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The endocrine regulation network of growth hormone synthesis and secretion in fish: Emphasis on the signal integration in somatotropes

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In teleosts, growth hormone (GH) production is governed by multiple neuroendocrine factors from the hypothalamus and other regulators from the pituitary and peripheral organs. Exploring the principles followed by pituitary somatotropes when differentiating and integrating the signals from these regulators at the cellular and intracellular level is essential for understanding the endocrine regulation network of growth hormone synthesis and secretion in fish. This paper discusses recent advances in the action mechanisms of GH regulation factors, including the neuroendocrine regulators, pituitary level factors and peripheral factors, primarily involved in their receptor systems as well as in post-receptor signal transduction pathways.

fish, growth hormone, receptor, signal transduction

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The growth status of fish is one of the primary economic considerations of aquaculture. The somatic growth of teleosts is controlled by the growth axis consisted of hypothalamus–pituitary–liver, i.e. the GH/IGF-I axis. GH released from the pituitary gland binds to its receptor and stimulates insulin-like growth factor-I (IGF-I) synthesis and secretion from the liver and other sites, evoking biological actions through IGF receptors [1].

GH, synthesized and secreted by the pituitary somatotrope, not only locates in the central position of the growth axis, but is also a pivotal factor in somatic growth in fish. However, the modulation patterns in the axis are not a basic point to point linear regulation and feedback, but rather are a kind of multifactorial and multiregulational manner, which makes up a regulation network of GH synthesis and secretion in fish. In the hypothalamus, a number of neuroendocrine factors directly act on somatotropes, including pituitary adenylate cyclase-activating peptide (PACAP), GH-releasing hormone (GHRH), gonadotropin-releasing hormone (GnRH), Neuropeptide Y (NPY), somatostatin (SS) [2]. In addition, these neuroendocrine factors have interactions controlling GH secretion [3] and are also affected by some of the peripheral factors [4]. Several peripheral factors, e.g. IGF-I and ghrelin have been confirmed to control growth hormone release either in an indirect way via their influence on the neuroendocrine factors or by exhibiting a direct effect on somatotrope GH synthesis and release [5,6]. At the pituitary level, GH is regulated by itself via an ultrashort feedback loop under the mechanisms of autocrine/paracrine [7]. No matter where the regulation factors originate and whatever they are, all signals must reach and integrate in somatotropes. The signal transduction mechanism of some neuroendocrine factors have been investigated in a number of fish species, e.g. GnRH in goldfish (*Carassius*

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auratus) [8], PACAP in grass carp (*Ctenopharyngodon idella*) [9] and SS in rainbow trout (*Oncorhynchus mykiss*) [10]. In this article, the receptor systems as well as the post-receptor signal transduction cascades mediating the actions of GH regulation factors in the somatotropes of fish were examined and discussed. The relevant regulators according to the GH/IGF-I axis involved 3 levels, the hypothalamus level (neuroendocrine factors, e.g. PACAP, NPY and SS), the pituitary level (GH and gonadotropin) and the peripheral level (ghrelin).

1 Neuroendocrine factors

1.1 Pituitary adenylate cyclase-activating polypeptide

Pituitary adenylate cyclase-activating polypeptide (PACAP), a member of the glucagon/secretin peptide family, has been well known as a potent GH-releasing factor in such fish species (for review [11]), as goldfish [12], grass carp [13], trout [14] and European eel (*Anguilla anguilla*) [15]. It has been reported that the PACAP recombinant peptides enhance the growth rates of catfish (*Clarias gariepinus*), tilapia (*Oreochromis niloticus*), carp (*Cyprinus carpio*) [16] and orange-spotted grouper (*Epinephelus coioides*) [17].

PACAP exerts biological actions by interacting with distinct G-protein-coupled receptors with a classical structure of 7 transmembrane domains (TMD) [18]. In mammals, 3 types of PACAP receptors with different pharmacological properties have been identified, 2 of them, VPAC1 and VPAC2, usually designated as the PACAP type II receptors, have similar affinities for PACAP and vasoactive intestinal polypeptide (VIP), and the third one is called the PACAP type I receptor which is a PACAP-specific receptor with high affinity to the two molecular forms of PACAP peptides (PACAP-38 and PACAP-27) but with a low affinity for VIP [19]. In teleosts, different types of PACAP receptors have been cloned, including zebrafish (*Danio rerio*) [20] and goldfish [21].

The GH-releasing actions evoked by PACAP mediated by the PACAP type I receptor have been verified to be dependent on adenylate cyclase, cAMP, protein kinase A and voltage-sensitive calcium channels (VSCC) in fish [21,22]. Wong *et al*. [9] have shown that PACAP is effective in stimulating GH production and *GH* gene expression by directly acting at the pituitary cell level. GH mRNA levels are elevated by PACAP via functional coupling of the Ca^{2+}/calmodulin (CaM)/CaM kinase II cascade with the adenylate cyclase (AC)/cAMP/protein kinase A (PKA) pathway [9]. At the pituitary level, the GH release responses to such neuroendrine factors as GnRH (sGnRH, cGnRH), dopamine (DA) and PACAP, have been well documented to be Ca^{2+}-dependent (for review [8]). They elevate the intracellular free Ca^{2+} concentration ($[Ca^{2+}]i$) in somatotropes, and their actions on GH release are sensitive to the inhibition of voltage-sensitivity and dependent on calmodulin (CaM) and CaM kinase II [23]. However, there are various differences in the Ca^{2+} pathway involved between various neuroendocrine factors. Using sarcoplasmic/endoplasmic reticulum Ca-ATPases (SERCA) inhibitors, Chang *et al*. [24] found that PACAP-evoked GH release responses are attenuated by BHQ and potentiated by thapsigargin (Tg). By contrast, the GH secretion response to sGnRH and cGnRH-II is not affected by either Tg or BHQ [25]. These observations provide the evidence that distinct multiple Ca^{2+} stores mediate the GH secretion response to different neuroendocrine regulators.

Although the function of PACAP in stimulating GH production and release has been extensively evaluated, its functional interactions with other GH regulators have not yet been fully characterized. Using grass carp, Wang *et al*. [26] have demonstrated that norepinephrine (NE) suppresses both basal and PACAP-stimulated GH release and *GH* gene expression by directly acting at the pituitary cell level. These inhibitory actions are mediated through α2-adrenoreceptors negatively coupled to the cAMP-dependent pathway and Ca^{2+} entry through L-type VSCC. Although previous findings indicated that PACAP induces GH synthesis by enhancing GH mRNA stability and *GH* gene transcription, α2-adrenergic inhibition of GH mRNA expression is mediated by reducing GH promoter activity and does not involve posttranscriptional modification of GH transcript stability [26]. In addition, using single morphologically identified somatotropes loaded with the Ca^{2+}-sensitive dye Fura-2, SS14 was found to inhibit PACAP-stimulated GH release in goldfish, but it did not couple with a Ca^{2+} signal decrease [27]. This observation implies that the Ca^{2+} signal may not be involved in the PACAP interaction with somatostatin concerning regulating GH release.

1.2 Neuropeptide Y

Neuropeptide Y (NPY) belongs to a pancreatic polypeptide family including NPY, peptide YY (PYY), pancreatic polypeptide (PP or PPY) and polypeptide Y (polypeptide tyrosine, PY). NPY and PYY are identified in all vertebrate classes from Agnatha to mammalia, whereas, PP and PY are found only in the pancreas of tetrapods and certain teleost fishes (for review [28]). By peptide purification or cDNA cloning or genomic DNA, NPY structures have been deduced and characterized from rainbow trout [29], goldfish [30], sea bass (*Dicentrarchus labrax*) [31], channel catfish (*Ictalurus punctatus*) [32], flounder (*Paralichthys olivaceus*) [33], tilapia [34] and zebrafish [35]. Almost all forms of NPY, PYY, PP and PY share 36 amino acids in length and an amidated C-terminal. The structures of prepro-NPY, prepro-PYY, prepro-PP and prepro-PPY are similar. They are all nearly 100 amino acids long, containing the sequences in order for the signal peptide, mature peptide, typical GKR sequence for cleavage and C-terminal amidation, as well as a C-terminal peptide.

The effects of NPY in vertebrates include stimulation of pituitary hormone release and appetite, anxiolysis, vasoconstriction, circadian rhythms and pain signaling [36]. NPY immunoreactivity has also been detected in the pituitary of some teleost species such as platyfish (*Xiphophorus maculates*) [37], lungfish (*Protopterus annectens*) [38] and Senegalese sole (*Solea senegalensis*) [39] suggesting its function at the pituitary level. The NPY family peptides exert their biological functions by binding to rhodopsin-like G protein-coupled receptors (GPCR). Different from being highly conserved in ligands, the NPY family receptor system is more diversified. Due to the duplication events that occurred before the origin of gnathostomes, 7 different NPY receptors have been described in tetrapods [40]. Based on sequence comparison, phylogenetic analysis and chromosomal positions, the NPY receptor family is divided into 3 subfamilies, the Y1 subfamily (Y1, Y4(Ya), Y6, and Y8(Yb/c)), the Y2 subfamily (Y2 and Y7) and the Y5 subfamily (with Y5 as the single representative). These 3 subfamilies, Y1, Y2 and Y5, possess approximately a 30% identity. Compared with mammals, Y2, Y4, Y7, and Y8 were identified in pufferfish and zebrafish, with a lack of Y1 and Y5 [41], and Y2 and Y7 in rainbow trout [42]. These differences are the results of differential loss of genes in the 2 lineages after the large-scale duplications in the gnathostome ancestor [43]. As a well-known neuroendocrine system of receptors and peptides with gene family members in both vertebrates and invertebrates, the NPY receptor family has been regarded as a research model for evolution and gene duplications before the gnathostome radiation. In the coelacanth *Latimeria chalumnae,* Y5 and Y6 have been cloned and characterized, which has a key evolutionary position at the divergence of bony fishes and tetrapods [44]. Larsson *et al*. [43] have identified 7 receptor genes orthologous to the Y1, Y2, Y4, Y5, Y6, Y7 and Y8 subtypes found in tetrapods and teleost fishes in the elephant shark, *Callorhinchus milii*. However, specific functions mediated by the corresponding receptors and their signaling mechanisms remain undetermined.

In fish, similar to mammals, direct actions of NPY on GH secretion from pituitary somatotropes have been reported. In goldfish, the GH release responses to 5-min pulses of NPY are relatively small in sexually regressed fish (July), intermediate in recrudescent fish (December), and maximal in sexually mature fish (May). Further implantation of testosterone significantly enhances NPY-induced GH release from perifused pituitary fragments in sexually regressed goldfish [45]. However, relatively few reports have been published about the signal transduction mechanism of NPY inducing GH secretion from somatotropes. NPY mediated ghrelin-inducing feeding and ghrelin signaling and its effect on the GH/IGF axis have been reported in goldfish [46] and tilapia [47]. It is essential to clarify the NPY's precise post-receptor signal mechanism in the control of somatotrope GH release.

1.3 Somatostatin

Somatostatin (SS) was originally isolated from the ovine hypothalamus as a 14-amino acid peptide and characterized as a physiological inhibitor of pituitary GH secretion [48]. To date, it has been well documented that SS possesses a wide variety of biological functions, including numerous secretotropic, developmental, and metabolic effects [49]. In mammals, there are 2 major biologically active SS forms: SS-14, and its NH_2-terminally extended form, SS-28, which both proceed from the cleavage of a larger precursor preprosomatostatin I (PPSS I). In fish, the inhibitory effect of SS on GH release *in vitro* or *in vivo* has been widely demonstrated in a number of teleost species. At the level of the pituitary, SS-14, SS-28, and [Pro2]-SS-14, but not salmonid SS-25, inhibit pituitary GH release [50]. SS-14 injection of rainbow trout disrupts the GH-IGF-1 axis (inducing GH and IGF-1 deficiency) and results in growth retardation [51]. In contrast with mammals, 3 precursors of preprosomatostatin, PPSS I, PPSS II, and PPSS III, have been cloned from such fish species as Russian sturgeon (*Acipenser gueldenstaedti* Brant), goldfish, African lungfish, zebrafish, rainbow trout and grouper [52–57].

SS exerts its inhibitory actions via binding to specific receptors. Five types of SS receptor have been identified by molecular cloning in several mammalian species, and later named as sst1 to sst5 according to the conventional lower case nomenclature [58]. All of these receptors belong to the rhodopsin family of guanine nucleotide binding G protein-coupled receptors, sharing a 39%–57% sequence identity among the various sst members, and contain a highly conserved sequence motif, YANSCANPI/VLY, in the 7th transmembrane domain, which serves as a signature sequence for this receptor family [58]. In mammals, although in the pituitary, all 5 types of sst are expressed in the major cell types, with sst5 and sst2 being as the principal subtypes expressed in the rat pituitary somatotrophes [59]. Further studies demonstrate that sst1, sst2 and sst5 are involved in the inhibition of GH release [60].

Four ssts (sst1, 2, 3, and 5) have been described in fish, including such isoforms as sst1A/1B (goldfish and rainbow trout), sst3A/3B (goldfish), and sst5A/5B/5C (goldfish) [10,53,61]. Two types of sst1 cDNA were respectively cloned from goldfish [62] and rainbow trout [63]. In COS-7 cells transiently expressing goldfish sst1A or sst1B, both SS-14 and [Pro2]-SS-14 significantly inhibit forskolin-stimulated cAMP release, suggesting coupling of the receptors to the inhibition of adenylate cyclase [62]. Lin *et al*. [64] cloned sst2 from goldfish brains. They found that the sst2 mRNA expression levels in the pituitary were significantly higher than those in brain regions, consistent with the findings in mammals that sst2 and sst5 are predominantly expressed in pituitary somatotrophs and involved in the direct regulation of GH secretion. The goldfish sst2 also binds with SS-14 and [Pro2]-SS-14 followed by coupling to the inhibition of adenylate cyclase [64]. A type three sst has

been identified from Apteronotid eels *(Apteronotus albifrons)* [65]. Both SS-14 and mammalian SS-28 potently inhibited forskolin-stimulated adenylate cyclase activity in CCL39 cells expressing sst3 receptors, which was blocked by pertussis toxin, suggesting coupling of the sst3 receptor to Giα and/or Goα G-proteins [66]. In mammals, in addition to the principal pathway of inhibition of adenylyl cyclase and cAMP formation [58], ssts also activate phospholipase A2, stimulate the phospholipase CPKC signaling system, inhibit voltage-gated Ca^{2+} channels (regulating GH release) and modify the mitogen-activated protein, MAP kinase (MAPK) signaling system [58,67]. Whereas in fish, the other signaling cascades remain undetermined.

The SS effect on GH secretion and SS itself have been observed to be affected by such other neuroendocrine and peripheral factors as dopamine, 17β-estradiol (E2) and IGF-I. In goldfish, the dopamine effect of inhibitory and/or stimulatory regulation of the three *SS* genes through both D1-like and D2-like receptors was found with differences based on the gonadal status, early or late stages of gonadal recrudescence, and gender [68]. E2 has effects on the PSS-I mRNA levels in sexually regressed goldfish [69]. In the goldfish forebrain, E2 increased the expression of PPSS I and PPSS III mRNA [70]. *In vivo* administration of E2 into rainbow trout decreased plasma SS-14 and SS-25-II levels and increased plasma GH [71]. Based on the observation that estradiol implantation resulted in elevated plasma GH levels and produced a down-regulation of *sst2* gene expression in the goldfish pituitary, Cardenas *et al*. [72] suggested that E2-regulated GH release extends from reduced pituitary responsiveness to SS-14 and [Pro2]-SS-14, resulting from decreased sst2 expression. We isolated and characterized *PSSI* gene promoter in grouper *Epinephelus coioides*. Sequence analysis showed that −848 to −373 bp in the 5′-flanking region of grouper *PSSI* gene contained 5 ERE half sites. Our functional analysis suggested that estradiol increase of the expression of PSSI may be mediated through the ERE half sites in the *PSSI* gene [73].

Using rainbow trout as an animal model, Melroe *et al*. [74] have demonstrated that GH and IGF-I stimulate the pancreatic islet expression of PPSS *in vitro*, as well as the plasma SS-14 levels after a 3 week implantation of ovine GH [75]. These findings suggest that GH-stimulated and IGF-1-stimulated SS production potentially reduce growth through several means, including inhibition of pituitary GH release, desensitization of target cells to GH, and reduced IGF-1 expression, which finally induce the inhibition of the GH/IGF-I axis and the reduction of organism growth [50]. By means of a mini osmotic pump technique to implant GH into rainbow trout, GH has been found to reduce the expression of all three receptor forms in the brain, sst1A and sst1B expression in the pancreas, and to affect sst1A and sst1B hepatic expression [76]. However, GH and IGF-I direct actions on the SS at the pituitary somatotrope level have not been identified. A direct molecular interaction between different sst subtypes has been demonstrated in humans [77]. The question as to whether or not there is a similar interaction between the sst subtypes in fish remains undetermined.

2 Peripheral factors

2.1 Ghrelin

Ghrelin is a peptide hormone that was identified as an endogenous ligand for a growth hormone secretagogue (GHS) receptor. Afer being initially isolated from rat stomachs by using a reverse pharmacological procedure in 1999 [78], ghrelin has been shown to be a pluripotent hormone with many physiological functions including regulation of food intake [79], gastrointestinal motility [80], energy metabolism [81], gastric acid secretion [80], cardiovascular function [82] and cell proliferation [83]. Among these actions, ghrelin's first known biological function was stimulating GH secretion from the pituitary both *in vivo* and *in vitro* [78].

In ghrelin, the unique *n*-octanoic acid modification for the N-terminal serine residue (Ser3) has been regarded as an essential structure for binding to its receptor GHS-R and subsequent biological activity [78]. This acyl modification of Ser is highly conserved among species not only in mammals [84] but also in such teleosts as goldfish [85], eels (*Anguilla japonica*) [86], tilapia [87], rainbow trout [88], channel catfish [89] and sea bream (*Acanthopagrus schlegeli*) [90]. Recently, the activities of octanoyl goldfish ghrelin12, 17 and des-acyl goldfish ghrelin17 were compared using GHS-R1a-expressing GHSR62 cells [91]. The acyl modification ghrelins tested activated GHSR62 cells and increased $[Ca^{2+}]i$ in a dose-dependent manner. By contrast, des-acyl ghrelin17 did not increase $[Ca^{2+}]i$ in GHSR62 cells, suggesting that acyl modification of ghrelin is essential for its function in fish models. Given that the enzyme involved in the acylation of ghrelin has recently been identified as GOAT (ghrelin O-acyltransferase) [92], and it was shown to be a member of a family of 16 hydrophobic membrane-bound acyltransferases that included Porcupine, which attaches long-chain fatty acids to Wnt proteins [92], the mechanism of acylation and its contribution to ghrelin binding with GHSR-1a have not yet been clearly defined.

Ghrelin's action has been recognized to be mediated via binding to its receptor GHS-R which preferentially couples to Gq and ultimately leads to increases in intracellular calcium. The GHS-R is a typical G-protein coupled (GPCR) seven-transmembrane (7-TM) receptor [93]. Two distinct ghrelin receptor cDNAs have been isolated, GHS-R 1a, which encodes a 7-TM GPCR with binding and functional properties consistent with its role as the endogenous receptor for ghrelin, and GHS-R 1b, which is produced by an alternative splicing mechanism without binding affinity with either ghrelin or synthetic ligands, whose biological function remains unclear [94]. Multiple signaling cascades of ghrelin-triggered GHS-R1a have been observed to be

involved in the signal transduction of ghrelin's action on somatotropes including phospholipase C (PLC) and protein kinase C (PKC), resulting in increasing inositol 1,4,5-triphosphate and intracellular Ca^{2+} levels. Ca^{2+} influx through L-type voltage-gated Ca^{2+} channels is also involved as well as the Na^{+} influx through Na^{+} channels [95]. A greater Ca^{2+} influx via voltage-gated Ca^{2+} channels, which induces a greater GH secretion, is probably due to ghrelin's ability to reduce transmembrane voltage-gated K^{+} currents, which are mainly responsible for the resting potential through the cGMP dependent protein kinase (cGMP/PKG) signaling pathway [96]. In fish, GHS-R was identified in pufferfish [97], black sea bream [98], tilapia [99] and grouper [100], and shared good homology with human GHS-R. In black sea bream, ghrelin is able to trigger an elevation of intracellular Ca^{2+} ion concentration in HEK293 cells expressing sbGHSR-1a and further demonstrates that stimulation of PLC, increases in cytosolic free Ca^{2+} levels ($[Ca^{2+}]i$), and activation of L-type voltage-sensitive Ca^{2+} channels (LVSCCs) are possible mechanisms linked to ghrelin-induced GH release in fish [98,101]. Based on the understanding of ghrelin's effect on stimulating GH release both *in vivo* and *in vitro* in goldfish [85,102], Grey and Chang [4] further confirmed that ghrelin induces GH release from goldfish pituitary cells by enhancing Ca^{2+} entry through L-type voltage-sensitive Ca^{2+} channels (LVSCCs) using perifusion GH release and fura-2/AM Ca^{2+}-imaging experiments.

Ghrelin as a peripheral regulator of GH has been observed to conduct interaction with neuroendocrine factors of GH secretion in recent reports. Sequential applications of goldfish ghrelin (gGRL19) (1 nmol/L) and salmon GnRH (100 nmol/L), a known Ca^{2+}-dependent stimulator of GH release, increased intracellular free Ca^{2+} levels ($[Ca^{2+}]i$) from the same identified somatotropes, suggesting co-expression of GRL and GnRH receptors of single cells [4], implying the interaction of GH secretion with ghrelin and GnRH. In addition, co-administration of ghrelin and growth hormone-releasing hormone (GHRH) results in greater GH release than that following administration of either GHRH or ghrelin alone [103,104]. This observation implies a synergistic effect of GHRH and ghrelin on GH secretion. GHRH induced cAMP production was increased by GHS co-treatment through a PKC- and PLC-independent mechanism in a homogeneous pituitary cell population expressing GHRH and GHS receptors [105], suggesting a direct interaction between GHRH-R and GHSR, although GHS-R1a/ GHRH receptor hetero-oligomers have not yet been identified.

3 Pituitary factors

3.1 GH and gonadotropin (GtH)

In mammals, it has been demonstrated that local interactions of autocrine/paracrine factors within the pituitary and the GH itself are involved in the regulation of GH release from somatotropes [106]. This ultrashort feedback of GH regulation has been supported by the observations that GH receptors are ubiquitously expressed in the pituitary of rats [107], mice [108] and humans [109], and GH treatment inhibits GH release in bovine pituitary cells [110]. GH exerts its biological actions by coupling to the Janus kinase-2 (JAK2)/signal transducer and activator of transcription (STAT), JAK2/MAPK, and/or JAK2/insulin receptor substrate (IRS)/phosphoinositide 3-kinase (PI3K) pathways [111] and GH receptor activation induces$[Ca^{2+}]$e influx via L-type voltage-sensitive Ca^{2+} channels in a protein kinase C-dependent manner [112]. However, hindered by two technical difficulties, crossreactivity of exogenous GH added to pituitary cell cultures by GH RIA and saturation/activation of pituitary GH receptors by endogenous GH [7], the mechanisms of GH feedback for its own secretion at the pituitary level have not yet been elucidated.

Using grass carp as an animal model, Zhou *et al*. [7] have shown that GH induces *GH* gene expression in grass carp pituitary cells through autocrine/paracrine mechanisms. They firstly reported that endogenously secreted GH serves as an intrapituitary autocrine/paracrine factor maintaining basal GH release, *GH* gene expression, and somatotrope sensitivity to stimulation by GH releasing factors, including GnRH, apomorphine, and PACAP-38. They also found that GH-induced *GH* gene expression is mediated by improving the stability of GH mRNA and activation of *GH* gene transcription. The signal pathway coupling of JAK2, PI3K, and MAPK to pituitary GH receptors has been shown to be involved in the post-receptor signaling mechanism in GH feedback for its own secretion at the pituitary level.

Gonadotropin (GtH) released from the anterior pituitary regulating reproductive functions, interacts with GH at multiple levels to respectively modulate the functions of the gonadotrophic and somatotrophic axes [113]. In mammals, at the pituitary level, transcripts of GH receptors [114] and GH-binding sites [115] are observed to appear in gonadotrophs, and the stimulatory actions of GnRH on LH and FSH release are inhibited by GH immunoneutralization [116], suggesting that endogenous GH may act in a paracrine manner regulating gonadotroph functions. Similarily, GH release is also under the influence of the gonadotrophic axis, especially via the release of sex steroids [117].

In the fish model, GH secretion increases with GTH-II levels during sexual recrudescence and the spawning period in goldfish. *Vice versa*, the preovulatory GTH-II surge occurs with a concurrent increase in GH release [118], and GnRH-stimulated GH have been reported in rainbow trout [119], common carp (*Cyprinus carpio*) [120] and tilapia [121]. Using a static incubation approach, exogenous GtH directly induces GH release and GH mRNA expression in carp pituitary cells [113]. Removal of endogenous GtH by immunoneutralization with GtH antiserum suppresses GH release, GH production, and GH mRNA levels [113]. Based

on these findings, Zhou *et al*. [113] proposed a novel mechanism regulating GH release and synthesis in fish where by the local interactions between gonadotrophs and somatotrophs may form an intrapituitary feedback loop for regulating GH release and synthesis. In this model, GTH released from gonadotrophs induces GH release and GH production in neighboring somatotrophs. GH secretion maintains somatotroph sensitivity to GtH stimulation, and simultaneously, inhibits basal GtH release in gonadotrophs. The intercellular communication among pituitary cells has been received extensively investigated [106], but the regulation mechanisms of GtH for GH synthesis and secretion remain unclear.

4 Conclusion

Although multiple kinds of GH regulators have been examined relating to their post-receptor signal pathways and their interplay effects at the pituitary somatotrope level, our understanding of the theory of the interplay/synergistic effect and the mechanism for the eventual integration result of GH synthesis and secretion in fish growth is far from complete. For further research, novel findings in mammals provide informative idea for the fish model. For example, PACAP serves as a hypophysiotropic factor evoking GH secretion from the pituitary via the adenylate cyclase (AC)/cAMP/protein kinase A (PKA) pathway. A novel signaling pathway of cAMP-dependent, protein kinase A-independent has been found to mediate the PACAP-induced neuritogenesis through Egr1 in PC12 cells [122]. Selected characteristics of the neuroendocrine organ and pituitary in teleosts are very different from mammals. For example, unlike mammals with a random pattern of cell distribution in the pituitary, a distinct zonation of individual cell types has been identified in the pituitary of fish, e.g. grass carp [21]. In this case, somatotrophs and gonadotrophs are restricted to the proximal pars distalis, and the close proximity between these two cell types provides the anatomical substrate for local interactions between gonadotrophs and somatotrophs. Taking advantage of this distinctive feature, more results have been achieved in the fish model than in other vertebrates. In addition, very different from mammals, in which a specialized area of the neurohypophysis median eminence becomes the principal terminus for many neurosecretory neurons, and portal blood vessels from this area carry and distribute the regulatory neuropeptides or neurohormones into the adenohypophysis [123], in teleosts, the anterior pituitary is under the direct innervation of the hypothalamus, i.e. hypothalamic neurons terminate very close to the adenohypophysial cells making the diffusional distance very short or the hypothalamic neurosecretory endings make synaptic contact with the adenohypophysial cells [123]. This innervation is regarded as a kind of paracrine mechanism within adenohypophysial cells including somatotropes, suggesting that the effect of neuropeptides on somatotropes in fish will significantly differ from that in mammals.

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