General and Comparative Endocrinology 211 (2015) 154–164

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

The acute salinity changes activate the dual pathways of endocrine responses in the brain and pituitary of tilapia

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ARTICLE INFO

Article history:
Received 18 May 2014
Revised 11 October 2014
Accepted 12 December 2014
Available online 20 December 2014

Keywords:
Salinity stress
Osmoregulation
crh
Corticosteroid receptors
igf-1

ABSTRACT

To analyze and compare the stress and osmoregulatory hormones and receptors in pituitary during acute salinity changes, the expression patterns of corticotropin releasing hormone (*crh*) in hypothalamus, prolactin (*prl*) releasing peptide (*pRrp*) in telencephalon and diencephalon, glucocorticoid receptors 2 (*gr2*), and mineralocorticoid receptor (*mr*), *crh-r*, pro-opiomelanocorticotropin (*pomc*), *pRrp*, *prl*, dopamine 2 receptor (*d2-r*), growth hormone (*gh*), *gh*-receptor (*gh-r*) and insulin-like growth hormone (*igf-1*) transcripts in pituitary were characterized in euryhaline tilapia. The results indicate that the *crh* transcripts increased in the hypothalamus and rostral pars distalis of the pituitary after the transfer of fish to SW. Similarly, the *pRrp* transcripts were more abundant in SW acclimated tilapia forebrain and hypothalamus. The *crh-r*, *gr2* and *mr* transcripts were more expressed in rostral pars distalis and pars intermedia of pituitary at SW than FW tilapia. The data indicate that the SW acclimation stimulates these transcripts in the specific regions of the brain and pituitary which may be related to the activation of the hypothalamic–pituitary–interrenal (HPI)-axis. The results of dual *in situ* hybridization reveal that the transcripts of *crh-r*, *gr2* and *mr* with *pomc* are highly co-localized in corticotrophs of pituitary. Furthermore, we demonstrate high expression of *pRrp* in the brain and low expression of *pRrp* and *prl* transcripts in the pituitary of SW fish. No *crh-r* and corticosteroid receptors were co-localized with *prl* transcripts in the pituitary. The *gh-r* and *igf-1* mRNA levels were significantly increased in SW acclimated tilapia pituitary whereas there was no difference in the *gh* mRNA levels. The data suggest that the locally produced *pRrp* and *d2-r* may control and regulate the expression of *prl* mRNA in pituitary. Therefore, the dual roles of *pRrp* are involved in the stress (via brain-pituitary) and osmoregulatory (via pituitary) pathways in tilapia exposed to acute salinity changes.

1. Introduction

The neuroendocrine aspects of the teleostean stress response are initiated by the release of hypothalamic peptide, corticotropin releasing hormone (*crh*), which is considered to be a neurotransmitter and/or neuromodulator (Rotllant et al., 2000). The *crh* mRNA expression has been studied under different physiological conditions in teleosts revealed that the involvement of *crh* in several physiological process including stress, osmoregulation, energy metabolism, locomotor control and reproduction (Ando et al., 1999; Lovejoy and Balment, 1999; Sawchenko et al., 1993; Van Enckevort et al., 2000; Westphal et al., 2009). In addition, the anatomical localization studies reported that most of the CRF-immunoreactive perikarya and nerve fibers have been found in nucleus lateralis tuberis (NLT) than in nucleus preopticus (NPO) in teleosts Batten et al., 1990; Olivereau and Olivereau, 1988, 1990; Yulis et al., 1986; Zupanc et al., 1999. However, there are fewer studies about the localization of *crh* mRNA in hypothalamus during salinity stress.

The biological actions of *crh* are mediated through the *crh*-receptor (*crh-r*). The *crh-r* is ubiquitously expressed in brain and anterior pituitary (Aruna et al., 2012b; Chalmers et al., 1995). In teleosts, the anterior pituitary synthesized eight different hormones including, prolactin (*prl*), adrenocorticotropic hormone (*acth*), growth hormone (*gh*), thyrotropin, gonadotrophins, melanotropin (*msh*), and somatolactin (Folleenius et al., 1978). During salinity stress, the *crh* from the hypothalamus stimulated the pro-opiomelanocorticotropin (*pomc*), a precursor peptide of several mature peptides including adrenocorticotropic hormone (*acth*) Slominski et al., 2000; Wendelaar, 1997. *pomc* has important roles

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http://dx.doi.org/10.1016/j.ygcen.2014.12.005
0016-6480/

in the stress response, skin pigmentation, thermoregulation, reproduction, food intake, and immunomodulation (Cerda-Reverter et al., 2003; Hadley and Haskell-Luevano, 1999; Harris and Bird, 2000; Kawauchi and Sower, 2006; Lee et al., 2008). The *pomc* gene is expressed predominantly in two types of cells in the pituitary gland: corticotrophs in the pars distalis and melanotrophs in the pars intermedia where the *pomc* gene is processed into *acth* and *msh*, respectively, followed by the interrenal secretion of glucocorticoids and subsequent physiological changes associated with the classic stress response.

The glucocorticoids are synthesized by steroidogenic enzymes in head kidney interregnal tissue of teleosts (Hirano et al., 1987; Jiang et al., 1996; Kusakabe et al., 2002). Glucocorticoids play an important role in the homeostasis of many biological systems, including stress response, energy metabolism, immune and inflammatory responses (Charmandari et al., 2005; De Kloet et al., 2005). In the brain, the corticosteroid has a neuromodulatory role, and it regulates the main neuronal populations of the caudal telencephalon/anterior preoptic region and diencephalon of the teleosts (Huang et al., 1999). Glucocorticoids regulate the function of many organ systems, including the brain, liver, pancreas, and muscles, via glucocorticoid receptors (*gr*s) and/or the mineralocorticoid receptor (*mr*) (Kino and Chrousos, 2004). Immunoreactivity for *gr* was detected in the anterior and posterior pituitary. *gr* mRNA was abundant in the anterior and intermediate pituitary cells but scattered sparsely in the mammalian posterior pituitary (Ozawa et al., 1999). However, the localization and distribution of *gr* and *mr* transcripts in FW and SW pituitary during salinity stress were poorly understood in teleosts.

In teleosts, prolactin releasing peptide (*pRrp*) has stimulatory effects on *prl* secretion (Moriyama et al., 2002; Seale et al., 2002) and expression (Fujimoto et al., 2006; Kelly and Peter, 2006; Sakamoto et al., 2003). Beyond *prl* release, *pRrp* is also known to play some important physiological roles in stress response (Matsumoto et al., 2000; Uchida et al., 2009). PrRP is suggested to have effects on CRH secretion in rat (Maruyama et al., 2001). In teleosts, there was no information regarding about the involvement of *pRrp* in stress response. The mechanisms of *pRrp* in the stress and osmoregulation remain unknown. More studies are needed on the localization of *pRrp* during FW and SW acclimation in teleosts. Furthermore, dopamine is known not only as an inhibitory factor for PRL secretion and expression (Ben-Jonathan and Hnsako, 2001) but also as a multi-functional molecule in mammals (Björklund and Dunnett, 2007). The regulation of *PRL* by dopamine is mediated by the *D2-R* in mammals (O'Connell, 1996).

The growth hormone (*gh*) stimulates the synthesis of insulin-like growth factor I (*igf-1*), predominantly from the liver, which carries out the physiological actions of *gh* (Green et al., 1985; Holly and Wass, 1989). *igf-1* in fish is produced not only in liver, but also in several extrahepatic sites indicating an important role of local *igf-1* via paracrine/autocrine mechanisms (Reinecke and Collet, 1998; Reinecke, 2006). The *igf-1* mRNA was detected in *Cottus kazika* (Inoue et al., 2003), and *igf-1* immunoreactivity was localized in adult tilapia in *acth* cells in pituitary of tilapia (Quérat et al., 1990). Furthermore, there was not much study about gh-receptor (*gh-r*) during salinity changes.

In this study, we hypothesize that the acute salinity changes will activate the stress and osmoregulatory responses in the brain and pituitary to enhance the homeostasis in tilapia. The expressions of stress mediators (*pRrp*, *crh*, *crh-r*, *pomc*, *gr* and *mr*) and the osmoregulatory mediators (*pRrp*, *gh*, *gh-r*, *igf-1*, *d2-r* and *prl*) were investigated in euryhaline tilapia brain and pituitary. The Q-PCR and *in situ* hybridization were the approaches to conduct. The possible interaction between stress and osmoregulatory pathways was also studied.

2. Materials and methods

2.1. Experimental fish

Hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*) (6–7 months age; $n = 30$; body weight = 28.05 ± 0.73 g; body length = 13.5 ± 0.45 cm) were cultured in FW at the university aquarium with a natural light system (water temperature ranged from 19 to 24 °C). The hybrid tilapia could provide all male fish in the experiments. The fish were fed with pelleted dry feed *ad libitum* at a daily ration of 1% of their estimated body weight. The experiments in the present study were conducted in accordance with the principles and procedures approved by the Institutional Animal Care and Use Committee, National Taiwan Ocean University, Taiwan.

2.2. Experimental design

The fish were randomly divided into two groups and maintained in FW. After an initial acclimation period (30 days), the fish ($n = 15$) maintained in FW were transferred directly to SW (20 ppt). The hybrid tilapia could not stand for salinity of 35 ppt SW. The control group ($n = 15$) was similarly transferred but without a change in the salinity. One day after FW–FW and FW–SW transfer, the fish were anesthetised with 1% glycophenol monophenyl ether and decapitated. Brain (telencephalon [Tel] and diencephalon [Dien]) and pituitary samples were collected from FW ($n = 10$) and SW ($n = 10$) and snap frozen in liquid nitrogen and stored at −80 °C. Brain and pituitary samples were collected from FW ($n = 5$) and SW ($n = 5$) were fixed in 4% paraformaldehyde in 1X PBS for histological analysis.

2.3. Experiment 1-gene expression profiles in tilapia brain pituitary during salinity stress

To study the mRNA expression pattern of *pRrp* in Tel and Dien and *gh*, *gh-r*, *igf-1*, *crh-r*, *pRrp*, *gr2*, *mr* and *d2-r* in tilapia pituitary and brain during salinity stress, we collected brain and pituitary samples from FW ($n = 10$) and SW ($n = 10$) at day 1. The mRNA expression of the genes was quantified by Q-PCR analysis.

2.4. Experiment 2-localization of stress hormone and receptors in tilapia by in situ hybridization analysis

To localize the mRNA expression of *pRrp* in forebrain and hypothalamus and *crh-r*, *pRrp*, *gr2* and *mr* in pituitary during FW and SW fish, we collected brain and pituitary samples from FW ($n = 5$) and SW ($n = 5$) at day 1. The localization of the transcripts was conducted by *in situ* hybridization analysis.

2.5. Experiment 3-colocalization of the transcripts in tilapia pituitary by dual in situ hybridization analysis

To co-localize the transcripts of *crh-r*, *gr2* and *mr* with *pomc* and *prl* in the pituitary, we collected the pituitary samples from SW ($n = 5$) at day 1. The co-localization of the transcripts was conducted by dual *in situ* hybridization analysis.

2.6. Experiment 4-co-localization of the pRrp transcripts and Blbp-ir by double staining

In order to confirm whether *pRrp* expressed in radial glial cells, we performed double staining of *pRrp* (*in situ* hybridization) and glial cell marker, brain lipid binding protein (Blbp) antibody (immunohistochemistry) in forebrain and hypothalamus of tilapia.