ATP RELEASE AND OSMOLYTE CHANNEL REGULATION IN HYPOTONICALLY STRESSED SKATE (*RAJA ERINACEA*) ERYTHROCYTE

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Cell volume regulation is a fundamental property in many cells. In a variety of cells, cell swelling activates a compensatory mechanism that induces the release of osmolytes out of the cell followed by osmotically obligated water to reduce cell volume, a process called regulatory volume decrease (Perlman et al., J.Exp.Zool. 283: 725-733, 1999). It has been shown that a variety of endothelial and epithelial cells, when exposed to hypotonic stress, release ATP as well as other nucleotides into the extracellular fluid (Lazarowski et al., Brit. J. of Pharm. 127: 1272-1278, 1999, Hisadome et al., J. Gen. Physiol. 119: 551-560, 2002, Oike et al., Am J. Physiol. 274: R1677-R1686, 2000, Sabirov et al., J. Gen. Physiol. 118: 251-266, 2001). It has also been shown that extracellular ATP (and possibly other nucleotides) inhibits hypotonically activated taurine transport via the swelling activated anion channel, $I_{Clswell}$ (Hisadome et al., J. Gen. Physiol. 119: 551-560, 2002). However, it is not yet known whether the transport of ATP occurs via the swelling activated organic osmolyte/anion channel or a different channel. Little skate (*Raja erinacea*) red blood cells transport organic osmolytes (e.g. taurine) via a different channel than $I_{Clswell}$ (Davis-Amaral et al., Am. J. Physiol. 271: R1544-R1549, 1996). The purpose of this study, therefore, was to determine whether ATP and other nucleotides inhibit the release of osmolytes (taurine) in hypotonically stressed skate erythrocytes.

Skate (*Raja Erinacea*) red blood cells were collected from the caudal vessel of little skates. The red blood cells were centrifuged and isolated, washed 2 times in isotonic solution (940 mM elasmobranch incubation media), then resuspended to 20% hematocrit in either isotonic (940 EIM) or hypotonic (460 EIM) medium. At the beginning of the experiment, samples were placed in a 15 degree C shaking water bath in a beaker with 3H-taurine (1.0 uCi/mL), and incubated for 0 or 30 minutes. At designated time points, aliquots of the samples were taken (in duplicate), samples were washed, centrifuged and the supernatant was separated from the pellet. The red blood cell pellets were lysed in 7% perchloric acid, vortexed and placed on ice for 10 minutes. The samples were then centrifuged and aliquots of the supernatant were placed in scintillation vials and analyzed by scintillation counting. For inhibitor studies, ATP, 5'-AMP, cAMP. ADP or UTP was added to the indubation medium at a final concentration of 5.0 mM before the experiment began. All washes were done in less than 20 seconds (on ice), just long enough to resuspend all the red blood cells.

Skate red blood cells were incubated in isotonic media, hypotonic media or hypotonic media with ATP, 5'-AMP, cAMP, ADP or UTP. Taurine transport was significantly inhibited by ATP, cAMP, 5'-AMP, ADP and UTP by ~85%, 93%, 77%, 39% and 37%, respectively ($p<0.05$ for all nucleotides). In addition, approximately 15% of the intracellular ATP was found to be released from hypotonically stressed skate red blood cells into the medium. However, it is not known whether the released ATP (and possibly other nucleotides) could reach a concentration high enough to exert an inhibitory effect on osmolyte transport. Our findings suggest that when ATP (and other nucleotides) is released from hypotonically stressed skate red blood cells, the extracellular ATP can potentially act as a regulator of osmolyte release in these cells during regulatory volume decrease.

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