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Dopamine inhibits somatolactin gene expression in tilapia pituitary cells through the dopamine D2 receptors

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ABSTRACT

Dopamine (DA) is an important neurotransmitter in the central nervous system of vertebrates and possesses key hypophysiotropic functions. Early studies have shown that DA has a potent inhibitory effect on somatolactin (SL) release in fish. However, the mechanisms responsible for DA inhibition of SL gene expression are largely unknown. To this end, tilapia DA type-1 (D1) and type-2 (D2) receptor transcripts were examined in the neurointermediate lobe (NIL) of the tilapia pituitary by real-time PCR. In tilapia, DA not only was effective in inhibiting SL mRNA levels *in vivo* and *in vitro*, but also could abolish pituitary adenylate cyclase-activating polypeptide (PACAP)- and salmon gonadotropin-releasing hormone (sGnRH)-stimulated SL gene expression at the pituitary level. In parallel studies, the specific D2 receptor agonists quinpirole and bromocriptine could mimic the DA-inhibited SL gene expression. Furthermore, the D2 receptor antagonists domperidone and (−)-sulpiride could abolish the SL response to DA or the D2 agonist quinpirole, whereas D1 receptor antagonists SCH23390 and SKF83566 were not effective in this respect. In primary cultures of tilapia NIL cells, D2 agonist quinpirole-inhibited cAMP production could be blocked by co-treatment with the D2 antagonist domperidone and the ability of forskolin to increase cAMP production was also inhibited by quinpirole. Using a pharmacological approach, the AC/cAMP pathway was shown to be involved in quinpirole-inhibited SL mRNA expression. These results provide evidence that DA can directly inhibit SL gene expression at the tilapia pituitary level *via* D2 receptor through the AC/cAMP-dependent mechanism.

1. Introduction

Dopamine (DA) is a neurotransmitter involved in a wide variety of physiological processes including motor coordination, emotions, memory, reward mechanism and regulation of the hypothalamic–pituitary axis (Beaulieu and Gainetdinov, 2011). The diverse physiological actions of DA are mediated through two major receptor subtypes, the D1-like (D1 and D5) and D2-like (D2, D3, and D4) receptors, which are G protein coupled receptors (Rangel-Barajas et al., 2015). These receptors are further characterized by their ability to stimulate (D1-like receptors) (Dearry et al., 1990; Sunahara et al., 1991) or inhibit (D2-like receptors) (Civelli et al., 1993; Grandy et al., 1989) adenylyl cyclase. Besides their distribution in the central nervous system, D1 and D2 receptors, which are also expressed in the pituitary gland (Beaulieu and Gainetdinov, 2011), can mediate DA regulation of hormone secretion in both mammals and teleosts at the pituitary level. It is well established that DA inhibits pituitary prolactin (PRL) release in rat (Lledo et al., 1992) or goldfish (Wong et al., 2002) and also suppresses PRL gene expression in tilapia (Poncelet et al., 1996) or GH4 (Liu et al., 2005) pituitary cells *via* D2 receptor functional inhibition of cAMP (Liu et al., 1994), or activation of Ca^{2+}-responsive signaling pathways (Lledo et al., 1992) and ERK1/2 cascades (Liu et al., 2005). Although still controversial, there is evidence that DA is also known to modulate growth hormone (GH) release in the anterior pituitary. It has notably been shown that DA inhibits GH release *in vitro* through activation of D2 receptors in rat (Cronin et al., 1984) or ovine (Law et al., 1984) pituitary cells, but stimulates GH release in tilapia (Melamed et al., 1995) or goldfish (Wong et al., 1993) pituitary cells *via* D1 receptors.

Somatolactin (SL), the novel member of GH/PRL family, is a pituitary hormone unique to teleost species. Both *in vivo* and *in vitro* studies have revealed that SL may be involved in gonadal maturation (Rand-Weaver et al., 1992), steroidogenesis (Planas et al., 1992), stress responses (Rand-Weaver et al., 1993), background adaptation (Zhu and Thomas, 1997), immune functions (Calduch-Giner et al., 1998), energy mobilization (Kaneko et al., 1993), and lipid metabolism (Fukamachi et al., 2005). Two SL subtypes, *i.e.*, SLa and SLb, have been identified in fish. SLa is present in all fish species, but SLb appears to be restricted to basal teleost species such as catfish, goldfish, salmonids and eel. The

Abbreviations: DA, dopamine; SL, somatolactin; GH, growth hormone; PRL, prolactin; PACAP, pituitary adenylate cyclase-activating polypeptide; sGnRH, salmon gonadotropin-releasing hormone; NIL, neurointermediate lobe; cAMP, cyclic adenosine monophosphate; AC, adenylate cyclase.

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SLb gene probably was lost in the diversification of teleosts (Lynn and Shepherd, 2007). According to genome databases, tilapia seem to have only one copy of SL (SLa), which shares around 70% identity with SLa of carp, goldfish and zebrafish (Uchida et al., 2009). Similar to other pituitary hormones, SL expression is under the control of hypothalamic regulators (*e.g.*, gonadotropin-releasing hormone (GnRH) (Canepa et al., 2008), pituitary adenylate cyclase-activating polypeptide (PACAP) (Jiang et al., 2008b)). A previous study has demonstrated that DA markedly inhibits SL release from the organ-cultured pituitary in rainbow trout (Kakizawa et al., 1997). It raises the possibility that SL gene expression may be under dopaminergic inhibitory control in fish. However, there has been no direct evidence to confirm the effect of DA on SL gene expression at the pituitary level so far and the mechanisms responsible for DA inhibition of SL gene expression are largely unknown.

Tilapia is the second largest species of fish farmed worldwide with 40% produced by China (Chiu et al., 2013). Since a better understanding of biological functions of DA in tilapia may have important implication in the improvement of the species culture, the tilapia was chosen as the animal model in our present study. As a first step, the expression patterns of D1 and D2 receptors were examined in tilapia pituitary and the roles of DA on SL gene expression in tilapia were investigated both *in vivo* and *in vitro*. Furthermore, the receptor specificity for DA actions was characterized in tilapia NIL cells using D1 or D2 receptors agonists and antagonists. The functional roles of the AC/cAMP pathway in dopaminergic regulation of SL mRNA expression were evaluated. In this study, we have demonstrated for the first time that DA can act at the pituitary level *via* D2 receptor to inhibit SL gene expression through the AC/cAMP-dependent mechanism.

2. Materials and methods

2.1. Animals

Sexually mature male Mozambique tilapia (*Oreochromis mossambicus*) (standard length: 11 ± 0.5 cm, body weight: 50 ± 5.0 g) were maintained in freshwater aquaria at 28 °C under 10 h dark/14 h light photoperiod. The fish were fed commercial diet (40% protein, 12% fat, 2% fiber, 8.5% moisture, 8% ash, Tongwei, China) to satiety twice a day at 10:00 and 16:00. During the process of tissue sampling, the fish were sacrificed by spinosectomy after anesthesia with 0.05% MS222 (Sigma, St Louis, MO) according to the procedures approved by the Animal Ethics Committee of Sichuan University.

2.2. Test substances

DA, ovine PACAP38 (oPACAP38), sGnRH, apomorphine, SKF38393, SKF77434, quinpirole, bromocriptine, SCH23390, SKF83566, domperidone and (−)-sulpiride were obtained from Sigma (St. Louis, Mo). 3-Isobutyl-1-methylxanthine (IBMX), 8-(4-chloro-phenylthio)-cAMP (CPT-cAMP) and forskolin were obtained from Calbiochem (San Diego, CA). For *in vivo* experiments, apomorphine, domperidone, and SCH23390 were first dissolved in a minimal amount of dimethyl sulfoxide (DMSO) and subsequently diluted to appropriate concentrations with propylene glycol. These pharmacological compounds have been used previously in goldfish (Wong et al., 1993) and tilapia (Levavi-Sivan et al., 2005), confirming that they are highly selective for respective targets in fish models. For *in vitro* experiments, dopamine and oPACAP38 were prepared in double-distilled deionized water and aliquots of sGnRH stock solution in 0.1 M acetic acid were frozen at −80 °C and diluted with the testing medium to appropriate concentrations immediately prior to use. All Dl or D2 drugs were first dissolved in DMSO. The final dilutions of DMSO were less than 0.1% and had no effects on SL gene expression (Jiang et al., 2008b).

2.3. Intraperitoneal injection

Male tilapia were anesthetized by submersion in with 0.05% MS222 (Sigma, St Louis, MO) before experimental handling. The time-course effects of DA on SL gene expression *in vivo* were investigated by intraperitoneal injection of a nonselective DA agonist apomorphine (20 μg/g body weight). Eight fish from each group were anesthetized and then sacrificed by spinosectomy at the following intervals: 6, 12, and 24 h postinjection. The DA receptor subtype mediating the SL responses to apomorphine was also examined. Apomorphine (20 μg/g body weight) was injected intraperitoneally in the presence or absence of 40 μg/g body weight of the D2 antagonist domperidone or the D1 antagonist SCH23390. Eight fish from each group were anesthetized and then sacrificed by spinosectomy at 24 h postinjection. The NIL of individual pituitaries was removed and immersed in liquid nitrogen for real-time PCR studies. In these experiments, RT-PCR of 18S rRNA mRNA was used as an internal control.

2.4. Primary culture of tilapia NIL cells

Tilapia pituitary cells were prepared by trypsin/DNase II digestion method as described previously (Jiang et al., 2011). For each batch of experiments, NIL cells were prepared from 40 individual fish. Briefly, the NIL of individual pituitaries was isolated by manual dissection under a stereomicroscope, diced into 0.6 mm fragments using a McILwain tissue chopper (Brinkmann, Mississauga, Ont.), and digested in type II trypsin (4 mg/ml, GIBCO) for 30 min at 28 °C with constant shaking. After that, the reaction was terminated by adding trypsin inhibitor (2.5 mg/ml, Sigma) and pituitary fragments were dispersed in Ca^{2+}-free MEM [S-MEM with 26 mM NaHCO3, 25 mM HEPES, 1% antibiotic–antimycotic, and 0.1% BSA; pH 7.7] with DNase II (0.01 mg/ml, Sigma). NIL cells were then harvested and cultured in 48-well culture plates (Costar, Corning Inc., N.Y.) at a seeding density of ~1×10^6 cells/well in M199 (Invitrogen Carlsbad, CA, USA). NIL cells were incubated overnight at 28 °C under 5% CO_2 and saturated humidity to allow for the recovery of membrane receptors after trypsin digestion. Total cell yield and percentage viability were estimated by cell counting in the presence of trypan blue using a hemocytometer. On the following day, culture medium was replaced with serum-free M199 and drug treatment was initiated for the duration as indicated in individual experiments.

2.5. Real-time PCR measurement of D1 receptor, D2 receptor and SL

Real-time PCR was used for quantitative measurement of transcript expression for the respective genes. For detection of D1 and D2 receptors mRNA expression at the pituitary level, the NIL and pars distalis (PD) of individual pituitaries were isolated by manual dissection under a stereomicroscope as described previously (Jiang and Wong, 2014). For pituitary cells, tilapia NIL cells were seeded at a density of ~1×10^6 cells/ml/well in 48-well culture plates and treated with hormones or drugs for the duration as indicated in individual experiments. After that, total RNA was isolated using RNAzol reagent (MRC, Cincinnati, OH, USA), digested with RNase-free DNase I to remove genomic DNA contamination, and reversely transcribed using M-MLV (TaKaRa, Dalian, China). After that, real-time PCR assays were performed on the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, CA, USA). PCR reactions were conducted with a SYBR Select Master Mix kit (Applied Biosystems) with the gene-specific primers for tilapia D1 receptor, D2 receptor and SL. The primers used for tilapia SL detection were (GenBank Accession No: AB442015) SL forward primer: 5′ CCCA CTCCCTTTGCGACTT 3′ and SL reverse primer: 5′ TAGCGGTCCAGTGTCG TCT 3′, whereas the primers used for D1 receptor detection were (GenBank Accession No: X81969) D1 receptor forward primer: 5′ AGGTTCGGCTGCGTGATGA 3′ and D1 receptor reverse primer: 5′ TCGG CG TTGAAGGCGTAGAT3′, and for D2 receptor detection were (GenBank Accession No: AY673985) D2 receptor forward primer: 5′ CAGTATGCCT