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Solute and fluid secretion mechanisms in ADPKD cells

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Fluid secretion is a major factor involved in renal cyst growth in patients with autosomal dominant polycystic kidney disease. We have begun an investigation into the nature of the solute transport systems that drive that secretion. The data we have collected thus far are compatible with the hypothesis that chloride secretion drives fluid transport by the ADPKD cystic tissue. Three preparations were used in this investigation; all are derived from kidneys removed from ADPKD patients. First, intact cysts were dissected from these kidneys and their ability to secrete fluid was measured. Second, monolayers of cells of primary cultures of cystic tissue were grown on permeable supports. Their ability to secrete fluid and their electrical properties were determined. Third, microcysts were formed by clonal growth of cells from primary cultures of cystic tissue. Single cysts were isolated and video and epifluorometric techniques were used to measure fluid secretion, cell volume and changes in cell potential and cell chloride concentration.

Individual superficial cysts were dissected from ADPKD kidneys, the cyst fluid was removed and replaced with culture medium, and a gravimetric technique was used to measure fluid loss or gain by the cyst in 24 hour periods. A series of experiments was performed to test the effect of the adenylate cyclase agonist, forskolin, and ouabain on fluid transport by the isolated cysts. Forskolin caused the cysts to secrete fluid. Ouabain, added to the cyst cavity (apical surface) in the presence of forskolin, did not affect secretion. Addition of the inhibitor to the bath (basolateral surface) completely blocked fluid secretion. The results of these experiments indicate that the location of functional Na/K-ATPase is on the basolateral surface of these cysts.

Primary cultures were grown from the inner walls of ADPKD cysts and confluent cultures of the resulting cells were grown on the permeable membrane of Transwell-Col culture chambers. Forskolin induced fluid secretion by these monolayers. The monolayers of these cells and the supporting membrane were also mounted in standard Ussing chambers and the transepithelial potential difference, V_{te}, the short-circuit current, SCC, and tissue resistance were measured. In 36 monolayers, the apical surface was negative with respect to the basolateral surface and V_{te} was hyperpolarized by the addition of forskolin. SCC measurements indicated a positive ionic current flowed from the apical to the basolateral surface and this current was increased by forskolin. The transepithelial resistance averaged 156 ohms $\cdot$ cm^2 and was reduced by forskolin. These measurements are not compatible with the thesis that the cystic tissue secretes Na. The effect of ouabain on the forskolin-treated monolayers was also tested. Apical application of ouabain did not affect V_{te} or SCC. However, basolateral application of ouabain depolarized the monolayers and reduced SCC nearly to zero. These data indicate that the location of functional Na-K, ATPase is on the basolateral surface of monolayers of cultured ADPKD tissue.

Since mislocation of Na/K-ATPase to the apical membrane and sodium secretion could not account for fluid secretion in intact and in cultured ADPKD tissue, we considered that secretion of chloride may drive fluid secretion much as it does in other secretory epithelia. We hypothesized that chloride enters the cell across the basolateral membrane via a Na-K-2Cl cotransporter in the basolateral membrane and exits the cell via a conductance pathway in the apical membrane activated by cAMP. We began our investigation of this hypothesis by applying the chloride channel blocker, diphenylamine-2-carboxylate, DPC, to the apical membrane of forskolin-treated monolayers. DPC depolarized the tissue and greatly decreased the SCC. These data suggest that a chloride channel in the apical membrane may be involved in fluid secretion. However, much more work is needed to verify that tentative conclusion. In the second series of experiments we examined the effects of bumetanide, an inhibitor of the Na-K-2Cl cotransporter. Bumetanide inhibited the forskolin-induced increase in SCC and depolarized V_{te}. These results are compatible with the hypothesis that a Na-K-2Cl cotransporter is present in the basolateral membrane of cystic tissue and participates in the fluid secretion initiated by forskolin.

Microcysts grown from single, cultured ADPKD cells seeded in a collagen matrix were isolated and placed in a superfusion chamber on the stage of an inverted microscope. Morphometric techniques were used to measure the rate of change in cavity volume and the volume of the cells forming the cyst. Epifluorimetric techniques were used to measure the changes in cell electrical potential as reported by changes in the fluorescence of bisoxonol, a lipophilic anion. Fluid secretion into the cystic cavity was induced by the application of 8-bromo-cAMP. The initiation of secretion was accompanied by a loss of cell volume and hyperpolarization of the cell potential. Bumetanide blocked the secretion, caused a further loss in cell volume and an additional hyperpolarization. In preliminary experiments, changes in cell chloride concentration were determined with the use of the fluorescent indicator, methoxy-ethyl quinolinium (MEQ). The application of bumetanide to a microcyst, stimulated to secrete fluid by cAMP, caused a sharp reduction in cell chloride concentration that was reversed when the inhibitor was removed. The results obtained with the microcysts were interpreted to support the hypothesis that chloride secretion drives fluid secretion by cystic tissue as it does in airway epithelia and in certain cultures of intestinal epithelia.

The data reported here did not confirm the thesis that mislocation of Na/K-ATPase to the apical membrane and the secretion of sodium is responsible for fluid secretion by ADPKD cysts. The data did indicate that an anion is secreted by ADPKD tissue and are compatible with the hypothesis that fluid secretion is driven by chloride secretion.

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Determinants of polarity in renal epithelial cells

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Epithelial cells carry out vectorial functions for which they require a highly polarized structure. Our laboratory is interested in uncovering the molecular mechanisms responsible for this polarity. Four mechanisms have been described: intracellular sorting by the Trans-Golgi Network (TGN), intracellular sorting by specialized sorting endosomes, domain-selective stabilization of surface molecules by the submembrane cytoskeleton and passive segregation by the tight junctional fence. Experiments with the renal kidney cell line MDCK have shown that Na/K-ATPase is not sorted preferentially to one surface domain; its surface distribution is, instead, stabilized by interaction with the ankyrin-fodrin submembrane cytoskeleton, which is induced to assemble by E-cadherin mediated cell-cell contact. However, in a different MDCK cell line, and in the FRT cell line derived from rat thyroid, Na/K-ATPase is targeted preferentially to the basolateral membrane, indicating that certain cells have the ability to segregate this enzyme intracellularly. Our laboratory has been studying the retinal pigment epithelium(RPE), which localizes *in situ* Na/K-ATPase to the apical surface, the site of contact with the neural retina. Na/K-ATPase becomes depolarized upon primary RPE growth *in vitro*, suggesting that apical localization depends on its interaction with the neural retina. We have generated a cell line derived from rat RPE that preserves polarity and the tight junction fence. This cell line, RPE-J, expresses Na/K-ATPase in a nonpolar fashion. A striking redistribution to the lateral membrane is observed upon transfection of E-cadherin. The colocalization of E-cadherin and Na/K-ATPase confirms a previous report by McNeil et al and supports the model of E-cadherin induced polarity of the Na pump.

Experimental models developed by our laboratory have allowed us to follow the intracellular post-synthetic route of apical and basolateral proteins, such as Na/K-ATPase, dipeptidyl-peptidase IV, viral membrane proteins and histocompatibility antigens. Apical and basolateral proteins are sorted in a distal Golgi subcompartment (TGN) into specific transport vesicles. These vesicles use specialized docking systems to fuse with apical and basolateral domains to deliver their cargo. Transfection of genes, which encode normal or mutant apical or basolateral proteins, have led to the identification of molecular sorting signals that direct delivery to either surface. We have identified a glycolipid, glycosylphosphatidylinositol (GPI), as an apical targeting signal in several epithelial cell lines, including MDCK, LLC-PK and Caco-2. Proteins attached to the membrane by the GPI, which is transfered to the protein in the ER, are incorporated in the TGN into detergent insoluble complexes with glycosphingolipids, synthesized in the Golgi apparatus, and cholesterol. A phosphorylated protein, VIP21/caveolin, was recently shown to form part of these complexes. FRT cells, which do not express caveolin, fail to target GPI-proteins to the apical surface and to incorporate them into detergent-insoluble aggregates.

Basolateral sorting signals have been discovered in the cytoplasmic domain of basolateral proteins. These signals resemble in sequence the endocytic determinants previously found to direct receptor endocytosis into coated pits. Thus, it may be presumed that the molecules that interact with these basolateral sorting signals are related to endocytic determinants and interact with similar molecules yet to be identified, to the two adaptor protein/coat complexes described to date, clathrin/adaptins and coatomers. Cross linking experiments with bi-functional reagents detect putative receptors for basolateral signals in the TGN. Recently, we have introduced a perforated cell model to reconstruct *in vitro* the process of sorting of apical and basolateral proteins and the assembly of transport vesicles in the TGN. Our objective is, ultimately, to identify the cytosolic and membrane factors that regulate the production, directionality, and selective fusion of these vesicles with polarized surface domains.

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Abnormalities in polarized molecular transport in PKD cyst epithelia

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Cyst formation in autosomal dominant polycystic kidney disease (ADPKD) results from the expansion of every segment of the nephron and the early separation of the cysts from the nephron of origin. Cysts are fluid filled sacs lined by a layer of epithelial cells typically as a single layer, although occasional hyperplastic regions are seen. Early in the disease process, microcysts can be seen in which the epithelia resemble proximal or distal tubules, but later their distinguishing morphological properties are modified resulting in a variety of structural characteristics of cyst lining epithelia, ranging from columnar, through cuboidal to flattened. Transmission electron microscopy shows that despite these alterations in their differentiated characteristics all cyst epithelial cells are polarized with different degrees of an apical brush border membrane distinct from basolateral membranes and distinct intercellular occluding tight junctions. These properties are also maintained *in vitro* in cultures of microdissected ADPKD and recessive (ARPKD) cyst epithelia which exhibit polarized morphology, membrane protein segregation and vectorial transport.

Alterations in the polarized distribution of specific membrane proteins is widespread in PKD cyst epithelia, and was first described for Na/K-ATPase in human ADPKD epithelia which is