General and Comparative Endocrinology 277 (2019) 3–8

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Recent studies of LPXRFa receptor signaling in fish and other vertebrates

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ARTICLE INFO

Keywords:
GnIH
LPXRFa
LPXRFa receptor
Signaling pathway
GnRH
Kisspeptin

ABSTRACT

The hypothalamo–pituitary–gonadal (HPG) axis plays a major role in coordinating the reproduction of fish and other vertebrates. Gonadotropin-releasing hormone (GnRH) is the primary stimulatory factor responsible for the hypothalamic control of gonadotropin secretion. In 2000, a previously unidentified hypothalamic neuropeptide was isolated from the brain of Japanese quail and termed gonadotropin-inhibitory hormone (GnIH) based on its ability to directly inhibit gonadotropin release from the cultured quail anterior pituitary gland. One year later, the cDNA sequence that encodes the quail GnIH precursor polypeptide was cloned and was found to encompass two further peptides (GnIH-related peptide (RP)-1 and GnIH-RP-2) besides GnIH. To date, GnIH orthologous have been detected in a variety of vertebrates from fish to humans. These peptides possess a characteristic-LPXRFa (X = L or Q) motif at the C-terminus and are designated as LPXRFa peptides. It is generally accepted that LPXRFa peptides act on GnRH neurons in the hypothalamus to inhibit gonadotropin synthesis and release in addition to affecting the pituitary function in birds and mammals. However, the exact physiological role of LPXRFa is still uncertain in fish and dual actions of LPXRFa on the HPG axis have been observed. Research aiming to elucidate the detailed signaling pathways mediating the actions of LPXRFa on target cells may contribute to understanding the functional divergence of the LPXRFa system in teleosts. Accordingly, this review will discuss the recent advances in LPXRFa receptor signaling, as well as the potential interactions on cell signaling induced by other factors, such as GnRH and kisspeptin.

1. Introduction

The neuroendocrine regulation of reproduction in vertebrates, including fish, is primarily mediated through the hypothalamo–pituitary–gonadal (HPG) axis with each component secreting specific neuropeptides or hormones. In 2000, a novel hypothalamic dodecapeptide with RFamide at the C-terminus was isolated from the brain of the Japanese quail and was shown to inhibit gonadotropin release from cultured anterior pituitary gland, thus this peptide was named gonadotropin-inhibitory hormone (GnIH) (Tsutsui et al., 2000). This was the first demonstration of a hypothalamic neuropeptide inhibiting gonadotropin release in any vertebrate. Subsequently, GnIH homologs have been identified in a number of vertebrates, including mammals, birds, reptiles, amphibians and fish (Munoz-Cueto et al., 2017; Ogawa and Parhar, 2014; Ubuka et al., 2016; Ullah et al., 2016). GnIH is also called RFamide-related peptide (RFRP) in mammals or LPXRFa in teleosts. All these peptides possess a characteristic C-terminal LPXRFa (X = L or Q) motif and are designated as LPXRFa peptides which form a new group of the RFamide peptide family (Tsutsui, 2009). In this review, LPXRFa will refer to all LPXRFa orthologous (GnIH, RFRP and LPXRFa) regardless of species unless otherwise stated.

It has been clearly established that LPXRFa serves as a key player in the regulation of reproduction across vertebrates, acting on the brain, pituitary and gonads to modulate reproductive physiology and behavior (Kriegsfeld et al., 2018; Munoz-Cueto et al., 2017; Ubuka and Parhar, 2018; Ubuka et al., 2018; Ubuka et al., 2016). Despite its functional significance, the molecular mechanism of LPXRFa actions on the target cells has not been fully elucidated and is just emerging in fish. This article primarily summarizes the advances made in our knowledge regarding the intracellular signal transduction pathways activated by LPXRFa in fish, but information from other vertebrates is included so as to provide a more complete picture, especially where data in fish are

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https://doi.org/10.1016/j.ygcen.2018.11.011
Received 15 August 2018; Received in revised form 17 October 2018; Accepted 17 November 2018
Available online 19 November 2018

minimal or absent.

2. Identification and physiological actions of LPXRFa in fish

2.1. Diversity of LPXRFa peptides in fish

The potential for the existence of piscine LPXRFa system was initially validated in goldfish where a cDNA encoding three LPXRFa peptides (gfLPXRFa-1, -2, and -3) was cloned, and gfLPXRFa-3 was identified as a mature peptide (Sawada et al., 2002). Subsequently, LPXRFa orthologs were identified and characterized in zebrafish (Zhang et al., 2010), grass puffer (Shahjahan et al., 2011), lamprey (Osugi et al., 2012), tilapia (Biran et al., 2014), orange-spotted grouper (Wang et al., 2015), the cichlid fish *Cichlasoma dimerus* (Di Yorio et al., 2016), common carp (Peng et al., 2016), sea bass (Paullada-Salmeron et al., 2016a), Senegalese sole (Aliaga-Guerrero et al., 2018) and tongue sole (Wang et al., 2018a). In most fish species, these LPXRFa genes encode a polypeptide that is possibly cleaved into three putative peptides (LPXRFa-1, -2, and -3), while only two putative sequences (LPXRFa-1 and -2) are present in other teleosts such as tongue sole, sea bass, stickleback and the puffer fish (Munoz-Cueto et al., 2017; Paullada-Salmeron et al., 2016a; Shahjahan et al., 2011; Wang et al., 2018a; Zhang et al., 2010). Notably, multiple amino acid sequence alignments of LPXRFa precursors from different fish species showed that in some teleosts LPXRFa-1 and LPXRFa-2 peptides have an MPXRFa (X = M, L, or Q) motif at their C-terminus instead of the conserved LPXRFa (X = L or Q) motif observed in other teleosts and tetrapods (Munoz-Cueto et al., 2017; Ogawa and Parhar, 2014; Ubuka and Parhar, 2018). It should be noted that the C-terminal LPXRFa structure appears critical for the binding of LPXRFa peptides to their cognate receptors (Yin et al., 2005). Thus, elevated structural diversity is present among LPXRFa peptides in bony fish which may contribute to the functional divergence of LPXRFa.

2.2. Functional roles of LPXRFa peptides in fish

The biological effects of LPXRFa on reproduction have been approached in a few fish species, and results obtained are in some cases conflicting. An initial study performed in sockeye salmon showed that three heterologous LPXRFa peptides (gfLPXRFa-1, -2 and -3) stimulated the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from cultured pituitary cells (Amano et al., 2006). Similarly, tilapia LPXRFa-2 positively increased LH and FSH release both in *vivo* and *in vitro* (Biran et al., 2014), and *Cichlasoma dimerus* LPXRF-2 could act as an FSH-releasing factor in intact pituitary cultures of this cichlid fish (Di Yorio et al., 2016). Additionally, gfLPXRFa-1 enhanced *lhβ* and *fshβ* gene expression in grass puffer pituitary *in vitro* (Shahjahan et al., 2011). On the other hand, intraperitoneal (ip) injection of zebrafish LPXRFa-3 reduced the plasma LH levels in goldfish (Zhang et al., 2010), and *Cichlasoma dimerus* LPXRFa-1 also inhibited LH and FSH release in intact pituitary cultures (Di Yorio et al., 2016). Zebrafish LPXRFa-3 downregulated pituitary *lhβ* and *gthα* expression *in vitro* (Spicer et al., 2017). Ip injection of gfLPXRFa-2 and gfLPXRFa-3 significantly reduced *fshβ* mRNA levels in female goldfish, while only gfLPXRFa-2 was able to decrease *lhβ* expression (Qi et al., 2013a). Similarly, ip injection of grouper LPXRFa-2 and common carp LPXRFa-3 also decreased *lhβ* transcript levels in these two species, respectively (Peng et al., 2016; Wang et al., 2015). Moreover, intracerebroventricular (icv) injection and peripheral implants of LPXRFa-2 reduced the expression of *lhβ* subunit in sea bass (Paullada-Salmeron et al., 2016b; Paullada-Salmeron et al., 2016c), and intramuscular (im) injection of LPXRFa-3 provoked a significant reduction in *lhβ* expression in sexually maturing Senegalese sole males (Aliaga-Guerrero et al., 2018). Interestingly, gfLPXRFa-3 exerted both stimulatory and inhibitory effects on pituitary LH release and gonadotropin subunit mRNA levels depending on maturational status and administration route (Moussavi et al., 2012, 2013).

Notably, the actions of LPXRFa on brain neuroendocrine systems also seem to differ among species. A recent study performed in Senegalese sole showed that im injection of LPXRFa-3 provoked a significant reduction in brain *gnrh3* expression (Aliaga-Guerrero et al., 2018), and LPXRFa-3 also reduced *gnrh3* transcript levels in brain slices of zebrafish (Spicer et al., 2017). Similarly, ip injection of gfLPXRFa-2 and gfLPXRFa-3 downregulated hypothalamic *gnrh3* mRNA levels in the same species (Qi et al., 2013a), but stimulatory effects of grouper LPXRFa-3 on *gnrh3* expression have been reported in ip-injected orange-spotted grouper (Wang et al., 2015). In tongue sole, LPXRFa-1 increased hypothalamic expression of *gnrh2* mRNAs, whereas LPXRFa-2 inhibited *gnrh3* mRNA expression *in vitro* (Liu et al., 2017). Interestingly, the effects of LPXRFa-2 on *gnrh2* expression in the brain of male sea bass appear dependent of the route of administration because icv injection of LPXRFa-2 reduced *gnrh2* mRNA levels, whereas im injection of LPXRFa-2 increased *gnrh2* expression (Paullada-Salmeron et al., 2016b; Paullada-Salmeron et al., 2016c). Taken together, the physiological and functional properties of LPXRFa peptides vary greatly in teleosts, depending on the species, the physiological status, the administered peptide, and the route of administration. For more detailed information of physiological actions of LPXRFa in fish, the reader is also referred to another two excellent, recent reviews (Munoz-Cueto et al., 2017; Ubuka and Parhar, 2018).

3. Mode of LPXRFa actions

3.1. Identification and localization of LPXRFa receptor in fish

Identification of LPXRFa receptor (LPXRFa-R) is critical to investigate the cellular mechanisms of LPXRFa actions which will give insight into the physiological significance of LPXRFa in vertebrates. Shortly after the discovery of GnIH in quail, a novel G-protein coupled receptor (GPCR) GPR147 cDNA encoding the putative receptor for GnIH was cloned in the same species (Yin et al., 2005). Subsequently, LPXRFa-R with characteristic seven transmembrane domains has also been found in other avian and mammalian species (Ogawa and Parhar, 2014; Ubuka et al., 2016). In teleosts, different situations have been observed concerning the number of paralogous LPXRFa-R genes. Three LPXRFa-R forms have been identified in zebrafish (Zhang et al., 2010), goldfish (Qi et al., 2013a) and common carp (Peng et al., 2016), while only one type of LPXRFa-R gene was obtained from grass puffer (Shahjahan et al., 2011), tilapia (Biran et al., 2014), orange-spotted grouper (Wang et al., 2015), cinnamon clownfish (Choi et al., 2016) and tongue sole (Wang et al., 2018b). All LPXRFa-Rs among different fish species contain an extracellular N-terminus, a seven transmembrane domain and a cytoplasmic C-terminus, which are characteristic of GPCRs (Naor, 2009; Yin et al., 2005).

Understanding of the localization and distribution of LPXRFa-R in the central and peripheral tissues is important to elucidate multiple action sites and potential functions of LPXRFa. The expression patterns of LPXRFa-R have been detected in various tissues of zebrafish (Zhang et al., 2010), grass puffer (Shahjahan et al., 2011), tilapia (Biran et al., 2014), orange-spotted grouper (Wang et al., 2015), cinnamon clownfish (Choi et al., 2016), common carp (Peng et al., 2016) and tongue sole (Wang et al., 2018b) by RT-PCR, which showed that the distribution of LPXRFa-R mRNA was in a species-specific manner. Generally, a substantial degree of LPXRFa-R mRNA expression was observed in the brain of all teleosts examined. Moreover, the expression of LPXRFa-R mRNA was also evident in the pituitary of zebrafish (Zhang et al., 2010), grass puffer (Shahjahan et al., 2011), tilapia (Biran et al., 2014), orange-spotted grouper (Wang et al., 2015), cinnamon clownfish (Choi et al., 2016), common carp (Peng et al., 2016), tongue sole (Wang et al., 2018b) as well as the gonads of these fish species excluding grass puffer. The predominant expression of LPXRFa-R mRNA in the brain, pituitary and gonads suggests that LPXRFa may serve as a key player of

reproduction in fish and regulate the reproductive axis by acting not only at the brain and pituitary levels but also on gonadal physiology (Munoz-Cueto et al., 2017). Notably, the precise cellular localization of LPXRFa-R has also been elucidated in the brain, pituitary and gonads of goldfish using *in situ* hybridization (Qi et al., 2013a; Qi et al., 2013b), and in the brain and pituitary of tilapia using a combination of *in situ* hybridization and immunohistochemistry (Biran et al., 2014; Ogawa et al., 2016), which provides the morphological evidence of LPXRFa actions on the target cells and further confirms the involvement of LPXRFa in the regulation of reproduction in fish.

3.2. LPXRFa-R signaling in fish

Members of the GPCR family typically couple to either Gαq, Gαs or Gαi, with coupling to Gαq resulting in stimulation of phospholipase C (PLC) and activation of Ca^{2+} channels, and coupling to Gαs or Gαi resulting in activation or inhibition of adenylyl cyclase (AC), respectively. In turn, PLC hydrolyses phosphatidyl inositol diphosphate to inositol triphosphate and diacylglycerol triggering cascades of activation of protein kinases, while AC regulates intracellular cAMP levels which activates cAMP-dependent protein kinase (PKA), and triggers the binding of cAMP response element binding protein (CREB) to cAMP response element (CRE) on target genes (Bedecarrats et al., 2009; Naor, 2009; Shimizu and Bedecarrats, 2010). In addition, the presence of phosphorylation sites in LPXRFa-R suggests that the receptor potentially conveys its signal via the PKA and/or PKC pathways (Biran et al., 2014; Ikemoto and Park, 2005; Shahjahan et al., 2011; Wang et al., 2018b). Multiple signals may underlie the functional diversity of the LPXRFa system, however the mechanisms through which LPXRFa exert its functions have not been fully elucidated in fish. Recently, the molecular mechanisms of LPXRFa actions have been investigated in few fish species using an mammalian cell line transfected with its cognate receptor and these results will be discussed below.

Response element (RE) assays, namely CRE-dependent luciferase (CRE-luc) as a reporter gene for PKA activation and serum responsive element-dependent luciferase (SRE-luc) as a reporter gene for the PKC pathway, were employed to clarify the possible signal transduction pathways involved in LPXRFa actions. Signal-transduction analysis of the tilapia LPXRF-R in COS-7 cells showed clear stimulation of CRE-luc activity and SRE-luc activity by LPXRFa-2, indicating that tilapia LPXRFa-R signals can be transduced via both PKA and PKC pathways (Biran et al., 2014). However, contradictory results were observed in orange-spotted grouper where all three grouper LPXRFa peptides significantly decreased forskolin-induced CRE-luc activity and only LPXRFa-1 inhibited SRE-luc activity in COS-7 cells expressing its cognate receptor (Wang et al., 2015). In zebrafish, all three LPXRFa peptides activated LPXRFa-R2 and LPXRFa-R3 via the PKA pathway (CRE-luc), while no activation of the PKC pathway (SRE-luc) was observed by any of the three LPXRFa peptides with any of the three LPXRFa-Rs (Spicer et al., 2017). In medaka, LPXRFa inhibited forskolin-induced CRE-luc activity in a dose-dependent manner when LPXRFa was applied at lower concentrations (from 10^{-13} to 10^{-9} M) to LPXRFa-R expressing HEK293-T cells. Surprisingly, at higher concentrations ranging from 10^{-9} to 10^{-5} M, this inhibition diminished in a dose-dependent manner. On the other hand, LPXRFa dose-dependently increased CRE-luc activity at concentrations of 10^{-8} M and higher in the absence of forskolin, demonstrating a possible switch of coupling of LPXRFa-R to Gαi and Gαs proteins in medaka (Akazome et al., 2015).

More recently, we also investigated tongue sole LPXRF-R signaling using COS-7 cells combined with a pharmacological approach (Wang et al., 2018b). No increase in CRE-luc activity was observed when COS-7 cells expressing tongue sole LPXRFa-R were stimulated with a wide range of concentrations of LPXRFa-1 or LPXRFa-2, suggesting that tongue sole LPXRFa-R dose not couple to Gαs protein. However, activation of tongue sole LPXRFa-R by LPXRFa-2 significantly inhibited forskolin-induced CRE-luc activity, implying that this receptor may couple to Gαi protein and inhibit AC activity. On the other hand, both LPXRFa-1 and LPXRFa-2 increased SRE-luc activity in COS-7 cells transfected with tongue sole LPXRFa-R in a dose-dependent manner. To further establish the possible participation of the PLC/PKC pathway in the action of tongue sole LPXRFa peptides, we employed the specific PLC inhibitor U73122 and the PKC inhibitor GF109203X. The LPXRFa-induced SRE-luc activity was significantly reduced by these two inhibitors. In sum, these results suggest that tongue sole LPXRFa-R may convey its signal through the PKA and PKC pathways (Wang et al., 2018b).

It is worth mentioning that all the signal transduction studies now were performed on the mammalian cell lines, which just demonstrated whether LPXRFa and its receptor are really functional in fish. Nevertheless, it is not yet clear whether these studies could reveal the authentic post-receptor signaling events evoked by LPXRFa using mammalian cell lines. Thus, further studies performed in primary cultures are urgently needed to confirm the signaling pathways activated by the LPXRFa/LPXRFa-R system, as did in mammals (Clarke et al., 2008; Li et al., 2013; Sari et al., 2009).

3.3. LPXRFa-R signaling in other vertebrates

In mammals, Hinuma and colleagues reported a specific receptor for RFRP and named it OT7T022, which was identical to LPXRFa-R (Hinuma et al., 2000). Moreover, no mobilization of intracellular Ca^{2+} or release of arachidonic-acid metabolites was detected in Chinese hamster ovarian (CHO) cells expressing rat LPXRFa-R after stimulation with human LPXRFa peptides (hRFRP-1 and hRFRP-3), whereas LPXRFa suppressed forskolin-induced production of cAMP. This result suggests that rat LPXRFa-R may couple to Gαi but not to Gαq protein in these cells (Hinuma et al., 2000). Similarly, human LPXRFa (hRFRP-1) lowered forskolin-induced cAMP production in CHO cells expressing human LPXRFa-R and it was abolished by pretreatment with an inhibitor of Gαi protein (pertussis toxin), suggesting that human LPXRFa-R also couples to Gαi protein in the signal transduction pathway (Fukusumi et al., 2001). In addition, hRFRP-3 inhibited gonadotropin- and forskolin-induced intracellular cAMP accumulation in human granulosa-lutein (hGL) cells via activation of Gαi (Oishi et al., 2012). On the other hand, ovine LPXRFa (RFRP-3) potently inhibited GnRH-stimulated mobilization of intracellular Ca^{2+} and GnRH-induced extracellular signal-regulated kinases (ERK) phosphorylation in cultured sheep pituitary cells (Clarke et al., 2008; Sari et al., 2009). Likewise, human LPXRFa (hRFRP-3) markedly reduced ERK phosphorylation in primary pig granulosa cells (Li et al., 2013). Whether and how Ca^{2+} and ERK routes mediate the action of LPXRFa have yet to be fully elucidated in fish.

In chickens, Ikemoto and Park initially reported that LPXRFa peptides reduced Gαi mRNA levels in COS-7 cells transiently transfected with chicken LPXRFa-R in a dose-dependent manner (Ikemoto and Park, 2005), but it did not address coupling of the G protein. Subsequently, Shimizu and Bédécarrats investigated the effect of LPXRFa on the activation of several second messengers indicative of possible coupling to Gαq, Gαs or Gαi in GH3 cells (a rat somatolactotrope cell line) transiently transfected with the chicken LPXRFa-R (Shimizu and Bedecarrats, 2010). No response in inositol phosphates accumulation was observed when GH3 cells transfected with chicken LPXRFa-R were stimulated with LPXRFa. Similarly, no increase in CRE-luc activity was observed, suggesting chicken LPXRFa-R does not couple to Gαq or Gαs. However, LPXRFa significantly reduced forskolin-induced CRE-luc activity, suggesting that chicken LPXRFa-R couples to Gαi to inhibit the production of cAMP (Shimizu and Bedecarrats, 2010).

Son and collaborators elaborated the signaling pathway triggered by LPXRFa and its possible interaction with GnRH signaling using a mouse gonadotrope cell line (LβT2) in which both endogenous receptors are expressed (Son et al., 2012). First, mouse LPXRFa peptides (mRFRP-1, mRFRP-3L and mRFRP-3S) effectively reduced GnRH-induced CRE-luc

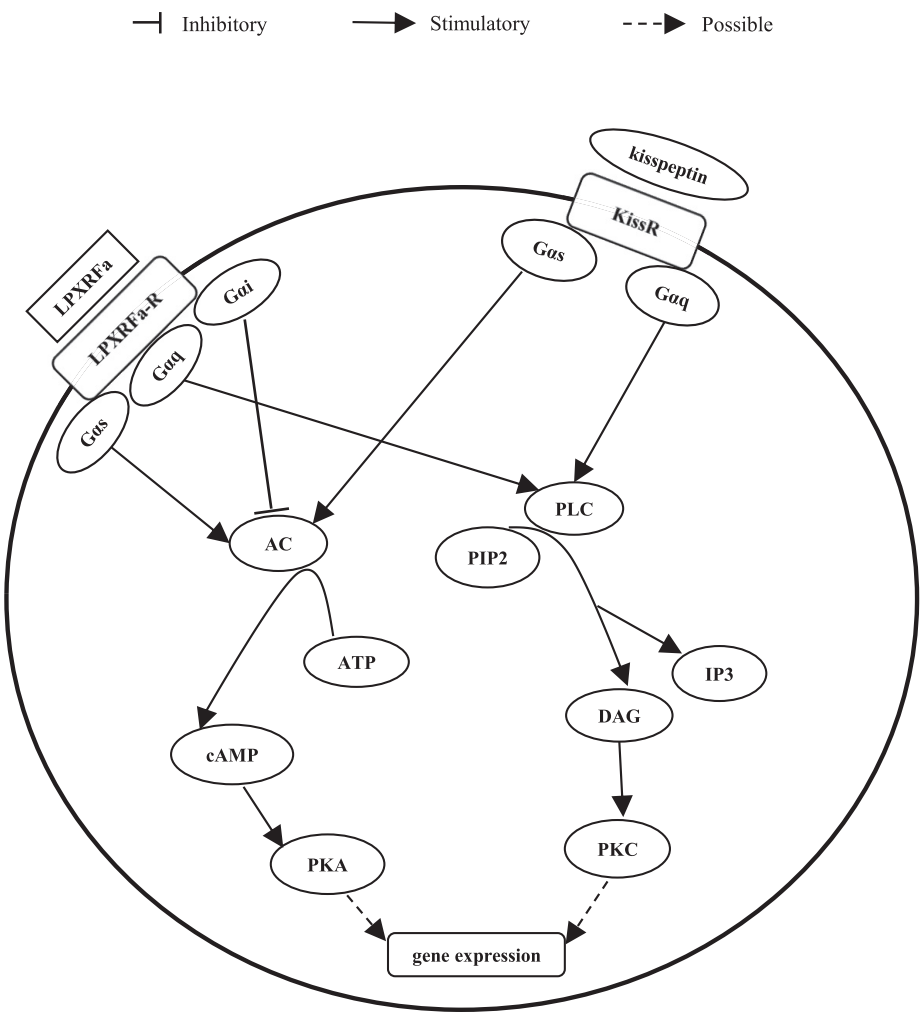

Fig. 1. A proposed model for LPXRFa receptor (LPXRFa-R) signaling and its possible interaction with kisspeptin receptor (KissR) signaling in fish. Upon binding to its specific receptor, LPXRFa activates different signal pathways. In tilapia and tongue sole, coupling to Gαq activates phospholipase C (PLC)/protein kinase C (PKC) pathway, possibly stimulating gene transcription. In tilapia, zebrafish and medaka, coupling to Gαs activates adenylate cyclase (AC)/protein kinase A (PKA) pathway, thus resulting in gene expression. However, in grouper, tongue sole and medaka, binding of LPXRFa to its receptor inhibites AC via Gαi. This inhibition results in a decrease in kisspeptin-induced cAMP response, and the down-regulation of target genes. Moreover, in zebrafish and tongue sole, LPXRFa exerts an inhibitory effect on kisspeptin activation of the PLC/PKC pathway. Please see the text for details. Gαq, Gαs and Gαi, heterotrimeric G proteins; DAG, diacylglycerol; PIP2, phosphatidylinositol 4,5-bisphosphate; IP3, inositol 1,4,5-trisphosphate.

activity and cAMP production in LβT2 cells, demonstrating the inhibitory effect of LPXRFa on GnRH-induced AC/cAMP/PKA activation. Next, mouse LPXRFa peptides also suppressed GnRH-elicited ERK phosphorylation and this effect was mediated by the inhibition of the AC/cAMP/PKA pathway. Finally, mouse LPXRFa peptides abolished GnRH-stimulated gonadotropin subunit gene transcriptions and LH release. Taken together, these results suggest that LPXRFa exerts an inhibitory action on GnRH-induced gonadotropin subunit gene transcriptions by suppressing AC/cAMP/PKA-dependent ERK pathway in LβT2 cells (Son et al., 2012). Furthermore, the potential signal transduction pathway that conveys the inhibitory action of LPXRFa in GnRH neurons was investigated by using a mouse GnRH neuronal cell line, GT1-7 (Son et al., 2016). Mouse LPXRFa peptides effectively eliminated the stimulatory effect of vasoactive intestinal polypeptide (VIP) on CRE-luc activity, p38 and ERK phosphorylation in GT1-7 cells. Notably, LPXRFa specifically inhibited VIP-induced p38 and ERK activation via the AC/cAMP/PKA pathway. Overall, these results suggest that LPXRFa exerts its inhibitory effect by specifically acting via the AC/cAMP/PKA pathway in GnRH neurons as in gonadotropes (Son et al., 2016).

3.4. Interactions between LPXRFa and other factors on cell signaling

As mentioned above, LPXRFa-R is a member of the GPCR family which couples to Gαi and, upon activation inhibits adenylyl cyclase activity, thus reducing intracellular cAMP levels. This implies that LPXRFa could interfere with signaling of any GPCR coupled to Gαs, such as GnRH and kisspeptin receptors (Naor, 2009; Pasquier et al., 2014). Indeed, when GH3 cells transfected with both chicken LPXRFa-R and GnRH-R were stimulated simultaneously with LPXRFa and GnRH peptides, a dose-dependent reduction in GnRH-induced CRE-luc activity was observed (Shimizu and Bedecarrats, 2010). Currently, no information exists about the inhibitory effect of LPXRFa on GnRH-R signaling in teleosts, which is urgently needed to be examined in futher studies.

Additionally, the intracellular mechanisms through which kisspeptin exerts its functions have been elucidated in several fish species, including zebrafish (Biran et al., 2008; Lee et al., 2009), goldfish (Chang et al., 2012; Jiang et al., 2014; Li et al., 2009), orange-spotted grouper (Shi et al., 2010), chub mackerel (Ohga et al., 2013), medaka (Kanda et al., 2013), sea bass (Felip et al., 2015), tongue sole (Wang et al., 2017), European eel (Pasquier et al., 2018), Southern bluefin tuna and yellowtail kingfish (Nocillado et al., 2012). Although piscine kisspeptin receptor (KissR) signals have been shown to be transduced via the PKA and PKC pathways as well as intracellular Ca^{2+} mobilization, studies on the potential interaction of LPXRFa with kisspeptin signaling are still scarce, even in other vertebrates. Our recent studies showed that tongue sole LPXRFa-R couples to Gαi protein while tongue sole Kiss2R is coupled to the Gαs-mediated signaling pathway, thus activation of LPXRFa-R by LPXRFa-2 significantly reduced Kiss2-evoked CRE-luc activity, demonstrating an inhibitory action of LPXRFa on Kiss2R signaling involved in the PKA pathway (Wang et al., 2017, 2018b). Interestingly, both tongue sole LPXRFa-R and Kiss2R are coupled to Gαq protein (Wang et al., 2017, 2018b), we hypothesized that a synergistic activity could be detected for LPXRFa and kisspeptin combined *in vitro*. However LPXRFa significantly reduced Kiss2-induced SRE-luc activity when compared to stimulation with Kiss2 alone,

indicating that LPXRFa may also antagonize the action of Kiss2R by inhibiting the PKC pathway (Wang et al., unpublished data). Similarly, although none of zebrafish LPXRFa peptides altered SRE-luc activity with any of the three LPXRFa-Rs, LPXRFa-2 and LPXRFa-3 suppressed Kiss2 activation of the PKC pathway (Spicer et al., 2017). In addition, LPXRFa-2 but not LPXRFa-3 also inhibited Kiss1 activation of the PKC pathway (Spicer et al., 2017). On the other hand, LPXRFa had no inhibitory effect on kisspeptin stimulation of SRE-luc and nuclear factor of activated T-cells response element (NFAT RE-luc) activities and ERK phosphorylation, indicating that LPXRFa may not directly inhibit kisspeptin signaling in GnRH neurons (Son et al., 2016). Future studies are needed to light previously unknown interactions of GnIH with GnRH and kisspeptin.

4. Conclusions

Athough the physiological relevance and functions of LPXRFa system were evaluated from fish to mammals, the intricate web of intracellular signals mediating the action of LPXRFa is still far from being fully understood. This review summarized the intracellular signal transduction pathways in response to LPXRFa, focusing chiefly on teleosts (Fig. 1). The information gathered hitherto clearly indicates differential involvement of signaling pathways in the action of LPXRFa, and multiple signals may contribute to the functional divergence of LPXRFa. Nevertheless, it must be taken into account that much more needs to be done to elucidate the signaling events occurring in response to the activation of LPXRFa at all levels of the reproductive axis in fish and other vertebrates.

Acknowledgments

This work was supported by grants from National Natural Science Foundation of China (31602133), Natural Science Foundation of Shandong Province (ZR2016CB02), Qingdao Municipal Science and Technology Bureau (17-1-1-61-jch), and China Agriculture Research System (CARS-47).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2018.11.011.

References

Akazome, Y., Yamamoto, E., Oka, Y., 2015. Ligand Dose-Dependent Switch in G-Protein Coupling (Gi and Gs) of Medaka (Oryzias latipes) Neuropeptide FF Receptors, NPFFR1 (GPR147) and NPFFR2 (GPR74). Endocrine Society's 97th Annual Meeting and Expo March 7, San Diego, California.

Aliaga-Guerrero, M., Paullada-Salmeron, J.A., Piquer, V., Mananos, E.L., Munoz-Cueto, J.A., 2018. Gonadotropin-inhibitory hormone in the flatfish, *Solea senegalensis*: molecular cloning, brain localization and physiological effects. J. Comp. Neurol. 526, 349–370.

Amano, M., Moriyama, S., Iigo, M., Kitamura, S., Amiya, N., Yamamori, K., Ukena, K., Tsutsui, K., 2006. Novel fish hypothalamic neuropeptides stimulate the release of gonadotrophins and growth hormone from the pituitary of sockeye salmon. J. Endocrinol. 188, 417–423.

Bedecarrats, G.Y., McFarlane, H., Maddineni, S.R., Ramachandran, R., 2009. Gonadotropin-inhibitory hormone receptor signaling and its impact on reproduction in chickens. Gen. Comp. Endocrinol. 163, 7–11.

Biran, J., Ben-Dor, S., Levavi-Sivan, B., 2008. Molecular identification and functional characterization of the kisspeptin/kisspeptin receptor system in lower vertebrates. Biol. Reprod. 79, 776–786.

Biran, J., Golan, M., Mizrahi, N., Ogawa, S., Parhar, I.S., Levavi-Sivan, B., 2014. LPXRFa, the piscine ortholog of GnIH, and LPXRF receptor positively regulate gonadotropin secretion in Tilapia (*Oreochromis niloticus*). Endocrinology 155, 4391–4401.

Chang, J.P., Mar, A., Wlasichuk, M., Wong, A.O., 2012. Kisspeptin-1 directly stimulates LH and GH secretion from goldfish pituitary cells in a Ca^{2+}-dependent manner. Gen. Comp. Endocrinol. 179, 38–46.

Choi, Y.J., Kim, N.N., Habibi, H.R., Choi, C.Y., 2016. Effects of gonadotropin inhibitory hormone or gonadotropin-releasing hormone on reproduction-related genes in the protandrous cinnamon clownfish, *Amphiprion melanopus*. Gen. Comp. Endocrinol. 235, 89–99.

Clarke, I.J., Sari, I.P., Qi, Y., Smith, J.T., Parkington, H.C., Ubuka, T., Iqbal, J., Li, Q., Tilbrook, A., Morgan, K., Pawson, A.J., Tsutsui, K., Millar, R.P., Bentley, G.E., 2008. Potent action of RFamide-related peptide-3 on pituitary gonadotropes indicative of a hypophysiotropic role in the negative regulation of gonadotropin secretion. Endocrinology 149, 5811–5821.

Di Yorio, M.P., Perez Sirkin, D.I., Delgadin, T.H., Shimizu, A., Tsutsui, K., Somoza, G.M., Vissio, P.G., 2016. Gonadotropin-inhibitory hormone in the cichlid fish *Cichlasoma dimerus*: structure, brain distribution and differential effects on the secretion of gonadotropins and growth hormone. J. Neuroendocrinol. 28. https://doi.org/10.1111/jne.12377.

Felip, A., Espigares, F., Zanuy, S., Gomez, A., 2015. Differential activation of kiss receptors by Kiss1 and Kiss2 peptides in the sea bass. Reproduction 150, 227–243.

Fukusumi, S., Habata, Y., Yoshida, H., Iijima, N., Kawamata, Y., Hosoya, M., Fujii, R., Hinuma, S., Kitada, C., Shintani, Y., Suenaga, M., Onda, H., Nishimura, O., Tanaka, M., Ibata, Y., Fujino, M., 2001. Characteristics and distribution of endogenous RFamide-related peptide-1. Biochim. Biophys. Acta 1540, 221–232.

Hinuma, S., Shintani, Y., Fukusumi, S., Iijima, N., Matsumoto, Y., Hosoya, M., Fujii, R., Watanabe, T., Kikuchi, K., Terao, Y., Yano, T., Yamamoto, T., Kawamata, Y., Habata, Y., Asada, M., Kitada, C., Kurokawa, T., Onda, H., Nishimura, O., Tanaka, M., Ibata, Y., Fujino, M., 2000. New neuropeptides containing carboxy-terminal RFamide and their receptor in mammals. Nat. Cell Biol. 2, 703–708.

Ikemoto, T., Park, M.K., 2005. Chicken RFamide-related peptide (GnIH) and two distinct receptor subtypes: identification, molecular characterization, and evolutionary considerations. J. Reprod. Dev. 51, 359–377.

Jiang, Q., He, M., Ko, W.K., Wong, A.O., 2014. Kisspeptin induction of somatolactin-alpha release in goldfish pituitary cells: functional role of cAMP/PKA-, PLC/PKC-, and Ca^{2+}/calmodulin-dependent cascades. Am. J. Physiol. Endocrinol. Metab. 307, E872–884.

Kanda, S., Akazome, Y., Mitani, Y., Okubo, K., Oka, Y., 2013. Neuroanatomical evidence that kisspeptin directly regulates isotocin and vasotocin neurons. PLoS One 8, e62776.

Kriegsfeld, L.J., Jennings, K.J., Bentley, G.E., Tsutsui, K., 2018. Gonadotropin-inhibitory hormone (GnIH) and its mammalian ortholog RFamide-related peptide-3 (RFRP-3): discovery and functional implications for reproduction and stress. J. Neuroendocrinol. 30, e12597.

Lee, Y.R., Tsunekawa, K., Moon, M.J., Um, H.N., Hwang, J.I., Osugi, T., Otaki, N., Sunakawa, Y., Kim, K., Vaudry, H., Kwon, H.B., Seong, J.Y., Tsutsui, K., 2009. Molecular evolution of multiple forms of kisspeptins and GPR54 receptors in vertebrates. Endocrinology 150, 2837–2846.

Li, S., Zhang, Y., Liu, Y., Huang, X., Huang, W., Lu, D., Zhu, P., Shi, Y., Cheng, C.H., Liu, X., Lin, H., 2009. Structural and functional multiplicity of the kisspeptin/GPR54 system in goldfish (*Carassius auratus*). J. Endocrinol. 201, 407–418.

Li, X., Su, J., Fang, R., Zheng, L., Lei, R., Wang, X., Lei, Z., Jin, M., Jiao, Y., Hou, Y., Guo, T., Ma, Z., 2013. The effects of RFRP-3, the mammalian ortholog of GnIH, on the female pig reproductive axis in vitro. Mol. Cell. Endocrinol. 372, 65–72.

Liu, Q., Wang, B., Liu, X., Xu, Y., Shi, B., Liu, Z., 2017. Effects of gonadotropin-inhibitory hormone peptides on the reproduction-related gene expression in the hypothalamus of half-smooth tongue sole (*Cynoglossus semilaevis*). Prog Fish Sci 38, 56–62.

Moussavi, M., Wlasichuk, M., Chang, J.P., Habibi, H.R., 2012. Seasonal effect of GnIH on gonadotrope functions in the pituitary of goldfish. Mol. Cell. Endocrinol. 350, 53–60.

Moussavi, M., Wlasichuk, M., Chang, J.P., Habibi, H.R., 2013. Seasonal effect of gonadotrophin inhibitory hormone on gonadotrophin-releasing hormone-induced gonadotroph functions in the goldfish pituitary. J. Neuroendocrinol. 25, 506–516.

Munoz-Cueto, J.A., Paullada-Salmeron, J.A., Aliaga-Guerrero, M., Cowan, M.E., Parhar, I.S., Ubuka, T., 2017. A journey through the gonadotropin-inhibitory hormone system of fish. Front. Endocrinol. (Lausanne) 8, 285.

Naor, Z., 2009. Signaling by G-protein-coupled receptor (GPCR): studies on the GnRH receptor. Front. Neuroendocrinol. 30, 10–29.

Nocillado, J.N., Biran, J., Lee, Y.Y., Levavi-Sivan, B., Mechaly, A.S., Zohar, Y., Elizur, A., 2012. The Kiss2 receptor (Kiss2r) gene in Southern Bluefin Tuna, *Thunnus maccoyii* and in Yellowtail Kingfish, *Seriola lalandi* – functional analysis and isolation of transcript variants. Mol. Cell. Endocrinol. 362, 211–220.

Ogawa, S., Parhar, I.S., 2014. Structural and functional divergence of gonadotropin-inhibitory hormone from jawless fish to mammals. Front. Endocrinol. (Lausanne) 5, 177.

Ogawa, S., Sivalingam, M., Biran, J., Golan, M., Anthonysamy, R., Levavi-Sivan, B., Parhar, I.S., 2016. Distribution of LPXRFa, a Gonadotropin-inhibitory hormone (GnIH) ortholog peptide and LPXRFa Receptor in the Brain and Pituitary of the Tilapia. J. Comp. Neurol. 524, 2753–2775.

Ohga, H., Fujinaga, Y., Selvaraj, S., Kitano, H., Nyuji, M., Yamaguchi, A., Matsuyama, M., 2013. Identification, characterization, and expression profiles of two subtypes of kisspeptin receptors in a scombroid fish (chub mackerel). Gen. Comp. Endocrinol. 193, 130–140.

Oishi, H., Klausen, C., Bentley, G.E., Osugi, T., Tsutsui, K., Gilks, C.B., Yano, T., Leung, P.C., 2012. The human gonadotropin-inhibitory hormone ortholog RFamide-related peptide-3 suppresses gonadotropin-induced progesterone production in human granulosa cells. Endocrinology 153, 3435–3445.

Osugi, T., Daukss, D., Gazda, K., Ubuka, T., Kosugi, T., Nozaki, M., Sower, S.A., Tsutsui, K., 2012. Evolutionary origin of the structure and function of gonadotropin-inhibitory hormone: insights from lampreys. Endocrinology 153, 2362–2374.

Pasquier, J., Kamech, N., Lafont, A.G., Vaudry, H., Rousseau, K., Dufour, S., 2014. Molecular evolution of GPCRs: kisspeptin/kisspeptin receptors. J. Mol. Endocrinol. 52, T101–117.

Pasquier, J., Lafont, A.G., Denis, F., Lefranc, B., Dubessy, C., Moreno-Herrera, A., Vaudry, H., Leprince, J., Dufour, S., Rousseau, K., 2018. Eel kisspeptins: identification, functional activity, and inhibition on both pituitary LH and GnRH receptor

expression. Front. Endocrinol. (Lausanne) 8, 353.
Paullada-Salmeron, J.A., Cowan, M., Aliaga-Guerrero, M., Gomez, A., Zanuy, S., Mananos, E., Munoz-Cueto, J.A., 2016a. LPXRFa peptide system in the European sea bass: a molecular and immunohistochemical approach. J. Comp. Neurol. 524, 176–198.
Paullada-Salmeron, J.A., Cowan, M., Aliaga-Guerrero, M., Lopez-Olmeda, J.F., Mananos, E.L., Zanuy, S., Munoz-Cueto, J.A., 2016b. Testicular steroidogenesis and locomotor activity are regulated by gonadotropin-inhibitory hormone in Male European Sea Bass. PLoS One 11, e0165494.
Paullada-Salmeron, J.A., Cowan, M., Aliaga-Guerrero, M., Morano, F., Zanuy, S., Munoz-Cueto, J.A., 2016c. Gonadotropin inhibitory hormone down-regulates the brain-pituitary reproductive axis of male European sea bass (*Dicentrarchus labrax*). Biol. Reprod. 94, 121.
Peng, W., Cao, M., Chen, J., Li, Y., Wang, Y., Zhu, Z., Hu, W., 2016. GnIH plays a negative role in regulating GtH expression in the common carp, *Cyprinus carpio* L. Gen. Comp. Endocrinol. 235, 18–28.
Qi, X., Zhou, W., Li, S., Lu, D., Yi, S., Xie, R., Liu, X., Zhang, Y., Lin, H., 2013a. Evidences for the regulation of GnRH and GTH expression by GnIH in the goldfish, *Carassius auratus*. Mol. Cell. Endocrinol. 366, 9–20.
Qi, X., Zhou, W., Lu, D., Wang, Q., Zhang, H., Li, S., Liu, X., Zhang, Y., Lin, H., 2013b. Sexual dimorphism of steroidogenesis regulated by GnIH in the goldfish, *Carassius auratus*. Biol. Reprod. 88, 89.
Sari, I.P., Rao, A., Smith, J.T., Tilbrook, A.J., Clarke, I.J., 2009. Effect of RF-amide-related peptide-3 on luteinizing hormone and follicle-stimulating hormone synthesis and secretion in ovine pituitary gonadotropes. Endocrinology 150, 5549–5556.
Sawada, K., Ukena, K., Satake, H., Iwakoshi, E., Minakata, H., Tsutsui, K., 2002. Novel fish hypothalamic neuropeptide. Eur. J. Biochem. 269, 6000–6008.
Shahjahan, M., Ikegami, T., Osugi, T., Ukena, K., Doi, H., Hattori, A., Tsutsui, K., Ando, H., 2011. Synchronised expressions of LPXRFamide peptide and its receptor genes: seasonal, diurnal and circadian changes during spawning period in grass puffer. J. Neuroendocrinol. 23, 39–51.
Shi, Y., Zhang, Y., Li, S., Liu, Q., Lu, D., Liu, M., Meng, Z., Cheng, C.H., Liu, X., Lin, H., 2010. Molecular identification of the Kiss2/Kiss1ra system and its potential function during 17alpha-methyltestosterone-induced sex reversal in the orange-spotted grouper, *Epinephelus coioides*. Biol. Reprod. 83, 63–74.
Shimizu, M., Bedecarrats, G.Y., 2010. Activation of the chicken gonadotropin-inhibitory hormone receptor reduces gonadotropin releasing hormone receptor signaling. Gen. Comp. Endocrinol. 167, 331–337.
Son, Y.L., Ubuka, T., Millar, R.P., Kanasaki, H., Tsutsui, K., 2012. Gonadotropin-inhibitory hormone inhibits GnRH-induced gonadotropin subunit gene transcriptions by inhibiting AC/cAMP/PKA-dependent ERK pathway in LbetaT2 cells. Endocrinology 153, 2332–2343.
Son, Y.L., Ubuka, T., Soga, T., Yamamoto, K., Bentley, G.E., Tsutsui, K., 2016. Inhibitory action of gonadotropin-inhibitory hormone on the signaling pathways induced by kisspeptin and vasoactive intestinal polypeptide in GnRH neuronal cell line, GT1-7. FASEB J. 30, 2198–2210.
Spicer, O.S., Zmora, N., Wong, T.T., Golan, M., Levavi-Sivan, B., Gothilf, Y., Zohar, Y., 2017. The gonadotropin-inhibitory hormone (Lpxrfa) system's regulation of reproduction in the brain-pituitary axis of the zebrafish (*Danio rerio*). Biol. Reprod. 96, 1031–1042.
Tsutsui, K., 2009. A new key neurohormone controlling reproduction, gonadotropin-inhibitory hormone (GnIH): biosynthesis, mode of action and functional significance. Prog. Neurobiol. 88, 76–88.
Tsutsui, K., Saigoh, E., Ukena, K., Teranishi, H., Fujisawa, Y., Kikuchi, M., Ishii, S., Sharp, P.J., 2000. A novel avian hypothalamic peptide inhibiting gonadotropin release. Biochem. Biophys. Res. Commun. 275, 661–667.
Ubuka, T., Parhar, I., 2018. Dual actions of mammalian and piscine gonadotropin-inhibitory hormones, RFamide-related peptides and LPXRFamide peptides, in the hypothalamic-pituitary-gonadal axis. Front. Endocrinol. (Lausanne) 8, 377.
Ubuka, T., Parhar, I.S., Tsutsui, K., 2018. Gonadotropin-inhibitory hormone mediates behavioral stress responses. Gen. Comp. Endocrinol. https://doi.org/10.1016/j.ygcen.2018.03.004.
Ubuka, T., Son, Y.L., Tsutsui, K., 2016. Molecular, cellular, morphological, physiological and behavioral aspects of gonadotropin-inhibitory hormone. Gen. Comp. Endocrinol. 227, 27–50.
Ullah, R., Shen, Y., Zhou, Y.D., Huang, K., Fu, J.F., Wahab, F., Shahab, M., 2016. Expression and actions of GnIH and its orthologs in vertebrates: current status and advanced knowledge. Neuropeptides 59, 9–20.
Wang, B., Liu, Q., Liu, X., Xu, Y., Shi, B., 2018a. Molecular characterization and expression profiles of LPXRFa at the brain-pituitary-gonad axis of half-smooth tongue sole (*Cynoglossus semilaevis*) during ovarian maturation. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 216, 59–68.
Wang, B., Yang, G., Liu, Q., Qin, J., Xu, Y., Li, W., Liu, X., Shi, B., 2017. Inhibitory action of tongue sole LPXRFa, the piscine ortholog of gonadotropin-inhibitory hormone, on the signaling pathway induced by tongue sole kisspeptin in COS-7 cells transfected with their cognate receptors. Peptides 95, 62–67.
Wang, B., Yang, G., Liu, Q., Qin, J., Xu, Y., Li, W., Liu, X., Shi, B., 2018b. Characterization of LPXRFa receptor in the half-smooth tongue sole (*Cynoglossus semilaevis*): molecular cloning, expression profiles, and differential activation of signaling pathways by LPXRFa peptides. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 223, 23–32.
Wang, Q., Qi, X., Guo, Y., Li, S., Zhang, Y., Liu, X., Lin, H., 2015. Molecular identification of GnIH/GnIHR signal and its reproductive function in protogynous hermaphroditic orange-spotted grouper (*Epinephelus coioides*). Gen. Comp. Endocrinol. 216, 9–23.
Yin, H., Ukena, K., Ubuka, T., Tsutsui, K., 2005. A novel G protein-coupled receptor for gonadotropin-inhibitory hormone in the Japanese quail (*Coturnix japonica*): identification, expression and binding activity. J. Endocrinol. 184, 257–266.
Zhang, Y., Li, S., Liu, Y., Lu, D., Chen, H., Huang, X., Liu, X., Meng, Z., Lin, H., Cheng, C.H., 2010. Structural diversity of the GnIH/GnIH receptor system in teleost: its involvement in early development and the negative control of LH release. Peptides 31, 1034–1043.