THE EFFECT OF MEDIUM COMPOSITION ON THE ANISOSMOTIC VOLUME RESPONSE OF DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND CELLS

Arnost Kleinzeller[1], George W. Booz[1] and Fuad N. Ziyadeh[2]
[1]Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104
[2]Department of Medicine, University of Pennsylvania, Philadelphia, PA 19104

The K^+-induced swelling of rectal gland cells (RGC) is associated with a reorganization (depolymerization?) of F-actin at the basolateral cell face (A.Kleinzeller & J.W.Mills, Biochim. Biophys. Acta 1014, 40, 1989). A similar action on RGC swelling and F-actin depolymerization is produced by p-chloromercuribenzene sulfonate (pCMBS) (A. Kleinzeller et al., Biochim. Biophys. Acta 1025, 21, 1990). We sugested (A. Kleinzeller & F.N. Ziyadeh (Comp. Physiol., Basel, Karger, Vol. 4, pp. 59-96, 1990) that F-actin at the cell membrane has a restraining effect on RGC swelling. This hypothesis was tested by examining effects of varying external osmolarity on the steady-state cell volume.

Tissue slices were incubated (2 h, air + 1% CO_2, 15°C) in elasmobranch saline containg only 135 mM NaCl; the saline osmolarity was then varied (550 mosM to 1300 mosM) by addition of the non-permeant N-methylglucamine sulfate. The salines were modified either by various additions or omissions, or by complete replacement of Na^+ by other cations. Tissue wet wt. and dry wt. (drying overnight at 95°C) was determined. The cell volume was assessed as intracellular H_2O (H_2O_i) after correction for the extracellular (^{14}C-poly-ethylene glycol) space (including the weight contribution of the osmotic agent). $(H_2O)_i$ was then plotted vs $1/\pi$, the reciprocal relative external osmolarity (vapor pressure osmometry); isotonic conditions (900 mosM) were taken as 1.0. All points represent triplicate measurements, $\pm$ S.D.

The Fig. shows that in controls (Na-saline) the plot was linear for $1/\pi$ = 0.82 - 1.1 (i.e. 810 - 1100 mosM), corresponding to the behavior of an osmometer; the slope of the plot reflects the membrane tension (J. Bereiter-Hahn, in Cytomechanics,pp. 3-30. Springer Verl., Berlin 1987). The cell volume increased less when the medium tonicity further decreased due to the pronounced regulatory volume decrease of RGC (Ziyadeh et al., The Bulletin 29, 68, 1990). Replacement of saline Na^+ by K^+ increased not only $(H_2O)_i$, but also the slope of the plot by 3.4 $\pm$ 0.8 (S.D.) (n = 5), indicating a decrease of the membrane tension. No effect of 0.5 mM ouabain in Na-saline was seen. A decrease in the membrane tension (1.4 fold, 2 experiments) was also found with 1 mM pCMBS. On the other hand, no effects were found by replacement of Na^+ by Li^+, or omission of saline Ca^{2+}, these conditions not affecting the F-actin organization. A variety of protein cross-linking reagents also did not affect the response of cell volume to external osmolarity. The above data are consistent with the advanced hypothesis that the organization of F-actin (and cytoskeletal proteins) at the RGC basolateral membrane are a determinant in the physical restraint to osmotic cell swelling.

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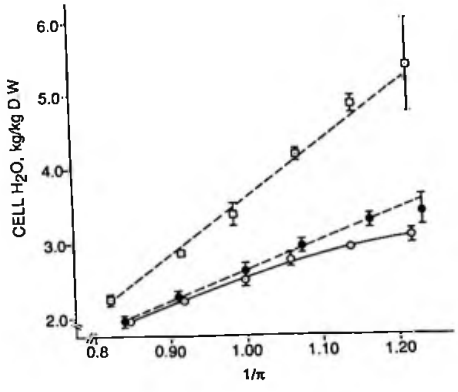

Relationship between $(H_2O)_i$ and $1/\pi$: o, Na-saline (135 mM NaCl, varied with N-methylglucamine sulfate); ●, 0.5 mM ouabain in Na saline; □ , K-saline (135 mM KCl and varying π).