Membrane Trafficking Factors are Involved in the Hypotonic Activation of the Taurine Channel of the Little Skate (*Raja erinacea*).

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Cell volume regulation is an important mechanism employed by most vertebrate cells to maintain homeostasis. In response to hypotonicity, little skate (*Raja erinacea*) erythrocytes initially swell; they then release small organic compounds and osmotically obligated water in what is called a regulatory volume decrease (RVD) to restore cell volume. This requires the anion exchanger, AE1, which has been shown to be inserted into the external plasma membrane with hypotonic stimulation.[2] Under isotonic conditions, skAE1 is found in intracellular lipid raft regions and is highly tyrosine phosphorylated. The purpose of this study was to examine factors that might be involved in the exocytosis of AE1 with the use of inhibitors previously shown to affect membrane trafficking.

Blood was collected from the caudal vessel of the skate. The blood was washed twice with 940mOsm/kgH_2O LiCl-EIM (isotonic) [in mM: 300 LiCl, 5.2 KCl, 5.0 $CaCl_2$, 2.7 $MgSO_4$, 15 Tris (pH 7.5), 370 urea]. The remaining red blood cells were resuspended to 20% in 940 LiCl-EIM. They were pre-incubated for 30 minutes in inhibitor before taurine uptake under hypotonic conditions (460 LiCl-EIM) was determined at 30 minutes by liquid scintillation spectroscopy, as previously described.[1]

Under hypotonic conditions, taurine uptake was significantly ($p<0.001$) inhibited after preincubation with 100μM wortmannin, known to inhibit phosphatidylinositol 3-kinase (PI3 kinase). Taurine uptake was 32±1.0 nmol/gRBC and it was 18±2.3 nmol/gRBC after preincubation with wortmannin, a 42 percent inhibition. A more specific inhibitor of PI3 kinase, LY294002 also resulted in significant ($p<0.001$) inhibition of taurine uptake at a concentration of 200μM. The taurine uptake was 38±1.3 nmol/gRBC without inhibitor but after preincubation with 200μM LY294002 it was 27±0.4 nmol/gRBC, a 28 percent inhibition.

There was a significant reduction of taurine uptake with the addition of Latrunculin B, an actin filament disruptor, at concentrations of 10, 20, and 40 μM, ($p<0.001$) 40±0.4 compared to 23±0.7nmol/gRBC [41% inhibition], ($p<0.01$) 42±3.0 compared to 29±1.7 nmol/gRBC [32%] and ($p<0.001$) 37.2±1.1 compared to 22±1.6 nmol/gRBC [39%], respectively. At 2 and 5μM, there was no significant inhibition. Preliminary results indicate that the F to G actin ratio changes are in agreement with the percent inhibition found in the uptake studies.

Inhibitors of AMP (adenosine monophosphate) kinase, myosin, kinesin, dynein, and microtubule disruptors showed no significant effect on taurine uptake. In sum, the results presented suggest that PI3 kinase and actin filaments may be involved in the exocytosis of AE1.

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1. **Goldstein L, DT Koomoa, MW Musch.** ATP release from hypotonically stressed skate RBC: Potenial role of osmolyte channel regulation. *J Exp Zool* 296A: 160-163, 2003.
2. **Musch MW, DT Koomoa, L Goldstein.** Hypotonically-induced exocytosis of the skate anion exchanger skAE1: Role of lipid raft regions. *J Biol Chem* 279: 39447-39453, 2004.