New Insights into the Cell Biology of Ischemic Acute Renal Failure

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ABSTRACT

Proximal tubule cells play an essential role in the reabsorption of ions, water, and solutes from the glomerular filtrate. This is accomplished, in large part, by having a surface membrane polarized into structurally, biochemically, and physiologically distinct apical and basolateral membrane domains separated by cellular junctional complexes. Establishment and maintenance of these unique membrane domains are essential for the normal functioning of the cell. Ischemia results in the duration-dependent loss of apical and basolateral surface membrane lipid and protein polarity. Loss of surface membrane polarity is preceded by disruption of the microfilament network and opening of cellular tight junctions. Surface membrane lipids and proteins are then free to diffuse laterally within the bilayer into the alternate membrane domain. Functionally, ischemia-induced loss of epithelial polarity has been shown to be responsible for reduced sodium and glucose reabsorption. Reduced Na⁺ reabsorption has been related to redistribution of Na⁺,K⁺-ATPase into the apical membrane. During recovery from ischemic injury, proximal tubule cells undergo remodeling of the surface membrane such that the unique apical and basolateral membrane domains are re-established, allowing for the return of normal cellular function.

Key Words: Epithelial polarity, apical membrane, basolateral membrane, ischemia, phospholipids, Na⁺,K⁺-ATPase, proximal tubule, actin, cytoskeleton

The purpose of this review is to use recent advances in the understanding of epithelial cell biology to explain, in part, the pathophysiology of ischemic acute renal failure. To accomplish this goal, I will discuss the establishment, maintenance, and importance of proximal tubule cell surface membrane polarity, and the effect, functional significance, and mechanism by which ischemia induces loss of proximal tubule surface membrane protein and lipid polarity. In an attempt to interrelate these areas, I will repeatedly use Na⁺,K⁺-ATPase to highlight certain fundamental cellular processes. Although Na⁺,K⁺-ATPase serves well to illustrate many aspects of epithelial cell biology, one must be careful to emphasize that many other surface membrane components and processes may be affected in a similar or entirely different manner.

RENAL PROXIMAL TUBULE CELLS AS A POLARIZED EPITHELIUM

Renal proximal tubule cells function to provide and regulate the efficient and vectorial movement of ions, water, and macromolecules transcellularly. This is accomplished, in large part, by having a surface membrane polarized into apical and basolateral membrane domains consisting of structurally, biochemically, and physiologically distinct membrane components including ion channels, transport proteins, enzymes, and lipids (Table 1). The apical membrane faces the urinary lumen (external compartment) and is composed of membrane proteins with specialized properties related to the reabsorption of water, electrolytes, and macromolecules. The basolateral membrane domain faces the internal milieu (blood compartment) and has a complement of intrinsic and extrinsic membrane proteins that are involved in the maintenance of the normal physiological state of the cell and also signal recognition and transduction (1,2). Extensive differences also exist between apical and basolateral membrane lipids, which...
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are responsible for large physicochemical differences between the two different membrane domains (3–6). Differences in membrane lipids also influence the function of numerous membrane proteins and enzymes (6).

To illustrate the importance of establishing and maintaining epithelial surface membrane polarity, consider the reabsorption of sodium. This involves the movement of Na\textsuperscript{+} down its electrochemical gradient across the apical membrane. Apical Na\textsuperscript{+} transport provides the energy for internalization of glucose, phosphate, and amino acids and for secretion of hydrogen. Sodium is then transported up its electrochemical gradient in an ATP-dependent reaction via Na\textsuperscript{+},K\textsuperscript{+}-ATPase, which is localized to the basolateral membrane. Therefore, the polar distribution of Na\textsuperscript{+},K\textsuperscript{+}-ATPase to the basolateral membrane is essential for efficient and vectorial Na\textsuperscript{+} reabsorption (Figure 1A). Consider what would occur if Na\textsuperscript{+},K\textsuperscript{+}-ATPase were randomly distributed over the entire surface membrane. Na\textsuperscript{+} that entered via the apical membrane could then be either pumped across the basolateral membrane, completing the reabsorptive process, or transported back across the apical membrane. This latter route would result in inefficient Na\textsuperscript{+} reabsorption and the uncoupling of ATP utilization and Na\textsuperscript{+} transport. The uncoupling of renal Na\textsuperscript{+} reabsorption and oxygen consumption has been reported after ischemic injury (7).

Establishment and maintenance of epithelial polarity is a complex process involving coordination of a number of cellular processes (Figure 2). Surface membrane proteins and phospholipids are synthesized in the endoplasmic reticulum, move to the cis aspect of the Golgi apparatus, undergo posttranslational modification, are sorted, and then, from the trans Golgi network, are targeted in a polar fashion to apical and basolateral membranes. For example, Caplan et al. (8) have shown that Na\textsuperscript{+},K\textsuperscript{+}-ATPase is targeted specifically to the basolateral membrane. Maintenance of surface membrane domain-specific characteristics is dependent upon three factors. First, a functionally intact zonula occludens or tight junction acts as a barrier to the lateral diffusion of surface membrane proteins and external leaflet phospholipids (1,2). The tight junction also regulates paracellular permeability (9). Second, maintenance of polarity is dependent upon the polar recycling of surface membrane components internalized via the endocytic route. Finally, certain surface membrane proteins, such as Na\textsuperscript{+},K\textsuperscript{+}-ATPase, are attached to the cortical (surface membrane-associated) cytoskeleton which is essential for both their recruitment and stabilization (10–12).

The cytoskeleton plays an essential role in the establishment and maintenance of surface membrane polarity. First, many of the aforementioned processes are dependent upon an intact cytoskeleton.
ISCHEMIA RECOVERY

Figure 1. Polarized proximal tubule cell (A). Sodium enters across the apical membrane down its electrochemical gradient which provides the driving force for hydrogen ion secretion and the cellular uptake of glucose, amino acids (A.A.), and phosphate via cotransport processes. Sodium is then transported up its electrochemical gradient by Na⁺,K⁺-ATPase, which is specifically localized to the basolateral membrane. This is an energy-requiring process. A nonpolarized renal proximal tubule cell after ischemic injury (B). After ischemic injury, Na⁺,K⁺-ATPase is found in both basolateral and apical membrane domains. In the apical membrane, Na⁺,K⁺-ATPase maintains its functional activity and is, therefore, capable of competing for intracellular sodium ions. Na⁺,K⁺-ATPase-mediated Na⁺ transport across the apical membrane markedly diminishes the efficiency of sodium reabsorption and results in a high urinary excretion of sodium. By the transport of sodium back across the apical membrane, a futile cycle is set up which may uncouple ATP utilization and sodium transport.

For example, endocytic and exocytic vesicles destined for the surface membrane travel in a polarized fashion via microtubules (13). The actin cytoskeleton, which is involved in the overall organization and maintenance of cellular integrity, plays a particularly important role in maintaining the structural and functional integrity of microvilli, cell-cell attachments at the junctional complex, and the recruitment, attachment, and stabilization of certain basolateral membrane proteins such as Na⁺,K⁺-ATPase (10–12,14). Linkage of Na⁺,K⁺-ATPase to the actin cortical cytoskeleton occurs through an association of the α subunit with ankyrin (15). The actin cortical cytoskeleton also interacts with cell adhesion molecules via ankyrin, providing cell-cell contact stabilization and a framework for maturation of the cortical cytoskeleton (12).

ISCHEMIA ALTERS SURFACE MEMBRANE PROTEIN AND LIPID POLARITY

Evidence that ischemia results in the loss of surface membrane protein and lipid polarity includes biochemical, histochemical, and immunocytochemical observations. Apical membrane fractions isolated after variable durations of ischemia showed significant elevations in Na⁺,K⁺-ATPase enzymatic activity (16). Redistribution of Na⁺,K⁺-ATPase into the apical membrane during ischemia occurred rapidly and in a duration-dependent fashion with differences being documented after only 10 min of ischemia (17,18). Histochemical and immunocytochemical studies have confirmed these biochemical results (18,19). Redistribution of Na⁺,K⁺-ATPase occurred in all segments of the proximal tubule but was not observed in distal nephron cells (19).

Large alterations in apical membrane lipid content also occurred during ischemia. Marked decreases in apical sphingomyelin and cholesterol content and increases in phosphatidylcholine and phosphatidylinositol content occurred within 5 min of ischemia, induced by renal pedicle clamping (17). The alterations in apical lipid content occurred rapidly during the first 15 min of ischemia and thereafter at a much slower rate (17,18). That lipids redistribute between apical and basolateral domains more rapidly than proteins is consistent with the increased lateral mobility of phospholipids within the bilayer (20). Biochemical and immunocytochemical studies have also documented that apical lipids and protein (leucine aminopeptidase) redistribute into the basolateral membrane during ischemia (18). In summary, ischemia induces the rapid, duration-dependent redistribution of apical and basolateral membrane domain-specific proteins and lipids into the alternate surface membrane domain.

Reestablishment of apical and basolateral membrane polarity occurs during the recovery phase of
Figure 2. Cellular components and processes involved in the establishment and maintenance of epithelial cell polarity. On the left, apical and basolateral protein synthesis and sorting and targeting pathways are shown. Apical (A)- and basolateral (C)-destined protein vesicles move from the trans-Golgi network (TGN) to their respective surface membrane domains. In hepatocytes (39) and some other cell types, apical proteins may be delivered first to the basolateral domain and then endocytosed and redistributed to the apical membrane (B). Apical endocytosis is also shown. Receptor-mediated endocytosis starts (a) with the formation of a clathrin-coated vesicle (2) from a clathrin-coated pit (1). Clathrin then disassembles leaving primary or early endosomes. From primary endosomes, membrane components can either recycle back to the surface membrane of origin (b), be transferred (c) to lysosomes (L) via multivesicular bodies (4), or undergo transcytosis (d). The junctional complex along the lateral membrane includes the zonula occludens (ZO), zonula adherens (ZA), maculae adherens (MA), gap junctions (GJ), and cell adhesion molecules. On the right, components of the cytoskeletal network are shown including actin microfilaments, which form the structural core within each microvillus and are held together by the "bundling" proteins villin and fimbrin (O). Microtubules (MT) form along the apico-basal axis of the cell, and intermediate filaments (IF) interconnect adjacent cells at macula adherens (MA) and the substratum at hemidesmosomes (HD). The inset shows the interactions of actin microfilaments and their associated cytoskeletal proteins. Na⁺K⁺-ATPase is associated with the cortical cytoskeleton by linkage of the α subunit to ankyrin. Ankyrin also serves to link the cortical cytoskeleton to cell adhesion molecules (CAM).

Ischemic acute renal failure. The rate at which repolarization of the surface membrane occurs is dependent on the severity of the injury. Fifteen minutes of ischemia (mild injury) requires only 24 h of reperfusion, whereas 50 min of ischemia (moderate to severe injury) requires several days for correction of apical and basolateral membrane alterations (21). In both cases, normal cell morphology is restored before the reestablishment of surface membrane polarity. This delay in the reestablishment of surface membrane polarity may explain the paradox of why proximal tubule function remains abnormal even though cellular morphology has normalized after ischemic injury.

The morphologic recovery of rat proximal tubule cells after variable lengths of ischemia has been extensively studied (22-24). S₁ and S₂ cells, after even up to 60 min of ischemia, completely recover by cellular repair (22-24). Cells of the S₃ segment undergo necrosis, and mitotic figures have been identified in this segment (23,24). However, the overall importance of cellular proliferation in repopulating this segment has never been quantified. Cuppage et al. (25), using an extremely severe model of ischemic injury (3 h unilateral or 1 ½ h bilateral), noted "tubular mitotic figures" during recovery but did not determine in which portion of the nephron they occurred. Cuppage et al. also used [³H]thymidine uptake in an attempt to quantify cellular proliferation after ischemia. There are, however, two important limitations of this technique when used in vivo. First, numerous nontubular (interstitial and vascular) cells were noted to take up the [³H]thymidine (25). Second, cellular DNA repair after ischemic injury also requires thymidine incorporation. Taken together, these data imply that in S₁ and S₂ segment cells, the surface...
membrane changes we have observed during recovery represent actual remodeling of surface membrane domains by recovering cells and are not due to cellular proliferation. The exact role of cellular proliferation in the S3 segment after ischemic injury remains to be determined.

**FUNCTIONAL SIGNIFICANCE OF ISCHEMIC-INDUCED LOSS OF EPITHELIAL POLARITY**

Loss of surface membrane polarity could lead to numerous alterations in the ability of the cell to conduct normal functions. Because ischemia leads to the duration-dependent redistribution of Na⁺,K⁺-ATPase into the apical membrane, we hypothesized that the redistributed enzyme could be functional and, therefore, able to transport intracellular sodium back into the nephron lumen (Figure 1B). This, in turn, would lead to reduced effective Na⁺ reabsorption and would also uncouple ATP and sodium reabsorption, resulting in inefficient utilization of an already limiting source of cellular energy. Two approaches were used to test this hypothesis. First, we used paired in vivo micropuncture techniques to quantify sodium reabsorption under physiologic situations and then after 15 min of ischemia and 2 h of reperfusion in the same nephron segment. After 2 h of reperfusion, there was a marked reduction in the reabsorption of both sodium (37.4 versus 23.0%; P < 0.01) and water (49.6 versus 36.9%, P < 0.01) in these paired tubule studies (24). This reduction in Na⁺ reabsorption was independent of cellular ATP content, morphology, and apical Na⁺ permeability as these variables had all normalized during the 2-h reperfusion period (26). In an additional in vivo study, after 50 min of ischemia, normalization of the transcellular transport of sodium and lithium (a specific marker of proximal tubule Na⁺ reabsorption) was dependent upon the reestablishment of surface membrane Na⁺,K⁺-ATPase polarity in proximal tubule cells (21).

Proximal tubule glucose transport was also reduced after ischemic injury (21,27). The defect was apparently due to reduced Na⁺-coupled glucose transport across the apical membrane. In brush border membrane vesicle (BBMV) studies, the Vₘₐₓ for Na⁺-dependent glucose transport decreased from 1,913 ± 251 to 999 ± 130 pmol/mg of protein/s (P < 0.01) after 15 min of ischemia (28). This was associated with a reduction in the number of phlorizin binding sites (390 ± 43 versus 146 ± 24 pmol/mg of protein; P < 0.01), indicating there was a decrease in the number of Na⁺-dependent glucose "carriers" within the apical membrane.

Glucose transport in BBMV was highly correlated with the sphingomyelin to phosphatidylcholine ratio (r = 0.96; P < 0.01) and was inversely correlated with membrane fluidity (r = 0.83; P < 0.01). In in vivo studies during recovery from ischemia, glucose transport increased to control levels concurrently with the normalization of the apical sphingomyelin:phosphatidylcholine ratio (21). These data, therefore, suggest that the rapid ischemia-induced alterations in apical phospholipid content were responsible for reduced glucose reabsorption after ischemic injury (28).

Taken together, these data indicate that the reabsorption of ions, water, and solutes by proximal tubule cells is dependent upon the establishment and maintenance of domain-specific lipid and protein polarity in apical and basolateral membranes. After ischemic injury, proximal tubule cells are unable to function properly until surface membrane lipid and protein polarity has been reestablished.

**MECHANISM OF ISCHEMIA-INDUCED LOSS OF EPITHELIAL POLARITY**

Ischemia could induce loss of epithelial polarity in proximal tubule cells by at least three different mechanisms. First, the random (nonpolar) delivery of newly synthesized membrane components to either the apical or the basolateral membrane would result in the loss of domain-specific differences. This, however, seems unlikely because both the synthesis and intracellular translocation of membrane components are ATP dependent and during ischemia cellular ATP drops rapidly (26,29) to levels not compatible with either event. Second, random migration and fusion of previously existing intracellular endocytic and exocytic vesicles with the surface membrane could also lead to the loss of surface membrane polarity. Studies from our laboratory, published in preliminary form, indicate that this mechanism was also not involved in the loss of epithelial polarity (30). The third, and most likely, mechanism involves the lateral migration of proteins and lipids within the bilayer from one membrane domain to the alternate domain.

Maintenance of surface membrane domain-specific characteristics is dependent upon an intact tight junction, and, for some proteins, attachment to the cortical cytoskeleton. Tight junctions in S1 and S2 proximal tubule cells consist of one discontinuous strand. Proximal tubules are classified as a "leaky" epithelium because the electrical resistance across a monolayer is low (31). Tight junctions of more distal nephron segments consist of numerous strands which are associated with a high transcellular electrical resistance. Disruption of tight junctions in vitro, by several mechanisms (calcium chelation, monoclonal antibodies), results in the loss of surface membrane lipid and protein polarity (32,33). To investigate whether ischemia resulted in disruption of tight junctions, in vivo micropuncture of early loops of proximal tubules with ruthenium red in glutaral-
dehyde was used to gain selective access to and outline the apical surface membrane and tight junctions. Ischemia resulted in a time-dependent increase in $S_1$ and $S_2$ tight junction penetration by ruthenium red from less than 10% under physiological conditions to 29, 50, and 62% after 5, 15, and 30 min of ischemia, respectively (17). Ischemia had an even greater effect on tight junctions of the $S_3$ segment but had no effect on tight junctions of more distal nephron cells (19). Therefore, ischemia rapidly diminishes the ability of proximal tubule cells to maintain tight junction integrity. After the dissociation of the tight junction, apical and basolateral lipids and unbound proteins could then diffuse laterally within the plane of the bilayer and redistribute into the alternate membrane domain.

As previously mentioned, Na$^+$.K$^+$.ATPase is tethered to the basolateral membrane by the actin cortical cytoskeleton (10-12,15). Therefore, before Na$^+$.K$^+$.ATPase can diffuse through an open tight junction and enter the apical membrane, it must first be released from its cytoskeletal attachment. To evaluate the effect of cellular ATP depletion on the attachment of Na$^+$.K$^+$.ATPase to the cytoskeleton, studies were conducted in confluent monolayers of a cloned line of LLCPK1 cells. Na$^+$.K$^+$.ATPase association with the cytoskeleton was determined by a functional assay in which cells were disrupted with the detergent Triton X-100 and then centrifuged. Cytoskeletal-associated Na$^+$.K$^+$.ATPase is Triton X-100 insoluble and, therefore, is found in the pellet after centrifugation. Noncytoskeletal-associated Na$^+$.K$^+$.ATPase is detergent soluble and is found in the supernatant. Under physiologic conditions, Na$^+$.K$^+$.ATPase was 93% associated with the cytoskeleton (34). However, during cellular ATP depletion, induced by using substrate free media and antimycin A, there was a rapid decrease in the amount of cytoskeletal-associated Na$^+$.K$^+$.ATPase and a concomitant increase in noncytoskeletal-associated Na$^+$.K$^+$.ATPase. The ratio of detergent-soluble to detergent-insoluble Na$^+$.K$^+$.ATPase increased dramatically during cellular ATP depletion from 0.074 to 0.504 (34). Similar results have been found, and reported in preliminary form, in in vitro studies with superficial cortical tissue (19). These data imply that during ATP depletion Na$^+$.K$^+$.ATPase dissociates from its cytoskeletal attachment. After dissociation, basolateral membrane Na$^+$.K$^+$.ATPase would then be free to redistribute into the apical membrane by lateral diffusion through an open tight junction.

**ROLE OF THE ACTIN CYTOSKELETON IN ISCHEMIC INJURY**

Actin exists within cells in either the monomeric (G) form or the polymeric (filamentous, F) form (35). The distribution of actin between these two forms is tightly regulated with the ratio being approximately 1:2 (36).

The actin cytoskeleton is essential for microvilli and tight junction integrity and for stabilizing Na$^+$.K$^+$.ATPase and other surface membrane proteins. To determine the effects of ischemia on the actin cytoskeleton of proximal tubule cells, we used an indirect immunofluorescence technique on renal cortical sections after ischemia. In normal proximal tubule cells, the majority of actin staining was associated with the apical pole of the cell where actin is known to form a circumferential terminal web with extensions of individual filaments out into individual microvilli (37). Fifteen minutes of ischemia caused disruption of the circumferential actin microfilament network. Fifty minutes of ischemic injury resulted in redistribution of stainable actin from the apical pole to throughout the cytoplasm. These alterations were not seen in distal tubule cells after ischemic injury.

To further investigate the effect of ischemia on actin microfilaments, we have used confluent monolayers of LLCPK1 cells to study the effect of ATP depletion on the actin cytoskeleton. Under physiologic conditions, rhodamine phalloidin-stained filamentous actin existed as a dense cortical cytoskeleton. After 30 min of cellular ATP depletion, small patchy accumulations of rhodamine staining were seen throughout the cell (34). After 1 h, large rhodamine-stained aggregates were noted in a perinuclear distribution. This apparent redistribution of actin from a cortical to a perinuclear location was associated with the rapid time-dependent conversion of G-actin to F-actin (34). In summary, the effect of ischemia on the actin cytoskeleton included the duration-dependent conversion of G-actin to F-actin, disrupting the actin cortical cytoskeleton, and redistribution of F-actin from a surface membrane distribution to a perinuclear location.

To determine if actin filament disruption played a fundamental role in the pathophysiology of ischemic cell injury, we studied the effect of cytochalasin D, which causes selective disruption of the actin cytoskeleton, in an isolated perfused kidney preparation. Cytochalasin D treatment for 60 min resulted in morphological alterations in the apical membrane similar to those seen during ischemia. These alterations included sloughing of the apical membrane, blebbing, vacuolization, fusion, and patchy loss of microvilli and enucleation. The mitochondria, endoplasmic reticulum, and Golgi apparatus were not affected by cytochalasin D treatment, indicating there was a selective effect on those cellular structures most closely associated with the actin cytoskeleton (37).

Cytochalasin D treatment also resulted in dramatic...
duration-dependent reductions in both Na\(^+\) and Li reabsorption. During 60 min of cytochalasin D treatment, sodium reabsorption decreased from 97.1 ± 0.7% to 64.3 ± 7% in a linear fashion (35). Lithium reabsorption also decreased linearly during treatment with cytochalasin D. A high degree of correlation existed in individual kidneys between sodium and lithium reabsorption, indicating the defect in Na\(^+\) reabsorption, induced by cytochalasin D-mediated microfilament disruption, was localized to the proximal tubule (37). In summary, selective actin microfilament disruption was associated with specific morphologic and physiologic events known to occur during ischemia. In addition, cytochalasin D-induced disruption of the actin cytoskeleton is also known to result in the opening of cellular tight junctions (38). Taken together, these data imply that disruption of the actin cytoskeleton during ischemia may play an important role in the early pathophysiology of ischemic injury.

In summary, ischemia leads to the duration-dependent loss of surface membrane polarity in proximal tubule cells. This appears to occur via the loss of the cells' ability to maintain the actin cytoskeleton and the opening of cellular tight junctions. This, in turn, allows for the rapid lateral diffusion of apical and basolateral domain-specific components into the alternate domain. After ischemic injury, proximal tubule cells function as nonpolar cells until cellular remodeling occurs, allowing for the return of unique apical and basolateral membrane domains which can then function together in a coordinated fashion to bring about the vectorial transport ions, water, and macromolecules transcellularly. Further understanding of ischemia-induced surface membrane polarity and cytoskeletal alterations will greatly enhance the understanding of the pathophysiology of ischemic injury and cellular recovery.

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