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Goldfish brain somatostatin-28 differentially affects dopamine- and pituitary adenylate cyclase-activating polypeptide-induced GH release and Ca^{2+} and cAMP signals

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ABSTRACT

Dopamine (DA) and pituitary adenylate cyclase-activating polypeptide (PACAP) stimulate goldfish growth hormone (GH) release via cAMP- and Ca^{2+}-dependent pathways while DA also utilizes NO. In this study, identified goldfish somatotropes responded to sequential applications of PACAP and the DA D1 agonist SKF38393 with increased intracellular Ca^{2+} levels ($[Ca^{2+}]_i$), indicating that PACAP and DA D1 receptors were present on the same cell. A native goldfish brain somatostatin (gbSS-28) reduced SKF38393-stimulated cAMP production and PACAP- and NO donor-elicited GH and $[Ca^{2+}]_i$ increases, but not PACAP-induced cAMP production nor the GH and $[Ca^{2+}]_i$ responses to forskolin, 8-bromo-cAMP and SKF38393. gbSS-28 might inhibit PACAP-induced GH release by interfering with PACAP's ability to increase $[Ca^{2+}]_i$ in a non-cAMP-dependent manner. However, DA D1 receptor activation bypassed gbSS-28 inhibitory effects on cAMP production and NO actions via unknown mechanisms to maintain a normal $[Ca^{2+}]_i$ response leading to unhampered GH release.

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1. Introduction

The adenylate cyclase /cAMP/protein kinase A (AC/cAMP/PKA) signalling cascade plays a major role in the regulation of pituitary somatotrope functions. It is critical for somatotrope ontogeny and proliferation, and mediates the effects of important neuroendocrine regulators on growth hormone (GH) release and synthesis (Chen, 2000; Mayo et al., 2000; Ramirez et al., 2002). GH-releasing hormone (GHRH), the major stimulatory neuroendocrine factor of GH secretion in mammals and birds, increases GH release via activation of this pathway (Mayo et al., 2000). The resultant activation of PKA causes phosphorylation of voltage-sensitive Ca^{2+} channels (VSCCs) leading to increased Ca^{2+} entry and elevations in intracellular Ca^{2+} ($[Ca^{2+}]_i$) that enhance GH secretion (Naumov et al., 1994; Sartin et al., 1996). Inhibition of basal and GHRH-induced GH secretion by somatostatin-14 (SS-14) is manifested, at least in part, via G_i/G_o-mediated inhibition of cAMP production and the subsequent result of K^+ channel activation, hyperpolarization and reduction in Ca^{2+} influx through VSCC (Chen and Clarke, 1996; Chen, 1998; Barnett, 2003). In addition, PKA-mediated phosphorylation of Ser_{133} of cAMP-response element binding protein has been proposed to play a pivotal role in the regulation of GH gene expression in normal, as well as tumoral, mammalian somatotropes (Rosenberg et al., 2002).

In teleosts, the stimulatory regulation of GH release is multifactorial and this is particularly well characterized in goldfish (Canosa et al., 2007; Chang and Wong, 2009). In this model, two endogenous gonadotropin-releasing hormones (GnRHs) act via protein kinase C (PKC) to stimulate GH secretion while dopamine (DA), acting via D1 receptors, and pituitary adenylate cyclase-activating peptide (PACAP), acting via type I PACAP receptors, are cAMP-dependent GH-releasing neuroendocrine factors (Chang et al., 2000; Chang and Wong, 2009). Like the cAMP-dependent GHRH actions in mammals, DA and PACAP stimulation of goldfish GH are Ca^{2+}-dependent and both DA and PACAP increase $[Ca^{2+}]_i$ in single, identified goldfish somatotropes (Chang et al., 2003; Yunker and Chang, 2004). Pharmacological studies using VSCC agonists and antagonists showed that this Ca^{2+}-dependent GH release elicited by PACAP and DA relied on both an intracellular Ca^{2+} mobilization and a VSCC-dependent extracellular Ca^{2+} component (Wong et al., 1994b; Chang et al., 1994; Wirachowsky et al., 2000). 8Br-cAMP enhanced VSCC currents record in perforated-patch, whole-cell voltage clamp of identified goldfish somatotropes, suggesting that VSCC activation by PACAP and DA are mediated, at least in part, by cAMP (Chang et al., 1994). Although both DA and PACAP mobilize intracellular Ca^{2+}, the pharmacological properties of intracellular Ca^{2+}-dependence of DA and PACAP action differ, suggesting that these two regulators utilize distinct suites of intracellular Ca^{2+}

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stores (Chang et al., 2003). In addition, DA D1, but not PACAP, uses a nitric oxide (NO) signalling component (Mitchell et al., 2008).

Inhibitory regulation of GH release in goldfish is also multifactorial in nature and includes the evolutionarily conserved SS-14, serotonin and norepinephrine (Kwong and Chang, 1997; Lee et al., 2000; Yu et al., 2008). In addition, multiple SS isoforms are potentially involved. Three SS mRNAs, each from a distinct gene, are expressed in the hypophysiotropic hypothalamus and each of these encodes for a distinct SS isoforms: SS-14, goldfish brain SS-28 (gbSS-28) and [Pro^2]SS-14 (Lin et al., 1999). Furthermore, mRNA for SS-14 and gbSS-28 are also expressed in the pituitary indicating that goldfish pituitary cells are exposed to multiple SS isoforms (Yunker et al., 2003). Although the mechanisms of action of SS isoforms in goldfish somatotropes are far from being fully understood, some information is available. All three SSs reduce basal GH secretion from, and cAMP production in, goldfish pituitary cells, but with different potencies and efficacies (Yunker et al., 2003). In particular, the potency of gbS-28 ($IC_{50} \sim 0.2$ nM) in inhibiting GH release is at least an order of magnitude greater than those of SS-14 and [Pro^2]SS-14 (IC_{50}s of ~ 1.7 and 6.7 nM, respectively). In addition, SS-14 is capable of inhibiting cAMP-mediated GH secretion at levels distal to cAMP production and $[Ca^{2+}]_i$ elevation but does not inhibit PKC-induced $[Ca^{2+}]_i$ signals (Yunker and Chang, 2001, 2004). On the other hand, gbSS-28 directly reduces basal, as well as PKC-induced increases in, $[Ca^{2+}]_i$ to inhibit GH secretion (Yu and Chang, 2010). However, whether gbSS-28 affects cAMP-dependent Ca^{2+} signalling is unknown.

The goal of the present study is to investigate the effects of gbSS-28 on Ca^{2+} signals involved in mediating cAMP-dependent GH secretion from goldfish somatotropes, and especially the actions of DA and PACAP. The D1 receptor agonist SKF38393 and mammalian $PACAP_{1-38}$ (mPACAP38), which have previously been shown to be effective in this system, were used to elucidate the DA and PACAP stimulatory actions (Yunker and Chang, 2004). GH release responses were examined in column perifusion experiments using dispersed goldfish pituitary cells and $[Ca^{2+}]_i$ changes in identified somatotropes in primary culture were monitored fluorometrically using the Ca^{2+}-sensitive Fura-2/AM dye. cAMP responses were quantified by a commercial ELISA. Results indicate that gbSS-28 differentially affects DA D1- and PACAP-induced GH release, as well as their cAMP and $[Ca^{2+}]_i$ signals.

2. Methods and materials

2.1. Animal

All animal studies were conducted in accordance with the Canadian Council on Animal Care Guidelines and Policies and approved by the University of Alberta Biological Sciences Animal Care and Use Committee. Male and female common goldfish (*Carassius auratus*, 4-6 inches, post-pubertal) were purchased from Aquatic Imports (Calgary, Canada). Fish were held for 2–4 weeks prior to use in flow-through aquaria (1800 l) at 18 °C on a simulated natural photoperiod (times of graded light on and light off adjusted to the time of sunrise and sunset in Edmonton, Alberta, Canada). Fish were normally used within 1 month of arrival. Serum GH levels undergo annual changes in goldfish in the temperate zone with the lowest levels occurring in the fall. This is followed by a gradual increase starting in mid-winter corresponding with the beginning of gonadal recrudescence and increased steroidogenesis. Serum GH levels remain high through spring during the final stages of gonadal recrudescence and spawning and into the summer months when somatic growth is maximal. GH responses to DA and PACAP can be observed at all times of the year although maximal responsiveness to DA occurs in the fall while that to PACAP occurs during winter and early spring (reviewed in Canosa et al., 2007; Chang and Wong, 2009). To facilitate future comparisons, the time(s) of year when experiments were performed have been described in the figure legends.

2.2. Reagents

Synthetic gbSS-28 was a gift from Dr. J. Rivier of the Salk Institute (La Jolla, CA). D1 receptor agonist 1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride (SKF38393), nifedipine, sodium nitroprusside (SNP), forskolin and 8-bromo-cAMP (8Br-cAMP) were obtained from Sigma Chemical Co (St Louis, MO). mPACAP38 (abbreviated as PACAP) was purchased from Peninsula Laboratories (Belmont, CA). The Ca^{2+}-imaging dye Fura-2/AM and its solvent, Pluronic F-127, were purchased from Invitrogen (Carlsbad, CA). Distilled, deionized water was used to prepare stock solutions of SNP, gbSS-28 and mPACAP38. Ethanol was used to dissolve nifedipine. SKF38393, forskolin and 8Br-cAMP were dissolved in dimethyl sulphoxide (DMSO). Aliquots of stock solutions were stored at −20 °C. Final concentrations of DMSO and ethanol were less than 0.1% and had no effect on basal GH release and $[Ca^{2+}]_i$ in identified goldfish somatotropes. Stock solutions of 10 mM Fura-2/AM was made up fresh in DMSO with 20% Pluronic F-127 and sonicated for 10 min prior to use. Concentrations of drugs used in this study have been shown to be maximally effective on GH release in this system in previous studies.

2.3. Pituitary cell preparation

Fish were anaesthetized in 0.05% tricane methanesulphonate (Syndel Lab., Vancouver, BC) prior to decapitation. Pituitaries were rapidly dissected out and pituitary cells were dispersed using a trypsin/DNase protocol as previously described (Chang et al., 1990). Following dispersion, cells were re-suspended in plating medium (Medium 199 with Earle's salts, Invitrogen, Grand Island, 1% horse serum, 25 mM HEPES, 2.2 g/l $NaHCO_3$, 100 000 U/l penicillin and 100 mg/l streptomycin, pH adjusted to 7.2 with NaOH), and cultured overnight under 5% CO_2 and saturated humidity at 28 °C.

2.4. Cell column perifusion experiments

Cell column perifusion experiments were performed as previously described (Chang et al., 1990; Wong et al., 1993). Briefly, dispersed goldfish pituitary cells cultured on preswollen Cytodex-I beads (Sigma) were loaded onto perifusion columns (1.5×10^6 cells/column) and perifused with testing medium (Medium 199 with Hank's salts, Invitrogen, Grand Island, NY, 1% horse serum, 25 mM HEPES, 2.2 g/l NaHCO3, 100 000 U/l penicillin and 100 mg/l streptomycin, pH adjusted to 7.2 with NaOH) at a rate of 13 ml/h. Following a 4-h resting period, fractions of perifusate were collected either every 1 min or 5 min and stored at −26 °C until GH levels were assayed by a previously validated radioimmunoassay (Marchant et al., 1987). GH content in perifusates from individual columns was normalized as a percentage of the average values obtained in the first four fractions collected prior to the drug application (% pretreatment). Hormone responses were quantified by determining the net change in GH levels (area under the curve, i.e., sum of the area of trapezoids, expressed as % pretreatment × time (s), of each of the observation periods across the response period with baseline subtracted; baseline = average value of the five fractions prior to drug application) as previously described (Wong et al., 1993; Yu and Chang, 2010). Treatments were performed in duplicate in each experiment and all experiments were repeated a minimum of three times using different cell preparations.

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