THE DEVELOPMENT OF A METHOD FOR MEASURING CELL VOLUME REGULATION IN SINGLE HEPATOCYTES FROM RAJA ERINACEA

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All cells in living organisms are constantly exposed to changing conditions in their extracellular environments. Changes in osmolarity cause cells to swell or shrink. In order to maintain homeostasis, cells undergo a process known as regulatory volume decrease (RVD) after cell swelling and regulatory volume increase (RVI) after cell shrinkage. Studies in our laboratory (Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992; Ballatori et al. Am. J. Physiol. 267:G285-G291, 1994; Ballatori et al, Mol. Pharmacol. 48:472-476, 1995) have examined the role of organic osmolytes in regulating the process of RVD. In previous studies volume changes have been measured by isotope dilution methods which provide an average measurement for these changes in large populations of cells in suspension. The present study was designed to measure volume regulatory responses in single cells maintained on cover slips and examined by optical techniques.

To develop this method, skate hepatocytes were isolated as previously described (Smith et al. J. Exp. Zool. 241:291-296, 1987) and allowed to settle on glass coverslips. A second coverslip was suspended above the first by two strips of plastic, then the hepatocytes in the space between the coverslips were perfused with elasmobranch buffer. This perfusion chamber was placed on the stage of a Zeiss IM35 inverted microscope and individual hepatocytes were observed directly. The preparation was maintained at approximately 15°C and cells were visualized using Nomarski optics. Images were captured by a Dage-MTI video camera connected to a Panasonic optical disk recorder. After selecting a field containing several isolated hepatocytes, the cells were subjected to 40% dilution in either Ringer's solution or 40% water and images were recorded at 0.25 minute intervals for one minute during perfusion with normal Ringer's, 0.5 intervals for 5 minutes after diluting with water, then 1 minute intervals for 5 minutes and 2.5 minute intervals for the remaining 20 minutes of the study.

To quantify the volume changes separately for each cell, the images were transferred to a MacIntosh 7100 PC. NIH Image software (version 1.55) was used to trace the outline of individual cells; the measurements were calibrated by measuring beads of known size on the optical disk recorder after calculating the area. The known relationship between area and volume for spheres, $V=(A)^{1.5} \times (.7523)$, was used to calculate an average cell volume for each cell.

The figure below illustrates the mean ± SEM for 20 cells normalized by expressing the volumes as a percentage of their control values. Cells swelled immediately after application of hypotonic medium, peaking to approximately 130% of the normal value after 1-2 minutes. Cells then spontaneously underwent regulatory volume decrease as observed in previous studies, approaching control values during the next 30-60 min.

As noted in the figure, the % swelling in response to 40% hypotonic media is significantly less than the predicted peak value of approximately 167%, characteristic of a perfect osmometer. This degree of swelling is also less than seen previously with isotopic dilution methods in isolated cell suspensions (Ballatori and Boyer, Am. J. Physiol. 262:G451-G460, 1992). The reason for the decreased volume response is not clear although cells adherent to cover slips might have a

restricted response compared to cells in suspension and may not behave as perfect spheres. Therefore this method may not reflect true volume response. Nevertheless this imaging technique allows independent data to be collected for single cells where measurements of changes in volume can be sequentially determined in real time. Supported by NSF ESI-9452682, ES-03828, DK-25636 and 34989.

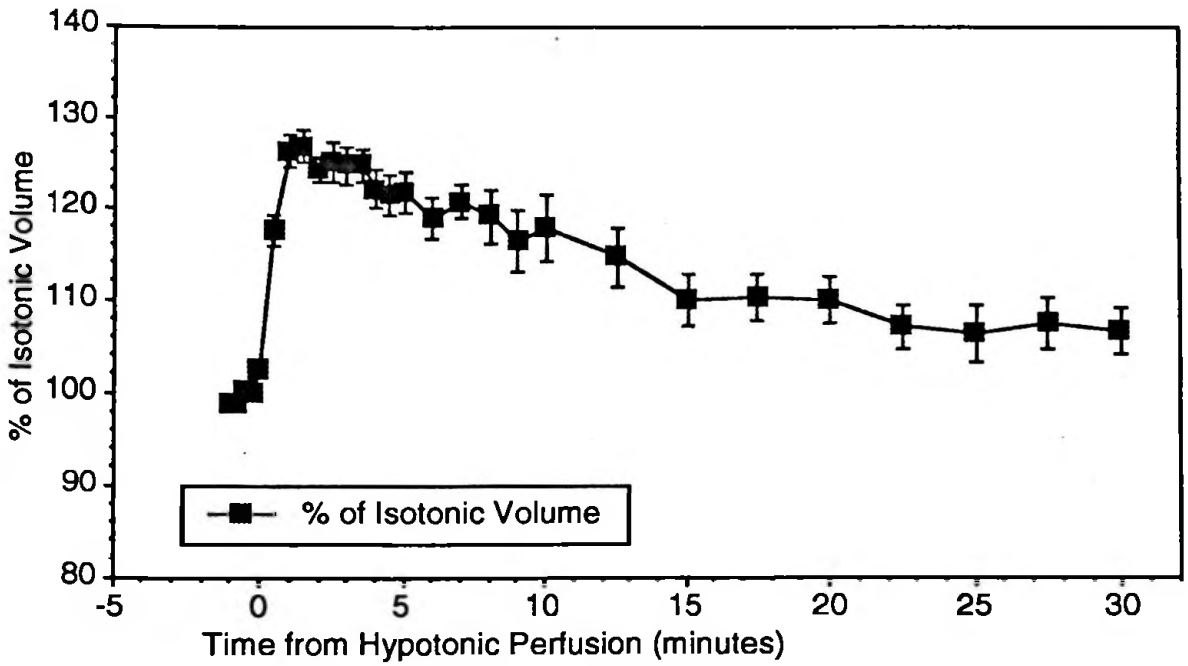

Fig. Effect of Hypotonic Shock on change in volume (±SE) of single isolated skate hepatocytes (n=20) in response to perfusion with a 40% dilution of Elasmobranch Ringer's as assessed by an image analysis technique.