CELL SWELLING CAUSES INCREASED DIDS BINDING IN ERYTHROCYTES OF THE LITTLE SKATE, RAJA ERINACEA

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Erythrocytes of the little skate respond to hypotonic swelling by releasing taurine, a free amino acid maintained at high intracellular concentrations. The taurine transport system is readily inhibitable by DIDS, as well as other Band-3 inhibitors, incdicating a possible involvement of Band-3 in the taurine efflux system (Goldstien and Brill, Am. J. Physiol. 260: R1014-R1020, 1991). Previous experiments have shown an increased affinity of Band-3 for H2DIDS in hypotonic media (460 mOsm) (Flanagan, et al. Bull. MDIBL, 30:75, 1991). The purpose of the present experiments was to repeat the hypotonic binding experiments and extend the experimental conditions tested to include 660 mOsm (a milder hypotonic stress), as well as ethylene glycol EIM (elasmobranch incubation medium), and NH_4Cl EIM, which have been previously shown to cause isosmotic swelling of the erythrocytes.

Blood was drawn from the skate and the erythrocytes were immediately isolated by centrifugation. The cells were brought up to 2% hematocrit in either 940 mOsm (control isotonic), 460 mOsm, 660 mOsm, ethylene glycol, or NH_4Cl EIM. Five ml of the RBC suspension was then transferred immediately to a flask for incubation with 5 uCi of 3H2DIDS (0.5 uM, sp. act. 2 Ci/mmol) in a shaking water bath at 16°C. Three 150 ul aliquots were removed from the incubations at 0,5,10,15,30, and 60 min. and transferred into 1.5 ml microcentrifuge tubes containing 850 ul of the corresponding EIM with 0.5% albumin. The samples were washed twice with 1000 ul of the albumin EIM to remove all unbound or reversibly bound 3H2DIDS. The samples were then solubilized with 400 ul of a 1:1 soluene, isopropanol mixture, transferred to scintillation vials, and decolorized with 200 ul of 30% H_2O_2. The 3H2DIDS bound to the cells was then determined by liquid scintillation counting in Hionic-fluor (Packard). A separate experiment with tritiated polyethylene glycol (m.w.=4000) showed that essentially none of the radioactivity in the medium was trapped in the pellet.

In the hypotonic media, 460 and 660 mOsm, 3H2DIDS binding at 60 min. was increased nearly 2-fold and 1.5-fold respectively. In the isosmotic media, a 2-fold increase of binding at 60 min. was seen with the ethylene glycol EIM but no increased binding was observed with the NH_4Cl EIM. The increases in 3H2DIDS binding were similar at 0.1 mM and 0.5 uM 3H2DIDS. Earlier published reports had shown that the affinity of Band-3 for H2DIDS was increased in hypotonic media (Flanagan, 1991). Our results, however, show no significant difference in initial rate of binding (affinity) in any of the conditions. We attribute this difference to the use of a higher specific activity 3H2DIDS, which has been shown by gel electrophoresis to bind more specifically to Band-3. The results do suggest an increased number of binding sites available for H2DIDS in 460, 660, and ethylene glycol EIM. This can be explained by either an increased number of Band-3 proteins being made available or a conformational change in each Band-3 which results in more available binding sites on each protein. The isosmotic results match data from taurine efflux experiments in which NH_4Cl caused only a 100% increase in efflux while ethylene glycol resulted in a 300% increase (Goldstein and Brill, J. Exp. Zool., 1990). However, the match is not as good when comparing 460 vs. 660 mOsm. Binding is increased only 50% more by 460 mOsm but taurine efflux is increased about 10 times more (Goldstein and Brill, 1991). This difference must be attributable to another factor which may regulate the use of the available transporters, and not just to the number of transporters available. These results support the hypothesis that Band-3 is involved in the taurine efflux response of skate erythrocytes to hypotonic and isosmotic swelling.

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