FRESHWATER ADAPTATION OF KILLIFISH INVOLVES MORPHOLOGICAL AND FUNCTIONAL ALTERATION IN BRANCHIAL CHLORIDE CELLS

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Introduction

The killifish, *Fundulus heteroclitus,* is a euryhaline species that can adapt to a wide range of salinities. Although chloride cells are more developed in seawater (SW) than in fresh water (FW) in most fishes examined to date (Uchida et al., 2000), it has been revealed that chloride cells in killifish are larger in FW than in SW. The apical membrane of branchial chloride cells in FW-adapted killifish showed projections with microvilli that expanded the apical surface area (Katoh et al., 2001), suggesting active ion uptake through chloride cells in FW. Thus, killifish is considered to serve as a suitable model for studies on chloride cell functions in FW. The model for active Na^+ uptake across the gill epithelia involves the vacuolar-type proton pump (V-ATPase) coupled with amiloride-sensitive Na^+ channel (Harvey and Wieczorek, 1997). In the present study, we investigated the effects of environmental Na^+ concentration on chloride cell morphology, and examined the implication of V-ATPase in Na^+ absorption through the gill epithelia in killifish.

Materials and methods

Killifish of the Arasaki strain (Shimizu, 1997) were kept in a tank with recirculating SW at the ambient temperature. The fish were first adapted to 50% SW for 1 week, and then kept in normal FW for 1 month. The FW-adapted fish were separated into three groups and reared for 1 week in defined FWs with 0.1 mM, 1 mM, or 10 mM NaCl and 0.5 mM $CaCl_2$. The fish were not fed and the water temperature was maintained at 25^{o}C during the experiment. The gills were fixed and observed by confocal laser scanning microscopy (LSM), and scanning (SEM) and transmission (TEM) electron microscopy. Furthermore, we cloned and sequenced cDNA encoding A subunit of V-ATPase and examined immunolocalization of V-ATPase in the gill epithelia.

Results

Plasma osmolality of the killifish adapted to defined FWs containing 0.1, 1 or 10 mM Na^+ was maintained within a physiological level, although there were significant differences among three experimental groups ($P<0.01$). There was no significant difference in gill Na^+, K^+-ATPase activity among three experimental groups (Table 1).

With decreasing environmental Na^+ concentration, chloride cells became significantly larger (Table 1), and extended their distribution toward the efferent-vascular side. In SEM observations, the apical membrane of chloride cells was located at the boundary of pavement cells. In 0.1 and 1 mM Na^+ groups, the apical membrane was equipped with microvilli on its surface. In 10 mM Na^+ group, on the other hand, the apical membrane of most chloride cells was invaginated to form an apical pit. The TEM observations showed that the chloride cells often formed multicellular complexes with accessory cells, and that chloride and accessory cells shared the apical pit in Na^+ 10 mM group. Such complexes were not observed in 0.1 and 1 mM Na^+ groups.

Table 1 Effects of ambient Na^+ concentration

	Na^+ 0.1 mM	Na^+ 1 mM	Na^+ 10 mM
Osmolality (mOsm kg^{-1})			
Gill Na^+, K^+-ATPase activity	12.1±0.8 (8)	10.6±0.8 (8)	9.0±0.5 (7)
(μmol ADP mg $protein^{-1}$ h^{-1})	305.3±4.7 (10)		
Gill chloride cells			
size (μm^2)	201.8±5.9 (5)	117.7±4.2 (5)*	96.5±3.9 (5)*
density (cells mm^{-2})	3374±554 (5)	3774±235 (5)	4329±702 (5)

*$p<0.01$ compared with Na^+ 0.1 mM group
†$p<0.01$ compared with Na^+ 1 mM group

A full length of cDNA encoding A subunit of V-ATPase (2573 bases) and the deduced amino acid sequence (618 amino acids) were obtained. These sequences showed high degrees of identity with V-ATPase from other animals. Immunocytochemistry with an antibody specific for killifish V-ATPase showed that the V-ATPase was distributed in the branchial chloride cells and pavement cells in FW-adapted fish, but the intensity of signal was faint in SW-adapted fish.

Conclusions

The development and ultrastructural alternations of chloride cells in lower Na^+ environments suggest that chloride cells are the sites for Na^+ uptake in FW-adapted killifish. This is also supported by the immunolocalization of V-ATPase in the gill epithelia with the homologous antibody.

References

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