CYTOSKELETAL REORGANIZATIONS FOLLOWING VOLUME CHANGES IN CULTURED SHARK (SQUALUS ACANTHIAS) RECTAL GLAND CELLS

John H. Henson[1], Colleen D. Roesener[1], Stephanie Capuano[1],
John N. Forrest, Jr.[2], and Arnost Kleinzeller[3]
[1]Department of Biology, Dickinson College, Carlisle, PA 17013
[2]Department of Medicine, Yale University School of Medicine, New Haven, CT 06510
[3]Department of Physiology, University of Pennsylvania, Philadelphia, PA 19104

Previous reports (Ziyadeh et al., Am. J. Physiol. 262: F468, 1992) have shown a correlation between the actin cytoskeleton and volume changes in cells from tissue slices of Squalus acanthias rectal gland. Using rhodamine phalloidin (RdPh) to label filamentous actin (F-actin) combined with conventional fluorescence microscopy, cells in tissue slices exposed to high external K^+ and hypotonically-induced cell swelling displayed a fading of the RhPh staining intensity, particularly at the basolateral cell borders. However, a RdPh-based spectroscopic measurement of the F-actin present in the treated rectal gland slices failed to demonstrate a net change in F-actin with the treatments (Hays et al., Bull. MDIBL 31: 150, 1992). In an effort to resolve the structural reorganizations of F-actin which may be occuring during high K^+ and hypotonic shock treatments, we have used cultured shark rectal gland cells in conjunction with confocal laser scanning microscopy to examine actin and tubulin cytoskeletal dynamics under these two experimental conditions.

Isolated shark rectal gland cells were grown as monolayer cultures on collagen treated glass coverslips using a Na^+-based shark Ringer's medium. Cultures were then treated with either control, high K^+ (300 mM), or hypotonic (600 mOsM) shark Ringer's. Cells were fixed in their appropriate culture medium containing 3% formaldehyde and 0.1% glutaraldehyde. Cells were then treated with blocking buffer composed of phosphate buffered saline (PBS) plus 2% goat serum, 1% bovine serum albumin (BSA) and either 100 mg/ml of lysolecithin, or 0.5% triton X-100 as a permeabilizing agent. Actin was labeled by staining with either RdPh or fluorescein phalloidin, while microtubules were labeled using an indirect immunofluorescent procedure employing a mouse monoclonal anti-alpha tubulin as the primary antibody and a fluorescein conjugated sheep anti-mouse IgG as the secondary antibody. Some cultures were double labeled for actin filaments and microtubules. The labeled cells were viewed with a 60X (NA 1.4) Nikon planapochromatic objective lens mounted on an epifluorescent Nikon Optiphot 2 microscope coupled to a Bio-Rad MRC 1000 confocal laser scanning unit. Frame averaged and contrast enhanced digitized images were displayed on a high resolution monitor for photorecording on 35 mm TMAX 100 film.

RdPh staining of control cultures of rectal gland cells indicated that actin filaments predominated in the cortices of the cells where they appeared to primarily be arranged in closely packed parallel bundles. Actin filaments were also observed in the cores of surface microvilli. Within 10-20 minutes of exposure to hypotonic shock these parallel arrays of cortical actin bundles had been transformed to punctate sources of filamentous actin. The hypotonically-induced changes in F-actin were transient in that the control pattern of actin bundles returned in cells fixed 2 hours after hypotonic shock. The control actin filament pattern was also restructured in cells exposed to high K^+ Ringer's. In these cells the parallel arrays of actin bundles were replaced with a dense network of actin filaments. Microtubules in the shark cells were distributed in an elaborate array in control cells which lacked a well-defined, focal microtubule organizing center. This distributional pattern of microtubules did not appear to be significantly altered by either of the experimental treatments.

The above results are consistent with and extend our previous reports on the effects of high K^+

and hypotonicity on the structural organization of actin filaments in shark rectal gland cells. The described reorganization of actin filaments demonstrated by confocal microscopy of cultured monolayers could appear as a fading of RdPh fluorescence in conventional fluorescence microscopy of tissue slices, but per se may not produce a net decrease in the amount of spectroscopically measurable F-actin. Future experimentation is planned to help further elucidate the role of the actin cytoskeleton in the regulation and maintenance of rectal gland cell size and shape.

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