Ischemia/reperfusion Injury in the Kidney: Alterations in Proximal Tubular Transport Function

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Renal ischemia produces significant changes in structure and function of the proximal tubule, a major site where organic compounds in the glomerular filtrate are mostly reabsorbed and where organic ions are actively secreted. It would be thus expected that an impairment in proximal tubular reabsorption and secretion of various substances may occur in the ischemic kidneys. In fact, *in vivo* and *in vitro* experiments have demonstrated that a transient ischemia causes a significant impairment in glucose reabsorption (Johnston *et al.*, 1984; Spiegel *et al.*, 1989) and the secretion of *p*-aminohippurate (PAH) (Phillips and Hamilton, 1948; Eisenbach *et al.*, 1974) in the proximal tubule. However, the underlying mechanisms of these changes have not been fully defined.

Changes in proximal tubular transport function were characterized in postischemic kidneys. Unilateral ischemia was induced by complete occlusion of the left renal artery with a ligature for appropriate duration. The right nonischemic kidney served as a paired control. In some studies, bilateral renal ischemia was induced. Once the period of renal ischemia was completed, the clamping was removed and blood reflow to the kidney was verified visually. Urine and blood samples were obtained following the induction of renal ischemia. Alterations in membrane transport function was measured in membrane vesicles and renal cortical slices.

Clearance data

Ischemia for 30 min caused a marked polyuria which was accompanied by an increase in excretion of Na^+, K^+ and Cl^-. A marked reduction in reabsorption of glucose and phosphate was also observed. When ischemia was extended to 60 min, these effects were more pronounced. Glomerular filtration rate (GFR) and PAH clearance (C_{PAH}) were not reduced by 30 min of ischemia, but significantly attenuated by 60 min of ischemia (Kim *et al.*, 1995a). Renal blood flow 10 min or 24 hr after 60-min ischemia was not significantly different from the preischemic value. These results indicate that ischemia induces defects in tubular function before an impairment in GFR, and that a marked defect in proximal tubular reabsorptive function is the early event in nonoliguric acute renal failure in rabbits. The present study also suggest that reduction of GFR in postischemic kidneys is not due to reduced renal blood flow.

Alterations in Proximal Secretion of Organic Ions

Secretion of organic ion PAH was significantly reduced after 60 min of ischemia. To characterize mechanisms underlying reduced secretion of organic ions, the uptake of PAH and organic cation tetraethylammonium (TEA) was measured in

cortical slices and plasma membrane vesicles isolated from the proximal tubule of ischemic rabbit kidney (Kim *et al.*, 1996). The uptake of PAH or TEA in cortical slices increased after 30 or 60 min of ischemia. When the kidneys were reperfused for 30 min, the uptake of organic ions returned to control level. When ischemic (60 min) slices were preincubated for 120 min in a oxygenated medium, the stimulatory effect of ischemia on organic ion uptake was not observed. PAH uptake by basolateral membrane vesicles (BLMV) and brush border membrane vesicles (BBMV) was not altered following 60 min of ischemia. However, TEA uptake by BBMV but not BLMV was significantly reduced. Dissipation rate of H^+ gradient across BBMV was similar in control and ischemic kidneys. These results indicate that the organic anion secretory system is not inhibited by 60 min of ischemia and this system is more resistant to ischemia than the organic cation secretory system in the rabbit renal proximal tubule.

Alterations in Proximal Reabsorption of Organic Compounds

Ischemia for 30 or 60 min induced glucosuria and phosphaturia. To examine whether such changes were due to an direct defect of membrane transport systems, the uptakes of various substances were measured in membrane vesicles. The Na^+-dependent uptakes of glucose, succinate, amino acid and phosphate by BBMV was not altered by 30 or 60 min of ischemia, while the H^+/TEA (Kim *et al.*, 1993) was significantly inhibited by 30 min of ischemia. When the duration of ischemia was extended to 120 min, the uptakes of glucose, succinate and phosphate by BBMV were also significantly attenuated, but the L-glutamate uptake was not altered. The uptakes of organic substances by basolateral membrane vesicles were not impaired even with 120 min of ischemia, suggesting that transport systems for organic compounds in the brush- border membrane are more sensitive to ischemia than those in the basolateral membrane. This is further supported by the data on the succinate uptake in brush-border and basolateral membranes, which is transported with a similar characteristics in both membranes (Kim *et al.*, 1992). The succinate uptake by BBMV was significantly impaired by 120-min ischemia, but the uptake by BLMV remained to be unaltered.

The first step of renal proximal tubular reabsorption of substances such as glucose, dicarboxylate, amino acid and phosphate involves the Na^+-dependent transport across the brush-border membrane. The Na^+ gradient across the brush-border membrane is generated by the active Na^+ pump in the basolateral membrane. Thus, ischemia-induced glucosuria or phosphaturia could be a consequence of a decrease in the Na^+-pump activity in the basolateral membrane. However, the Na^+-K^+-ATPase activity measured chemically remained unchanged in kidneys subjected to 30 min of ischemia, and functional pump activity (estimated by ouabain-sensitive oxygen consumption) was also not reduced by 30 or 60 min of ischemia. Thus, it seems unlikely that an alteration of Na^+-pump activity is totally responsible for the glucosuria and phosphaturia caused by a transient ischemia.

Indirect lines of evidence have suggested that reperfusion may actually result in an enhancement of the cellular injury that occurred during the ischemic period (Paller *et al.* 1984). Thus, glucosuria or phosphaturia observed from ischemic kidneys could be due to the reperfusion injury rather than ischemia *itself.* To examine the possibility, the kidneys were reperfused for 60 min following 60 min of ischemia. However, the uptakes of glucose and succinate by BBMV were not altered. The H^+/TEA antiport which was

reduced by 60 min of ischemia returned to the value of the control kidneys. These results suggest that the function of brush-border transporters is not further impaired for 60 min of reperfusion. No evidence for progressive tubular damage during reperfusion period might be due to minor changes in hemodynamics and nonoliguria in our experimental model. This hypothesis is based on the data that the drugs increasing renal blood flow and inducing diuresis during the reperfusion phase can attenuate the tubular injury induced by ischemia (McDougal, 1988). However, we recently observed a marked reduction in GFR and increase in Na excretion 24 hr after 60 min of ischemia, a result showing deterioration of renal function during the later phase of reperfusion. Such changes may largely attributed to obstruction of tubular lumen by the formation of casts, since renal blood flow was not significantly different from the preischemic value 24 hr after 60 min of ischemia.

Morphological changes in the proximal tubules of the kidneys subjected to 60 min of ischemia. A loss of brush border microvilli and plasma membrane was observed after 5 or 60 min of reflow in the proximal convoluted tubule. After 3 hr of reflow, focal necrosis appeared although the microvilli were partially regenerated (Kim *et al.*, 1995b).

These results indicate that renal ischemia induces selective impairments of transport systems in the rabbit renal proximal tubule, and that the glucosuria and phosphaturia induced by a transient ischemia are resulted, at least in part, from a diminution of brush border surface area rather than a direct defect in function of membrane transport systems.

Concluding Remarks

Although the present study shows that impairment in proximal tubular reabsorption is, at least in part, attributed to a diminution of brush border surface area, other mechanisms could be involved. These include an increase in epithelial permeability and changes in transport systems by intracellular signalling. Molitoris *et al.* (1989) demonstrated that proximal tubule tight junction opens during ischemia. If the tight junction is disrupted during reperfusion, reduction of tubular reabsorption could be expected.

Reabsorption of various substances in the proximal tubules is regulated by second messengers involved in intracellular signal transduction. Interestingly, recent studies provide evidences that oxygen free radicals play a role as bioregulators in signal transduction and in the generation of second messengers in vascular endothelium (Natarajan, 1995). Since oxygen free radicals have been suggested to be generated by reperfusion following ischemia, tubular reabsorptive function may be altered through the oxidant-induced signal transduction. Phospholipase A_2, which has been reported to be involved in the regulation of renal Na^+-pump and Na^+/H^+ antiporter (Satoh *et al.*, 1992), is activated by ischemia (Weinberg, 1991). These results suggest that intracellular signalling may be responsible for reduction in proximal tubular reabsorption induced by ischemic/reperfusion. Johnston *et al.* (1984) also reported that transport defects for glucose and Na^+ during reperfusion following brief ischemia persist even when morphological changes are restored to normal. This functional and morphological disparity might result from an intracellular signalling activated by reperfusion. However, little information is available regarding a role of signal transduction pathway in reperfusion injury.

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