IMMUNOLOGICAL LOCALIZATION OF ENDOTHELIN RECEPTORS IN THE GILL OF *FUNDULUS HETEROCLITUS*

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Our laboratory has demonstrated that endothelin (ET) can inhibit the short-circuit current (I_{sc}) across the killifish opercular epithelium (Evans, D.H. et al. Bull. MDIBL 39: 17, 2000), the standard model for the teleost chloride cell (e.g., Evans, D.H. et al. J. Exp. Zool. 283: 641-652, 1999). The effect is concentration dependent, and also is produced by the ET_B-specific agonist, Sarafotoxin S6c, suggesting the presence of ET_B-type receptors in the gill (Evans et al., Bull. MDIBL 41: 8, 2002).

To localize these putative receptors, we fixed killifish gills for 60 minutes in 3% parafomaldehyde, 0.05% glutaraldehyde and, 0.05% picric acid. We then used the immunohistochemical methods from Piermarini et al. (Am J Physiol 283: R983–R992, 2002), to localize the ET_B receptors using anti-ET_B (1:500 dilution, Assay Designs) and double labeled-slides for ET_B and Na^+, K^+-ATPase (1:2000 dilution--α5, Developmental Studies Hybridoma Bank, see Piermarini, P. and Evans, D.H. J. Exp Biol. 203:2957-66, 2000).

ET_B receptors are obvious in the walls of what appears to be the afferent filamental artery of the gill filament as well as the afferent lamellar arterioles, which carry blood from the filament into the lamellae (Fig. 1). This suggests that ET may be involved in modulating vascular tone and thereby blood flow into the gill lamellae. In the gill epithelium, the ET_B staining was found in cells different from those containing Na^+, K^+-ATPase. The ET precursor, Big ET, has been localized in neuroendocrine cells of the dogfish, sea conger eel and catfish (Zaccone et al., Neuropeptides 30:53-57, 1996), so we hypothesize that the ET_B receptors localized here are in these neuroendocrine cells. This would suggest a direct autocrine function of the hormone and potentially an indirect paracrine effect on neighboring cells such as the mitochondrion-rich cells. Future studies using markers for neuroendocrine cells will test this hypothesis. (Supported by NSF IBN-0089943 to DHE)

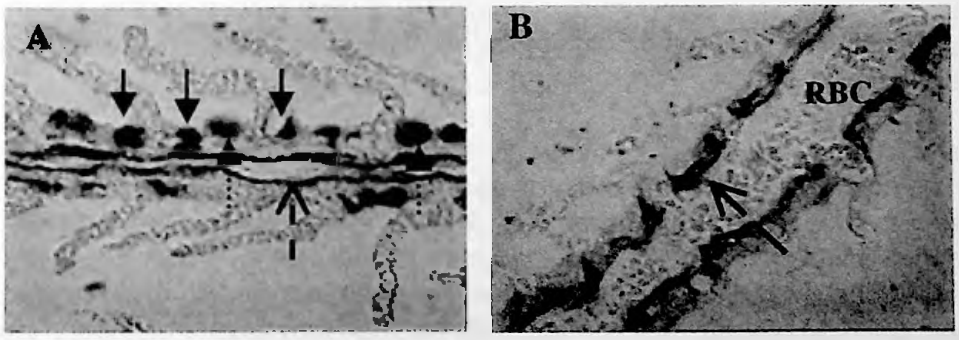

Figure 1. Solid arrows point to mitochondrion-rich cells stained for the Na^+,K^+-ATPase, dotted arrows point to ET_B receptor cells which are not in the Na^+,K^+-ATPase cells, and the dashed arrows point to the vascular ET_B staining. Note in B the red blood cells (RBC) entering the lamella. Magnification 200X, light microscopy.