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Grass carp prolactin: Molecular cloning, tissue expression, intrapituitary autoregulation by prolactin and paracrine regulation by growth hormone and luteinizing hormone

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ABSTRACT

Prolactin (PRL), a pituitary hormone with diverse functions, is well-documented to be under the control of both hypothalamic and peripheral signals. Intrapituitary modulation of PRL expression via autocrine/paracrine mechanisms has also been reported, but similar information is still lacking in lower vertebrates. To shed light on autocrine/paracrine regulation of PRL in fish model, grass carp PRL was cloned and its expression in the carp pituitary has been confirmed. In grass carp pituitary cells, local secretion of PRL could suppress PRL release with concurrent rises in PRL production and mRNA levels. Paracrine stimulation by growth hormone (GH) was found to up- regulate PRL secretion, PRL production and PRL transcript expression, whereas the opposite was true for the local actions of luteinizing hormone (LH). Apparently, local interactions of PRL, GH and LH via autocrine/paracrine mechanisms could modify PRL production in carp pituitary cells through differential regulation of PRL mRNA stability and gene transcription.

1. Introduction

Prolactin (PRL) is a pituitary hormone released from lactotrophs within the anterior pituitary. It is a ~ 23 kD protein with four α helixes arranged in an "up-up-down-down" manner typical of the members of PRL/growth hormone (GH) family (Brooks, 2012). Although the pituitary is the major site for PRL production, extrapituitary expression of PRL is also well documented, e.g., in the brain, gonad, placenta and intestine in human (Marano and Ben-Jonathan, 2014). Similar findings have also been reported in lower vertebrates, including fish species (Santos et al., 1999; Zhang et al., 2004). In mammals, PRL plays a key role in reproductive functions including milk production, mammary differentiation, paternal behavior, implantation and luteotropic activity in early phase of pregnancy (Bouilly et al., 2012; Egli et al., 2010). It is also involved in other biological functions including osmoregulation, immune responses, cell proliferation/migration, organogenesis and fetal development, body metabolism, and neurotransmission/neuromodulation (Ben-Jonathan et al., 2008; Freeman et al., 2000; Shelly et al., 2012). In bony fish, in contrast to the functional role of GH in hyperosmotic regulation (e.g., for seawater adaptation; Sakamoto and McCormick, 2006), PRL is essential for osmoregulation under hypoosmotic condition, especially during freshwater adaptation (Breves et al., 2014). In representative species, e.g., goldfish (Tse et al., 2000), tilapia (Sandra et al., 2000), flounder (Higashimoto et al., 2001) and pufferfish (Lee et al., 2006), high levels of PRL receptor (PRLR) can be identified in osmoregulatory organs including the gills, kidney and intestine. In general, PRL can elevate plasma Na^+ and Cl^- levels, inhibit Na^+/K^+-ATPase activity in the gills, trigger differentiation of freshwater-type chloride cells, and reduce permeability of branchial and intestinal epithelia in fish adapting to a hypoosmotic environment (Manzon, 2002; Sakamoto and McCormick, 2006). Similar to GH, PRL is also known to have somatotropic activity in fish model (e.g., tilapia; Shepherd et al., 1997), probably by up-regulation of IGF-I expression at the hepatic level (Murphy et al., 1988).

In vertebrates ranging from fish to mammals, PRL secretion from the pituitary is regulated by stimulatory (e.g., TRH and PRL-releasing peptide) and inhibitory input (e.g., dopamine) from the hypothalamus as well as the endocrine signals from peripheral tissues (e.g., sex steroids) (Ignacak et al., 2012; Seale et al., 2013). Besides the neuroendocrine factors, osmolality changes in extra- cellular fluid can also serve as a "regulatory signal" to modify PRL release and gene expression directly at the lactotroph level, e.g., during freshwater adaptation in fish species (for recent review, see Seale et al., 2012). In recent years, the autocrine/paracrine actions of PRL has

Abbreviations: PRL, prolactin; GH, growth hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; hCG, human chorionic gonadotropin; RPD, rostral pars distalis; PPD, proximal pars distalis; NIL, neurointermediate lobe; LCM, laser capture microdissection; $T_{1/2}$, transcript half-life.

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received a lot of attention, mainly due to the findings that high levels of PRL produced locally in human tissues can be associated with tumorigenesis (Bernichtein et al., 2010), and in general, considered to be a risk factor for cancer development (e.g., breast cancer) (Muthuswamy, 2012). In mammals (e.g., rodents), PRL autoregulation by negative feedback is well documented. Besides its "short-loop" feedback by increasing dopamine input into the pituitary via stimulating neuronal activity in the tuberoinfundibular dopaminergic nerve tract of the hypothalamus (Fitzgerald and Dinan, 2008), an "extra-short loop" feedback via local actions of PRL acting at the pituitary level has also been reported (Denef, 2008). This idea is supported by the findings that (i) PRLR expression was located in pituitary lactotrophs (e.g., rat, Morel et al., 1994), and (ii) PRL treatment could suppress PRL secretion in pituitary cells (e.g., rat, Bentley and Wallis, 1987) and lactotroph cell lines (e.g., GH3 cells, Melmed et al., 1980). Unlike the inhibitory effect on PRL release, no consensus has been reached for the autocrine actions of PRL on PRL synthesis, as no effect (Melmed et al., 1980) and inhibition on PRL production/gene expression (Devost and Boutin, 1999) have been reported. To date, the local actions of other pituitary hormones, e.g., GH and luteinizing hormone (LH), on PRL secretion/gene expression are still unclear. Although GH receptor (GHR) can be identified in pituitary lactotrophs, e.g., in human (Mertani et al., 1995), PRL regulation by paracrine actions of GH has not been examined. Given that PRL mRNA levels were found to be much lower in the pituitary of dwarf rat with GH deficiency (Nogami et al., 1989), the possibility of PRL regulation by GH cannot be excluded. In mammals, PRLR expression in gonadotrophs is well documented, e.g., in rat (Morel et al., 1994) and sheep (Tortonese et al., 1998), but the effects of PRL on LH regulation at the pituitary level are still controversial. In ovine (Gregory et al., 2004) or equine pituitary cells (Hodson et al., 2010a), PRL treatment could reduce basal and block GnRH-induced LH secretion. However, similar treatment in mouse gonadotrophs was found to up-regulate LH release with no effects on LHβ mRNA levels (Hodson et al., 2010b). Of note, LH receptor (LHR) expression in lactotrophs has not been reported and it is still unclear if LH released locally can play a role in paracrine regulation of PRL at the pituitary level.

In grass carp, a commercial fish with high market value in Asian countries, somatotrophs are found clustering with gonadotrophs and located in close proximity to lactotrophs in the anterior pituitary (Wong et al., 1998), which provides the anatomical basis for local interactions among the three cell types. In our previous studies, intrapituitary autoregulation of GH (Zhou et al., 2004a) and somatolactin (Jiang and Wong, 2013) via autocrine/paracrine mechanisms has been reported in grass carp pituitary cells. In the same animal model, an "intrapituitary feedback loop" for GH regulation has also been documented (Wong et al., 2006). In this feedback loop, LH released by gonadotrophs acts as a local stimulatory signal to trigger GH secretion from nearby somatotrophs (Zhou et al., 2005). Local release of GH, interestingly, can amplify its own signal by GH-induced GH synthesis and secretion in somatotrophs (Zhou et al., 2004a) with concurrent inhibition on LH release in neighboring gonadotrophs (Zhou et al., 2004b). In carp pituitary cells, LH treatment can also induce GH gene expression via cAMP/PKA, MAPK and PI3K cascades (Sun et al., 2014) but inhibit LHR expression at the somatotroph level via activation of phosphodiesterase III (Sun et al., 2013). Since (i) PRL, GH and somatolactin are all members of the GH gene lineage (Kawauchi and Sower, 2006) and (ii) spatial distribution of lactotrophs in the carp pituitary can allow for local interactions with other pituitary cell types (Wong et al., 1998), we speculate that PRL regulation in grass carp may also involve autocrine/paracrine modulation similar to other pituitary hormones. As a first step to test our hypothesis, grass carp PRL was cloned and its pituitary expression was confirmed both at the transcript level as well as protein level. Using static incubation of grass carp pituitary cells, the direct effects of PRL, GH and LH on PRL release, cell content, total production and transcript expression were tested and the results obtained were further confirmed by reciprocal experiments with immunoneutralization of respective hormones. In this study, the mechanisms for PRL regulation by local interactions of the three pituitary hormones were also examined by testing the possible modifications in PRL transcript stability and primary transcript production. Our investigation for the first time shed light on the novel mechanisms for modulation/fine tuning of lactotroph functions by somatotropic and gonadotropic signals produced locally in the pituitary of a carp species, which may play a key role in setting up the "basal levels" of PRL synthesis and secretion related to the spawning cycle/seasonal growth in fish models.

2. Materials and methods

2.1. Animals

One-year-old grass carp (*Ctenopharyngodon idellus*) with body weight of 1.5–2.0 kg were acquired from local markets and kept in a well-aerated 200-l aquaria under 12L:12D photo-period at 18 ± 2 °C. Since the grass carp at this stage was prepubertal (gonadosomatic index ≤ 0.2%) and sexual dimorphism was not apparent, fish of mixed sexes were used for tissue sampling and pituitary cell preparation. During the process, the fish were sacrificed by anesthesia in 0.05% MS222 (Sigma, St. Louis, MO) followed by spinosectomy according to the protocol CULATR 3355-14 approved by the committee for animal use in research and teaching at the University of Hong Kong.

2.2. Reagents and test substances

Ovine prolactin (PRL) and growth hormone (GH), human chorionic gonadotropin (hCG), and equine luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were acquired from Sigma. These mammalian hormones were used in our studies as they have been previously shown to activate or bind to the respective receptors in fish models, e.g., zebrafish (PRLR, Breves et al., 2013), grass carp (LHR, Sun et al., 2013; GHR, Zhou et al., 2005), tilapia (PRLR, Dauder et al., 1990) and goldfish (GHR, Marchant and Moroz, 1993; PRLR, Tse et al., 2000). The antisera for carp PRL, GH and LH were generous gift from Prof. J.P. Chang (University of Alberta, Canada) and have been validated previously to be specific for grass carp PRL, GH and LH, respectively (Wong et al., 1998). Actinomycin D was obtained from Calbiochem (San Diego, CA) whereas TRIZOL, Superscript II, DNase I, oligo(dT) and poly-D-lysine were purchased from Invitrogen (Grand Island, NY). Stock solutions of PRL and GH (in 1 mg/ml stock) and hCG, LH and FSH (in 5 IU/μl stock) were prepared in small aliquots, "snap-frozen" in liquid N_2 and stored at −80 °C until used. On the day of experiments, stock solutions of test substances were thawed on ice and diluted to appropriate concentrations with pre-warmed culture medium 15 min prior to drug treatment.

2.3. Molecular cloning of grass carp PRL

Molecular cloning of PRL was conducted in grass carp by nested PCR coupled to 5'/3' RACE according to the procedures described previously (Huo et al., 2005). Primers were designed based on the conserved regions of PRL cDNAs reported in goldfish, common carp and zebrafish. PCR was then performed using RT sample prepared from the pituitary as a template to pull out a partial fragment of carp PRL cDNA. Based on the sequence obtained, specific primers were designed and 5'/3'RACE were carried out using a GeneRacer™ RACE cDNA Kit (Invitrogen). The full-length PRL cDNA obtained was analyzed using MacVector 6.5 (Oxford Molecular, Madison, WI). Phylogenetic analysis (using unrooted model) based on the open-reading frame (ORF) of