# ATP CONTENT AND RELEASE IN SKATE (*RAJA ERINACEA*) HEPATOCYTES

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Cell volume regulation involves the activation of regulatory pathways which move solutes across the plasma membrane in response to osmotic changes. Skate (*Raja erinacea*) hepatocytes express a volume-sensitive organic osmolyte/anion channel (VSOAC), which is thought to be involved in the release of organic solutes (Jackson, et al. Am. J. Physiol. 270: C57-C66, 1996). VSOAC activity is regulated by intracellular ATP and can be inhibited by the nucleotide analog pyridoxal 5-phosphate (P5P), and by glibenclamide, an inhibitor of the cystic fibrosis transmembrane conductance regulator (CFTR).

In mammalian systems, expression of P-glycoprotein, an ATP-permeable channel related to CFTR (Abraham, et al., Proc. Natl. Acad. Sci. USA 90: 312-316, 1993) has been shown to regulate a volume activated chloride conductance (Hardy, et al., EMBO J. 14: 68-75, 1995). It was postulated, therefore, that ATP release in skate hepatocytes could regulate cell volume activity. In this study we began a characterization of ATP content and release in the skate hepatocyte.

Skate hepatocytes were kindly provided to us by Dr. Nazzareno Ballatori, and were isolated as previously reported (Ballatori et al., Molecular Pharmacology 48: 472-476, 1995). A 1:60 dilution of the cell suspension was made in elasmobranch Ringer (ER), which contained, in mM: 270 NaCl, 4 KCl, 3 $MgCl_2$, 0.5 $Na_2SO_4$, 1 $KH_2PO_4$, 8 $NaHCO_3$, 350 urea, 5 D-glucose and 5 N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES)- tris(hydroxymethyl) amino methane (Tris) at pH 7.5. Cells were then incubated with or without P5P (1 mM). Cells were washed with ER and centrifuged at 500 rpm for one minute at 10 $^oC$ and the supernatant was discarded.

ATP content was measured with the luciferin-luciferase assay using a luminometer (Monolight 2010, Analytical Luminescence Labs). 15 microliters of cell suspension were placed in 0.5 ml ER and 0.1 ml of luciferin/luciferase assay mix (Firelight LB, Analytical Luminescence Labs, Ann Arbor, MI). To determine the total amount of ATP per sample, the cells were permeablized by addition of alamethicin (10 μM) followed by sonication for 15 sec. Using a calibration curve made using known concentrations of ATP in ER, the concentration of ATP per cuvette was determined. Cell volume was obtained by micrometry (average measurement of the cell diameters), and calculated as an oblate spheroid with the formula $V=4/3\ \pi\ (a/2)^2\ (b/2)^2$ where a= width of cell and b= length of cell (in μm). By knowing the cell

volume/ml and the concentration of ATP per cuvette, the concentration of ATP per cell was calculated. The concentration of intracellular ATP in skate hepatocytes was 2.42 ± 4.2 mM (n=8).

Hypotonic shock was induced by a 33.3% dilution of the assay solution with distilled water. ATP release was again monitored for several minutes. Following a transient increase in ATP release, intracellular ATP decreased 50.4% ($p<0.05$) after hypotonic shock to 1.2 ± 2.5 mM (n=8). ATP levels in cells preincubated with the VSOAC inhibitor P5P, however, showed no statistical difference with values of control cells.

Under hypotonic stress, VSOAC activity in the skate hepatocyte increases in conjunction with a decrease in intracellular ATP. In addition, VSOAC activity can be inhibited by P5P and glibenclamide (Jackson, et al., op. cit.). However, our results indicate that intracellular ATP was not affected by P5P, suggesting that VSOAC itself was not the pathway for ATP release in our study. It is possible, therefore, that ATP released to the extracellular milieu may regulate VSOAC and other volume-regulatory activity.

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