CLONING OF sAE3 ANION EXCHANGER OF SKATE (*RAJA ERINACEA*) ERYTHROCYTE

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The anion exchangers family consists of integral membrane proteins of ubiquitous occurence catalyzing exchange of Cl^- and HCO_3^-. Up to now, four different genes are known°: AE1, AE2, AE3 and AE4 coding for multiple forms of anion exchangers that are specifically expressed in different tissues. Associated with their ubiquitous occurence, anion exchangers are involved in many different processes such as cellular and organismal aging, neurological diseases and structural integrity of the cell. In fish erythrocytes the anion exchanger is proposed to mediate the volume-sensitive loss of taurine and other small organic compounds. Cloning of tAE1, the anion exchanger of trout erythrocytes, and expression of this protein in *Xenopus* oocytes led to the conclusion that it is able to form an anion channel permeable to taurine as well as to some organic solutes (choline, urea and sorbitol) and inorganic cations (Na^+ and K^+). These permeabilities are activated in trout red cell in response to a decrease in intracellular ionic strength (Fi vet et al. EMBO J. 14°:5158-5169,°1995; Guizouarn et al. J. Physiol.°535°:2, 497-506, 2001). Volume regulation of hyposmotically swollen skate red cells induces activation of a taurine permeability that was also shown to involve the anion exchanger (Musch et al°; J. Biol. Chem. 274°: 7923-7928, 1999). To investigate the role of skate AE in swelling-induced taurine pathway, AE cloning in skate red cells was initiated two years ago (MDIBL Bulletin, 38,p.23,2000). Surprisingly, we found three different isoforms of AEs expressed in skate erythrocytes: AE1, AE2°, and AE3. The present paper reports the cloning of sAE3 isoform.

The membrane spanning domains of sAE3 were amplified by PCR using cDNA derived from skate erythrocytes. RACE PCR (Rapid amplification of cDNA ends by PCR) done on cDNA of skate erythrocytes failed to give specific products. Thus it was decided to clone the 5 and 3 ends of sAE3 in a cDNA library of skate brain hypothezing that this tissue expresses predominantly the AE3 isoform. Skate brain poly-A RNA purified by affinity chromatography (Qiagen kit, Valencia CA 91355) were retrotranscribed in cDNA using the Marathon amplification kit (Clontech, Palo Alto CA 94303) procedure. 3 RACE PCR gave a main product of about 600 bp coding for the C terminal part of sAE3, cloned in pGEMT-easy vector (Promega, Madisson WI 53711-5399).To get the N-terminal part of sAE3, four different 5 RACE PCR runs were needed, none of the 5 RACE PCR done being able to amplify directly in one piece the 2100 coding bp corresponding to the N-terminal cytoplasmic domain of AE3. sAE3 isoform is about the same size as sAE2 with the same organization°: a large N-terminal cytoplasmic domain (about 700 aa) and a membrane spanning domain of about 500 aa with a long connecting loop between spans 5 and 6. It differs from sAE1 which has a shorter N-terminal cytoplasmic domain (400 aa) and a spanning domain devoid of the long connecting loop between spans 5 and 6. Future experiments are aimed at determining the physiological functions of the three isoforms. Supported by NSF grant IBN 9974350.