TYROSINE KINASE INHIBITION EFFECTS DIFFERENTIALLY ALTERS CELLULAR EVENTS FOLLOWING VOLUME EXPANSION IN LITTLE SKATE (*RAJA ERINACEA*) ERYTHROCYTES

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Upon volume expansion, cells swell upon the entry of water. Cells achieve decreased volume through regulatory volume decrease mediated through the stimulated efflux of various solutes. Red blood cells (RBC) from the little skate *Raja erinacea*, accomplish this due to the efflux of the β-amino acid taurine. Volume expanded-stimulated taurine efflux requires one or more anion exchangers (AEs). Upon volume expansion, the AE of the skate RBC oligomerize into tetrameric structures from their predominant dimer conformation in isotonic conditions. A number of signal transduction events occur, including activation of the tyrosine kinases syk and lyn, decreased binding of band 4.1, and increased affinity binding of ankyrin. To determine which event might lead to subsequent oligomerization, the tyrosine kinase inhibitor piceatannol was used. In addition, to determine if the skate AE accumulated in a specialized area of the membrane, cholesterol/sphingolipid-rich lipid rafts were isolated from cells under different conditions and analyzed for oligomerized AE

RBC were drawn from the tail vein of little skates into a heparinzed syringe. Cells were washed in isotonic elasmobranch incubation medium (940mosm/l) and resuspended at 50% hematocrit. Cells were diluted five fold into either isotonic or hypotonic medium (460mosm/l). When appropriate, cells were treated with piceatannol in isotonic EIM for 15 min prior to dilution. At varying times after dilution to 10% hematocrit in isotonic or hypotonic buffer, aliquots were removed and the cell pellet frozen in a dry ice/alcohol bath. Methodologies for the crosslinking studies as well as binding of ankyrin and band 4.1 have been published previously (Musch, M.W., et al. J. Biol. Chem. 271:21221-5, 1996 and Musch, M.W. et al., J. Biol. Chem. 269:19683-6, 1994). To determine if the AE was moved into lipid rafts, cells were treated and frozen as described. Upon thawing, a detergent-insoluble fraction was made using 1% Triton X-100. Insoluble material was pelleted at 20,000g and the pellet was resuspended in 40% sucrose and lipid rafts isolated on a discontinuous 5-30% sucrose gradient layered over the material after centrifugation at 32,000g for 18 hours.

Inhibition of tyrosine kinases did not alter skate AE oligomerization nor accumulation in lipid rafts. Piceatannol treatment decreased band 4.1 binding and the formation of high affinity ankyrin binding was nearly abolished by this treatment. Upon volume expansion, a large fraction of the skate AE was found in the lipid raft fraction. This detergent insoluble fraction contained a majority of the oligomerized skate AE.

The present results suggest a possible model. Upon volume expansion, the interaction of skate AEs with band 4.1 may be interrupted, allowing their entry into the detergent insoluble raft regions. In this region, the AEs associate into higher oligomers. Structural changes may take place, promoting association with tyrosine kinases. Tyrosine phosphorylation of the skate AEs or other proteins is required for high affinity binding to ankyrin. The precise nature of the AE complex that is formed which mediates taurine efflux is, however, unknown.

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