General and Comparative Endocrinology 160 (2009) 67–75

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Effect of extracellular osmolality and ionic levels on pituitary prolactin release in euryhaline silver sea bream (*Sparus sarba*)

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ARTICLE INFO

Article history:
Received 23 April 2008
Revised 1 September 2008
Accepted 22 October 2008
Available online 6 November 2008

Keywords:
PRL
Abrupt transfer
Pituitary cells
Silver sea bream
Osmosensing

ABSTRACT

In many euryhaline fish, prolactin (PRL) plays a key role in freshwater adaptation. Consistent with this function, the present study showed a remarkable reduction in pituitary PRL content of silver sea bream abruptly transferred to low salinity (6 ppt). This reduction in pituitary PRL content followed closely the temporal changes in serum osmolality and ion levels. Serum osmolality, Na^+ and Cl^- levels of silver sea bream abruptly transferred to hyposmotic salinity (6 ppt) were markedly reduced 2 h after the transfer. The decline in pituitary PRL content lagged behind the serum changes implying that reduction in pituitary PRL content is a response to the drop in serum ion levels and osmotic pressure. Silver sea bream pituitary cells were dispersed and exposed to a medium with reduced ion levels and osmolality in vitro, and PRL released from pituitary cells was significantly elevated. In hyposmotic exposed anterior pituitary cells, cell volume exhibited a 20% increase when exposed to a medium with a 20% decrease in osmolality. The enlarged pituitary cells did not shrink until the surrounding hyposmotic medium was replaced, a phenomenon suggesting an osmosensing ability of silver sea bream PRL cells for PRL secretion in response to a change in extracellular osmotic pressure. The decrease in pituitary PRL content in vivo and stimulated pituitary PRL release in vitro under reduced osmolality together suggest hyposmotic exposure triggers PRL release from the pituitary.

1. Introduction

Prolactin (PRL) is an important osmoregulatory hormone involved in freshwater acclimation of fish. According to previous studies in euryhaline teleosts, pituitary or plasma PRL level was higher in freshwater (FW)-adapted fish than those adapted to seawater (SW) (Nagahama et al., 1975; Sakamoto et al., 1991; Ayson et al., 1993). However, the mechanism for translating a hyposmotic (or FW) signal to PRL secretion is still not well understood although many neural or hormonal factors are implicated in the process. PRL cell secretory activity was shown to be controlled by hypothalamic factors including dopamine, serotonin, thyrotropin-releasing hormone, somatostatin, and gonadotropin-releasing hormone (Wigham et al., 1977; Batten and Wigham, 1984; Wigham and Batten, 1984; Barry and Grau, 1986; Williams and Wigham, 1994a,b; James and Wigham, 1984; Grau et al., 1987; Weber et al., 1997). Another factor which controls PRL secretion is cortisol, as cortisol is shown to inhibit PRL release from tilapia pituitary in vitro (Uchida et al., 2004). However, there is considerable evidence showing that blood and tissue fluid osmotic pressure may also contribute significantly to the regulation of PRL secretion. Experiments involving ectopic pituitary transplants in euryhaline SW gobiid fish (Nagahama et al., 1975), sailfin molly (Wigham and Ball, 1977) and tilapia (Shepherd et al., 1999) demonstrated lowering of plasma osmolality, in response to exposure to low environmental salinity, had a direct effect on pituitary PRL secretion in the absence of hypothalamic innervations. In addition to in vivo studies, various in vitro studies have demonstrated an increase in PRL release from tilapia rostral pars distalis (RPD) exposed to hyposmotic conditions suggesting a direct stimulatory effect of osmotic pressure on PRL cell activity (Wigham et al., 1977; Grau et al., 1981, 1987; Helms et al., 1991; Yoshikawa-Ebesu et al., 1995). More recent studies used a dispersed RPD cell preparation for hyposmotic exposure and revealed that the PRL cell volume change, stretch-activated ion channels of the PRL cells and extracellular Ca^{2+} were important components for the osmotic control of PRL secretion in tilapia RPD (Seale et al., 2003a,b, 2006; Weber et al.,2004). Results from these studies suggested that hyposmotic exposure could increase PRL cell volume, which activated the stretch-sensitive Ca^{2+}-permeant channels on the cell membrane, leading to a rise in intracellular Ca^{2+} via extracellular Ca^{2+} entry. Such a rise in intracellular Ca^{2+} induced subsequent PRL release from the PRL cells.

Demonstration of a direct effect of extracellular osmolality on PRL secretion has been limited to works on only a few fish species, with most detailed studies being carried out with tilapia. The tilapia is a euryhaline teleost of freshwater origin and the osmoregu-

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doi:10.1016/j.ygcen.2008.10.024

latory mechanisms of this species may not be universally applicable to other euryhaline fish species, especially those of marine origin. Silver sea bream (*Sparus sarba)*, the animal model of the present study, is a euryhaline marine teleost capable of survival in a wide range of salinities. Silver sea bream treated with PRL exhibited a reduction in branchial Na–K-ATPase activity (Kelly et al., 1999a), which is in contrast to studies in tilapia where PRL had no effect on branchial Na–K-ATPase activity (Herndon et al., 1991). In fact, there is no general consensus regarding the effect of PRL on gill Na–K-ATPase activity in different teleost species, which is either decreased (Shepherd et al., 1997; Madsen et al., 1997; Sakamoto et al., 1997; Kelly et al., 1999a; Mancera et al., 2002), increased (Leena and Oommen, 2000; Eckert et al., 2001; Boeuf et al., 1994) or unchanged (Herndon et al., 1991; Madsen et al., 1995; Seidelin and Madsen, 1999; Zhou et al., 2003). In a previous study, we have demonstrated that PRL mRNA expression was upregulated in pituitaries of silver sea bream adapted to hyposmotic salinities of 0–6 ppt (Kwong and Woo, 2008), corroborating the importance of PRL in hyposmotic adaptation in euryhaline teleost of marine origin. In view of these findings, it would be interesting to assess whether direct exposure to reduced ionic content and osmolality can also lead to stimulated PRL secretion in pituitaries of marine euryhaline silver sea bream. The present study provides in vivo and in vitro evidence to support that low ionic concentration and osmolality are direct stimulators of pituitary PRL secretion in silver sea bream.

2. Materials and methods

2.1. Experimental animals

Silver sea bream (*Sparus sarba*) weighing between 250 and 400 g were obtained from a local fish farm and held in recirculating seawater [33 parts per thousand (ppt); 28 ± 1 °C] in Simon FS Li Marine Science Laboratory, The Chinese University of Hong Kong. Fish were fed daily with a formulated diet (Woo and Kelly, 1995) and maintained in fully aerated seawater for one month before experiments.

2.2. Abrupt transfer of fish to hyposmotic salinity and sample collection

Salinity of the experimental tanks was reduced to 6 ppt through addition of fully aerated tap water. Fish were then abruptly transferred from seawater (33 ppt) holding tanks (1 m^3) to experimental tanks (1 m^3) containing hyposmotic seawater (6 ppt). Fish were sampled just before abrupt hyposmotic transfer and 2, 6, 9 and 24 h after abrupt transfer into 6 ppt (n = 10 per group). Different groups of fish in different sampling times and salinities were sampled in separate tanks to avoid stress of capture. Corresponding control groups were transferred from the original 33 ppt seawater tank to another 33 ppt seawater tank and sampled at the same time intervals as the fish transferred to 6 ppt.

Blood was taken via a syringe inserted into the caudal vessels and fish were killed by spinal transection. Blood was allowed to clot at room temperature for 30 min and centrifuged at 3000g for 5 min to obtain serum. Pituitaries were removed quickly, frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.3. Measurement of serum osmolality, Na^+ and Cl^- levels

Serum osmolality was measured using a vapor pressure osmometer (5520 Vapro®, Wescor, Logan, UT). Serum [Na^+] was determined by Polarized Zeeman atomic absorption spectrophotometry (Z-2300, Hitachi, Tokyo, Japan). Serum [Cl^-] was determined using a chloridometer (Corning EEL 920, Corning, Halstead, UK).

2.4. Primary pituitary cell static culture and hyposmotic exposure

A control medium and a hyposmotic medium were formulated for incubating the primary pituitary cell static cultures. Control medium was prepared by firstly diluting nine volumes of Leibovitz's L15 medium (320 mmol/kg) (Invitrogen, Carlsbad, CA) with one volume of sterilized deionized water to result in a solution having the following composition: Na^+: 125 mM; Cl^-: 132 mM; Ca^{2+}: 1.1 mM; osmolality: 290 mmol/kg. As the serum osmolality of 33 ppt-adapted sea bream was hyperosmotic (380 mmol/kg) to the diluted L15 medium, Na^+ and Cl^- concentration of the control medium for sea bream primary cell static culture was adjusted by adding NaCl and Na_2SO_4 to the diluted L15 medium to result in a final composition of Na^+: 198 mM; Cl^-: 163 mM; Ca^{2+}: 1.1 mM; osmolality: 380 mmol/kg. A hyposmotic medium was also prepared by adding Na_2SO_4 to the diluted L15 medium so as to result in a medium with the following composition: Na^+: 140 mM; Cl^-: 132 mM; Ca^{2+}: 1.1 mM; osmolality: 305 mmol/kg. Osmolality and ionic concentrations of the hyposmotic medium approximate the lowest levels attained in the serum of sea bream following abrupt transfer to hyposmotic medium (6 ppt).

Pituitaries removed from silver sea bream were placed in control medium before cell dispersion. Pituitaries were washed in the same medium and then diced into small fragments of 0.5 mm in thickness using a McILWAIN tissue chopper (Brinkmann, Westbury, NY). Diced pituitaries were washed using control medium and then exposed to 0.25 % trypsin (Sigma, St. Louis, MO) at 28 °C with shaking for 45 min. After incubation, cells were incubated in control medium containing 10% fetal bovine serum (FBS) for 5 min and then in medium containing DNase II (10 μg/ml) for 5 min, followed by mechanical dispersion and filtration by cell strainer (40 μm, BD Biosciences, Bedford, MA). The dispersed pituitary cells were centrifuged at 300g for 10 min, resuspended in control medium and plated in BD Falcon® 24-well cell culture plate (Becton Dickinson, Franklin Lakes, NJ) at a density of 0.6 million cells per well. Total cell count was estimated using a hemocytometer and viability was scored to be above 90% with trypan blue checking. The primary pituitary cells were allowed to recover overnight at 28 °C in a humidified atmosphere.

After overnight incubation, the pituitary cells were preincubated in replenished control medium for 15 min and the control medium was subsequently replaced by hyposmotic medium. After 10 and 30 min of hyposmotic exposure, the medium was collected for measurement of prolactin content by enzyme-linked immunosorbent assay (ELISA). For control groups, control medium instead of hyposmotic medium was used for the incubation.

2.5. Measurement of PRL secretion by ELISA

2.5.1. Preparation of anti-PRL antiserum

The antigen for the preparation of polyclonal antibody was a 16-amino acid peptide (CHTSSLQTPNDKEQAL) designed from black porgy PRL amino acid sequence (GenBank Accession No. ABW 05297). Antiserum was raised in rabbits using the complete antisera package service (Sigma–Genosys) including peptide synthesis, conjugation of peptides to keyhole limpet hemocyanin (KLH) as carrier protein, immunization, blood withdrawing from rabbits, ELISA checking of antiserum titers and affinity purification of the polyclonal antibody.

2.5.2. Western Blot analysis for checking antiserum specificity

One sea bream pituitary was homogenized in extraction buffer (4 M urea, 0.5% w/v SDS, 10 mM EDTA and 2 mM PMSF), incubated