General and Comparative Endocrinology 161 (2009) 216–228

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Extracellular calcium-sensing receptor distribution in osmoregulatory and endocrine tissues of the tilapia

Christopher A. Loretz [a,b,*], Catherine Pollina [a,b], Susumu Hyodo [b], Yoshio Takei [b]

[a] Department of Biological Sciences, University at Buffalo, 109 Cooke Hall, Buffalo, NY 14260-1300, USA
[b] Laboratory of Physiology, Ocean Research Institute, The University of Tokyo, Nakano, Tokyo 164-8639, Japan

ARTICLE INFO

Article history:
Received 29 October 2008
Revised 24 December 2008
Accepted 29 December 2008
Available online 14 January 2009

Keywords:
Extracellular calcium-sensing receptor
Calcium homeostasis
Osmoregulation
Mozambique tilapia
Japanese eel

ABSTRACT

The extracellular calcium-sensing receptor (CaSR) serves an important detector function in vertebrate Ca^{2+} homeostasis. In this study, we surveyed using immunohistochemistry the tissue and cellular distribution of the CaSR protein in the Mozambique tilapia (*Oreochromis mossambicus*) and the Japanese eel (*Anguilla japonica*). Specifically, we examined receptor expression in ion-transporting barrier tissues that may be directly responsive to extracellular Ca^{2+} levels, and in tissues that are implicated in endocrine signaling to homeostatic effectors such as Ca^{2+}-transporting epithelia. In tilapia osmoregulatory tissues, CaSR protein is strongly expressed in proximal segments of renal tubule, but not in distal segments (where Na^+,K^+-ATPase is prominently expressed) or in glomeruli. The receptor was also localized in the ion-transporting mitochondria-rich cells of gill and in ion- and nutrient-transporting epithelia of middle and posterior intestine. Consistent with our earlier RT-PCR assessment of mRNA expression in tilapia, CaSR protein expression was salinity dependent in some osmoregulatory tissues. In tilapia pituitary gland, CaSR expression was observed in the rostral pars distalis (containing prolactin-secreting cells, and in the pars intermedia (containing somatolactin-secreting and melanocyte-stimulating hormone-secreting cells), with notably greater expression in the latter. In the eel, weak immunostaining was seen in the stanniocalcin-secreting cells of the corpuscles of Stannius. Olfactory lobe CaSR expression suggests an environment-sensing role for the receptor. Altogether, these findings support the involvement of CaSR in piscine Ca^{2+} homeostasis at the levels of environmental sensing, of integrative endocrine signaling through both hypercalcemic (prolactin, and perhaps somatolactin) and hypocalcemic (stanniocalcin) hormones, and of direct local regulation of Ca^{2+}-transporting tissues.

1. Introduction

Homeostatic regulation of extracellular calcium concentration ($[Ca^{2+}]_o$) in animals is especially important in light of the key role that ionic calcium (Ca^{2+}) plays in many physiological events and processes, such as Ca^{2+}-based action potentials, stimulus–secretion coupling, and excitation–contraction coupling, to name but a few (Berridge et al., 2000; Berridge et al., 2003). And in vertebrates, especially, growth and maintenance of the bony endoskeleton that is functionally important in multiple ways places another demand on organismal calcium acquisition and balance. In addition to the sites and avenues for Ca^{2+} uptake, loss and storage that are present in all vertebrates (namely, the alimentary tract, kidney and bone), aquatic species additionally face the challenge and the potential of Ca^{2+} exchange with the external medium across the skin and gills (Flik et al., 1995). Further, the challenging aspects of Ca^{2+} exchange are exacerbated in euryhaline fishes where the calcium gradient between the fish and the exterior reverses as the external medium changes between fresh water and seawater. Central to the homeostatic process, regardless of habitat, is the sensing of extracellular Ca^{2+} ($Ca^{2+}{}_o$) by cells that play a direct role in maintaining or modifying the Ca^{2+} concentration in the extracellular spaces (through vectorial transport across barrier epithelia, or through deposition and release at stores such as bone, for example), or by cells that affect changes in extracellular Ca^{2+} levels indirectly via endocrine, neurotransmitter-based or other chemical signaling to effector cells and tissues.

We previously cloned, sequenced and functionally expressed the extracellular calcium-sensing receptor (CaSR, a divalent cation-sensing receptor) from the Mozambique tilapia (*Oreochromis mossambicus*), a euryhaline teleost fish (Loretz et al., 2004). CaSRs are members of G protein-coupled receptor (GPCR) family C that also includes pheromone (PherR) and odorant (OdorR), metabotropic glutamate (mGluR), type B γ-aminobutyric acid ($GABA_BR$) and some other receptors. Recently, the close evolutionary relationships among CaSRs and olfactory and vomeronasal-type odorant

* Corresponding author. Address: Department of Biological Sciences, University at Buffalo, 109 Cooke Hall, Buffalo, NY 14260-1300, USA. Fax: +1 716 645 2975.
E-mail address: loretz@buffalo.edu (C.A. Loretz).

0016-6480/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygcen.2008.12.020

receptors of fishes have been carefully evaluated (Alioto and Ngai, 2006; Dukes et al., 2006; Hashiguchi and Nishida, 2006).

CaSRs are modular proteins. They are characterized by possessing a large extracellular domain (ECD), the N-terminal part of which forms a bilobed Venus flytrap (VFT) structure with which the primary physiological ligand, Ca^{2+}, interacts (Fig. 1; Chang and Shoback, 2004; Loretz, 2008). The modular VFT feature is widely shared with other family C GPCRs, including PherRs and OdorRs, mGluRs, $GABA_B$-Rs, and others. Following the VFT module of the CaSR protein are a cysteine-rich segment of the ECD (the so-called "nine-cysteines" domain), the heptahelical transmembrane domain (TMD), and the C-terminal intracellular domain (ICD) tail (Loretz, 2008). Interactional G protein coupling of the ICD to multiple signal transduction pathways, including intracellular calcium messenger and mitogen-activated protein kinase cascades and others, functionally links receptor activation to target cell responses (Ward, 2004). Originally characterized from bovine parathyroid gland (Brown et al., 1993) and demonstrated to detect and to signal changes in $[Ca^{2+}]_o$, CaSR expression has been reported in a wide spectrum of tetrapod tissues including parathyroid gland, kidney, brain and olfactory tissues, alimentary tract, and elsewhere (reviews by Brown and MacLeod (2001); Hofer and Brown (2003); Bai (2004); Chang and Shoback (2004)). By a variety of assessment techniques, including reverse transcription-polymerase chain reaction (RT-PCR), quantitative or real-time PCR (Q-PCR), Northern blotting and in situ hybridization, recent evidence points to a similarly broad pattern of tissue expression in fishes as well (Loretz, 2008). Specifically, based on RT-PCR analysis, we showed tilapia CaSR (tCaSR) mRNA expression to be strong in gills, kidney, intestine, and brain (including pituitary gland), compared with lower expression levels in other tissues such as stomach, urinary bladder and heart (Loretz et al., 2004). Based on these and other data, the receptor is linked to multiple distinct aspects of organismal calcium homeostasis in the fishes through three categories of tissue expression (Loretz, 2008). CaSR is expressed in: (1) effector tissues such as transporting epithelia, where CaSR activity may influence vectorial ion-transport processes at a local scale, and at store tissues such as bone where receptor activity may influence calcium deposition and release; (2) nervous and endocrine tissues, where $[Ca^{2+}]_o$ sensing may be coupled to integrated systemic calcium regulation; and (3) olfactory tissues, where the receptor senses external, environmental Ca^{2+} concentrations. The CaSR may be not only an essential component in calcium homeostasis in fishes, but it also may be importantly integrated into osmotic stress response and nutrition through its proposed roles as salinity and broad-spectrum amino acid sensor, respectively (Quinn et al, 1998; Nearing et al., 2002; Hebert et al., 2004; Riccardi and Maldonado-Perez, 2005; Alioto and Ngai, 2006; Conigrave and Brown, 2006; Conigrave and Hampson, 2006; Fiol and Kültz, 2007; Loretz, 2008).

In the several contexts introduced above, direct measurement of tissue and cell expression patterns of receptor mRNA and protein can be employed to test hypotheses for CaSR involvement in homeostatic processes. The several epithelial tissues separating inside from outside, namely the gills, intestine and kidney are demonstrated or proposed to be key components in organismal Ca^{2+} homeostasis, as well (Takei and Loretz, 2006; Loretz, 2008). And the pituitary gland, particularly through its hormones prolactin (PRL, a hypernatremic and hypercalcemic factor) and somatolactin (SL, a proposed hypercalcemic factor), the corpuscles of Stannius, through the hormone stanniocalcin (STC, a hypocalcemic factor), and the ultimobranchial gland, through the hormone calcitonin, have been linked to calcium homeostasis in teleost fishes (Suzuki et al., 1999; Takei and Loretz, 2006). The objective of the studies reported herein was the characterization of CaSR protein expression in some tilapia osmoregulatory and endocrine tissues that are implicated in calcium homeostasis, and in the Japanese eel (*Anguilla japonica*) corpuscles of Stannius that due to their large size are particularly well suited to microscopical analysis. To that end, we developed antibodies directed against the tCaSR protein and applied these in a homologous immunohistochemical study of CaSR expression in freshwater (FW)- and seawater-acclimated (SW) tilapia, and in heterologous fashion to eel kidney and corpuscles of Stannius.

Some of these findings were reported elsewhere in preliminary abstract form (Loretz et al., 2007).

2. Materials and methods

2.1. Fish and tissues

Cultured Mozambique tilapia (*Oreochromis mossambicus*; body mass 20–100 g) for these studies were obtained from a standing

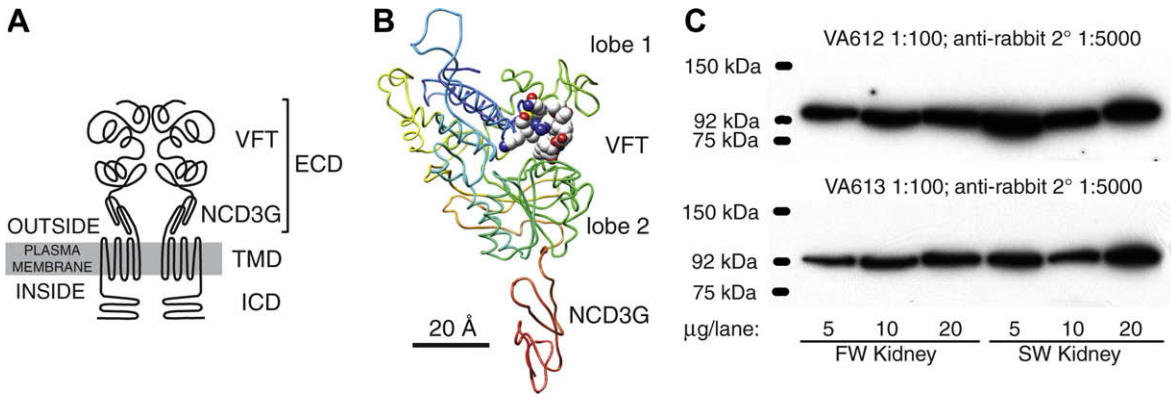

Fig. 1. Anti-tCaSR antibody development and evaluation. (A) The tilapia extracellular calcium-sensing receptor (tCaSR) is a glycosylated 940-amino acid (aa) modular protein comprising: a large N-terminal extracellular domain (ECD, ~600 aa in length) with signal sequence (removed in mature receptor), Venus flytrap (VFT), and nine-cysteines domain of family 3 GPCR (NCD3G) modules; a heptahelical transmembrane domain (TMD, ~250 aa); and a C-terminal intracellular domain (ICD, ~90 aa). At the plasma membrane, the functional receptor is a dimer. (B) Three-dimensional homology modeling of the tCaSR ECD (aa residues 27–584) reveals the bilobed structure of the VFT module and the position of the NCD3G module as a connector between VFT and TMD. In this rendition, the ECD protein backbone appears as a simple strand. The ECD epitope for the VA612 antibody (tCaSR aa residues 46–60; pictured in space-filling format) is located on the surface of VFT lobe 1. The ICD epitope for the VA613 antibody (aa residues 901–917) is not illustrated since there is no three-dimensional structural model for the CaSR ICD. (C) A tilapia kidney membrane protein immunoblot using affinity-purified VA612 and VA613 anti-CaSR antibodies shows identical immunoreactive bands for both antibodies at ~100 kDa molecular mass. Amounts of membrane protein loaded onto the original polyacrylamide gel are indicated beneath the respective lanes. Similar immunoblot patterns are observed for kidney tissues from freshwater (FW)- and seawater-acclimated (SW) tilapia.