

cDNA CLONING OF MOZAMBIQUE TILAPIA (*Oreochromis mossambicus*) EXTRACELLULAR Ca^{2+} -SENSING RECEPTOR: PRIMARY STRUCTURE AND TISSUE DISTRIBUTION

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Summary

Using PCR cloning, the complete cDNA sequence of the Mozambique tilapia (*Oreochromis mossambicus*) extracellular Ca^{2+} -sensing receptor (CaR) was determined. Including 5'- and 3'-UTR, the overall transcript length is 3.3 kbp. The tilapia (t) CaR cDNA encodes a 940 amino acid, 7-transmembrane domain protein, consistent in its structural features with known mammalian and piscine CaRs. Tissue mRNA expression was examined by RT-PCR using specific primer pairs for tCaR and β -actin (as internal control). Brain, gill and kidney showed strong expression; weaker expression was observed in heart, stomach, intestine and urinary bladder. This distribution pattern for the tilapia CaR is consistent with possible physiological roles in barrier epithelia and excitable tissues.

Introduction

Calcium-dependent biological processes are numerous and include hormone secretion (stimulus-secretion coupling), cell motility (excitation-contraction coupling) and neurotransmission (Ca^{2+} -based action potentials); this critical involvement of ionic calcium in numerous cellular functions is unique among ions. Calcium is simultaneously a controlled variable in physiological systems, a fluid-borne extracellular messenger in multicellular organisms and an intracellular mediator in a variety of effector cells.

In terrestrial vertebrates, calcium homeostasis depends upon appropriate dietary intake, renal output, and osseous storage and mobilization of Ca^{2+} ; these are regulated by parathyroid hormone, calcitonin and the active form of vitamin D_3 acting on kidney, intestine and bone. Activation of the calcium-sensing receptor (CaR) by extracellular Ca^{2+} (Ca^{2+}_o) alters parathyroid hormone and calcitonin secretions, and inhibits renal 1-hydroxylase to retard the synthesis of active vitamin D_3 (1). Interestingly, in fishes, the calcium homeostatic process involves ion-transporting tissues not represented in other vertebrate classes (*viz.*, the mitochondria-rich “chloride cells” of the gills and skin) and a different panel of hormones [prolactin and somatolactin from the pituitary, and stanniocalcin

from the piscine corpuscles of Stannius; (2)]. Maintenance of a stable calcium concentration in the body fluids of fishes is complicated further in euryhaline species that encounter changes in the composition of the external environment.

We cloned and sequenced a cDNA from kidney of Mozambique tilapia that encodes a CaR, deduced its protein structure, and determined its tissue distribution.

Materials and Methods

Animals. Freshwater-adapted (FW; <10 mosmol/l osmotic pressure, 0.4 mM calcium) Mozambique tilapia (*Oreochromis mossambicus*: Teleostei) were used for tissue harvesting. Fish were maintained and handled in accordance with the University of Tokyo Guidelines for Care and Use of Animals.

RNA Extraction and Purification. The brain, pituitary, gills, heart, kidney, urinary bladder, stomach, intestine, liver, skeletal muscle and skin were isolated from FW fish after anesthesia in 0.02% 3-aminobenzoic acid ethyl ester. Tissues were immediately frozen in liquid N₂, and were stored at -80 °C. Total RNA was extracted using ISOGEN (Nippon Gene). Poly (A)+ RNA was isolated from total RNA from the kidney of one FW tilapia using Oligotex-dT30 Super (Japan Synthetic Rubber).

cDNA Cloning and Sequencing. For cloning and sequencing, double-stranded cDNA pools were prepared from 0.5 µg poly(A)+ RNA (SMART cDNA Library Construction Kit; Clontech). A partial cDNA fragment was amplified by PCR using a degenerate sense and antisense primer pair based on known vertebrate CaR cDNA sequences. The PCR product was ligated and subcloned into pT7Blue T-vector (Novagen), and sequenced. Complete sequencing of tCaR cDNA beginning with the original PCR fragment was accomplished stepwise, requiring four sequencing steps in the 5' direction and a single reaction in the 3' direction, including terminal 5'- and 3'-RACE PCR, respectively.

Tissue Distribution. tCaR mRNA expression was examined by RT-PCR using a specific sense and antisense primer pair. Total RNA from the tissues of 2 FW fish was treated with DNase (DNase I; TaKaRa) to remove genomic DNA, and was reverse transcribed (Superscript First Strand Synthesis System; Invitrogen). The expression of tilapia β-actin mRNA was used as internal control.

Results

The overall tCaR transcript length is 3.3 kbp, including 5'- and 3'-untranslated regional (UTR). The coding sequence for the tCaR cDNA is

2823 nucleotides in length (with stop codon), and encodes a 940 amino acid (aa) protein. The deduced protein sequence comprises a 599 aa extracellular domain (ECD) that begins with an 18 aa signal sequence, a 245 aa 7-transmembrane (7-TM) domain (TMD) core, and a 96 aa intracellular domain (ICD). RT-PCR analysis of total mRNA revealed strong expression in brain, gill and kidney; weaker expression was observed in heart, stomach, intestine and urinary bladder.

Discussion

The cDNA sequence and deduced tCaR protein structure confirm identity as a CaR, rather than a closely related fish pheromone receptor (PherR) or other 7-TM domain protein of the G protein-coupled receptor superfamily. By aa identity, the tCaR was more similar to CaRs of other teleost fishes (>90%) than to either CaRs of mammals (*ca.* 65%), which are more than 100 aa longer at the C-terminal end, or PherRs (30-50%), which are more than 70 aa shorter. The conspicuous presence of an ICD distinguishes tCaR from PherRs, with their very short (*ca.* 15-20 aa) ICDs. Interestingly, there is greater similarity among CaRs and PherRs in their TMD compared with their corresponding ECD, which in CaRs contains the calcium-binding domain. The tissue mRNA distribution pattern for the tCaR is consistent with possible physiological roles in barrier epithelia and excitable tissues, including perhaps calcium-sensitivity in endocrine cells of the kidney (corpuscles of Stannius) or the brain and pituitary gland (prolactin- and somatolactin-secreting cells).

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