CELL VOLUME-DEPENDENT POTASSIUM TRANSPORT IN SKATE (RAJA ERINACEA) RED BLOOD CELLS

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When red blood cells (RBC) are placed in hypotonic medium, cell volume initially increases and then gradually returns to normal values, a process referred to as regulatory volume decrease (RVD). The RVD observed in hypotonically swollen cells is thought to be mediated by a net loss of intracellular solute content, which then secondarily results in osmotically obliged cell water loss and subsequent restoration of cell volume. One of the more common intracellular solutes participating in hypotonic cell volume regulation is K^+; net K^+ loss in response to hypotonic swelling has been reported in RBC derived from a variety of species ranging from trout (Bourne and Cossins, J. Physiol. 347:361-375, 1984) to human (Berkowitz and Orringer, Am.J.Physiol. 252:C300-C306, 1987). In addition to K^+, intracellular amino acids such as taurine appear to play an important role in RBC volume regulation, particularly in those derived from marine vertebrates. Leite and Goldstein (J.Exp.Zool. 242:95-97, 1987) recently reported that taurine efflux from skate RBC significantly increased upon acute exposure to hypotonic medium (950 vs 660 mOsM). Furthermore, both phorbol esters and A23187 mimicked the effects of hypotonicity on taurine efflux, suggesting that protein kinase C and calcium may play roles in volume regulatory processes. The present study was undertaken to determine whether, in addition to taurine, K^+ transport pathways also participate in skate RBC volume regulation.

The effects of graded hypotonicity and treatment with phorbol esters or A23187 on K^+ transport in skate RBC were examined with the use of ^{86}Rb tracer uptake and efflux studies. Detailed methodology regarding similar experiments on skate RBC amino acid transport have been previously published (Goldstein and Boyd, Comp.Biochem.Physiol. 60:319-325, 1978). RBC were preincubated for 3 h at 15°C in a shaking water bath in isotonic (950 mOsM) medium composed of (mM): 300 NaCl, 5 KCl, 3 $MgSO_4$, 5 $CaCl_2$, 370 urea, 5 glucose, and 15 Tris-HCl (pH 7.5). For efflux studies, 5 uCi/ml $^{86}RbCl$ were included in the preincubation medium. Following preincubation, efflux or uptake experiments were initiated by addition of RBC (final hemotocrit of 2%) to either isotonic or experimental (A23187, phorbol esters or hypotonic) media. For uptake experiments, these media also contained 5 uCi/ml $^{86}RbCl$. A23187 and phorbol ester effects were studied under isotonic conditions. Hypotonic media had compositions similar to that of isotonic (950 mOsM) medium except that NaCl was reduced to 200 mM and urea to 250 mM in 660 mOsM medium; NaCl was 100 mM and urea 250 mM in 460 mOsM medium. Uptake and efflux rates were calculated as previously described (Goldstein and Boyd, 1978) with the exception that data were normalized to hemoglobin (Hb) content rather than wet weight. Uptake rates were linear over a 30 min sampling period; efflux rates were linear for a least 1 h. Cell volume was indirectly determined by hematocrit measurement; data for experimental treatments are expressed a % change in volume relative to that observed in isotonic medium. Cell and medium Na^+ and K^+ contents were measured on perchlorate extracts by flame photometry. All data are presented as mean±SEM, n=3-6 preparations.

K^+ uptake and efflux rates averaged 0.36±0.08 and 0.46±0.11 nmol/min/mg Hb respectively under isotonic (950 mOsM) conditions. Reduction of medium osmolality to 660 mOsM, a maneuver which markedly stimulates

taurine efflux (Leite & Goldstein, 1987), had no detectable effect on either K^+ uptake or efflux. However, further reduction of extracellular osmolality to 460 mOsM caused K^+ efflux rate to triple (1.47±0.31 nmol/min/mg Hb) and K^+ uptake rate to double (0.79±0.11 nmol/min/mg Hb). Thus, in hypotonic 460 mOsM medium, the average rate of net K^+ efflux can be estimated as 0.7 nmol/min/mg Hb, which corresponds to a 10% reduction in cell K^+ content over a 1 h period. Experiments were also performed to characterize the nature of skate RBC K^+ transport systems. Removal of extracellular K^+ had no significant effect on K^+ efflux in either isotonic or hypotonic medium, suggesting that K^+/K^+ exchange diffusion is absent in skate RBC under isotonic conditions and not activated by hypotonic treatment. K^+ transport was, however, sensitive to the anion transport inhibitor DIDS (0.1 mM). The DIDS-sensitive and -insensitive components of K^+ efflux averaged 0.27±0.09 and 0.19±0.02 nmol/min/mg Hb respectively in isotonic 960 mOsM medium and 0.75±0.26 and 0.72±0.23 nmol/min/mg Hb respectively in 460 mOsM medium. Thus both DIDS-sensitive and -insensitive K^+ transport systems appear to be activated by exposure to hypotonic medium.

In order to correlate K^+ transport with volume regulation in skate RBC, cell volume as a function of medium osmolality was examined. Incubation in 600 mOsM medium caused cell volume to increase by 25±2% within 5 min; this swollen state was maintained for at least 1 h. The notable absence of rapid RVD in 660 mOsM medium correlates with the lack of an effect of this hypotonic medium on K^+ transport. In contrast, incubation in 460 mOsM medium caused cells to swell by 60±1% within 5 min. The initial swelling phase was followed by a continuous decline in cell volume (RVD) such that after 60 min the increase in cell volume was reduced to 37±4%. The RVD was totally abolished in the presence of 0.1 mM DIDS. The observation that 460 mOsM medium elicits both a RVD and net K^+ efflux, coupled with the DIDS-sensitivity of these two processes, supports the notion that K^+ participates in cell volume regulation in skate RBC.

To further investigate the role of K^+ in skate RBC volume regulation, the effects of the phorbol ester phorbol 12-myristate 13-acetate (PMA) and the calcium ionophore A23187 on K^+ efflux and cell volume were examined. Previous studies (Leite and Goldstein, 1987) reported that both of these treatments stimulate taurine efflux in skate RBC under isotonic conditions. In the present study, treatment with 0.5 uM A23187 for 40 min had no detectable effect on either cell volume or K^+ efflux. However, treatment with 0.5 uM PMA resulted in continuous cell swelling such that after 40 min cell volume was increased by 66±3%. Cell swelling was accompanied by a 75% increase in K^+ efflux (0.88±0.15 vs 0.50±0.10 nmol/min/mg Hb) when averaged over the 40 min exposure period. Consistent with previous reports on PMA-incuced cell swelling via activation of Na^+/H^+ exchange, cell Na^+ content increased dramatically from 135±20 to 1022±131 nmol/mg Hb. Simultaneous treatment with 0.1mM dimethyl amiloride severely inhibited the PMA-induced increases in cell volume, K^+ efflux and Na^+ content. While this observation suggests that K^+ efflux is stimulated in the presence of PMA as a secondary response to cell swelling, a primary effect of phorbol ester on K^+ transport cannot be ruled out since this concentration of dimethyl amiloride may directly inhibit both Na^+/H^+ exchange and protein kinase C (Besterman et al, J.Biol.Chem. 260:1155-1159, 1985).

In summary, we have demonstrated the presence of volume-sensitive K^+ transport pathway(s) in skate RBC. The transport system has an anion-dependent component as judged by DIDS-sensitivity. Several differences were noted in the ability of a variety of stimuli to activate K^+ and taurine fluxes, suggesting that these two transport systems may be independently regulated.

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