HYPOTONIC STRESS STIMULATES DIDS BINDING BY ERYTHROCYTES OF THE LITTLE SKATE, RAJA ERINACEA

Katherine Flanagan, Mark Musch, Michelle Guertin and Leon Goldstein
Section of Physiology, Brown University
Providence, RI 02912

Erythrocytes of the little skate previously have been shown to adjust to hypo-osmotic stress by activating β-amino acid efflux via a DIDS inhibitable transport system. Recent evidence (Goldstein & Brill, Am. J. Physiol., in press) suggests that Band 3 mediates the volume activated β-amino acid release. The purpose of the present experiments was to gather further evidence in support of such a hypothesis, using the Band 3 inhibitor tritiated dihydroDIDS (3H2DIDS).

Blood was drawn from a caudal vessel of the skate and the erythrocytes were isolated by centrifugation. The cells were brought to an 80% hematocrit in either 940 mOsm (isotonic) or 460 mOsm (hypotonic) Elasmobranch Incubation Medium (EIM) (Goldstein, et al. J. Exp. Zool. 254: 114-118, 1990). Aliquots (0.3 ml) of each of these preparations were incubated for 30 min. at 15^{o}C with 1 uCi/ml of 3H2DIDS (HSC, Toronto) in 1.7 ml of the appropriate EIM and 6x10-7M to 1x10-4M H2DIDS. After incubation, a 1.0 ml aliquot was removed from each sample, the remainder being used to assay the amount of hemoglobin per sample (Sigma Kit #525-A). The aliquot was centrifuged and the pellet was washed twice in the appropriate EIM containing 0.5% albumin, in order to remove unbound 3H2DIDS. The washed pellets were treated with 5% perchloric acid, kept for 10 min on ice and centrifuged. The pellets were dissolved in Soluene (Packard), isopropanol and hydrogen peroxide, and counted in Hionicfluor (Packard).

At 6x10-7M H2DIDS, approximately 3-4x more 3H2DIDS was bound to skate erythrocytes incubated in 460 mOsm EIM compared to binding of the agent by erythrocytes incubated in 940 mOsm EIM. The concentration of H2DIDS binding sites on the erythrocytes and the affinity of the binding sites for H2DIDS were determined using Lineweaver-Burke plots. The mean ($\pm$ S.E.) total binding protein and H2DIDS binding affinity (Kd) of erythrocytes bathed in 940 mOsm (n=6) were 5.00 ($\pm$ 1.94) μmol/gHb and 31.5 ($\pm$ 18.2) μM, respectively. The same values for erythrocytes bathed in 460 mOsm (n=4) were 3.94 ($\pm$ 1.79) μmol/gHb and 9.1 ($\pm$ 5.20) μM. The results show that hypotonic stress increases H2DIDS binding affinity, but does not change the maximal amount of H2DIDS bound by the cell membrane.

The increased H2DIDS binding affinity in volume expanded cells supports the hypothesis that Band 3 is involved in the response of skate erythrocytes to hypotonic stress. Volume expansion may increase the affinity of Band 3 for β-amino acids or it may induce a conformational change in the protein permitting the passage of β-amino acids through a volume induced channel in the transporter. Stilbene disulfonates have been shown to bind to adjacent monomers of Band 3 in a 14A^{o} space (Beth et al. Biochemistry 25: 3824-3832, 1986). Change in conformation of this space during volume expansion may create a channel for β-amino acids to efflux from the erythrocyte. Supported by NSF grant DCB-8801370.