CHLORIDE AND TAURINE EFFLUXES OCCUR BY DIFFERENT PATHWAYS IN RAJA ERINACEA ERYTHROCYTES

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We have been engaged in long-term study of volume-activated osmolyte (e.g. taurine) transport systems in skate RBC. The exact mechanism of this transport has yet to be determined and has sparked controversy. Our previous studies [Goldstein and Davis, Am. J. Physiol., 267:R426-R431, 1994] showed that volume-activated taurine transport occurs via a Na^+-independent, bi-directional transporter which has the properties of a size-limited channel. Evidence suggests that band 3 is involved in either formation or regulation of this channel. However, Kirk et al. [J. Biol. Chem., 267:23475-23478, 1992] believe that volume-activated Cl^- channels are involved in the transport of osmolytes across the fish RBC membrane. Their hypothesis is based upon studies with anion channel blockers. The aim of this study was to examine and to compare Cl^- efflux to taurine efflux in skate RBC under isotonic and hypotonic conditions.

Cl^-, K^+ and taurine effluxes were measured in isotonic and hypotonic media in which Cl^- and Na^+ were replaced by gluconate and mannitol; isotonic (940 mosmol/l) in mM: 510 mannitol, 2.7 Mg^{+2} D-gluconate, 5.0 Ca^{+2} D-gluconate, 15.0 Tris, 370 urea, pH 7.5; hypotonic (460 mosmol/l): mannitol was reduced to 185mM and urea was reduced to 250mM. All incubation media contained 0.1mM methazolamide to minimize HCO_3^- formation and Cl^-/HCO_3^- exchange. Final concentration of RBC in incubation media was 5%. ^3H-taurine was added to incubation media at final concentration of 0.1mM (2μCi/ml). Medium aliquots were taken and analyzed for Cl^- coulombmetrically, K^+ by flame photometry, and taurine efflux by liquid scintillation counting. Dry weight (DW) was obtained by heating a RBC aliquot overnight at 80°C. RBC pH was measured by freezing a RBC pellet in an EtOH/dry ice bath, the cells warmed in 15°C water bath and pH taken [T. McManus, personal communication].

Cl^- efflux showed no change in isotonic (mean± S.E.,(11) = 2.8±0.6 μmol/g•DW RBC•min) vs. hypotonic (3.5±0.9 -- $P>0.05$) stimulated RBC while taurine efflux rose from 0.045± 0.02 μmol/g•DW RBC•min (n=6) to 2.1±0.05 -- $P<0.01$. We sought to characterize the nature of Cl^- efflux. We found that there was no net K^+ efflux in either 940 or 460 media. Thus, Cl^- efflux was not accompanied by a conductive K^+ flux. To further clarify the nature of the Cl^- efflux, inhibitor studies were conducted. NPPB, quinine and arachidonic acid-- known inhibitors of volume-activated Cl^- channels-- had no significant effect on the Cl^- efflux, while DIDS-- a band 3 inhibitor-- completely inhibited Cl^- flux suggesting that Cl^- efflux was due to Cl^-/OH^- exchange. Therefore, the pH of the RBC was assayed after an hour incubation with and without DIDS. In the absence of DIDS, the pH went from (mean± S.E., n=6) 7.6±0.06 to 8.4±0.04 -- $P<0.01$, while in the presence of 0.1mM DIDS the pH rose only to 7.95±0.04 -- $P<0.01$. These results are consistent with Cl^- efflux via Cl^-/OH^- exchange. Bisognano et al. [J. Gen. Physiol., 102:99-123,1993] showed the presence in human RBC of Cl^-/OH^- exchange (or Cl^-/H^+ cotransporter). Similarly, their system was inhibited by DIDS. In conclusion, we showed that Cl^- flux is <u>not</u> volume-activated nor conductive. Taurine and Cl^- fluxes are apparently under different pathway influences: taurine diffuses via a channel while Cl^- is transported by exchangers (or cotransporters). Research supported by NSF: DCB 9102215 (L.G.) and ESI-9452682 (MDIBL).