UREA TRANSPORT IN SKATE (RAJA ERINACEA) ERYTHROCYTES

Erin M. Davis-Amaral, Sarah R. Carlson and Leon Goldstein
Department of Physiology, Brown University, Providence, RI 02912

Previous studies from our laboratory have shown that non-ionic organic solutes (e.g. polyols and amides) are transported across the skate erythrocyte plasma membrane via a channel (non-ionic organic solute transporter or NOST) (Russell et al., J. Exp. Zool. 267:628-632, 1993). The aim of this study is to show that urea, a major organic solute in elasmobranchs, can permeate the skate red cell membrane by the same channel.

We used a radioisotopic tracer technique to measure urea transport across the cell membrane. Blood was drawn from the caudal vessel of the skate and from the caudal sinus of the hagfish in a heparinized syringe and the erythrocytes were isolated by centrifugation. The cells were resuspended to 20-25% hematocrit in isotonic Ringer's [skate: elasmobranch incubation medium (EIM), 940 mOsm, hagfish: hagfish incubation medium (HIM), 1040 mOsm (Brill et al., J. Exp. Zool. 264:19-25, 1992)]. Flasks were prepared containing 1 ml isotonic or hypotonic medium [skate: 460 mOsm, hagfish: 537 mOsm], 1 µCi/ml ^{14}C-urea, and an inhibitor [(mM) 0.1 DIDS, 0.1 PCMBS, 2.0 P5P (pyridoxal-5-phosphate), 0.1 phloretin]. A 0.25 ml aliquot of RBC suspension was added to each flask and incubated in shaking water bath at 15°C. A 0.5 ml aliquot was removed from the incubations at 0, 2, 4 and 8 min. and transferred into 1.5 ml microcentrifuge tubes containing 0.4 ml dibutyl phthalate (DBT). Each sample was immediately microcentrifuged for 5 sec, supernatant and DBT were removed, the pellet was lysed over 10 min. with 7% perchloric acid (PCA) and centrifuged. The resulting supernatant was counted in 4.0 ml Optifluor. Urea uptake is linear for at least 4 min. under the assay conditions employed; measurements were routinely done at this point.

Kaplan et al. [Am. J. Physiol. 226(6):1327-1332, 1974] had shown that urea could be transported across the cell membrane of vertebrate erythrocytes by simple diffusion or carrier-mediated transport, and that the two could be distinguished by the use of phloretin, an inhibitor of the carrier-mediated process. Phloretin (0.1 mM) had no effect ($P > 0.05$, $n=4$) on urea transport (0.04 ± 0.005 min^{-1}) across the skate erythrocyte membrane consistent with transport occurring via a diffusive process. PCMBS, an inhibitor of cell membrane water channels, activated urea transport. The activation may be caused by swelling of the cells in the presence of PCMBS since cell swelling itself activates urea transport two-fold. The skate red blood cell taurine channel blockers P5P and DIDS had no effect on urea transport suggesting the urea does not cross the red cell membrane via the taurine channel. Furthermore, urea transport across hagfish erythrocytes (which lack taurine channels [Brill et al., 1992]) was at a rate similar to that in skate erythrocytes. Although these results rule out carrier-mediated transport, water channels and taurine channels as sites of urea transport across skate erythrocytes, definitive proof for transport via the non-ionic organic solute transporter is still lacking. However, the size (average diameter 4.3 A) of the neutral urea molecule indicates that it would readily penetrate the NOST channel.

Supported by NSF/DCB 9102215 to LG.