PARTIAL CLONING AND APICAL MEMBRANE LOCALIZATION OF AN ANION EXCHANGER (AE-2) IN SHARK (*SQUALUS ACANTHIAS*) RECTAL GLAND TUBULES: PRELIMINARY STUDIES

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Electroneutral anion exchangers are involved in the regulation of cell volume, intracellular pH and chloride concentration. The first plasma membrane anion exchanger (AE-1) to be identified, cloned and sequenced, was the erythroid band 3, a transporter which mediates one to one exchange of HCO_3 for Cl^-. By Northern blot analysis and immunohistochemical localization, a Band 3 related protein (AE-2) was identified in a non-erythroid tissue, the mammalian kidney collecting duct (Alper et al., J. Biol. Chem. 263:17092-99, 1989). AE-2 cDNAs have been characterized in mouse and human kidney, rat stomach, (Alper, Ann. Rev. Physiol. 53:549-564, 1991) and rabbit ileal enterocytes (Chow et al., Am. J. Physiol. 263:G345-G352, 1992). Highest expression of AE-2 is seen in several well differentiated cell types including choroid plexus, acid secreting parietal cells of the mammalian stomach, osteoclasts, and renal medulla. Xenopus oocytes lack endogenous AE-2 but have an endogenous sodium-hydrogen exchanger (NHE). When AE-2 is overexpressed in Xenopus oocytes, tight coupling of endogenous NHE and AE-2 occurs permitting regulatory volume increase (RVI) through net transport of NaCl (Jiang et al., Amer. J. Physiol. 272:C191-202, 1997).

We have been using PCR based strategies to clone membrane proteins involved in regulation of NaCl secretion in the shark (*Squalus acanthias*) rectal gland (SRG). Using highly degenerate primers to 7 transmembrane G protein coupled receptors, we unexpectedly amplified a 555 bp product from SRG which had 88% homology to human AE-2. In preliminary immunohistochemical studies using an antibody to AE-2 (provided by Dr. Seth Alper, Beth Israel Hospital, Boston, MA) we detected apical membrane localization of the AE-2 protein in SRG tubules by confocal microscopy (figure 1). Immunofluorescence was abolished by preincubation with the antigen peptide used to raise the antibody (data not shown). We have also used a ^{32}P-labeled AE-2 shark specific probe to carry out library screening of a SRG cDNA library and have isolated 25 positive plaques which will be sequenced in pursuit of the full length gene product.

Figure 1. Apical localization of shark AE-2 in rectal gland cells by confocal immunohistochemistry.

In human tissues, AE-2 has been localized by immunohistochemical techniques to apical membranes of small intrahepatic bile ducts and gall bladder (Scoazec et al., J. Hepatol. 26:543-53, 1997). We report here the first identification and partial cloning of an AE-2 exchanger in a marine species. The protein appears to be localized primarily to apical membrane domains of rectal gland tubules. The function of AE-2 in the shark rectal gland tubule is presently under study.

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