$Na^+/K^+/2Cl^-$-cotransporter immunoreactivity in *Myoxocephalus octodecimspinosus*

Justin S. Catches[1], Julie M. Burns[2], and James B. Claiborne[1]
[1]Department of Biology, Georgia Southern University, Statesboro, GA 30460
[2]Mount Desert Island High School, Bar Harbor, ME 04609

Chloride cells were first described in eel gills by Keys and Willmer in 1932.[4] Their main function in seawater teleosts has been shown to be Cl^- secretion.[3] The model for Cl^- secretion requires coordination among three transporters: basolateral Na^+/K^+-ATPase generates and maintains an inwardly directed Na^+ gradient that drives Cl^- into the cell by the basolateral $Na^+/K^+/2Cl^-$-cotransporter (NKCC); and an apical Cl^- channel that mediates diffusion of Cl^- into the environment. NKCC proteins have been found in the gills of European eel[2], mummichog[5] and shark[6]. Our study describes immunoreactivity of NKCC in longhorn sculpin gill.

Longhorn sculpin gills were prepared for immunohistochemistry according to the methods of Catches and Claiborne.[1] Sections were incubated in anti-NKCC (T4; 1/5000 with 2.5% NHS, Developmental Studies Hybridoma Bank) overnight at 4°C in a humidified chamber. Anti-NKCC staining was visualized using the Vectastain Elite ABC kit (Vector Labs). After 5 min incubation in Vector VIP (purple, Vector Labs), slides were washed in running tap water, dried and permanently mounted with a coverslip (Permount, Fisher Scientific).

Immunopositive cells demonstrated diffuse staining throughout the cell characteristic of staining along the basolateral membrane (Fig. 1). NKCC appears to colocalize in some but not all cells positive for Na^+/K^+-ATPase based on stained serial sections (data not shown). Na^+/K^+-ATPase was used as a marker for chloride cells in the interlamellar region of the gill.[3] There was no staining along the lamellae.

Fig. 1. Representative micrograph of anti-$Na^+/K^+/2Cl^-$-cotransporter staining in longhorn sculpin gill (T4:1/5000) magnified 400X. Staining occurred along epithelium in interlamellar region.

Our study supports the model of a basolateral NKCC in the gill epithelium. The antibody used detects both NKCC1 and NKCC2, thus we are unable to determine which isoform is present. Further work is needed to determine whether a Cl^- channel is present in sculpin gill and the role it would play with NKCC in Cl^- secretion. Funding was provided by a NSF IBN-0111073 to JBC, a GSU Professional Development Grant to JSC and a Hancock County Scholars award to JMB.

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