HOMOLOGS OF AN EXTRACELLULAR CALCIUM/POLYVALENT CATION-SENSING RECEPTOR (CaR) ARE LOCALIZED TO THE APICAL SURFACES OF SPECIFIC EPITHELIAL CELLS IN ORGANS CRITICAL FOR IONIC HOMEOSTASIS IN THE ELASMOBRANCHS, SQUALUS ACANTHIAS AND RAJA ERINACEA, AS WELL AS TELEOSTS (PLEURONECTES AMERICANUS), (ONCORHYNCHUS MYKISS) AND FUNDULUS HETEROCLITUS

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Molecular cloning and characterization of a cell surface receptor called the calcium/polyvalent cation sensing receptor or CaR has demonstrated that it responds to or "senses" extracellular Ca^{2+} and Mg^{2+} concentrations of 1-5 mM Ca^{2+} and 5-20 mM Mg^{2+} respectively. CaR is expressed by mammalian parathyroid and C cells as well as several tubule epithelial cells of the kidney including thick limb of Henle (TAL). CaR allows these cells to respond to alterations in serum Ca^{2+} and Mg^{2+} by modulation of intracellular signal transduction pathways (Brown, E.M. et al. New Eng. J. Med. 333:234, 1995). Recent data (Baum, M. et al. J. Am. Soc. Neph. 6: 319A, 1995) have shown that a CaR is also present on the apical membranes of rat inner medullary collecting duct where it modulates vasopressin-elicited water permeability. Since fish encounter alterations in the ambient and urinary concentrations of both Ca^{2+} and Mg^{2+} during fresh to seawater transitions, we studied both the distribution and expression of CaR homologs in marine and euryhaline species. The distribution of CaR protein was determined using an anti-CaR antiserum and immunohistochemistry of tissue sections while CaR expression was surveyed by RNA blotting using CaR specific cDNA probes.

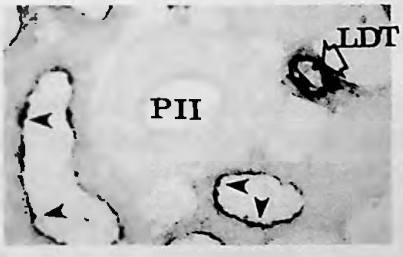

FIGURE 1: Localization of CaR in S. acanthus kidney tubules using anti-CaR specific antiserum. CaR protein (shown as the dark reaction product indicated by arrowheads) is present on the apical membrane of epithelial cells of the collecting duct (CD) and late distal tubule (LDT) but not PII segment (PII) responsible for Mg^{2+} secretion. (Mag 400X).

In both skate and dogfish, CaR protein was localized to the apical membranes of selected epithelial cells in kidney tubules, rectal gland and gill. In the kidney, CaR protein was confined to the CD and LDT (Figure 1). These cells are in close proximity to and receive the luminal fluid of the PII segment that is responsible for renal Mg^{2+} secretion (Hentschel, H. and K. Zierold Eur. J. Cell Biol. 63:32, 1994). The presence of CaR in these elasmobranch nephron segments suggests that CaR may play a role in

regulation of renal divalent metal ion secretion by sensing alterations in urinary Mg^{2+} and Ca^{2+} concentrations.

In the flounder (Pleuronectes americanus), CaR protein was localized to the apical membranes of epithelial cells in kidney tubules, gill, urinary bladder and intestine as well as specific regions of brain. In the fresh water trout (Onchorhynchus), CaR staining was present in the urinary bladder.

CaR protein was also localized to the apical membranes of epithelial cells in kidney tubules in the killifish, Fundulus heteroclitus. To determine if CaR expression was modulated by adaptation of Fundulus to either fresh or salt water, killifish collected in the local estuary were first fresh or salt water adapted for an interval of 18 days (chronic adaptation). Selected individuals from each group were then adapted to the corresponding salinity (fresh to salt; salt to fresh) for an interval of 7 days (acute adaptation). As shown in Figure 2, chronic adaptation to seawater results in an increase in steady state levels of CaR mRNA in 3 tissues. In a similar manner, we also observed increases in both the staining intensity of CaR as well as the number of epithelial cells possessing CaR in kidneys of fish adaptated chronically as well as acutely to salt water as compared to fresh water conditions.

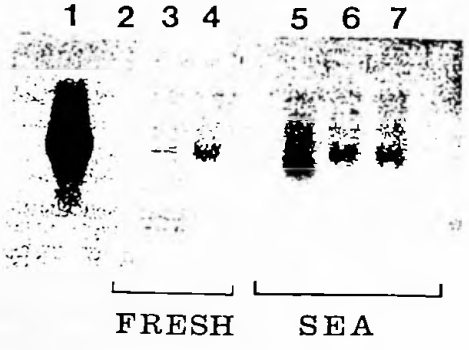

Figure 2: CaR expression in tissues of Fundulus heteroclitus after an 18 day interval of fresh (lanes 2-4) or sea water (lanes 5-7) adaptation. A blot containing RNA (40 µg/lane) prepared from control Xenopus kidney (lane 1) or Fundulus heart (containing ultimobranchial tissue) (lanes 2, 5), kidney (lanes 3, 6) and gill (lanes 4, 7) was probed with a ^{32}P-labeled Xenopus CaR cDNA, washed (0.1 X SSC, 65°C) and autoradiographed. As compared to control mRNA (lane 1), steady state levels of CaR mRNA are larger in tissues from sea water adapted fish (lanes 5-7) vs those in fresh water (lanes 2-4).

We conclude that homologs of the CaR protein present in mammals are also present in selected tissues of elasmobranch and teleost fish. The distribution of CaR protein on the apical membranes of epithelial cells in the gill, intestine, urinary bladder, rectal gland and kidney tubules as well as brain suggests the involvement of CaR in modulation of epithelial ion and water transport and perhaps endocrine function. Alterations in the steady state levels of CaR mRNA in ultimobranchial tissue, kidney and gill that accompany adaptation of Fundulus to either fresh or salt water further suggests a role for CaR in fish osmoregulation.

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