## Skate (*Raja erinacea*) anion exchanger, skAE1 expression in *Xenopus laevis* oocytes.

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Volume regulation is a fundamental property of most cells. In skate ( *Raja erinacea*) erythrocytes, cell swelling activates the release of small organic compounds (e.g. taurine) out of the cell followed by osmotically obligated water. The membrane protein AE1 has been suggested to be involved in this volume regulatory response and when the trout or skate AE1 (trAE1 or skAE1) was expressed in *Xenopus laevis* oocytes, anion exchange and organic osmolyte channel activity was induced. However, there is controversy as to whether the expressed AE1 (skate or trout) acted as the channel or not, due to the fact that when expressed in *Xenopus* oocytes, the channel was active in both hyposmotic (ND58, ~135 mOsm) and isosmotic (ND96, ~210 mOsm). However, previous studies have shown that the osmolarity of *Xenopus laevis* is plasma is ~235 mOsm. The purpose of this study was to determine whether hypotonic stimulation is part of the mechanism for channel activation in skAE1 expressing oocytes, as observed in skate erythrocytes.

Total mRNA was isolated and purified from skate erythrocytes, reverse transcribed and the cDNA for skAE1 was purified. The skAE1 cDNA was cloned into pSP64, linearized with ApaI and transcribed (Promega). The capped skAE1 cRNA was recovered, resuspended and an aliquot was analyzed by agarose-formaldehyde gel electrophoresis. The oocytes were removed from anesthetized *Xenopus laevis* and defolliculated by collagenase treatment, then injected with 50 nL of skAE1 cRNA (6.0 ng/oocyte) or water. The oocytes were maintained at 18°C in ND109 supplemented with penicillin (10 U/mL) and streptomycin (10 ug/mL). For taurine uptake experiments, 8-12 oocytes were incubated in 0.4 mL ND109 (isosmotic , 235 mOsm) or ND 96 (hyposmotic , 210 mOsm) containing 3H-taurine with a specific activity of 40,000 cpm/nmol taurine. For inhibitor studies, 0.1 mM niflumic, 0.1 mM piceatannol 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 was 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 skAE1 was expressed in *Xenopus* oocytes, the transport of organic osmolytes ( taurine) increased significantly when incubated in hyposmotic ND96 media compared to isosmotic ND109 media and water injected oocytes incubated in hyposmotic media, 27.5±3.2, 6.5±1.2, 2.4±0.8 pmol taurine/oocyte/h, respectively. Pharmacological blockers, DIDS, niflumic acid and piceatannol, known to inhibit the volume activated organic osmolyte channel in skate erythrocytes, were tested on oocytes expressing skAE1. DIDS, niflumic acid and piceatannol inhibited the organic osmolyte channel in hyposmotic media, decreasing taurine transport significantly by 50%, 60% and 53%, respectively. These results suggest that the expression of skAE1 in *Xenopus* oocytes formed an organic osmolyte channel activity and the mechanism of activation and characteristics of the channel are similar in skate erythrocytes and skAE1 expressing oocytes.

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