et al., 2016). Moreover, the expression patterns of NMB and NMBR mRNA along the reproductive axis for female pigs across the estrous cycle and for male pigs at postnatal development stages, support the suggestion that NMB may control the release of GnRH through the regulation of NMBR secretion (Ma et al., 2018). It has previously been shown that NMB can stimulate the HPG axis via hypothalamic GnRH in male rats (Boughton et al., 2013). However, the mechanism and physiological function of the NMB/NMBR system on porcine reproduction are currently not fully understood, requiring further research and analysis.

### **Neuromedin S (NMS)**

Neuromedin S (NMS) has been reported to have many physiological functions in mammals and has been identified as an endogenous ligand for two orphan G protein-coupled receptors, FM-3/GPR66 (NMU1R) and FM-4/TGR-1 (NMU2R) (Roesler et al., 2012). The distribution and location of NMS have mainly been identified in the pig's hypothalamic region, including in the periventricular nucleus (PEN), PVN, SCN, supraoptic nucleus (SON), VMH, and ARC (Mori et al., 2005). NMU2R is widely distributed in male pig hypothalamic cells, anterior pituitary cells, and Leydig cells, suggesting that the NMS/NMU2R system existed in the male pig reproductive axis and may play a significant role in the regulation of gonadotropin secretion in the brain and testis, which was similar to its function in other mammals (Yang et al., 2012; Ma et al., 2017). At present, evidence has revealed that NMS increases the release of LH and FSH from anterior pituitary cells and testosterone from Leydig cells as well as the expression of NMU2R and GnRH mRNAs in hypothalamic cells, NMU2R, LH, and FSH mRNAs in anterior pituitary cells; moreover, it downregulated the expression of GnRH mRNA in hypothalamic cells (Jin et al., 2019). Interestingly, lateral ventricle injection of NMS could significantly decrease LH response including serum LH level and LH mRNA expression in ovariectomized pigs, which is partially consistent with the previous results on rats (Yang et al., 2010). The inhibitory effect of NMS on LH contradicts previous reports of a positive role of NMS on the reproductive axis in vitro, but these results indicated that NMS may play an important role in the regulation of reproductive function via the NMU2R or GnRH.

### **Nesfatin-1**

The hypothalamic peptide, Nesfatin-1, derived from the precursor NEFA/nucleobindin 2 (NUCB2), was identified as anorexigenic signal, acting in a leptin-independent manner (Garcia-Galiano et al., 2010). Nesfatin-1 is expressed in the ventrolateral medulla (VLM), dorsal vagal complex (DVC), PVN, ARC, and SON of the pig brain (Gaigé et al., 2013). Accumulating evidence indicated that Nesfatin-1 regulates glucose metabolism, insulin secretion, gastrointestinal motility, stress response, development, cardiovascular functions, anxiety, and the onset of puberty (Aydin, 2013). Nesfatin-1 stimulates the secretion of GnRH and LH $\beta$  in vitro, raising the possibility of Nesfatin-1 acting directly on hypothalamic neurons and gonadotropes (Kalló et al., 2012).

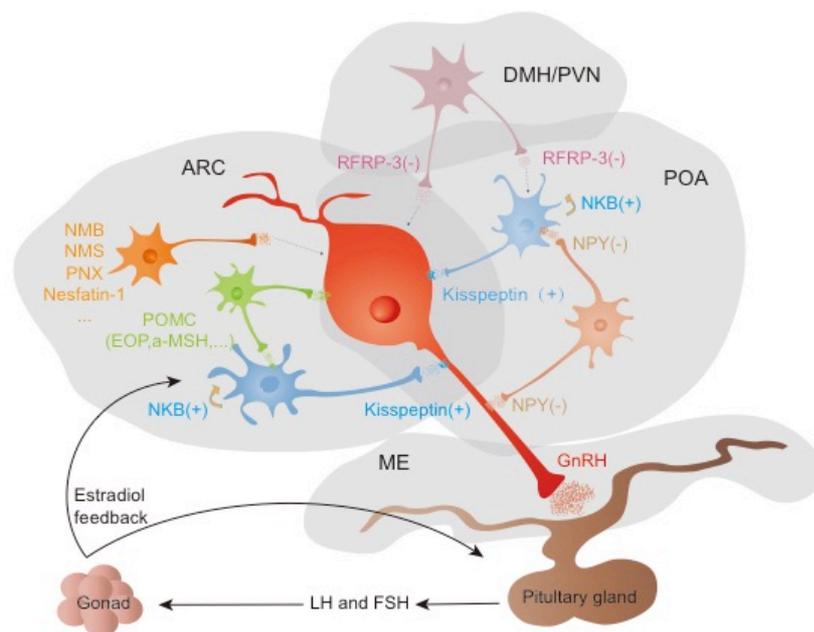
### **Phoenixin (PNX)**

Phoenixin (PNX) is a recently discovered hypothalamic neuropeptide, first identified in 2013 (Yosten et al., 2013). Phoenixin acts through its receptor, G protein-coupled receptor 173 (GPR173), to activate the cAMP/PKA pathway resulting in the phosphorylation of CREB (pCREB)

(Treen et al., 2016). PXN potentiates GnRH-stimulated LH release and increases GnRH and KISS1 gene expression, respectively, and it also raises the expression of GnRH receptor gene (Clarke and Dhillon, 2019). Alternatively, compromise of PXN in vivo using siRNA led to a reduction in GnRH receptor expression in the pituitary and the delayed appearance of oestrus (Nguyen et al., 2019). Until now, it is expected that PNx may have important roles in the regulation of porcine reproductive function, yet to be delineated (Lepiarczyk et al., 2020).

### Conclusion

In this review, beginning from the anatomic distribution and pharmacological function of neuropeptides, we follow the neuroendocrine pathways and mechanisms that control reproduction in pigs. GnRH neurons play a particularly critical role in the function of the reproductive central nervous system and act as the intermediate factor between the hypothalamus and hypophysis. The activity of GnRH neurons is regulated by different neuropeptides, forming a central control network. Kisspeptin neurons along with neurokinin B in the POA regulate GnRH cell bodies and in the ARC act on GnRH terminal axons in the median eminence, which is essential for GnRH neurons to stimulate LH secretion in pigs. Rather, it is speculated that RFRPs act as the essential upstream regulators in the control of GnRH secretion with an inhibitory effect in pigs, as has been proposed for rats and nonhuman primates, but remains unclear. NPY and POMC cells function as metabolic sensors for the activation of GnRH secretion, acting as inhibitory and excitatory signals, respectively. Additionally, EOP inhibition of GnRH secretion in pigs involves the direct suppression of noradrenergic neurons, which may come about with increasing sexual maturity. Several neuropeptides may play an important role in the regulation of reproductive functions via hypothalamic GnRH, such as NMB, NMS, PNx, GAL, Nesfatin-1, and as such, require further research and analysis (Figure 1).



**Figure 1.** Schematic illustration of the reproductive neuroendocrine pathways in pigs. The reproduction of pigs is operated by the HPG axis, in which GnRH neurons act as the intermediate factor between the hypothalamus and hypophysis. GnRH neurons directly or indirectly receive regulatory inputs from a wide array of regulatory signals and pathways, involving numerous neuropeptides and neurotransmitters. Kisspeptin neurons with neurokinin B in the POA regulate GnRH cell bodies and in the ARC act on GnRH terminal axons in the median eminence, stimulating GnRH secretion. NPY and POMC cells function as metabolic sensors for the activation of GnRH secretion, acting as inhibitory and excitatory signals, respectively. Rather, it is speculated that RFRP-3, NMB, NMS, PNx, GAL, and Nesfatin-1 act as the essential upstream regulators in the control of GnRH secretion, but remains unclear.

The list of neuropeptides known to modulate or potentially modulate GnRH neuronal activity and GnRH secretion in gilts will likely grow as the effects on GnRH neurons of additional neuropeptides, including those of neuropeptides that are yet to be discovered, are investigated. Although it is clear, based mostly on pharmacological and immunohistochemical studies, that some of the neuropeptides investigated thus far impact directly GnRH neurons and the receptors to which they bind on GnRH neurons, further research is required to understand their signaling mechanisms in GnRH neurons and to determine whether other known and yet undiscovered neuropeptides affect GnRH neurons directly or indirectly.

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#### Author contributions

SZ: Conceptualization, Investigation, Data curation, Writing – original draft; WX : Conceptualization, Supervision, Writing – review & editing; ZYG: Resources, Supervision, Writing – review & editing; PQW: Funding acquisition, Project administration.