Expression of the polymeric immunoglobulin receptor and excretion of secretory IgA in the postischemic kidney

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Rice, James C., Jeff S. Spence, Judit Megyesi, Randall M. Goldblum, and Robert L. Safirstein. Expression of the polymeric immunoglobulin receptor and excretion of secretory IgA in the postischemic kidney. Am. J. Physiol. 276 (Renal Physiol. 45): F666–F673, 1999.—The humoral mucosal immune response of the kidney involves the transport of secretory IgA (S-IgA) through renal epithelial cells by the polymeric immunoglobulin receptor (pIgR). The pIgR is cleaved and released as free secretory component (FSC) or attached to IgA (S-IgA). We examined the effects of an ischemic model of acute renal failure (ARF) on the expression of pIgR and the secretion of FSC and S-IgA in the urine. Kidney pIgR mRNA levels decreased in ischemic animals by 55% at 4 h and by 85% at 72 h compared with controls. pIgR protein expression in the medullary thick ascending limb (TAL) decreased within 24 h and was nearly undetectable by 72 h. Urinary S-IgA and FSC concentrations decreased by 60% between days 3 and 6. pIgR mRNA and pIgR protein in the kidney returned to near control levels on day 3. Urinary S-IgA concentrations returned to near control levels by day 7. We demonstrate that ischemic ARF decreases renal mucosal S-IgA transport in vivo and may contribute to the increased incidence of urinary tract infections.

secretory component; mucosal immunity

The secretions that bathe mucosal epithelial surfaces contain an array of host defense factors, including polymeric immunoglobulins (pIg), of which IgA is the major class. These secretory immunoglobulins (S-IgA and S-IgM) are transported from the basolateral to the apical surface of mucosal epithelial cells by the polymeric immunoglobulin receptor (pIgR). As in humans, the rat pIgR is a 118- to 120-kDa glycoprotein composed of an amino-terminal immunoglobulin-binding portion, termed secretory component (SC), a membrane-spanning element, and a carboxyl-terminal cytoplasmic domain (20, 34). After transcytosis of the pIgR to the apical region, both pIgR bound to immunoglobulin and unbound pIgR accumulate in endosomal vesicles called “apical recycling endosomes” (3). The pIgR-containing endosomal vesicles fuse with the apical membrane, where most of the ectoplasmic segment of pIgR is proteolytically cleaved and either secreted as free SC (FSC) or bound to polymeric immunoglobulins as secretory Ig (S-Ig).

The pIgR is expressed in renal tubule epithelial cells of humans (1) and rodents (27), both with and without IgA (1, 27). During urinary tract infections (UTI), secretion of SC is increased. This includes SC that is attached to IgA (12, 33), where it may function to prevent degradation of secretory IgA (16), and FSC (12), which may inhibit adhesion of some bacteria to cell membranes (9). Thus the concentration of S-IgA and FSC in the urine may influence susceptibility to UTI. Consistent with this view, bacterial growth is more rapid in urine obtained from animals undergoing water diuresis (13), and bacterial growth rates are higher in the kidneys of diuretic animals, compared with antidiuretic controls, in some models of pyelonephritis (7).

The effects of ischemia on the mucosal immune response in the kidney are of interest because UTI are the most common infectious complications during acute renal failure (ARF) (40) and in the early (postischemic) renal transplant period (29). We have shown recently that renal pIgR levels are influenced by hydration status, urine flow rate, and arginine vasopressin (27); factors that are altered in ischemic renal failure (2, 25).

In the present study, we investigated the effect of renal ischemia and reperfusion on pIgR expression in the rat kidney and both SC and secretory IgA (S-IgA) secretion in the urine. We speculated that ischemic ARF might decrease pIgR expression and impair the mucosal immune response. We found that renal ischemia and reperfusion resulted in a rapid and progressive decline in pIgR mRNA expression in the kidney. Immunohistology demonstrated that staining for pIgR in the thick ascending limb (TAL) was decreased in ischemic animals (compared with sham-operated controls) on day 1 and fell to nearly undetectable levels on day 3.

The urine concentration of both FSC and S-IgA fell significantly by day 3, due primarily to the increased urine flow. The rapid decrease in pIgR mRNA, the decline in intracellular pIgR protein levels, and the fall in urine SC and S-IgA concentrations may impair the mucosal immune response of the urinary tract and predispose the postischemic kidney to infection.

METHODS

Animals. Male Sprague-Dawley rats (Harlan, Houston, TX) weighing between 250–350 g were used for all experiments. We placed rats in individual metabolic cages for an equilibration period of 3–6 days before starting the study. Animals received food and water ad libitum until 3 days before surgery, after which they were pair fed (FormulaLab diet 5008; PMI Feeds, St. Louis, MO). Animals were randomized to two surgical study groups: 1) bilateral renal artery ischemia, in which rats underwent decapsulation of both kidneys and clamping of the renal vessels for 50 min, and 2) sham-ischemia (control) animals, which underwent decapsulation of...
both kidneys and manipulation of renal vessels without clamping. Rats were anesthetized with pentobarbital sodium (−65 mg/kg injected intraperitoneally). Renal ischemia was induced by simultaneous clamping of the renal hilum of both kidneys using 50 mm “bulldog-type” serrated vascular clamps (Kent Scientific, Litchfield, CT). We visually documented reperfusion of both kidneys after removing the vascular clamps at the end of the ischemic period and before closing the abdomen with 4-0 silk suture. All animals were placed in metabolic cages, after recovery from the anestheisia, until the completion of the study periods. Before harvest, we perfused the kidneys in situ by clamping the aorta and inferior vena cava above the renal arteries, venting the left renal vein, and injecting 20 ml 0.9% saline at 4°C via the infrarenal aorta using a 21-gauge “butterfly” needle (Sureflo winged infusion set, Terumo, Elkton, MD). mRNA was isolated from one kidney; immunohistology and plgR quantification was performed on the other kidney.

Antibodies. The monospecific polyclonal rabbit antibody against the SC portion of the rat plgR utilized in this study was described previously (27). Aliquots of the IgG fraction of this anti-rat SC antibody were conjugated with horseradish peroxidase (Sigma) (21) for use in ELISA. A monoclonal mouse anti-rat IgA antibody used for S-IgA quantification was obtained from Zymed Laboratories (San Francisco, CA). Horseradish peroxidase-conjugated sheep anti-rat IgA (α-chain) was obtained from The Binding Site (San Diego, CA). S-IgA, used as a standard in quantification, was isolated from rat bile on an HPLC Superdex 200HR 10/30 column (SMART Systems Pharmacia Biotech, Piscataway, NJ). The purity of the S-IgA was confirmed by electrophoresis of the individual fractions on 8% SDS-PAGE. These gels were subsequently either stained with Coomassie blue or used for Western blots, utilizing sheep anti-rat IgA (The Binding Site) and horseradish peroxidase-conjugated antibody (Vector Labs, Burlingame, CA) and avidin-biotin peroxidase complex with 0.01% dianobenzenidine (VectaStain Elite ABC; Vector Labs) were utilized to detect the primary antibody. Nonspecific staining was blocked with 5% normal goat serum (Sigma) in 0.1% Triton X-100 (Sigma), and endogenous peroxidase was inactivated by 1% H2O2 in PBS:methanol (1:1). Additional tissue sections were stained with the horseradish peroxidase-conjugated sheep anti-rat IgA (The Binding Site) to assess the distribution and relative amounts of IgA in the rat kidney. In addition, we incubated whole kidney sections with preimmune rabbit sera to assure that the staining was specific.

Statistical analysis. Differences between and within the ischemic and control groups were assessed by two-way ANOVA (32). When a significant difference was present between groups, additional analyses were performed using either the Tukey’s method for all comparisons or the unpaired t-test assuming equal variances for planned comparisons (Microsoft Excel, Redmond, WA). P < 0.05 was considered statistically significant.

RESULTS

plgR mRNA expression is decreased in the kidney postischemia. ARF induced by 50 min of renal artery clamping, followed by reperfusion, caused the plgR mRNA level to decrease rapidly to 53% of control (sham-ischemia) by 4 h postischemia (P < 0.02; Fig. 1). The plgR mRNA level fell to a nadir of 23% of control levels at 72 h (P < 0.001), then returned to near control levels at 7 days postischemia (Fig. 1).

plgR staining of the TAL is decreased postischemia. In sham-ischemia (control) animals, plgR protein was expressed in the epithelium of the TAL and appeared darker in the apical region (Fig. 2A). A smaller amount of plgR protein was also observed in the distal convoluted tubule and on the basolateral surface of proximal tubule cells (Fig. 2B). We have reported similar results for untreated animals (27). In the ischemic animals, the staining for plgR was decreased throughout the medullary TAL at 24 h postreperfusion (data not shown) and declined to nearly undetectable levels by 72 h (Fig. 2C). The morphology of the cells lining the medullary TAL, where plgR protein is predominantly located (27), was essentially unchanged after ischemia, suggesting that...
cells, proximal tubule cells, or glomeruli at any time point in control or ischemic animals, suggesting that the majority of IgA was delivered to the renal tubules via the renal blood flow.

Urinary flow rates increased and urine FSC concentration fell in postischemic rats. The daily urine volume in ischemic animals was increased by fourfold on day 2 (from 5.3 ± 0.7 to 23.2 ± 2.3 ml/24 h) compared with controls, and remained at this level through day 6 (Fig. 4). Urine FSC concentration was significantly less in ischemic animals than in controls from day 1 through day 6 (Fig. 4; P < 0.002). Urine FSC concentration fell to <40% of preischemic levels from day 2 through day 6 in the ischemic group (P < 0.001) and remained below preischemic values at day 7 (P < 0.01; Fig. 4). Within the control group, urine FSC concentration was decreased from pre-sham ischemia only on day 1 (the first 24 h postsurgery; P < 0.001) and day 5 (P < 0.05). Although the concentration of SC was significantly different between the ischemic and sham-ischemic (control) groups on day 1 through day 6, the total (daily) urinary SC (µg SC/24 h) did not differ. The ability to maintain the daily SC urine excretion in the ischemic group appeared to result in the depletion of stored intracellular pIgR/SC, as noted by immunohistology (Fig. 2).

Urinary S-IgA concentration was decreased postischemia. The S-IgA concentration in the urine of ischemic animals was significantly different from control (sham-ischemia) animals on day 4 through day 6 (Fig. 5; P < 0.05). In the ischemic group, urinary S-IgA concentration fell to 40% of control levels at 72 h and remained at that reduced level through day 6 after ischemia and reperfusion (Fig. 5). Urine S-IgA concentration in the ischemic animals returned to 63% of urine S-IgA concentration of controls on day 7. Daily S-IgA excretion (total µg S-IgA/24 h) in the ischemic animals did not differ significantly from controls, except when S-IgA excretion increased transiently during the onset of the diuresis, between 24 h and 48 h (P < 0.01, day 2, data not shown).

Increased urine S-IgA-to-SC ratio after ischemia-reperfusion. The ratio of S-IgA concentration to FSC concentration (µg S-IgA/µg FSC), measured on the same sample of urine, increased early after ischemia-reperfusion (Fig. 5). The S-IgA-to-FSC ratio in the urine of ischemic animals increased threefold from preischemic values on day 2 (P < 0.05; ischemic vs. control). These results suggest that ischemia affected urinary FSC excretion to a greater extent than urinary S-IgA excretion. There was no significant change in the S-IgA/FSC ratio in the urine of control animals.

DISCUSSION

To our knowledge, this is the first study to evaluate the effects of ischemic stress on pIgR expression in the kidney and FSC and S-IgA excretion in the urine. We demonstrated that renal ischemia, followed by reperfusion, decreased pIgR mRNA levels in the kidney, pIgR staining in the TAL, and FSC and S-IgA concentrations in the urine.
Fig. 2. Ischemia decreases plgR staining in kidney. Representative tissue sections from inner stripe of outer medulla (A, C, and E) and cortex (B, D, and F) of kidneys isolated from ischemic and controls animals. plgR staining at apical surface of thick ascending limb (TAL; *) is decreased at 72 h after ischemia-reperfusion (C), compared with controls (A). plgR staining of proximal (arrows) and distal tubules (arrowheads) is also decreased at 72 h after ischemia-reperfusion (D), compared with controls (B). However, decreases in plgR staining of the cortical segments are less than in TAL at same time postischemia (C). By day 7, staining for plgR returned to control levels in both TAL (E) and proximal and distal tubules of cortex (F). There is no staining of glomeruli (g), vessels, or inner medulla for plgR at any time point. Magnification, ×160.
plgR mRNA declines rapidly after ischemia. The rapid (4 h) and sustained (72 h) decrease in plgR mRNA levels postischemia (Fig. 1) cannot be explained by the simple loss of cells that produce plgR. The predominant sites of plgR expression in the kidney are the TAL and distal convoluted tubule, where, unlike the proximal tubule, the morphological changes induced by ischemia are moderate and reversible (8). The decline in plgR mRNA levels after ischemia follows a pattern similar to that observed for prepro-epidermal growth factor (prepro-EGF) mRNA and Tamm-Horsfall mRNA in the TAL, as reported by our group (30). The decrease in plgR mRNA levels after ischemia is not due to a nonspecific decline in TAL mRNA, as the expression of other genes that localize to the TAL and distal tubule are upregulated after ischemia (19). The changes in the plgR/GAPDH ratio in the kidney after ischemia-reperfusion are not due to changes in GAPDH mRNA levels, as we have previously noted that GAPDH mRNA levels in the kidney are not affected by ischemia (31).

We have previously suggested that plgR mRNA levels in the kidney in vivo are regulated, in part, by cAMP (27). Specifically, we found that arginine vasopressin administration increased plgR mRNA levels fourfold by 24 h, compared with controls. The vasopressin-V2 receptor is localized to the collecting duct and TAL in the rat (23) and is coupled, through adenylyl cyclase, to the production of cAMP (11, 17). Despite higher vasopressin levels in rats during the polyuric phase of acute tubular necrosis (25), the outer medulla of the postischemic kidney has a decreased adenylyl cyclase response to vasopressin (2). The administration of dibutyryl cAMP ameliorates the increase in serum creatinine and the fall in urine volume after acute ischemic renal failure in rats in vivo (15). It is possible, therefore, that decreased cAMP levels resulting from the decreased adenylyl cyclase response to vasopressin in the postischemic kidney may contribute to the decrease in plgR mRNA expression. Local cytokine (10, 36) and chemokine expression (30) are also altered in the postischemic kidney. For example, gamma interferon (IFN)-γ, which increases plgR mRNA in colon adenocarcinoma cells in vitro (24), is increased in the kidney by day 3 postischemia (10, 36). Hence, the extent to which the plgR mRNA level falls in the postischemic kidney may depend on the effects of multiple factors, including decreased cAMP levels and changes in local cytokine expression.

Fig. 3. IgA distributed in distal tubule and TAL. Representative tissue section of renal cortex from control animals reveals low levels of IgA staining in apical aspect of distal convoluted tubules (arrowheads). High-power view of outer stripe of outer medulla (inset) demonstrates localization of IgA in subapical surface of TAL (*). There was no staining of IgA-containing lymphocytes in interstitium or tubular basement membrane. Magnification, ×140; inset, ×350.

Fig. 4. A: ischemia increases urine flow rate. There was a significant increase in urine volume per 24 h in ischemic (○) vs. control (■) animals; n = 9–11 animals per group; *P < 0.001, ischemic vs. control. B: urine free secretory component (FSC) concentration is decreased postischemia. Urine FSC decreased from day 1 through day 6 in ischemic group (○) vs. controls (■); n = 9–11 animals per group; *P < 0.002, ischemic vs. control.
plgR protein levels decrease in the kidney after ischemia. The plgR protein, either free or bound to IgA, is stored in subapical vesicles before its transport across the apical membrane and excretion into the lumen of mucosal tissues (3, 35). We have previously shown that intact plgR is preferentially expressed near the luminal surface of the TAL in normal rat kidney (27), presumably in “apical-recycling endosomes” (3). In this report, we demonstrate that renal ischemia, followed by reperfusion, caused a significant decrease in immunohistochemical staining for plgR in the TAL by 24 h, which continued to decrease up to 72 h after reperfusion (Fig. 2).

The loss of staining that occurred primarily in the TAL of ischemic animals suggests depletion, or “washout”, of plgR-stored protein. Although the mechanisms that result in loss of stored plgR are unclear, we have demonstrated a similar decline in apical staining in normal animals during the diuresis induced by water loading (27). The increase in urinary FSC excretion during the polyuric phase may reflect the ischæmia-induced decline in ATP (38) and cAMP levels (37). cAMP has been suggested, by some authors, to inhibit FSC secretion in plgR-transfected Madin-Darby canine kidney (MDCK) cells in vitro (5). Hence, the decreased cAMP levels in the kidney during ischemia may facilitate the depletion of stored plgR. We are unable to determine, at the present time, whether the decrease in plgR staining in the TAL, specifically the loss of darker staining on the apical surface of these cells, is due to loss of plgR stored in apical vesicles.

We have estimated that, in normal animals, >60% of the total intracellular plgR in the kidney is secreted into the urine per day (unpublished observations). The depletion of intracellular plgR in ischemic animals noted by 72 h (Fig. 2), therefore, is likely due to the combined effects of continued SC excretion and a decrease in plgR mRNA levels (Fig. 1). Although we did not directly measure the production of plgR/SC protein after ischemia, the unchanged daily SC excretion, coupled with the immunological evidence of plgR depletion and the decreased plgR mRNA levels, suggests that plgR production is decreased by ischemia. These effects of renal ischemia would result in a decrease in the amount of plgR available for IgA transport into the tubular lumen of the kidney.

Urine SC concentration decreases early postischemia. Urine FSC concentrations are constant throughout a wide range of urine flows in untreated animals (27). In contrast, we found that urine FSC concentration fell significantly with increased urine flow in ischemic animals (Fig. 4). Hence, SC excretion did not increase in proportion to the increase in urine flow after ischemia. The lack of a proportional increase in urine SC excretion during the diuresis after ischemia is different than the proportional increase in urine SC excretion that occurs during the diuresis induced by water loading in normal animals (27).

Although the SC concentration in the urine of ischemic animals was significantly below control levels on day 1 through day 6, there was no difference in total SC excretion (µg/24 h) between the ischemic and control groups. The similar urine SC excretion between groups, during a time of decreased plgR mRNA in the ischemic animals, may account for the loss, or washout, of stored plgR/SC in the kidneys after ischemia-reperfusion (Fig. 2). The decrease in SC concentration in the urine may be biologically significant, as recent evidence suggests that SC can inhibit bacterial adhesion to cell membranes (9). Hence, the decline in SC concentration after ischemia may, in part, account for the increased susceptibility of the postischemic kidney to infection.

Urine S-IgA concentration is decreased postischemia. Total S-IgA excretion was similar between groups, except for an increase in S-IgA excretion that occurred in the ischemic group during the onset of diuresis (between day 1 and day 2). This may have contributed to the depletion of stored S-IgA in the kidney, as evidenced by immunohistochemistry on day 3 (data not shown). The more striking and perhaps biologically relevant result was the significant decline in S-IgA concentration in the urine on day 4 through day 6. The concentration of S-IgA decreased in the urine by 35–
70% between day 3 and day 7 in the ischemic group (Fig. 5), primarily due to the increase in urine flow rate (Fig. 4). Previous reports emphasize that S-IgA is important in the mucosal defense of the urinary tract (18, 28) and may be the primary factor in the urine that inhibits the binding of bacteria to urinary tract epithelial cells (33). The lowering of S-IgA concentration in the urine of ischemic animals from day 4 through day 6 correlates with a time period that UTI frequently complicates ARF (40). The significance of urine SC and S-IgA concentrations in preventing UTI is suggested by the observation that bacterial growth is more rapid in urine obtained from animals undergoing water diuresis (13). In addition, bacterial growth rates are higher in the kidneys of diuretic animals, compared with antidiuretic controls in some models of pyelonephritis (7).

In summary, we demonstrated that ischemia decreases both S-IgA and FSC concentrations in the urine. These findings may help to explain the frequent association of UTI with ARF (40).

REFERENCES


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