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Co-localization of Na^+/K^+-ATPase and the Peptide Hormone Receptor Guanylate Cyclase in Chloride Cells of *Fundulus heteroclitus*

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Fundulus heteroclitus (also called mummichog and killifish), a teleost widely distributed in estuarine waters of North America, has served as both a test organism for environmental toxicology studies and a physiological model for elucidating mechanisms of salt secretion by gills and opercular epithelia [1]. Chloride cells (mitochondria-rich cells) in gills and opercular epithelia of *Fundulus* and other salt-tolerant fish enable fish to adapt readily to extreme changes of salinity, which regularly occur in their natural habitat. The two objectives of the present study were to determine 1) any quantitative and/or qualitative differences in the location and morphology of chloride cells during 24-hr seawater (SW) adaptation and 2) the cellular location of guanylyl cylase (GC) isoform C in the gills and any quantitative and/or qualitative change in the isoform during the 24-hr SW adaptation.

For the 24-hr SW adaptation experiments, *Fundulus* were transferred from dilute seawater (DSW) to either DSW (3.5 ppt Instant Ocean) or SW (35 ppt Instant Ocean). After 24 hrs, the gills were dissected from the fish and fixed overnight at 4° C in a fixative containing 4% paraformaldehyde and 0.8 % phosphate buffered saline (PBS). Gill filament rows were dissected from the arch, decalcified for 48 hrs in a 10% EDTA solution, dehydrated in an ethanol series, cleared, and embedded in paraffin. Serial sections, parallel to the gill filaments, were cut at 8 μ using a rotary microtome. For immuno-cytochemistry, we modified the procedure of Vector Laboratories (Burlingame, CA). Sections were blocked for 1 hr using diluted normal horse serum. The primary antibody for the α subunit of Na^+/K^+-ATPase, a monoclonal antibody α5 (diluted 1:175 in PBS), was obtained from the Developmental Studies Hybridoma Bank at the University of Iowa. The primary antibody Z659 (diluted 1:300 in PBS) was used for identifying GC isoform C. After rinsing, sections were exposed to a diluted biotinylated "universal" secondary antibody, and then incubated in Vectastain Elite ABC Reagent. We used peroxidase substrate 3,3'-diaminobenzidine (DAB; Vector Laboratories) for Na^+/K^+-ATPase, and Vector SG for GC-isoform C. Sections were then dehydrated, cleared, and mounted. Sections were photographed under differential interference microscopy.

Gill sections of fish transferred to SW exhibited a different pattern of immunostaining (FIG. 1, A and C) for Na^+/K^+-ATPase α-subunit from sections of DSW (control) fish. As expected, the staining intensity was densest in chloride cells irrespective of salinity. SW chloride cells appeared to develop extensions of the basolateral membrane, penetrating into the primary lamellae, a condition which was not observed in DSW controls. A morphometric analysis indicated that chloride cell volume was greater in the SW specimens relative to DSW controls. GC-isoform C immunostaining of chloride cells resembled that of Na^+/K^+-ATPase (FIG. 1, B and D). However, in contrast to Na^+/K^+-ATPase staining, chloride cells in the DSW specimen stained more intensely than those in the SW specimen. Secondary lamellae did not stain because they contained no chloride cells (FIG.1).

Previous studies have established that chloride cells of *Fundulus* [2-4] are enriched in Na^+/K^+-ATPase. Although the number of chloride cells in *Fundulus* does not vary during salinity adaptation, the

cells undergo a change in shape and increase in size in SW, as confirmed in the present study. The present study is the first to localize GC-isoform C to a specific cell type in the *Fundulus* gill. In mammals, the cloned intestinal STa receptor, a peptide hormone receptor referred to here as GC-isoform C, is responsible for much of the particulate GC activity in intestinal brush border membranes and for the effects of heat stable enterotoxins (STa) on intestinal water secretion and absorption (5). The physiological ligand for the STa receptor tentatively identified in *Fundulus* chloride cells remains an open question. While natriuretic peptides (NPs) are likely to mediate osmoregulatory changes during SW adaptation of euryhaline fish, the available evidence (6) suggests that atrial, brain and C-type NPs do not stimulate GC-isoform C. However, the co-localization of GC-isoform C and Na^+/K^+-ATPase found in this study suggests that an endogenous peptide mediator, acting through cGMP, might regulate chloride cell sodium pump activity in an autocrine fashion.

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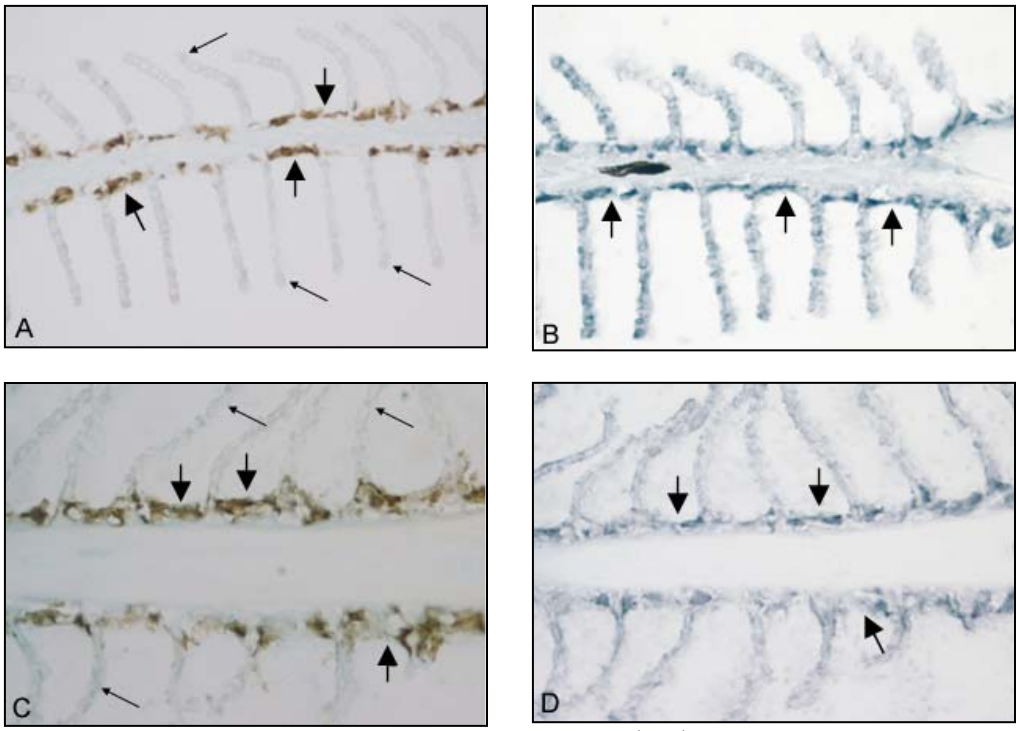

FIG. 1. Light micrographs of immunolocalization of Na^+/K^+-ATPase α-subunits (A and C) and guanylyl cyclase isoform C in gills of *Fundulus heteroclitus* (B and D). A and B, dilute seawater; C and D, seawater. Large arrows indicate immunopositivity; small arrows, no immunopositivity.

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