EFFECT OF HIGH KCl ON THE LOCALIZATION OF F-ACTIN IN TUBULE CELLS OF THE SHARK (SQUALUS ACANTHIAS) RECTAL GLAND.

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We have previously demonstrated that slices of dogfish rectal gland swell when incubated in high K^+ solutions. When returned to normal Na^+-saline the cells recover volume and this recovery phase is characterized by the appearance of vesicles in the early stages. The vesicles are retained, however, when the tissue is allowed to recover in the presence of cytochalasins (Mills and Kleinzeller, Bull. Mt. Des. Isl. Biol. Lab. 25:50-53, 1985). This indicates that an intact actin filament system may be involved in the processes that result in a return to normal morphology, especially that component that leads to the loss or removal of the vesicles. Since incubation with drugs which disrupt actin filaments was associated with vesicle retention, we investigated the possibility that the transient vesicle formation, seen at the onset of volume recovery, may be related to a disruption of the filaments during the initial incubation in K-saline.

Tissue slices were prepared and incubated as previously described (Mills and Kleinzeller, Bull. Mt. Des. Isl. Biol. Lab 25:50-53, 1985). After 60 min. incubation in either Na-saline or K-saline the slices were fixed in 1% formaldehyde in the respective saline. Subsequent processing and reaction to reveal the distribution of F-actin were done as previously described (Mills et al., Bull. Mt. Des. Isl. Biol. Lab 26:13-14, 1986).

As shown in Figure 1, F-actin is predominantly associated with the apical and basolateral plasma membranes of the rectal gland cells. The apical localization is most likely related to the central core filaments in the microvilli that are present on this surface. The basolateral association may be related to filaments that are present within the numerous interdigitating folds of the plasma membrane that exist at this pole of the cell. A sixty minute incubation in K-saline results in a reduction of F-actin associated with the basolateral membranes of the rectal gland cells. The localization at the apical surface does not appear altered at this point. A longer incubation results in the loss of apical staining in some tubules, but others still retain a bright fluorescent pattern.

The reduction in basolaterally-associated F-actin after incubation in K-saline is consistent with the hypothesis that the transient vesicle formation seen during recovery from this condition may be related to the alteration in the actin filaments from the control state. If recovery of the normal cytoskeletal arrangement is interrupted by incubation with cytochalasin, then the vesicle population is retained. It then follows that loss of vesicles during the recovery phase should coincide and not preceed return to a normal distribution of F-actin in the rectal gland cells. This possibility is being pursued.

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Figure 1. Localization of F-actin in shark rectal gland cells. Bright fluorescence indicates sites of binding of labeled phallacidin, a drug that binds with high specificity to F-actin. A. Frozen sections of glands incubated in normal Na-saline. Luminal surface of tubule cells is heavily reactive. Basolateral membranes are also clearly outlined (arrows). B. Sections from gland tissue incubated in KCl-saline for sixty minutes. Apical fluorescence is retained, whereas that associated with the basolateral membranes is greatly reduced.