

EXTRACELLULAR CALCIUM-SENSING RECEPTOR OF TILAPIA (*Oreochromis mossambicus*): cDNA SEQUENCE AND TISSUE EXPRESSION

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Using PCR cloning, 5'- and 3'-RACE, and beginning with degenerate primers designed according to known extracellular calcium-sensing receptor (CaR) nucleotide sequences from mammals and fishes, the complete cDNA sequence of the tilapia (*Oreochromis mossambicus*) CaR was determined. The coding sequence for the tilapia CaR cDNA is 2823 nucleotides in length (with stop codon). Including the 5'- and 3'-UTR, the overall transcript length is approximately 3.3 kb. The deduced protein sequence of 940 amino acids (aa) comprises a 600 aa extracellular domain (ECD) that begins with a 20 aa signal sequence, a 250 aa seven-transmembrane domain (TMD) core, and a 90 aa intracellular domain (ICD). The deduced tilapia CaR protein structure is consistent with those of known CaRs. By amino acid identity, the tilapia CaR was more similar to that of other teleost fishes (>90%) than to that of mammals (*ca.* 65%), which are also typically about 100 aa longer at the C-terminal end. The tilapia CaR is a member of the G protein-coupled receptor superfamily. The tilapia cDNA characterized in these studies was confirmed to be a CaR, rather than a closely related fish pheromone receptor (PherR). Overall amino acid identity was only *ca.* 40%. Additionally, tilapia CaR possesses the longer ICD that is typical for CaRs compared with the short ICD of fish PherRs. Interestingly, there is greater similarity among both CaRs and PherRs in their TMD compared with their corresponding ECD, which in CaRs contains the calcium-binding domain. Tissue mRNA expression was examined by RT-PCR using specific primer pairs for tilapia CaR and β -actin (as internal control). Brain, gill and kidney showed strong expression; weaker expression was observed in heart, stomach, intestine and urinary bladder. Using an antiserum to the extracellular domain of bovine CaR, protein expression was detected in brain, intestine and kidney. This distribution pattern for the tilapia CaR is consistent with possible physiological roles in barrier epithelia and excitable tissues.

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