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Loss of Epithelial Polarity: A Novel Hypothesis for Reduced Proximal Tubule Na^+ Transport Following Ischemic Injury

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Summary. Ischemia results in the marked reduction of renal proximal tubule function which is manifested by decreased Na^+ and H_2O reabsorption. In the present studies the possibility that altered Na^+ and H_2O reabsorption were due to ischemia-induced loss of surface membrane polarity was investigated. Following 15 min of renal ischemia and 2 hr of reperfusion, proximal tubule cellular ultrastructure was normal. However, abnormal redistribution of NaK-ATPase to the apical membrane domain was observed and large alterations in apical membrane lipid composition consistent with loss of surface membrane polarity were noted. These changes were associated with large decreases in Na^+ (37.4 *vs.* 23.0%, $P < 0.01$) and H_2O (48.6 *vs.* 36.9%, $P < 0.01$) reabsorption at a time when cellular morphology, apical Na^+ permeability, Na^+-coupled cotransport, intracellular pH and single nephron filtration rates were normal. We propose that the abnormal redistribution of NaK-ATPase to the apical membrane domain is in part responsible for reduced Na^+ and H_2O reabsorption following ischemic injury.

Key Words renal ischemia · epithelial polarity · NaK-ATPase · Na^+ transport

Introduction

Renal epithelial cells function to provide and regulate the vectorial (net) transport of selected compounds transcellularly. This is accomplished in large part by having a surface membrane polarized into apical and basolateral components separated by occluding junctions [11, 20, 24]. In renal proximal tubular cells, these two surface membrane components are known to be functionally different with respect to transport processes, hormone receptors, enzymes, electrical resistance, and membrane lipid composition [12, 18, 26]. We have recently advanced the hypothesis that ischemia results in the loss of proximal tubule surface membrane polarity [19]. Additional data from our laboratory indicates that surface membrane lipid and protein polarity is lost rapidly during ischemia [16]. Specifically, 15 min of renal pedicle clamping resulted in redistribution of basolateral membrane (BLM) lipids and NaK-ATPase to the apical membrane. Ischemia is also known to cause marked reversible alterations in renal proximal tubular cell function including net reductions in Na^+, H_2O, and glucose transport [8, 14]. Since epithelial polarity is required for unidirectional reabsorption and/or secretion, we questioned whether a loss of polarity played a role in altered Na^+ and H_2O reabsorption following ischemic injury. To evaluate this hypothesis we have established and characterized a model of mild ischemic injury induced by only 15 min of renal pedicle clamping. This model minimized or excluded many of the potential cellular mechanisms known to affect Na^+ reabsorption.

Materials and Methods

Membrane Preparation and Characterization

Bilateral renal ischemia was induced in male Sprague-Dawley rats for 15 min and cortical apical membrane fractions were isolated using Mg^{2+} precipitation as described previously [18, 19]. Enzymatic characterization of apical membrane functions was conducted as previously described [18, 19]. Specifically, leucine aminopeptidase (LAP) was quantitated using a kinetic assay monitoring the conversion of L-leucine-4 nitroanilid at 380 nm and 37°C. NaK-ATPase was quantitated using an assay system coupled to pyruvate kinase, phosphoenolpyruvate and lactate dehydrogenase at 340 nm and 37°C [18, 19].

Marker enzyme enrichments for the apical membrane marker, alkaline phosphatase, were unaltered by ischemia (8.5 ± 0.9 *vs.* 9.2 ± 0.8, $n = 6$). Intracellular organelle contamination of apical fractions, measured as previously described [18, 19], was also unaltered by ischemia. Specific activities of the organelle marker enzyme succinic dehydrogenase, KCN-resistant NADH dehydrogenase, cytochrome *c* reductase and glucosaminidase in apical membrane functions were all less than specific activities

measured in their corresponding homogenate. Phospholipids and cholesterol were determined as previously reported [18, 19].

Micropuncture Studies

Fractional reabsorption of Na^+, H_2O and α-methylglucoside (αMG) by proximal convoluted tubules prior to and following 15 min of left kidney ischemia was examined by micropuncture techniques as previously described for this laboratory [4, 5]. Briefly, male Sprague-Dawley rats maintained without foot overnight but allowed ab lib water were anesthetized as described above. Animals were placed on a thermostatically controlled heated surgical table (37°C). Tracheostomy was performed and polyethylene catheters placed in the right jugular vein and femoral artery. The femoral artery catheter was attached to a pressure transducer and blood pressure was recorded on a direct-writing recorder. The left kidney was then exposed through a flank incision, dissected free of surrounding connective tissue and placed in a Lucite cup affixed to the surgical table. The surface of the kidney was bathed with physiologic saline warmed to 37°C. A PE-50 catheter was inserted into the ureter for collection of urine samples. An initial loading dose of ^{3}H-inulin (130 μCi) was given through the jugular vein catheter followed by a continuous infusion of 100 μCi/hr in 2 ml of Ringer's solution. In one group of rats (n = 5) 150 μCi $^{22}Na^+$ was given simultaneously with the loading dose of ^{3}H-inulin. In a second group of five rats ^{14}C-αMG (100 μCi) was given with the loading dose of ^{3}H-inulin and 10 μCi/hr ^{14}C-αMG was added to the maintenance ^{3}H-inulin infusion. Five last surface loops of proximal convoluted tubules were identified with a micropipette (outside diameter 5 μm) containing 1% FD&C green. Microdissection studies indicate that last accessible surface loops represent sites between three-fourths and four-fifths of the total length of the proximal convoluted tubule. Separate micropipettes (outside diameter 10 μm) containing sudan black were inserted into the previously identified last loop puncture sites. After injecting an oil block of four to five tubular diameters in length, complete 2-min tubular fluid collections were initiated by gentle aspiration, but continued spontaneously. Completeness of collection was assured by observing that the oil block remained stationary just distal to the tip of the micropipette during the collection. The collected tubular fluid samples were maintained under oil until analyzed. Blood samples (10 μl) were taken from the femoral artery prior to and following the series of tubular fluid collections. After surface drawings were made to record the collection sites, an atraumatic clip was placed on the left renal artery to completely obstruct blood flow for 15 min. Thereafter, the clip was removed and blood flow reestablished for 2 hr. Timed fluid collections were again obtained from the previous proximal tubular puncture sites and blood samples taken from the femoral artery. The collected tubular fluid samples were transferred from the collection micropipette to a previously calibrated constant-based Pyrex pipette where the volume of tubular fluid was measured. The tubular fluid and plasma samples from femoral blood were transferred to counting vials and respective radioactivity of ^{3}H, ^{14}C and ^{22}Na was measured using standard scintillation techniques.

Single nephron glomerular filtration rate (SNGFR) was calculated as

$$SNGFR = TF/P_{IN}\ V \quad (1)$$

where TF/P_{IN} is the ratio of counts per minute (CPM) per nanoliter of ^{3}H-inulin in tubular fluid and plasma (corrected for plasma water) and V is the tubular fluid flow rate in nanoliters per minute.

Proximal tubular fractional reabsorption of H_2O to the site of tubular fluid collection was determined from the relationship.

$$FR\ H_2O = (1 - P/TF_{IN}) \times 100. \quad (2)$$

Proximal tubular fractional reabsorption of Na and ^{x}MG to the site of tubular fluid collections were calculated from the following formula, respectively:

$$FR_{Na} = 1 - (TF/PNa/IN) \times 100 \quad (3)$$

where TF/PNa/IN is the ratio of tubular fluid to plasma CPM per nl for $^{22}Na^+$ and ^{3}H-inulin, and

$$FR^xMG = 1 - (TF/P^xMG/IN) \times 100 \quad (4)$$

where $TF/P^xMG/IN$ is the ratio of tubular fluid to plasma CPM per nl for ^{14}C-^{x}MG and ^{3}H-inulin.

Previous studies have shown blood pressure, SNGFR, absolute reabsorption and TF/P inulin remain constant over the study interval [4].

Nuclear Magnetic Resonance Studies

^{31}P NMR studies were performed using a 1.89 Telsa 30 cm horizontal-bore cryomagnet (Oxford Instruments) and a Biospec NMR spectrometer (Bruker Instruments and Oxford Research Systems). The spectrometer was operated in the Fourier transform mode and was interfaced with an Aspect 3000 Computer. A double-tuned 1.5 cm solenoid, 32.6 MHz for ^{31}P and 80.55 MHz for ^{1}H, was used for in vivo kidney studies [23, 27]. T_1 determination of phosphate compounds and protons were determined in the in vivo rat kidney using the inversion recovery method [22]. All scans employed 90° pulse angles for both proton and phosphorus and relaxation delays of greater than 4 T_1. The receiver gain, sweep width, acquisition time and array size for proton and phosphorus were held constant during all studies. ^{31}P NMR spectra were obtained with 200 scans in each block prior to exponential multiplication using 10 Hz line-broadening and Fourier transformation. Peak areas were calculated following baseline correction of the spectra. Based on this calibration, absolute quantification of ATP was obtained using the proton spectra as an internal standard [1, 23, 27]. Intracellular pH was estimated by the chemical shift of inorganic phosphate [1, 23].

Morphology

Surface proximal tubules under direct visualization were perfusion-fixed in vivo by infusing in order 2% glutaraldehyde in 0.1 M Na^+ cacodylate buffer (pH 7.4), 2% O_5O_4 in 0.1 M Na^+ cacodylate buffer each for 15 min. Individual tubules were dissected, washed in distilled water, dehydrated in graded ethanol solutions and embedded in epon. Sections for light microscopy (1–2 μm) were stained with toluidine blue and photographed using a Leitz Orthoplan scientific widefield microscope. Ultrathin sections were viewed on a Phillips CM-12 electron microscope.

Results and Discussion

Prior to initiating extensive studies into cellular function following ischemic injury and reperfusion, it was first essential to evaluate cellular morphology

Table 1. Effect of ischemia (15 min) and reperfusion (2 hr) on renal cortical apical membrane enzymes[a]

	Leucine aminopeptidase			NaK-ATPase	
	n	SA[b]	*E*[c]	SA	*E*
Control	7	30.2 ± 10.9	11.0 ± 2.2	13.0 ± 3.5	1.1 ± 0.2
15 min ischemia, 2 hr reflow	11	23.7 ± 7.6	9.3 ± 2.0	21.8 ± 6.1	2.3 ± 0.9
P value[d]		NS	NS	<0.01	<0.01

[a] Values are the mean ± 1 SD.
[b] Specific activity (SA) is reported in μmol/hr/mg protein.
[c] Enrichment (*E*) is the membrane fraction specific activity divided by the homogenate specific activity.
[d] Statistical significance was determined using an unpaired Student's *t*-test.

Table 2. Effect of ischemia (15 min) and reperfusion (2 hr) on renal cortical apical membrane lipids[a]

	n	SPH[b]	PC	PI	PS	PE	SPH/PC	Chol/PL
Control	6	34.1 ± 2.1	17.1 ± 2.7	3.6 ± 0.5	20.2 ± 2.5	25.1 ± 1.3	2.1 ± 0.5	0.81 ± 0.05
15 min ischemia, 2 hr reflow	8	30.7 ± 4.6	20.5 ± 2.0	3.3 ± 0.8	17.4 ± 2.0	26.2 ± 2.4	1.5 ± 0.3	0.66 ± 0.02
P value[c]		NS	<0.05	NS	<0.05	NS	<0.05	<0.01
Control BLM[d]		14.1 ± 0.9	38.6 ± 3.6	7.7 ± 0.5	10.6 ± 1.1	26.1 ± 1.9	0.4 ± 0.1	0.40 ± 0.05

[a] These data represent the mean ± 1 SD.
[b] *Abbreviations:* SPH, sphingomyelin; *P*, phosphatidyl; C, choline; I, inositol; S, serine; E, ethanolamine; Chol/Pl, choleserol to phospholipid ratio.
[c] Statistical significance was determined using an unpaired Student's *t* test.
[d] Control basolateral membrane (BLM) lipids are shown for comparisons.
Individual phospholipids are reported as percent of total phospholipids.

to determine whether it had returned to normal following 15 min of ischemia and 2 hr of reperfusion. Figure 1 shows the extent of cellular damage induced by 15 min of ischemia. For comparison, in vivo perfused control proximal tubules are shown (Fig. 1*A*). Fifteen minutes of ischemia resulted in an abnormal irregular apical surface with microvilli clumping together. In addition, protrusion of cytoplasm into the lumen (open arrows) and release of cellular contents (solid arrows) into the lumen was noted. With 2 hr of reperfusion, however, proximal tubules appeared normal with complete reorganization of the apical surface (Fig. 1*C*). Electron microscopic evaluation of cellular ultrastructure following 15 min of ischemia revealed (Fig. 2) apical microvilli that were disorganized. Formation of cytoplasmic protrusions were also visualized. Following 15 min of ischemia and 2 hr of reperfusion (Fig. 2*B*), apical microvilli now appear normal and could not be differentiated from control proximal tubules (*not shown*).

Cellular morphology following graded levels of ischemic injury has been previously investigated. Fifteen minutes of renal ischemia caused only minimal focal alterations in the morphology of proximal convoluted tubular cells [6, 7]. Furthermore, even renal ischemia of longer duration (25 min) was without effect on endoplasmic reticulum, ribosomes, mitochondria, nuclei or the Golgi apparatus [21, 28]. In our 15-min model, we have confirmed these results and have also observed that the apical membrane of proximal convoluted tubular cells appears morphologically normal following 15 min of ischemia and 2 hr of reperfusion.

Fifteen minutes of ischemia is known to disrupt proximal tubule surface membrane lipid and protein polarity [16]. To determine whether this loss of surface membrane polarity was maintained during reperfusion, apical membrane fractions were isolated following 15 min of ischemia and 2 hr of reperfusion. As is shown in Table 1, although the specific activity and enrichment of the apical marker enzyme LAP was unaltered, both apical NaK-ATPase specific activity and enrichment remained elevated ($P < 0.01$) similar to values seen following ischemia alone [16]. The partial loss of apical lipid polarity was also observed following 15 min of ischemia and 2 hr of reperfusion (Table 2). Significant decreases were seen in both the sphingomyelin to phosphatidylcholine and cholesterol to phospholipid ratios. Phosphatidylcholine increased and phosphatidylserine decreased, but the change in SPH did not reach statistical significance.

Taken together these data indicate that, al-

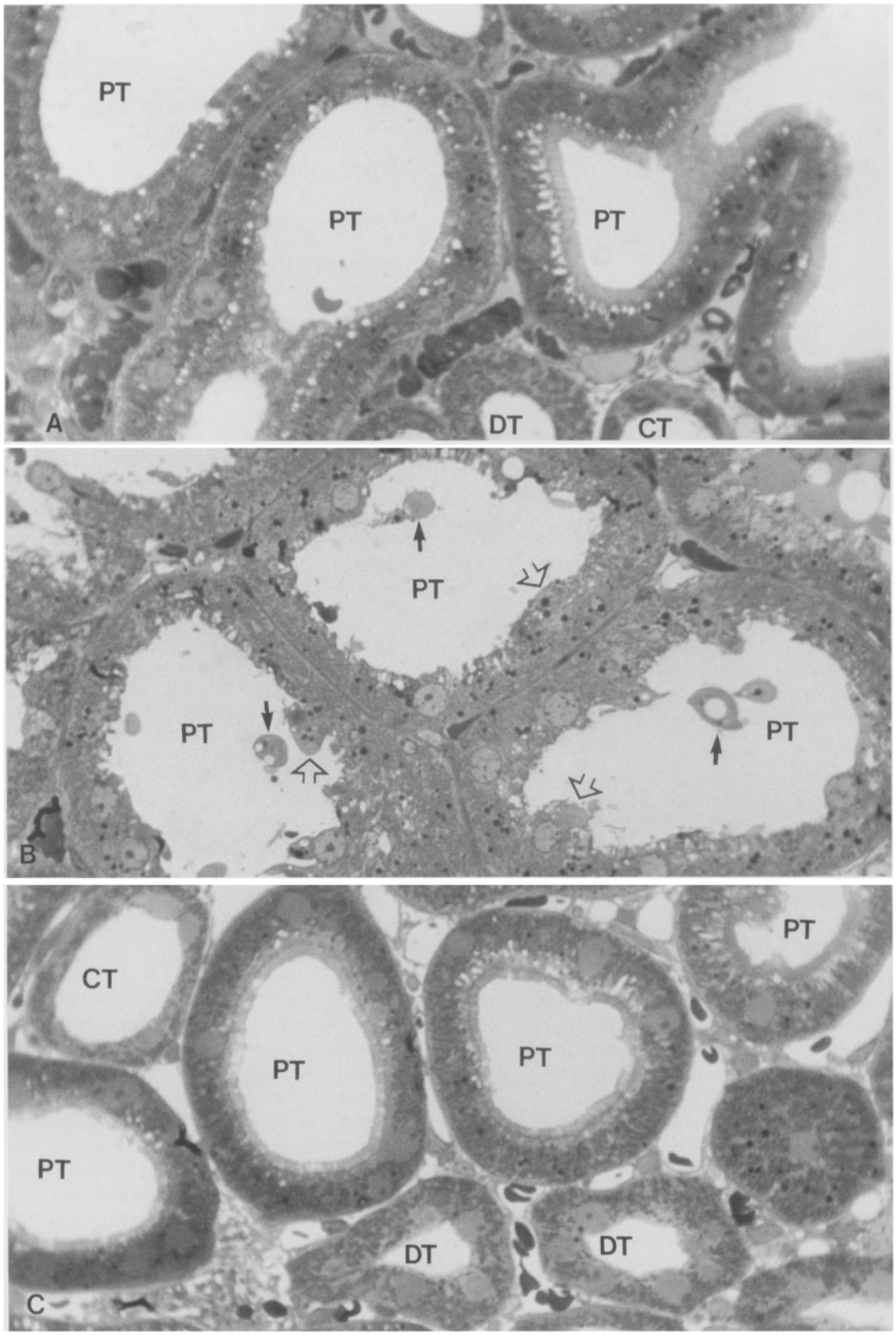

Fig. 1. Effect of mild ischemia (15 min) and reperfusion (2 hr) on proximal tubule morphology. (*A*) In vivo perfusion-fixed control proximal tubules showing normal cellular morphology (540×). (*B*) In vivo perfusion-fixed proximal tubules following 15 min of ischemia induced by renal artery clamping. *Note* protrusion of the cytoplasm into the lumen (open arrows) and blebbing of cytoplasmic contents into the lumen (solid arrows) (540×). (*C*) In vivo perfusion-fixed proximal tubules following 15 min of ischemia and 2 hr of reperfusion. Cellular morphology has returned to normal. (540×). Proximal tubules (*PT*) distal tubules (*DT*) and cortical collecting tubules (*CT*) are labeled

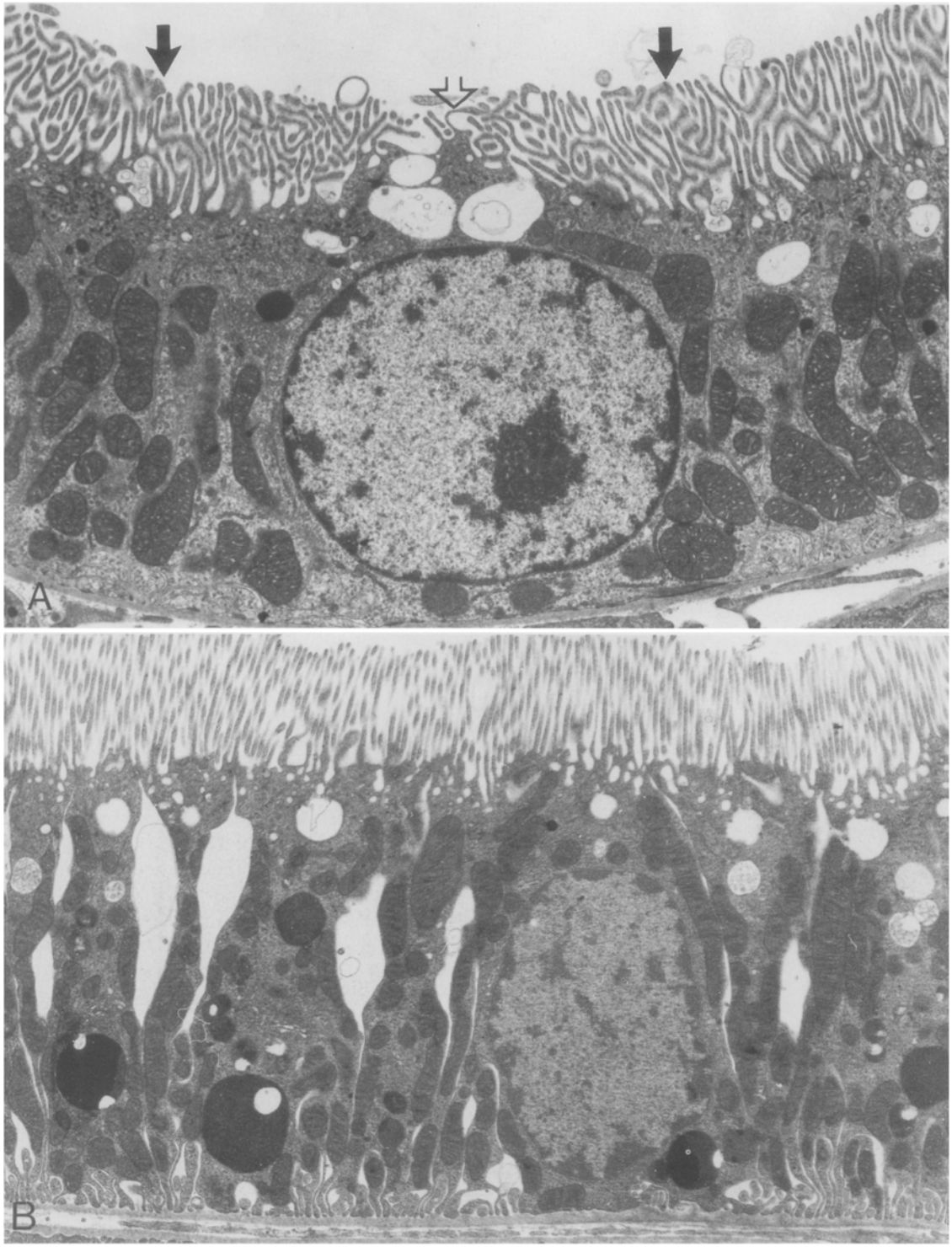

Fig. 2. Effect of mild ischemia (15 min) and reperfusion (2 hr) on proximal tubule ultrastructure. (*A*) Proximal tubule cellular ultrastructure (S_2 segment) following 15 min of ischemia. Apical microvilli were disorganized (solid arrows) and areas of early cytoplasmic protrusions (open arrows) were noted (12,000×). (*B*) Proximal tubule cellular ultrastructure (S_1 segment) following 15 min of ischemia and 2 hr of reperfusion. Note the normal appearance of the apical surface. (10,000×)

though cellular ultrastructure had returned to normal, surface membrane lipid and protein polarity has remained abnormal. This model then would allow us to test the hypothesis that apically localized NaK-ATPase may in part be responsible for reduced Na^+ reabsorption following ischemic injury.

To determine the functional significance of 15 min of ischemia and the abnormal redistribution of

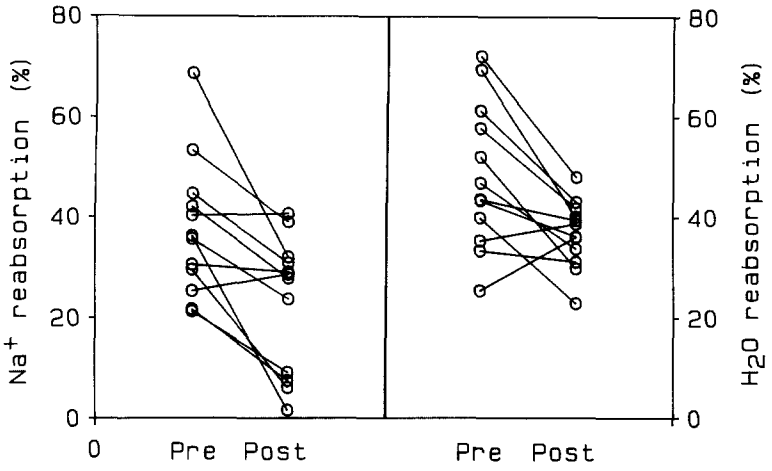

Fig. 3. Effect of ischemia on the fractional reabsorption of Na^+ and H_2O by proximal tubules. The fractional reabsorption of Na^+ and H_2O by proximal tubules prior to and 2 hr after 15 min of total clamping of the left renal pedicle were determined in vivo by micropuncture techniques as previously described [5]. The data are from paired studies of the same tubule with control and post ischemic injury indicated by *pre* and *post*, respectively. $n = 11$

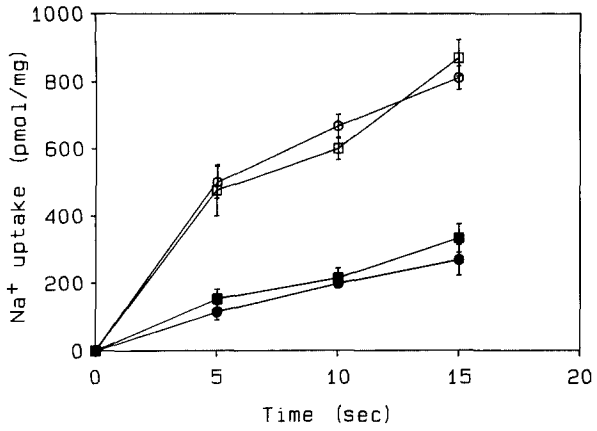

Fig. 4. Effect of ischemia on renal cortical apical membrane Na^+ uptake. Na^+ uptake by control (open, filled circles) and 15 min ischemic apical vesicles (open, filled squares) was quantitated in the presence (filled symbols) and absence (open symbols) of 10 mM harmaline using previously described millipore filtration techniques at 25°C [17]. Apical membranes were resuspended in buffer containing 200 mM mannitol, 20 mM HEPES with Tris used to adjust the final pH to 7.4. Apical vesicles (20 μl, 3–5 mg protein/ml) were added to 40 μl of transport buffer which contained 1 mM Na, 200 mM mannitol, 20 mM HEPES (pH 7.4 with Tris) and 1 μCi $^{22}Na^+$ per transport vial. The stop solution was identical to the transport buffer except there was no Na^+ present and it was ice cold. All determinations were done in duplicate, and these data represent the mean ± 1 SD of three different membrane preparations. $n = 3$

NaK-ATPase to the apical membrane, paired micropuncture studies of proximal convoluted tubules [5] were conducted in control situations and following 15 min of ischemia with 2 hr of reperfusion (Fig. 3). Ischemia resulted in a marked reduction in both Na^+ transport (37.4 *vs.* 23.0%, $P < 0.01$) and water transport (48.6 *vs.* 36.9%, $P < 0.01$). This 39% decrease in Na^+ and 24% decrease in H_2O reabsorption following minimal ischemic injury indicates that proximal convoluted tubules (S_1 and S_2) are extremely sensitive to ischemic injury. In addition, the time course for loss of surface membrane polarity and reduced Na^+ reabsorption were similar.

Numerous steps are involved in the transcellular movement of Na^+ across proximal tubular cells. Once filtered, Na^+ moves across the apical membrane by several different pathways including diffusion down its electrical and chemical gradients, Na^+-coupled cotransport and Na^+-H^+ exchange. After internalization Na^+ must be pumped across the basolateral membrane into the extracellular fluid against its electrochemical gradient by the primary active process, NaK-ATPase. Thus several potential mechanisms, in addition to the loss of surface membrane polarity, exist whereby ischemia could alter Na^+ transport. These include reductions in either apical membrane Na^+-permeability, ATP supply, apical membrane surface area or the lumen to cellular Na^+ gradient. In addition, an increase in single nephron glomerular filtration rates or a reduction in Na^+-coupled cotransport could also reduce Na^+ reabsorption. It was therefore necessary to evaluate each of these potential mechanisms.

Apical membrane Na^+ permeability was directly determined in control and ischemic membrane fractions. Na^+ uptake was quantitated using Millipore filtration techniques [17] in the presence and absence of harmaline (10 mM), a selective competitive inhibitor of renal apical Na^+ transport sites [2]. Neither total Na^+ uptake nor uptake in the presence of harmaline (binding) was affected by ischemia (Fig. 4). Thus, apical membrane Na^+ permeability was unaltered and could not explain reduced Na^+ reabsorption following ischemic injury. In addition, in the absence of organic solutes the Na^+/H^+ exchanger is primarily responsible for Na^+ movement into the apical vesicles [2]. The lack of a difference in Na^+ movement between control and ex-

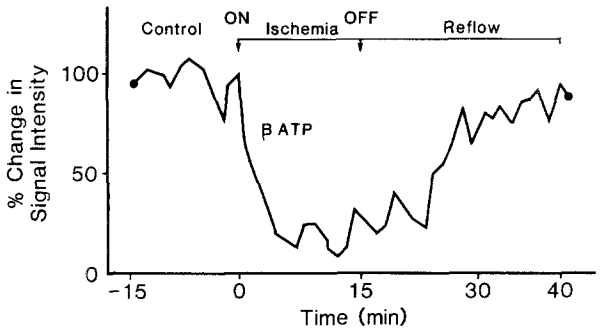

Fig. 5. Time-course of renal ATP changes during 15 min of ischemia and 50 min of reperfusion. Changes in ATP concentration during ischemia and early reperfusion were obtained by comparing the peak area of B phosphate of ATP in the ^{31}P NMR spectra before, during and after 15 min of renal ischemia [3]. These data represent the ATP time course obtained from an integration across the B resonance of the spectra obtained from the left kidney of one rat

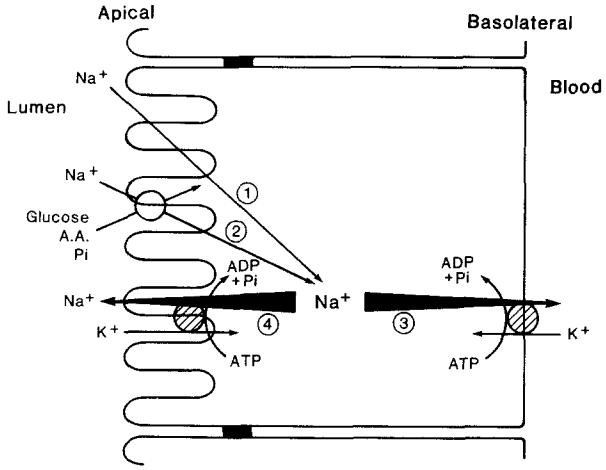

Fig. 6. Proposed mechanism for reduced Na^+ and H_2O reabsorption following ischemic injury. Na^+ enters the cell normally (*1–2*) but now, due to the abnormal localization of NaK-ATPase to apical membrane domain, Na^+ can be pumped either across the BLM into the blood (*3*) or back across the apical membrane into the urinary lumen (*4*)

perimental brush border membrane vesicles (BBMV), therefore, indicates the Na^+/H^+ exchanger is not affected by ischemia.

To determine if a reduction in Na^+-coupled cotransport was responsible for reduced proximal Na^+ reabsorption, the uptake of αMG was studied by micropuncture techniques as described for $^{22}Na^+$. αMG is a nonmetabolizable analogue of D-glucose, which is transported by the phlorizin inhibitable Na^+-coupled carrier in proximal tubules. Since the [17] BBMV Na^+-dependent uptake capacity (V_{max}) of D-glucose is reduced following ischemic injury, we felt it important to evaluate the in vivo proximal tubule Na^+-dependent glucose transport in this model. Following 15 min of ischemia and 2 hr of reperfusion, the reabsorption of αMG was unaltered (94.4 ± 1.8 *vs.* 93.8 ± 2.6%, $n = 5$), while the reabsorption of water was reduced 44.3% ($P < 0.01$). These data indicate reduced proximal tubule Na^+-coupled D-glucose transport is not responsible for the observed reduction in Na^+ transport. Since D-glucose uptake by BBMV is more sensitive to ischemic injury than Na^+-dependent alanine transport [17], these data suggest that proximal tubule Na^+-dependent cotransport does not explain the reduction in Na^+ reabsorption.

The kinetic changes in cellular ATP during ischemia and reperfusion were evaluated using phosphorus nuclear magnetic resonance (^{31}P NMR) spectroscopy [1, 3, 23, 27]. Renal cellular ATP declined rapidly (Fig. 5) to less than 20% of control levels during the first 5 min of ischemia and then to levels below quantitation for the remainder of the ischemic interval. Following reinitiation of blood flow, cellular ATP returned rapidly to near normal levels and was not statistically different from control values following 40 min of reperfusion (2.22 ± 0.20 *vs.* 2.01 ± 0.34 μmol/g wet wt, $n = 4$, $P > 0.05$). Thereafter cellular ATP remained stable and at 2 hr of reperfusion was 2.04 ± 0.36 μmol/g wet wt. In addition, intracellular (pH_i), which decreased during ischemia to 6.70 ± 0.12, also normalized during 40 min of reperfusion (7.35 ± 0.10 *vs.* 7.39 ± 0.14, $n = 4$, $P > 0.05$) and thereafter remained constant. These data indicate that neither alterations in cellular ATP supply nor pH could explain reduced Na^+ and H_2O transport following ischemic injury.

The reductions in Na^+ and H_2O transport were also not due to alterations in single nephron glomerular filtration rates (SNGFR) (46.2 ± 15.7 *vs.* 45.3 ± 13.3 nl/min). Finally, intracellular Na^+ and K^+ have been shown to be normal in a similar ischemic model [15], indicating the lumen-to-cellular Na^+ gradient was probably not responsible for the observed decreases in Na^+ reabsorption. Therefore, Na^+ and H_2O reabsorption by proximal convoluted tubules (Fig. 3) was markedly reduced, even though apical Na^+ permeability, Na^+/H^+ exchange, proximal tubule αMG uptake, cellular ATP supply, intracellular pH, cellular morphology, and SNGFR were all normal. Taken together, these results strongly suggest the abnormal redistribution of NaK-ATPase to the apical membrane is in part responsible for the reduced reabsorption of Na^+ and water following ischemic injury (Fig. 6). In our studies, however, we did not evaluate for a reduction in the BLM Na^+/HCO_3^- transporter. Decreased function of this carrier could result in reduced transcellular Na^+ movement, but its contribution to total

Na^+ movement is small and could not account for the large decrease in Na^+ reabsorption we observed.

The abnormal localization of NaK-ATPase to the apical membrane could decrease Na^+ transport via two mechanisms. First, apical NaK-ATPase could pump cytosolic Na^+ back into the urinary lumen. That the apical enzyme remains functional was indicated by several different lines of evidence. These include the cytochemical localization technique [16, 19] (which requires functioning enzyme units), ouabain inhibitability of the cytochemical process [16, 19], and enzymatically quantitatable NaK-ATPase in isolated apical membrane fractions. Our data, however, fail to provide direct evidence that the apically located NaK-ATPase can pump Na^+ into the lumen. In preliminary studies, we have quantitated efflux of $^{22}Na^+$ out of preloaded BBMV in the presence of intravesicular ATP, with and without external K^+ (5 mM) using the method of Hruska et al. [10]. Using this technique, $^{22}Na^+$ efflux (5 sec) increased 50% when K^+ was present in the external media, consistent with K^+ stimulation of the NaK-ATPase present in BBMV following ischemic injury. These studies, however, are difficult as ATP is rapidly consumed by phosphatases [10] and resealing of hypotonically ruptured BBMV occurs over an extended period (30–60 sec). In addition, inhibition of the process by ouabain was not possible, probably due to the limited and slow binding of ouabain to rat renal NaK-ATPase.

The pumping of Na^+ back into the lumen would not only reduce Na^+ reabsorption but would uncouple Na^+ transport and cellular energy utilization as ATP would be used without net transcellular Na^+ transport. Such a phenomenon, the uncoupling of Na^+ transport and oxygen utilization following ischemic injury, has recently been reported [9].

Second, the abnormal redistribution of NaK-ATPase to the apical membrane could result in reduced Na^+-transport by limiting the number of available pump sites on the basolateral membrane. We have observed no alteration in total cortical homogenate NaK-ATPase activity following 15 min of ischemia (15.7 ± 5.7 *vs.* 13.4 ± 3.8 μmol/mg protein/hr). Since no reduction in total cortical NaK-ATPase activity was seen, the presence of NaK-ATPase in apical membranes would imply a reduction in the number of basolateral membrane enzyme units.

The increase in apical NaK-ATPase could have resulted from the inability to isolate representative membranes following ischemic injury. This potential problem was evaluated thoroughly and excluded in our previous studies [16, 19]. Finally, our data are also consistent with the recently reported lateral mobility of surface membrane NaK-ATPase in Madin-Darby Canine kidney cells [13]. In the studies by Jesaitis and Yguerabide 50% of the BLM NaK-ATPase was capable of rapid lateral mobility. In our studies a large portion of BLM NaK-ATPase was capable of rapid movement into the apical membrane during ischemia.

These findings provide new insight into the understanding of abnormal Na^+ and water transport following ischemic injury. Reduced Na^+ and H_2O reabsorption are hallmarks of ischemic tubular injury and can result in clinical deterioration secondary to volume depletion. The present findings also help to clarify the paradox of reduced Na^+ and H_2O reabsorption in the presence of normal cellular morphology during the recovery of ischemic-cellular injury [14]. Clearly, for Na^+ reabsorption to normalize following ischemia-induced loss of surface membrane polarity, the apical membrane would have to undergo remodeling that resulted in NaK-ATPase removal. Recent data from our laboratory indicates this is indeed the case [25]. Whether the NaK-ATPase removed is redistributed back to the BLM or undergoes lysosomal degradation was not determined in our studies.

In summary, ischemia leads to a loss of epithelial polarity, which is manifested by large alterations in apical lipid composition and the abnormal redistribution of NaK-ATPase to renal cortical apical membranes. The loss of epithelial polarity is associated with reduced proximal tubule Na^+ and H_2O reabsorption. We propose that apical NaK-ATPase functions to pump Na^+ back into the lumen, thereby reducing proximal tubule Na^+ and H_2O reabsorption and uncoupling Na^+ reabsorption and cellular energy utilization.

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