THE RESPONSE OF THE ACTIN CYTOSKELETON OF DOGFISH (SQUALUS ACANTHIAS) RECTAL GLAND CELLS TO HYPOTONICITY, HIGH POTASSIUM AND MERCURIALS

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Hypotonic stress of dogfish shark (Squalus acanthias) rectal gland cells produces rapid cell swelling followed by a regulatory volume decrease. In addition, a disappearance of F-actin at the basolateral membrane of rectal gland cells was observed by fluorescence microscopy of rhodamine phalloidin labeled F-actin over the 5 - 10 minute period of cell swelling (Ziyadeh et al., Amer. J. Physiol., in press). This was followed by a gradual recovery of the F-actin organization after a 30 - 60 minute period, during the regulatory volume decrease. High medium K^+ reduced basolateral fluorescence (A.Kleinzeller and J.W. Mills, Biochim.Biophys.Acta 1014:40,1981), and organic mercurials reduced overall F-actin fluorescence in the rectal gland cell. (A.Kleinzeller et al., Biochim. Biophys. Acta 1025,21,1990).

We have asked whether the changes in basolateral F-actin are accompanied by a reduction in F-actin in the entire cell. We employed the rhodamine phalloidin binding assay to measure whole cell F-actin in slices of rectal gland which were incubated either in standard isotonic elasmobranch incubation medium (900 mosM/kg H_2O) or in hypotonic medium in which 160mM NaCl was removed, giving a final osmolality of approximately 600mosM/kg H_2O. High K^+ incubation medium, in which Na was equivalently replaced by K^+ was used in a second series of experiments. In addition, the effects of p-chloromercuri benzene sulfonate (pCMBS) and phenylmercuric acetate (pMA) were determined. For the rhodamine phalloidin binding assay (Ding et al., Amer.J.Physiol. 260,C9, 1991), slices were weighed, then incubated at 15°C for varying time periods in control or hypotonic medium, fixed with 3.7% formaldehyde in calcium-free dogfish medium containing 0.1mM EGTA, and then incubated for 1 hour in calcium-free dogfish medium containing 0.3uM rhodamine phalloidin. The slices were then washed, the fluorescence extracted with methanol, and read in a spectrofluorimeter. Results were expressed as fluorescence per mgm tissue wet weight, and in all cases, the ratio of the test sample to the simultaneously determined control sample was determined, (Table 1).

Table 1:Effect of Hypoosmolality, High K^+ and Mercurials on F-Actin

Time (min)	Hypotonicity	High K^+	pCMBS	pMA
		% of Control		
10	100±14 (3)*			
60	99± 8 (2)			
120		97±12 (3)	60±7 (3)**	27 (1)

* number of experiments

** p< 0.02

At 10 minutes exposure to hypotonic medium, there was no change in the F-actin content compared to the control. This was also the case at 60 minutes incubation. In 4 rectal glands, the affect of high K^+ was determined at 120 minutes. Again, there was no effect on total cell F-actin, although, as previously reported (Kleinzeller and Mills, loc. cit.) the basolateral pool of F-actin is considerably diminished by high K^+. In contrast 10^{-3}M pCMBS significantly decreased F-actin at 120 minutes. 10^{-4}M pCMBS decreased F-actin by 15% at 120 minutes in a single experiment. 10^{-3}M pMA decreased F-actin by 73% in a single experiment.

Our results show that overall cellular F-actin is not decreased by hypotonicity or high K^+, despite the decrease in basolateral F-actin as seen by fluorescence microscopy. This suggests that changes in actin cytoskeleton following hypotonic stress or high K^+ are regional in nature, with basolateral depolymerization presumably being offset by apical polymerization or polymerization elsewhere in the cell. It is also possible that actin filaments are significantly shortened during hypotonicity and high K^+ and yet retain the ability to bind rhodamine phalloidin, but are not readily seen in the conventional fluorescence microscope.

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