

**INTRACELLULAR INTEGRATION OF MULTIFACTORIAL
NEUROENDOCRINE REGULATION
OF GOLDFISH SOMATOTROPE FUNCTIONS**

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Abstract

Cellular functions of somatotropes are controlled by multiple stimulatory and inhibitory neuroendocrine factors. In goldfish, known stimulatory regulators for growth hormone release include two endogenous gonadotropin-releasing hormones (GnRHs), dopamine (DA), and pituitary adenylate cyclase-activating polypeptide (PACAP). Inhibitory regulators include somatostatin, of which three endogenous isoforms are known. We have used primary cultures of goldfish somatotropes as a study model to investigate how agonist- and function-specificity can be mediated at the level of intracellular signal transduction. Extracellular Ca^{2+} entry, mobilization of Ca^{2+} from TMB8-sensitive intracellular stores, and activation of calmodulin kinase are all involved in the GH release responses to the two endogenous GnRH forms, DA and PACAP. In contrast, cAMP/PKA-dependent pathways selectively mediate DA and PACAP stimulation of GH release, whereas Na^+/H^+ antiport and PKC-dependent mechanisms participate in GnRH-induced GH secretion. How GnRH actions on hormone secretion are coupled to the nitric oxide and cGMP signalling cascades also differ from those of DA and PACAP. Similarly, the

differential ability of three endogeneous somatostatin isoforms to inhibit basal and stimulated GH release is reflected in their selective ability to modulate the GH response to individual signalling pathways. Other pharmacological studies reveal the presence of several intracellular Ca^{2+} compartments. Selective manipulation of these stores differentially modulates basal GH secretion, cellular contents and mRNA levels, as well as the release responses to the two GnRHs. The multiplicity of signal transduction cascades and the complexity of Ca^{2+} stores provide the basis upon which functional and ligand specificity of multiple neuroendocrine signals may be integrated at the intracellular level. Together, these mechanisms may allow for the selective control of somatotrope functions appropriate for a particular situation in growth and metabolism.

Introduction

Growth hormone (GH) is a key regulator of growth, metabolism, and reproduction in teleost fishes (Peter and Chang, 1999). In goldfish, both stimulatory (gonadotropin-releasing hormone (GnRH), dopamine (DA), GHRH, thyrotropin releasing hormone, pituitary adenylate cyclase activating polypeptide (PACAP), neuropeptide Y (NPY), bombesin, cholecystokinin (CCK), galanin, activin, and inhibin) and inhibitory (somatostatin (SS), serotonin (5HT), and norepinephrine (NE)) neuroendocrine regulators control GH secretion at the level of the pituitary (Peter and Chang, 1999; Chang et al., 2000). In addition to having a number of neurosecretory factors, diversity in ligand isoforms, receptors and post-receptor signalling allow selective modulation of the pathway from synthesis to secretion of hormone.

Diversity of peptides and receptors subtypes

In goldfish, two isoforms of GnRH (salmon (sGnRH) and chicken-II (cGnRH-II)) play a critical role in GH secretion (reviewed in Chang et al., 2000). Moreover, two receptor isoforms for the goldfish GnRH receptor have been recently cloned and shown to be present in goldfish pituitary (Yu et al., 1998; Illing et al., 1999). Both receptors recognize both endogenous GnRH's. However, the partially selective nature of their signal transduction cascades in somatotropes suggests that there must either be some selectivity in receptor-mediated G-protein activation or that a third pituitary cGnRH-II-specific receptor, which has been found among other vertebrates, may exist in goldfish.

The somatostatin (SS) system also exhibits diversity in both peptides and receptors (Lin et al., 2001; reviewed in Lin and Peter, 2001). Three forms of brain SS mRNA (SS₁₄, [Pro²]SS₁₄ and gfSS₂₈) have been detected in the hypothalamus. Three SS receptor types (Sst₁, Sst₂, Sst₅) are expressed in goldfish pituitary and exhibit selectivity for the SS isoforms. For example, SS₁₄ and [Pro²]SS₁₄, but not gfSS₂₈ appear to activate Sst₂ receptors (Lin et al. 2000). Moreover, gfSS₂₈ appears to be slightly more potent in binding Sst₅ receptors than either SS₁₄ or [Pro²]SS₁₄ (Lin et al., 2001). This variable affinity of receptors for the different SS isoforms clearly offers mechanisms of selectivity within the SS regulatory system (see below).

Divergence of signal transduction pathways

GH secretion in goldfish is under the control of two minimally overlapping signalling cascades, which display selective activation by specific neuroendocrine regulators. Divergences between these pathways are outlined in Figure 1. For example, the two endogenous GnRH's (sGnRH & cGnRH-II) utilize the protein kinase C (PKC) pathway and display a dependence on the Na⁺/H⁺ antiporter. In contrast, both DA-D1 and PACAP-PVR1 receptors activate the adenylate cyclase-cyclic AMP (cAMP)-protein kinase A (PKA) system, as well as arachadonic acid signalling (Chang et al., 2000). In addition, extracellular Ca²⁺ entry through voltage-sensitive Ca²⁺ channels (VSCC's) and calmodulin kinase activation appear to play a role in both GnRH/PKC and DA/PACAP/cAMP signalling. However, whether the link between these two signalling pathways, VSCC's, and secretion is direct or indirect (i.e. through Ca²⁺ stores) remains to be explored.

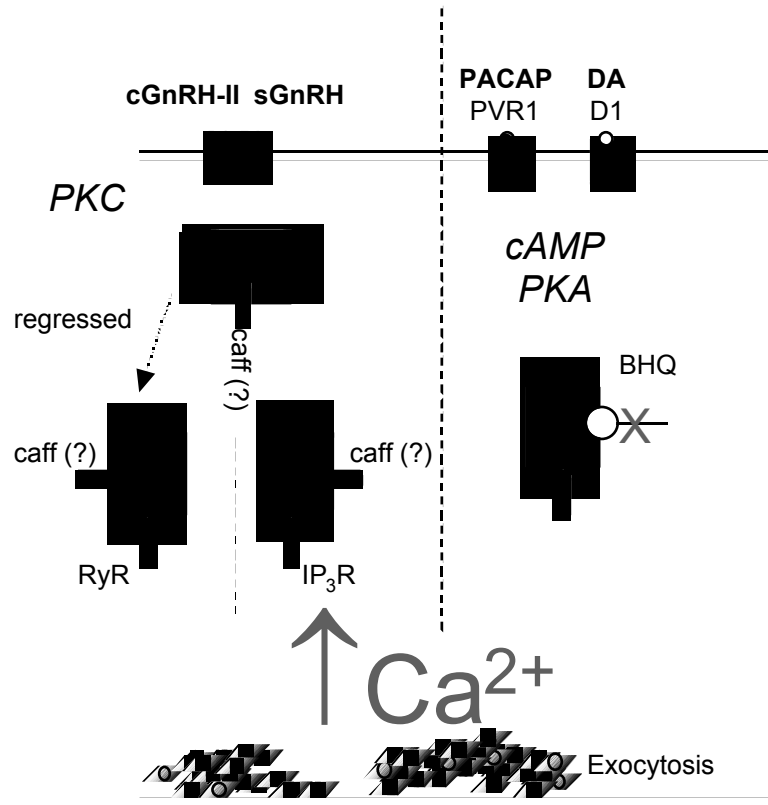


Figure 1 – Divergence in Ca²⁺ stores and major signalling cascades for stimulated growth hormone release in goldfish.

Recently, we have demonstrated the presence of a number of pharmacologically distinct intracellular Ca^{2+} stores, which differentially participate in mediating the secretory response elicited by neuroendocrine regulators (Johnson and Chang, 2000). The involvement of IP_3 Ca^{2+} -release channels in secretion stimulated by sGnRH, but not cGnRH-II, suggests divergence in pathways between the two GnRH's (Johnson and Chang, 2002). The participation of caffeine-sensitive stores in GnRH, but not DA, action on GH secretion also suggests that there is differential coupling of PKC and cAMP signalling cascades to pharmacologically distinct intracellular Ca^{2+} stores (Wong et al., 2001).

Further divergence among pathways, has been shown by using classical inhibitors of SERC ATPases (e.g., thapsigargin and BHQ). In particular, BHQ-sensitive stores do not appear to be involved in GnRH-evoked release (Johnson and Chang, 2000), which is in contrast with their participation in both PACAP- and DA-stimulated release (unpublished observations). However, there is some shared pharmacology between the two stores. In particular, the general intracellular Ca^{2+} channel blocker, TMB-8 appears to inhibit both GnRH and PACAP/DA signalling.

The nitric oxide synthase (NOS)/cGMP signalling pathway also appears to be involved in GnRH signalling yet plays only a minor role in DA signalling (Uretsky and Chang, 2000). Moreover, NOS signalling may be even less involved in PACAP stimulation of GH secretion (unpublished observations).

Divergence of signalling pathways is also present among inhibitors of GH release. For example, NE appears to inhibit cAMP signalling (Yunker et al., 2000), whereas 5HT does not (unpublished observations). Similarly, whereas both SS_{14} (Kwong and Chang, 1997) and $[\text{Pro}^2]\text{SS}_{14}$ abolish cAMP-induced GH secretion, gfSS_{28} has no effect on cAMP-induced GH release. Such specificity provides a mechanism for selective inhibition of individual GH releasers. Indeed, gfSS_{28} appears to be less effective in inhibiting endogenous secretagogues that are cAMP-dependent than are other SS isoforms (unpublished observations). Surprisingly, neither NE nor SS_{14} , both of which inhibit basal and stimulated secretion, do not reduce resting $[\text{Ca}^{2+}]_i$ (Yunker et al., 2000; Yunker and Chang, 2001). This would suggest that stimulated and basal secretion are quite different in their reliance on $[\text{Ca}^{2+}]_i$ and signalling cascades.

Control of synthesis

Exploration of the control of GH mRNA expression by a variety of groups has revealed that GH mRNA levels are subject to a variety of very specific control mechanisms which are not necessarily linked, one-to-one, with hormone secretion. For example, SS, which is a potent inhibitor of secretion, does not alter GH mRNA in tilapia and trout (Melamed et al., 1998). Similarly, we have recently shown that perturbations in Ca^{2+} homeostasis appear to dissociate secretion of GH from synthesis of GH (Johnson et al., 2002). Activation of VSCC's stimulates GH secretion but is accompanied by a reduction in GH mRNA. However, activation of ryanodine receptors rapidly stimulates GH release and increases $[\text{Ca}^{2+}]_i$, but does not affect GH mRNA levels. Conversely, selective inhibition of thapsigargin-sensitive Ca^{2+} ATPases elevates intracellular Ca^{2+} , does not affect GH mRNA, yet inhibits short-term secretion. Moreover, cellular GH protein content can be differentially affected by manipulations of ryanodine- and thapsigargin-sensitive stores, without altering GH mRNA levels (Johnson et al., 2002).

Seasonal variation in GH release

The regulation of GH release is subject to tremendous seasonal variation (Figure 2) displaying differential activity throughout the animal's reproductive cycle (Peter and Chang, 1999; unpublished observations). DA, CCK, and GHRH are most effective in sexually regressed animals whereas PACAP acquires prominence during stages of sexual recrudescence. Finally, during latter stages of recrudescence, maturation and spawning, GnRH, TRH, and NPY are at their most effective. Similarly, the inhibitory tone of SS, which is prominent among sexually regressed fish, decreases during recrudescence out of phase with NE, which is most effective in sexually mature animals. These seasonal variations in the control of GH release are paralleled by variations in intracellular Ca^{2+} stores (Johnson and Chang, 2002). In particular, ryanodine-sensitive stores, which selectively participate in cGnRH-II signalling, appear to be present only among somatotropes from sexually regressed fish.

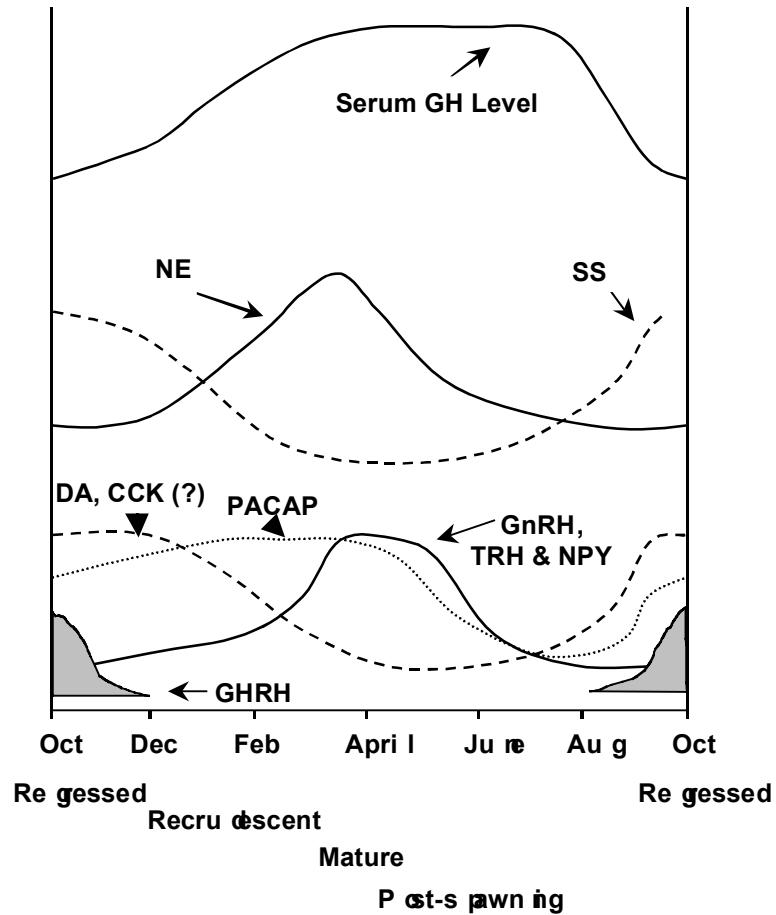


Figure 2 – Seasonal variations in neuroendocrine regulation of growth hormone release in goldfish.

Conclusions

Goldfish somatotropes are clearly subject to a variety of neuroendocrine modulators, which provide considerable complexity in regulation of cell function. While there is evidence to support the presence of somatotropes that

respond to multiple stimulatory regulators, we cannot discount the possibility that there may be somatotropes that are responsive to only a select few regulators. Nonetheless, the framework of transmitters and regulatory peptides, receptors and intracellular signalling pathways, including Ca^{2+} stores, provides highly selective regulation of cellular function. As a result, basal GH release, stimulated GH secretion, GH protein synthesis and GH mRNA expression, can be individually fine-tuned by neuroendocrine regulators to meet the continually changing demands for growth and metabolism throughout the reproductive cycle of the animal.

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