Ischemic injury induces ADF relocalization to the apical domain of rat proximal tubule cells

SHARON L. ASHWORTH,1 RUBEN M. SANDOVAL,1 MELANIE HOSFORD,1 JAMES R. BAMBURG,2 AND BRUCE A. MOLITORIS1

1Renal Epithelial Biology Experimental Laboratories, Indiana University Department of Medicine, and Roudebush Veterans Administration Medical Center, Indianapolis, Indiana 46202-5116; and 2Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, Colorado 80523-1870

Received 11 August 2000; accepted in final form 2 February 2001

Ischemic injury induces ADF relocalization to the apical domain of rat proximal tubule cells. Am J Physiol Renal Physiol 280: F886–F894, 2001.—Breakdown of proximal tubule cell apical membrane microvilli is an early-occurring hallmark of ischemic acute renal failure. Intraacellular mechanisms responsible for these apical membrane changes remain unknown, but it is known that actin cytoskeleton alterations play a critical role in this cellular process. Our laboratory previously demonstrated that ischemia-induced cell injury resulted in dephosphorylation and activation of the actin-binding protein, actin depolymerizing factor (ADF); Schwartz, N, Hosford M, Sandoval RM, Wagner MC, Atkinson SJ, Bamburg J, and Molitoris BA. Am J Physiol 280: F544–F551, 1999). Therefore, we postulated that ischemia-induced ADF relocalization from the cytoplasm to the apical microvillar microfilament core was an early event occurring before F-actin alterations. To directly investigate this hypothesis, we examined the intracellular localization of ADF in ischemic rat cortical tissues by immunofluorescence and quantified the concentration of ADF in brush-border membrane vesicles prepared from ischemic rat kidneys by using Western blot techniques. Within 5 min of the induction of ischemia, ADF relocalized to the apical membrane region. The length of ischemia correlated with the time-related increase in ADF in isolated brush-border membrane vesicles. Finally, depolymerization of microvillar F-actin to G-actin was documented by using colocalization studies for G- and F-actin. Collectively, these data indicate that ischemia induces ADF activation and relocalization to the apical domain before microvillar destruction. These data further suggest that ADF plays a critical role in microvillar microfilament destruction and apical membrane damage during ischemia.

actin depolymerizing factor; coflin; acute renal failure; actin cytoskeleton

ISCHEMIA-INDUCED ACUTE RENAL failure has drastic and duration-dependent effects on renal proximal tubule cells (21, 36). These changes initiate rapidly, and the extent of cell injury depends on length and severity of the ischemic insult. The structural, biochemical, and physiological properties of proximal tubule cells are particularly sensitive to ischemia. Maintaining the integrity of their distinctly polarized apical and basolateral domains is crucial for normal functioning of the kidney because proximal tubule cells play a critical role in ion, water, and solute reabsorption from the glomerular filtrate (35). In response to ischemia, the apical membrane breaks down with simultaneous internalization, fragmentation, and blebbing. Release of membrane vesicles into the tubular lumen can lead to tubular obstruction. Also, cell-cell junctional complexes, involved in separating the basal-lateral and apical membrane domains, dissociate, permitting protein and lipid interdiffusion and loss of surface membrane polarity (23).

It has been established that alterations in the cortical actin network of the proximal tubule cell occur before, or in conjunction with, the distinct morphological membrane changes induced by ischemia (14, 20). Evidence suggests rearrangement of the actin cytoskeleton may play an important role in surface membrane alterations. Under physiological conditions, the actin cortical cytoskeleton, actin-associated proteins, and proximal tubule cell surface membrane interact to establish and maintain crucial cellular structures that facilitate specific functions of proximal tubule cells. These include apical microvilli, terminal web, junctional complexes, cell-substratum attachment, and anchoring of basolateral membrane proteins. The onset of ischemia results in disruption of these cytoskeletal-membrane associations, with subsequent breakdown of their specific cellular structure and functions. For example, the fingerlike brush-border microvilli degenerate in response to ischemia (13). Furthermore, the complex F-actin meshwork present in the microvillar core and in the terminal web region located beneath the microvilli is disrupted, and junctional complexes dissociate (8, 22, 37). Both events contribute to the loss
of specific membrane domains and apical surface membrane stability.

The intracellular mechanisms responsible for actin dysregulation during ischemic cell injury remain unknown. Both Ca\(^{2+}\)-independent and -dependent processes have been postulated. Our recent studies suggested that a 19-kDa actin-associated protein, actin depolymerizing factor (ADF), may play a central role in this process (33). In the previous study, we observed that ischemia induced a duration-dependent activation of ADF and accumulation of ADF and G-actin in intraluminal vesicles shed from the apical surface of proximal tubule cells. These findings, in conjunction with known ADF interactions with actin, suggested that, during ischemia, ADF relocalized to the apical region of proximal tubule cells and mediated F-actin disruption via severing and depolymerization. Therefore, the present studies were undertaken to further test this hypothesis by determining the cellular localization of ADF in response to ischemia in vivo. We hypothesized an ischemia-induced ADF relocalization to the apical membrane in a time-dependent fashion consistent with its role in F-actin breakdown. Therefore, we used indirect immunofluorescence to characterize ADF localization in proximal tubule cells under physiological and ischemic conditions. In addition, we biochemically quantified the amount of ADF in brush-border membrane vesicles (BBMV) isolated following ischemia.

MATERIALS AND METHODS

**Tissue preparation.** Renal ischemia was induced in male Sprague-Dawley rats (200–250 g) by clamping the left renal pedicle for variable lengths of time as previously described by our laboratory (24). The right kidney of each animal was not clamped and functioned as the control for each experiment.  

**Immunofluorescence.** Kidneys were perfusion-fixed with 4% paraformaldehyde in PBS, pH 7.4, and then excised. The excised kidneys were placed in 4% paraformaldehyde overnight at 4°C. The following morning, the fixed tissue was washed with PBS three times for 15 min. The tissue was sectioned with a vibrotome into 40- to 75-μm sections. These tissue sections were then incubated for 1–2 h at 4°C in a PBS blocking-extraction buffer (PBS containing 2% defatted BSA, with or without 0.5% dry nonfat milk, and 0.1% Triton X-100, pH 7.4). After the specimens were blocked, they were incubated overnight at 4°C with either the primary rabbit anti-chick ADF antibody (1:200) or the primary mouse monoclonal anti-G-actin antibody, JLA 20 (1:100; Amersham, Piscataway, NJ) diluted in PBS blocking buffer. This primary incubation was followed by three 1-h rinses or one 6-h rinse in PBS. The tissue sections were then incubated overnight at 4°C with the respective secondary antibodies diluted in the PBS blocking buffer, with or without Texas red-phalloidin (1:200) (Molecular Probes, Eugene, OR). The specimens were washed three more times for 1 h or once for 6 h in PBS before being mounted in 50:50 glycerol-PBS with 1% 1,4-diazabicyclo(2,2,2)octane (DABCO; Sigma, St. Louis, MO). These samples were examined by using a MRC-1024 confocal microscope (Bio-Rad, Hercules, CA) with a ×60 NA 1.2 water-immersion lens.

**Brush-border membrane vesicles.** The left kidneys of Sprague-Dawley rats were clamped as described above for 5, 15, and 25 min, and the right kidneys were left untreated to be used as the control. The kidneys were excised, and the cortical region was recovered and processed as previously described (24). The BBMV were mixed 1:1 with SDS-sample buffer, vortexed, resuspended through a 25-gauge needle three times, and boiled for 5 min. Both the ischemic treated and untreated BBMV (10 μg total protein) were electrophoresed on 14% polyacrylamide gels, and the separated proteins were transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were blocked for 1 h at 4°C in TTBS (0.01 M Tris, 0.1 M NaCl, and 0.05% Tween 20, pH 7.5) containing 10% newborn-calf serum. After being washed three times with TTBS, the blots were incubated in the primary antibody to chick ADF (1:10,000) for 1 h at room temperature. Again, the blots were washed in TTBS and then incubated for 1 h at room temperature in the secondary antibody, goat anti-rabbit horseradish peroxidase (Fisher Scientific, Itasca, IL) at a 1:20,000 dilution. The blots were washed overnight at 4°C to remove nonspecific antibody binding. The proteins were detected by using enhanced chemiluminescence (Pierce, Rockford, IL) and quantified with Bio Image Intelligent Quantifier Software (Bio Imaging Systems, Ann Arbor, MI). The total protein loaded in each lane was the same for each sample tested (5 or 10 μg).

**Tissue preparation.** Male rats were anesthetized with Metafane, followed by a peritoneal injection of a Nembutal-sodium solution. The abdominal cavity was surgically opened, and the urinary bladder was gently massaged to release urine for control samples. The renal pedicle of both kidneys was clamped for 25 min to induce ischemia. After the clamps were removed, the abdomen was repeatedly irrigated with warm saline solution. The bladder was then gently massaged, and urine was collected for 2 h and kept on ice. Urine samples were centrifuged for 10 min at 14,000 rpm in an Eppendorf centrifuge at 4°C. Pellets were fixed in 4% paraformaldehyde in PBS, pH 7.4, containing 0.1% glutaraldehyde for 1 h at room temperature or 4°C overnight. Pellets were then washed three times at 4°C. For each wash, the pellets were vortexed in 100 μl of PBS, incubated for 5 min, and then centrifuged for 5 min at 14,000 rpm to pellet the urine sediment. After being washed, the pellets were incubated in 0.1% Triton X-100 in PBS for 10 min to maximize antibody penetration of the plasma membrane. The pellets were again washed as described above and further incubated in 2% BSA in PBS for 60 min to block nonspecific antibody binding. Primary antibodies, rabbit anti-chick ADF and mouse monoclonal anti-G-actin (JLA20, Amersham), were diluted in blocking buffer (1:100) and incubated with the urinary pellets for 2 h at room temperature or overnight at 4°C. The pellets were again washed and subsequently incubated with the secondary antibodies (FITC-goat anti-rabbit and Cy5-goat anti-mouse; Jackson Immuno Research Laboratories, West Grove, PA) diluted 1:400 in blocking buffer in addition to a 1:10 dilution of Texas red-phalloidin. After being washed, the pellets were suspended in 50 μl of PBS and mounted in a 50:50 solution of glycerol-PBS containing 1.0% DABCO.

**RESULTS**

**Effect of ischemia on ADF localization.** To determine the effect of ischemia on the intracellular distribution of ADF in rat kidney proximal tubule cells, ADF localization was observed by indirect immunofluorescence techniques in both normal and ischemic tissue. Under physiological conditions (Fig. 1, A–D), ADF demonstrated a diffuse cytoplasmic localization. Occasionally, a slightly higher concentration of ADF signal was observed at the subapical membrane in the region of
Fig. 1. Under physiological conditions, proximal tubule cells are characterized by rich F-actin containing microvilli and diffuse cytoplasmic actin-depolymerizing factor (ADF) localization. With the use of Texas red-phalloidin, the microvillar central microfilament core stained prominently for F-actin (A and C). The cytoplasm of the proximal tubule cell was diffusely stained with FITC-labeled anti-ADF, but no ADF staining was observed in the apical microvilli (B and D). Arrows in C and D, apical microvillar region. Bars, 10 μm.

Fig. 2. Ischemia induced relocalization of ADF to the apical microvillar region of proximal tubule cells. Within 15 min of the onset of ischemia, the F-actin circumferential band is no longer continuous but has numerous regions where F-actin staining is absent. In addition to F-actin changes, the localization pattern of ADF was altered. In response to ischemia, ADF relocalized, in part, to the apical microvillar region. A and C: F-actin stained by Texas red-phalloidin. B and D: indirect immunofluorescence of ADF using a FITC-labeled secondary antibody. Arrows in A–D: regions of microvillar breakdown and markedly increased ADF staining. Bars, 10 μm.
the terminal web. The ADF signal in the microvillar brush-border region was minimal or absent. Elements present in the interstitial space also stained with anti-ADF and represent fibroblast and vascular tissue that are known to contain ADF (39). The secondary antibody alone control showed no significant staining (data not shown). When ischemia was induced (Fig. 2, A–D, arrows), the distribution of ADF staining was distinctly different. Fifteen minutes of clamp-induced ischemia (Fig. 2, A–D) resulted in ADF signal detected throughout the disintegrating apical microvillar region and in the luminal vesicles.

Ischemia induced an increase in ADF in BBMV. To further confirm the immunofluorescent microscopy localization studies, BBMV were isolated from normal and ischemic rat kidneys and analyzed biochemically to determine whether the concentration of ADF increased in the apical brush-border region during ischemia (Fig. 3). After isolation, the BBMV underwent Western blot analysis, and the ADF protein bands were quantified as described in MATERIALS AND METHODS. All lanes were loaded with equal protein concentrations. Under physiological conditions, a small amount of ADF was found in the isolated membrane fraction (Fig. 3, inset). However, ischemia induced a duration-dependent increase in ADF concentration in the BBMV vs. control values.

Ischemia induced an increase in apical membrane G-actin. The next study was undertaken to determine whether we could document F-actin breakdown to G-actin in the apical region of proximal tubule cells during ischemia (Figs. 4 and 5). Monomeric actin was distinguished from the F-actin by using the G-actin-specific monoclonal mouse antibody JLA-20 (15) and Texas red-phalloidin staining. Under physiological conditions, neither ADF nor G-actin localized to the apical microvilli of proximal tubule cells (A and B, arrowheads). However, within 15 min of the induction of ischemia, the concentration of G-actin and ADF increased throughout the apical region of the proximal tubule cells, often concentrating and colocalizing in regions where the apical microvilli have begun to degenerate (C and D, arrows). Bar, 10 μm.
conditions, G-actin localized to the cytoplasm with a diffuse distribution pattern (Figs. 4A and 5, A and C). Texas red-phallidin primarily stained the apical region of proximal tubule cells (Fig. 5, B and C). Ischemia induced a significant change in the apparent F- and G-actin distribution, as microvillar F-actin staining was significantly reduced (Fig. 5, E and F) and an increase in cytoplasmic F-actin staining was detected. In contrast, ischemia resulted in increased G-actin staining in the apical microvillar regions (Fig. 4C, D and F) and F-actin staining was observed proximal to intact F-actin-stained microvillar cores in the presumed location of the F-actin core rootlet pointed ends (Fig. 5F, arrowheads). This observation suggests that ADF may bind the F-actin pointed ends to induce depolymerization. G-actin staining was also observed in the cell nuclei of some ischemic proximal tubule cells (Fig. 5, D and F, arrows).

Urine sediments of ischemic rats contained ADF-stained vesicles. To determine whether the vesicles formed in the proximal tubule lumen, in response to ischemia, were passed through the tubule and excreted, the urine from rats after renal ischemia was analyzed. Urine from ischemic rats was sedimented...
and analyzed by transmitted light and immunofluorescence microscopy to determine whether the membrane vesicles were present and whether they contained ADF, G-actin, or F-actin. These studies demonstrated that numerous vesicles of variable shapes and sizes were passed into the urine from ischemic rats (Fig. 6A, arrows) compared with an absence of urinary vesicles in the urine of rats under physiological conditions (data not shown). Urinary vesicles (B) were stained for ADF by using indirect immunofluorescence (green arrows) and for F-actin by using Texas red-phalloidin (red arrowheads). A cortical network of F-actin was observed in some of these vesicles, and actin bundles and aggregates were also noted. A triple-stained immunofluorescent image shown in C–E further demonstrates some urinary vesicles contain all 3: F-actin (C, asterisk), as detected with Texas red-phalloidin; ADF (D, asterisk), as detected with chick anti-ADF antibody; and G-actin (E, asterisk), as detected with the anti-G-actin antibody JLA-20. Bar, 10 μm.

**DISCUSSION**

Proximal tubule cells are characterized by distinct apical and basal membranes that are separated by junctional complexes. Surface membrane polarization contributes to the unique ability of these cells to reabsorb ions, water, and macromolecules from the glomerular filtrate. The apical membrane is distinguished by microvilli supported by a polarized bundle of parallel F-actin filaments extending from the electron dense plaque at the apical tip (barbed filament end) to the terminal web (pointed filament end) (25). Several key proteins involved in the structural integrity of the F-actin core have been identified (10). The actin-associated proteins, villin and fimbrin, bundle the parallel actin filaments together, whereas erzin and a myosin l-calmodulin complex tether the filaments to the overlying plasma membrane of the microvilli (4, 10). Although progress has been made in describing the structural components of the microvilli, the dynamics of the F-actin core within the microvillus are not well understood. In intestinal microvilli, the bundled filaments are thought to be stabilized through capping of the barbed filament end by unidentified molecules found in the apical electron dense plaque and/or through tropomyosin binding along the filaments and the pointed end-capping protein tropomodulin binding to the pointed filament end (10).

Renal ischemia has devastating effects on the structural, biochemical, and functional properties of proximal tubule cells are characterized by distinct apical and basal membranes that are separated by junctional complexes. Surface membrane polarization contributes to the unique ability of these cells to reabsorb ions, water, and macromolecules from the glomerular filtrate. The apical membrane is distinguished by microvilli supported by a polarized bundle of parallel F-actin filaments extending from the electron dense plaque at the apical tip (barbed filament end) to the terminal web (pointed filament end) (25). Several key proteins involved in the structural integrity of the F-actin core have been identified (10). The actin-associated proteins, villin and fimbrin, bundle the parallel actin filaments together, whereas erzin and a myosin l-calmodulin complex tether the filaments to the overlying plasma membrane of the microvilli (4, 10). Although progress has been made in describing the structural components of the microvilli, the dynamics of the F-actin core within the microvillus are not well understood. In intestinal microvilli, the bundled filaments are thought to be stabilized through capping of the barbed filament end by unidentified molecules found in the apical electron dense plaque and/or through tropomyosin binding along the filaments and the pointed end-capping protein tropomodulin binding to the pointed filament end (10).
mal tubule cells. In response to ischemia, the distinct apical and basolateral membrane domains lose their unique protein and phospholipid compositions, resulting in the loss of cellular polarity. Several studies have implicated actin cytoskeleton alterations as the mediators of these surface membrane changes (13, 14, 20). Chen et al. (7–9) demonstrated in a series of publications the dephosphorylation of ezrin and loss of ezrin-mediated actin surface membrane tethering in proximal tubule microvilli (7–9). However, the mechanism(s) underlying actin changes is not known. Investigators postulated that the breakdown of the F-actin core was secondary to the action of the Ca\(^{2+}\)-dependent actin severing proteins gelsolin and villin (30). Studies by Nurko et al. (30) demonstrated that villin could sever actin filaments in response to hypoxia in the presence of the Ca\(^{2+}\) ionophore ionomycin and increasing concentrations of Ca\(^{2+}\). However, two lines of evidence make this unlikely in normal and ischemic cells. First, Ca\(^{2+}\) levels would have to reach 10 \(\mu\)M for villin severing to occur (5). This is abnormally high even for ischemic cells. Second, during hypoxia, severing does not occur as quickly or as severely in the absence of ionomycin (34). Finally, evidence suggested that the Ca\(^{2+}\)-dependent actin severing protein gelsolin was not present in human proximal tubule cells, although it was observed in murine proximal tubule cells (16). Therefore, we believe another mechanism must be involved.

The actin-binding protein family ADF/cofilin is uniquely positioned to mediate destruction of the microvillar F-actin core in response to ischemia. First, ADF is known to be primarily responsible for actin dynamics under physiological and stimulated conditions in many cell types (1, 6). Second, there are four ischemia-induced cellular changes that strongly support the involvement of ADF in apical microvillar F-actin breakdown. These cellular alterations include a rapid and duration-dependent activation of ADF through dephosphorylation; ADF relocation to the apical microvillar region; increased depolymerized actin at the apical membrane concurrent with microvillar destruction; and colocalization of ADF with depolymerized actin in intraluminal and urinary vesicles. Collectively, these changes position ADF in its activated form at the microvillar region of the cell in a time frame consistent with mediation of the characteristic microvillar F-actin core destruction induced by ischemia.

Dephosphorylation of ADF/cofilin occurs not only in proximal tubule cells in response to ischemia, but also in response to a number of cellular stimuli known to induce rapid F-actin remodeling in other cell types (1). For example, activation of cofilin occurs rapidly in neutrophils in response to opsonized zymosan (28). In proximal tubule cells of rat kidneys clamped to induce ischemia, we have noted that the concentration of activated ADF increased 28% within 5 min and 81% by 15 min (33). Because the unphosphorylated or activated form of ADF binds actin with a much higher affinity than does phosphorylated ADF (3, 26), the actin cytoskeletal restructuring observed in response to these cellular stimuli was linked to the interaction of ADF with actin (1). Therefore, we propose that ischemia-induced activation of ADF in proximal tubule cells mediates alterations of the actin cytoskeleton in the apical microvilli. Although our observations suggest that ADF activation resulted in increased F-actin alterations, it is also important to remember that under physiological conditions unphosphorylated ADF was present and localized to the cytosol (33). What prevents
this fraction of ADF from localizing to the apical cell membrane and inducing F-actin alterations under physiological conditions is not known.

In addition to the induction of ADF activation, ischemia also resulted in ADF accumulation in the apical membrane region of the proximal tubule cell just before or in conjunction with major actin alterations of the F-actin core. This study is the first example of ADF relocalization to the epithelial cell apical brush border, although translocation of activated ADF/cofilin to cellular regions of intense actin reorganization has been observed in other stimulated cells (1). For instance, opossumed zymosan treatment of macrophage-like cells resulted in a transient relocalization of cofilin, an ADF homolog, to the actin-rich phagocytic-forming membrane, and it remained there until a stable phagocytic vesicle was formed (28).

Subsequent to microvillar ADF accumulation, a dramatic change in the microvillar F-actin core was observed. By 15 min of ischemia, the intense apical F-actin staining seen under physiological conditions was significantly decreased concomitantly with an increase in apical G-actin staining. In consideration of the in vitro biochemical characteristics of ADF (1), these observations strongly suggest that ADF may bind, sever, and depolymerize the microvillar F-actin core. To mediate F-actin depolymerization, ADF may initially locate to and preferentially bind the ADF-actin-rich domain toward the pointed end of the filaments in the terminal web region. Binding of ADF to ADP-actin is a pH-dependent and cooperative event. Binding induces a filament twist that may mediate filament severing and dramatically accelerates the pointed end-off rates, resulting in filament depolymerization (1, 6, 11, 12, 18, 19, 27). ADF severing is also directly related to the length of the filament (18, 31, 32); therefore, the extremely long microvillar F-actin filaments would be prone to ADF-mediated severing.

The final cellular change we observed indicated that ADF localized with G-actin in intraluminal and urinary vesicles. Colocalization of these two proteins further supports a role for ADF in active depolymerization of F-actin by binding to and sequestering the depolymerized G-actin subunits. Two additional pieces of evidence support a role for ADF in binding to and sequestering the actin subunits. First, ischemia decreases the concentration of cellular ATP and increases that of cellular ADP; therefore, the actin in the cell would primarily be loaded with ADP (35). Second, ADF preferentially binds ADP-actin and inhibits nucleotide exchange (17, 29), a substantial amount of actin could possibly be bound by ADF. The presence of F-actin in the urinary vesicles presents a dilemma because F-actin was not observed in luminal vesicles. It is possible that, in the presence of high concentrations of ADP and ADP-G-actin, G-actin polymerizes as it passes from the proximal tubule to the urinary bladder and is excreted into the urine.

Although these observations strongly point toward ADF-mediated F-actin core severing and depolymerization, the mechanism responsible for translocation to the apical region is still unclear. Three potential mechanisms may contribute to the changes in ADF in response to ischemia. These include changes in cytoplasmic pH, competition of ADF with F-actin-binding proteins, and ADF binding to phosphoinositol lipids. Localized changes in cytoplasmic pH may contribute to ADF binding and depolymerization of F-actin, because ADF binding to F-actin is enhanced by acidosis (11) and acidosis occurs early during ischemia. The second potential mechanism would address the binding competition between ADF and other F-actin-interacting proteins. In particular, the actin-stabilizing protein tropomyosin is known to compete with or limit ADF binding to F-actin (2). Although tropomyosin has been localized to the terminal web region in enterocytes and may limit ADF binding, it has not been localized in proximal tubule cells in vivo. ADF, along with many other actin-binding proteins, also binds phosphoinositol lipids (38). Binding to phosphoinositol lipids prevents the binding of ADF to actin. Therefore, a possible third mechanism to support the relocalization of ADF to the apical membrane may operate through its high affinity for binding to phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-bisphosphate and the known increase in apical concentrations of these phospholipids during ischemia (23, 24).

Our proposed model of the pathophysiology of microvillar F-actin destruction in response to ischemia is summarized in Fig. 7. For purposes of simplicity, we have divided the ADF events into three steps, although it is likely that all three proceed simultaneously. First, ADF is dephosphorylated during ischemia. Then, the activated form of ADF relocalizes to the apical membrane and binds to F-actin. Last, the F-actin core is destroyed through ADF-mediated severing and depolymerization. Although there is no direct evidence as yet to indicate that ADF is responsible for microvillar F-actin destruction, all observations to date are consistent with this hypothesis.

We especially thank Yolanda Burgener for expert technical assistance.

This work was supported by grants to B. A. Molitoris from the National Institute of Diabetes and Digestive and Kidney Diseases (DK-41126) and the Veterans Affairs Research Service.

REFERENCES

Ischemia induces ADF relocation.


