PROPERTIES AND REGULATION OF THE VOLUME-SENSITIVE ORGANIC OSMOLYTE/ANION CHANNEL VSOAC IN HEPATOCYTES OF THE MARINE SKATE RAJA ERINACEA.

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Cell swelling induces rapid efflux of taurine and other organic osmolytes via an anion channel termed VSOAC (Volume-Sensitive Organic osmolyte/Anion Channel).

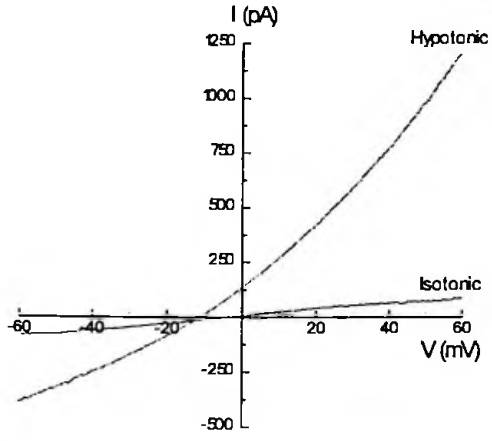

Figure 1. Current-to-voltage plots of whole cell current in a single hepatocyte before and 3 min after reduction of bath osmolality. Bath and patch pipet solutions contained 155 mM CsCl. Non-zero reversal potential is due to osmotic water flow across the cell membrane and/or through the channel itself (Jackson, Churchwell, Ballatori, Boyer, and Strange, J. Physiol., submitted).

We used patch clamp methods to detect and characterize volume-sensitive anion channels in hepatocytes isolated from the little skate Raja erinacea, an osmotically tolerant marine elasmobranch. Cells were patch clamped with symmetrical CsCl solutions as described previously (Jackson and Strange, Am. J. Physiol. 265: C1489-C1500, 1993). A 2 fold increase in hepatocyte volume increased whole cell Cl^- conductance at +60 mV from 0.3 pA/pF to 30 pA/pF (Figure 1). The conductance was outwardly rectified and had a Cs^+ permeability relative to Cl^- (i.e., P_{Cs}/P_{Cl}) of 0.22. Relative Cs^+ permeability was increased by reductions in the CsCl concentration in the patch pipet solution. The conductance had a broad anion selectivity ($SCN^- > I^- > NO_3^- > Br^- > Cl^-$ > pyruvate > F^- > isethionate > aspartate). The relative taurine permeability (i.e., $P_{taurine}/P_{Cl}$) of the conductance was 0.17. Extracellular application of 100 μM DIDS or 10 mM ATP inhibited the conductance in a voltage-dependent manner. The characteristics of the hepatocyte conductance are similar to its mammalian counterpart suggesting that the channel arose early in vertebrate evolution.

Activation of the conductance was blocked in cells treated with the metabolic inhibitor azide and dialyzed with an ATP-free patch pipet solution. Normal channel activation occurred in metabolically poisoned cells that were dialyzed with a solution

containing 4 mM ATP or 4 mM AMP-PCP (adenylyl (β,γ-methylene)-diphosphonate), a nonhydrolyzable ATP analog. Pre-incubation of hepatocytes for 15-30 min with 2 mM pyridoxal-5-phosphate, a drug that binds irreversibly to ATP binding sites, inhibited swelling-induced current activation by 92%. These results indicate that swelling-induced channel activation requires nonhydrolytic ATP binding to the channel itself or accessory/regulatory proteins. The ATP dependence of the channel is most likely a primitive adaptation that allows cells to conserve organic osmolytes and cell metabolites when cell metabolism is compromised (Jackson, Morrison, and Strange, Am. J. Physiol. 267:C1203-C1209, 1994).

Channel activation was also sensitive to the concentration of CsCl in the patch pipet solution. Normal channel activation occurred when cells were dialyzed with 20-50 mM CsCl. Increases in CsCl concentration above 50 mM dramatically reduced the rate of channel activation while the rate and extent of hypotonically-induced cell swelling were unaffected. Channel activation was blocked completely during a 7 min exposure to hypotonic medium by dialysis of cells with a solution containing 20 mM CsCl plus 135 mM NaBr. Replacement of NaBr with sodium gluconate or sodium isethionate partially restored normal channel activation. These results indicate that channel activation is sensitive to cell anion levels and possibly ionic strength. Reduction of patch pipet CsCl concentration to 5-10 mM caused spontaneous channel activation without apparent cell swelling. The effects of intracellular anion composition on channel activation suggest that increases in cell Cl^- levels increase the volume set-point of the channel. The Cl^- sensitivity of VSOAC allows cells to conserve organic osmolytes if Cl^- levels are sufficient to mediate recovery from a given volume increase. In addition, it prevents further increases in intracellular ionic strength if cells are swollen by elevated salt uptake.

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