SKATE (RAJA ERINACEA)ANION EXCHANGER, sAE1, EXPRESSION IN *XENOPUS LAEVIS* OOCYTES.

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Volume regulation is a fundamental property of most cells. In the red blood cells of trout and skates (Raja erinacea), cell swelling activates the release of small organic compounds (e.g. taurine) out of the cell followed by osmotically obligated water, effectively restoring cell volume. The membrane protein AE1 (band 3) has been suggested to be involved in this regulatory volume response in skates erythrocytes (Perlman, D., L. Goldstein. J. of Exper. Zool 283: 725-733, 1999). Previous studies have shown that expression of trout anion exchanger (trAE1) in *Xenopus laevis* oocytes induces band 3 anion exchange activity and organic osmolyte channel activity (Fi vet et al. EMBO J. 14:5158-5169,1995). The expression of skate AE1 in *Xenopus laevis* oocytes has been shown to induce anion exchange activity (H. Guizouarn, personal communication). The purpose of this study was to determine whether expression of skate AE1 in *Xenopus* oocytes induces organic osmolyte channel activity in these cells.

Total mRNA was isolated and purified from skate erythrocytes, reverse transcribed and the cDNA for skate AE1 was purified. The skate AE1 cDNA was cloned into pGEMTeasy, linearized with SacII and transcribed (Promega). The capped sAE1 cRNA was recovered, resuspended and an aliquot was analyzed by agarose-formaldehyde gel electrophoresis. The oocytes were removed from ice-anesthetized *Xenopus laevis* and defolliculated by collagenase treatment. Then, they were injected with 50 nL of sAE1 cRNA (6.0 ng/oocyte) or water. The oocytes were maintained at 18° C in ND96 supplemented with penicillin (10 U/mL) and streptomycin (10 ug/mL). For taurine uptake experiments, 6-10 oocytes were incubated in 0.4 mL gluconate containing 3H-taurine with a specific activity of 40,000 cpm/nmol taurine. For inhibitor studies, 1.0 mM quinine, 0.1 mM DNDS, 0.1 mM niflumic, 0.1 mM NPPB acid or 0.1 mM DIDS were added to the incubation medium. Oocytes were washed in ice cold media, quickly transferred to scintillation vials and 50uL of 20% SDS were added. Liquid scintillation fluid was added to tubes. Tubes were vortexed then placed in liquid scintillation counter to determine the taurine uptake of each oocyte.

When skate AE1 was expressed in *Xenopus* oocytes, the transport of organic osmolytes (taurine) increased significantly (11.6±2.5 pmol 3H-taurine/oocyte/hr) compared to that of control oocytes (1.6±0.6pmol 3H-taurine/oocyte/hr). Pharmacological blockers (DIDS, DNDS, NPPB, niflumic acid and quinine), known to inhibit the volume activated organic osmolyte channel in skate erythrocytes, were tested on oocytes expressing sAE1. In oocytes injected with sAE1, DIDS, DNDS, NPPB, niflumic acid andquinine inhibited the organic osmolyte channel, decreasing taurine transport significantly, 91%, 67%, 79%, 92% and 89%, respectively . These results suggest that the expression of sAE1 in *Xenopus* oocytes induces organic osmolyte channel activity and that this channel has pharmacological sensitivity similar to that in the skate red blood cells.

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