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In Vivo Functional Assay in Fish Gills: Exploring Branchial Acid-Excreting Mechanisms in Zebrafish

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Abstract:

Molecular and physiological analyses in ionoregulatory organs (e.g., adult gills and embryonic skin) are essential for studying fish ion regulation. Recent progress in the molecular physiology of fish ion regulation was mostly obtained in embryonic skin; however, studies of ion regulation in adult gills are still elusive and limited because there are no direct methods for in vivo functional assays in the gills. The present study applied the scanning ion-selective electrode technique (SIET) in adult gills to investigate branchial H^+-excreting functions in vivo. We removed the opercula from zebrafish and then performed long-term acid acclimation experiments. The results ofWestern blot and immunofluorescence showed that the protein expression of H^+-ATPase (HA) and the number of H^+-ATPase-rich ionocytes were increased under acidic situations. The SIET results proved that the H^+ excretion capacity is indeed enhanced in the gills acclimated to acidic water. In addition, both HA and Na^+/H^+ exchanger (Nhe) inhibitors suppressed the branchial H^+ excretion capacity, suggesting that H^+ is excreted in association with HA and Nhe in zebrafish gills. These results demonstrate that SIET is effective for in vivo detection in fish gills, representing a breakthrough approach for studying the molecular physiology of fish ion regulation.

Keywords: scanning ion-selective electrode technique, in vivo functional assay, H^+ excretion, adult gills, zebrafish