ORGANIC SOLUTE PERMEABILITY IN ERYTHROCYTES OF THE LITTLE SKATE, RAJA ERINACEA

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Erythrocytes of the little skate respond to hypotonic swelling mainly by releasing the B-amino acids, taurine and B-alanine, a process which is partially inhibited by DIDS and other inhibitors of the anion exchanger Band-3. Previous research suggests that the amino acid transporter is a channel, not a carrier, and amino acids containing 8 or less major atoms are able to pass through the channel during volume-activation. The purpose of the present experiments was to determine if other neutral organic molecules of similar size would also pass through the channel during volume-activation.

Blood was drawn from the skate and centrifuged 3 min. at top speed in a tabletop IEC Clinical Centrifuge. Plasma and buffy coat were removed. The RBC were washed once with elasmobranch incubation medium (EIM): 300 mM NaCl, 5.2 mM KCl, 2.7 mM $MgSO_4$, 5.0 mM $CaCl_2$, 15.0 mM Tris-HCl, 370 mM urea, pH = 7.5, osmolality = 940 mOsm, and pelleted by centrifugation. The pellet was resuspended to a 45% hematocrit in 940 EIM. Flasks were prepared containing 500 ul of either 940 Control EIM, 460 Control EIM (100 mM NaCl, 250 mM urea, osmolality = 460 mOsm), 940 Substituted EIM (same as 940 Control EIM, with 200 mM of organic solute and 200mM NaCl), or 460 Substituted EIM (same as 460 Control EIM with 200 mM of organic solute and 0 NaCl). A 250 ul aliquot of the RBC suspension was added to each flask and incubated in a shaking water bath at 15° C: final osmolarities were 940 and 620 mOsm. Samples were collected in heparinized glass capillary tubes at specific time points and hematocrits determined. Final values were expressed as a % of control (940 EIM at t=o). The increase in hematocrit due to the organic solute substitution was calculated as follows: 940 Substituted - 940 Control; 620 Substituted - 620 Control.

In the isosmotic medium, neutral molecules with less than 7 major atoms were found to penetrate the cell membrane (increase hematocrit). These were, with initial rate (hematocrit increase/minute (% control), mean + S.E. of 4-6 experiments) of entry: lactamide (11.34 ± 1.15), 1,3 dimethyl urea (9.70 ± 1.21), glycerol (1.82 ± 0.41). Molecules with 7 or more major atoms (malonamide, erythritol, choline) did not significantly penetrate the cell membrane. (Note: choline is not a neutral molecule). In the hypotonic medium approximately the same order of penetration was found: dimethyl urea (34.55 ± 2.14), lactamide (24.19 ± 2.14), glycerol (3.98 ± 0.37). Malonamide, erythritol and choline did not significantly penetrate the cell membrane. The volume-activated transport of both glycerol and malonamide was not found to be inhibited by 0.1mM DIDS, suggesting that the transport of these organic molecules occurs through a different channel than that responsible for volume-activated amino acid transport. The results from these experiments indicate that neutral molecules containing less than 7 major atoms are able to pass through the channel. During volume activation, these same molecules penetrated the cell membrane at a faster rate, but those molecules with 7 or more major atoms did not. Therefore, it appears that the pore size does not increase during hypotonic swelling. One possible explanation for the increase in penetration during volume activation is that the channel's "open time" is significantly increased. Supported by NSF/DCB9102215.