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Renal work, glutathione and susceptibility to free radical-mediated postischemic injury

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Renal work, glutathione and susceptibility to free radical-mediated postischemic injury. Studies were performed to determine whether renal glutathione (GSH) is an important free-radical scavenger following ischemia and reperfusion, whether alterations in renal transport work affect renal GSH levels, and whether a decrease in renal work decreases susceptibility to postischemic renal injury via the first two effects. Following administration of either intravenous GSH to increase renal GSH or intraperitoneal diethylmaleate to decrease renal GSH, Sprague-Dawley rats underwent 60 minutes of renal ischemia. In animals with high renal GSH following GSH infusion, GFR 24 hours after ischemia was 0.43 ± 0.08 ml/min compared to 0.15 ± 0.02 ml/min in saline-infused control animals ($P < 0.01$). When renal GSH was decreased by the administration of diethylmaleate postischemic renal dysfunction was accentuated. Twenty-four hours after ischemia GFR was 0.05 ± 0.02 ml/min in diethylmaleate-treated animals and 0.28 ± 0.06 ml/min in control animals ($P < 0.005$). To test whether a decrease in renal transport work alters renal GSH the filtered load of sodium was reduced by producing unilateral renal artery stenosis. Alternatively, renal work was lessened when sodium reabsorption was interfered with by the infusion of a combination of natriuretic agents. Renal artery stenosis produced a 37% decrease in GFR. Renal GSH was 0.435 ± 0.089 nmol/mg protein in intact kidneys and 0.804 ± 0.239 nmol/mg protein in stenotic kidneys ($P < 0.05$). The infusion of natriuretic agents produced no change in GFR or renal plasma flow but resulted in a striking elevation in renal GSH. Kidneys from rats infused with saline for two hours had a renal GSH level of 0.833 ± 0.084 nmol/mg protein, whereas kidneys from rats infused with the natriuretic agents for two hours had a GSH level of 1.669 ± 0.418 nmol/mg protein ($P < 0.01$). To determine whether a decrease in renal work alters susceptibility to ischemic renal injury, rats with 24 hours of unilateral renal artery stenosis were subjected to 60 minutes of bilateral renal artery occlusion. Although kidneys that had been stenotic had a lower GFR in the basal state, 24 hours after ischemia GFR was higher in previously stenotic kidneys (0.31 ± 0.06 ml/min vs. 0.18 ± 0.05 ml/min in previously intact kidneys, $P < 0.025$). Therefore, GFR fell by 84% in previously intact kidneys but only 55% in previously stenotic kidneys. Kidneys which had previously been stenotic also underwent less lipid peroxidation after ischemia and reperfusion as evidenced by significantly lower renal malondialdehyde content after ischemia plus 15 minutes of reperfusion. In addition, renal histologic examination revealed significantly less injury in previously stenotic kidneys compared with previously intact kidneys from the same animal. These studies are consistent with previous findings of protection against free radical-mediated injury in hypothyroid animals and suggest that a decrease in the base line level of renal transport work results in higher renal GSH levels. Since renal GSH is an important free radical scavenger during postischemic reperfusion, alterations in renal transport work affect susceptibility to free radical-mediated postischemic injury through changes in renal GSH.

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An imbalance between energy supply and energy requirements contributes to cellular dysfunction in acute renal failure. The inability of the kidney to synthesize adequate amounts of high energy phosphates after an ischemic or toxic insult prevents recovery from and may even potentiate the initial insult [1, 2]. Postischemic levels of adenine nucleotides correlate with functional and histologic impairment [1, 2] and administration of exogenous adenine nucleotides improves function [2–5].

Recently, Brezis, Rosen, Silva, Epstein and colleagues have reported studies in the isolated, perfused rat kidney suggesting a link between cellular transport work and metabolic substrate supply and the susceptibility to renal injury, particularly in the medullary thick ascending limb of Henle's loop [6–9]. In that nephron segment, because of limited blood flow and oxygen supply, oxygen demand can exceed supply and cause an hypoxic-type of injury [6]. When furosemide or ouabain was administered to diminish transport activity in this segment, injury was reduced [7]. When substrates were added, injury was greater [8]. A similar phenomenon may occur in the S_3 segment of the proximal tubule [9].

We became interested in another possible link between renal transport work and the susceptibility to acute renal injury. Does an increase in renal transport work predispose the kidney to free radical-mediated injury after an ischemic insult? Oxygen free radicals are now recognized to contribute to kidney damage after ischemia and reperfusion [10, 11]. Oxygen radical-mediated damage can be limited if there are adequate supplies of free radical scavengers of either exogenous or endogenous origin [11–16]. The supply of endogenous free-radical scavengers might, however, depend upon the basal production of oxygen free radicals.

Free radicals are produced during normal mitochondrial metabolism. Although the bulk of oxygen is completely reduced to water in the mitochondria by accepting four electrons per O_2 molecule, a small fraction (between 1 and 10%) is reduced by only one or two electrons to yield superoxide radical (O_2^-) or hydrogen peroxide (H_2O_2) [17–19]. These reactive oxygen species are deleterious to the DNA, proteins and lipids with which they readily react. Mitochondrial or cytoplasmic superoxide dismutase, GSH, glutathione peroxidase, catalase and other antioxidants all play a role in the "detoxification" of these oxygen radicals. For instance, reduced glutathione (GSH), a major intracellular scavenger, is oxidized to glutathione disulfide (GSSG) in the process of inactivating free radicals. GSSG is then regenerated to GSH by glutathione reductase. The ratio of

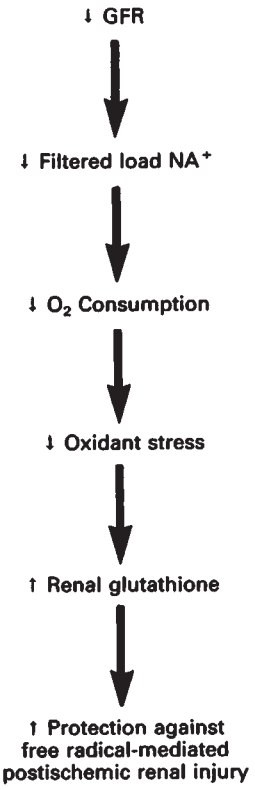

Fig. 1. *Possible relationship among filtered load of sodium, renal O_2 consumption, oxidant stress, renal glutathione (GSH) and susceptibility to postischemic, free radical-mediated injury.*

GSSG to GSH is thus believed to be an excellent marker for intracellular oxidant stress [20]. If the flux of electrons through the electron transport chain is increased, then the production of oxygen free radicals also increases [20]. However, since under normal conditions adequate supplies of free radical scavengers exist, little damage ensues.

Kidney work is dependent upon an adequate supply of ATP and there is a feedback system which regulates ATP production to match ATP consumption. ATP production in turn regulates the flux of O_2 through the mitochondria [21]. Therefore, an increase in kidney transport work increases O_2 consumption [21], but would also, as a consequence, increase the production of O_2- and H_2O_2 by mitochondria. To protect the cell against oxidant injury GSH would be consumed, as discussed above. Depending on the kidney's ability to synthesize GSH and maintain it in its reduced form, an increase in kidney transport work could ultimately lead to a decrease in cellular GSH stores. Such a sequence of events would not be harmful per se but would leave the kidney more susceptible to severe oxidant stresses, such as occurs after renal ischemia and reperfusion. We sought to test this hypothesis (Fig. 1) by determining whether renal GSH is an important free radical scavenger during ischemia and reperfusion, whether alterations in renal transport work affect renal GSH levels, and whether a decrease in renal work decreases susceptibility to postischemic renal injury via the first two effects.

Methods

Model of ischemic acute renal failure

Male Sprague–Dawley rats (Harlan, Madison, Wisconsin, USA) weighing 225 to 275 g were used. They were allowed free access to food and water until the time of study. Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.), and a femoral vein catheter (PE-50) inserted for administration of drugs. Bilateral flank incisions were made and the right kidney removed. The left kidney was exposed, the perirenal fat removed, and the left renal artery exposed. A non-traumatic vascular clamp was then placed across the renal artery for 60 minutes. After removal of the clamp the animal was sutured and allowed to recover for study 24 hours later.

The effects of renal ischemia on renal function were assessed by measurement of inulin clearance (C_{In}) and fractional excretion of sodium (FE_{Na}), an index of tubular ability to reabsorb sodium. To measure glomerular filtration rate by C_{In}, 24 hours after renal ischemia animals were anesthetized with pentobarbital and placed on a heated, temperature-controlled table. Catheters were placed in the femoral artery and vein and ureter. An infusion of 10% inulin in saline was begun at 0.06 ml/min for 15 minutes, followed by 0.02 ml/min for the duration of the study. After a 60 minute equilibration period three, 20-minute urine collections were made. Blood for plasma inulin determination was obtained at the midpoint of each collection. The concentration of inulin in urine and blood was determined by the anthrone method and inulin clearance was calculated by the standard formula. Urine for measurement of FE_{Na} was obtained during clearance studies.

For histologic evaluation of the effect of renal ischemia, kidney tissue was examined by light microscopy. After ischemia and 24 hours of reflow, kidneys were fixed by perfusion at mean arterial pressure with 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer (pH 7.4, osmolality 300 mOsm/kg) followed by immersion in fixative for three hours. Sections were embedded in paraffin and stained with hematoxylin and eosin. Slides were reviewed in a blinded manner and scored with a semiquantitative scale to evaluate the presence and extent of tubular epithelial cell flattening, brush border loss, cell membrane bleb formation, cytoplasmic vacuolization, cell necrosis, interstitial edema and tubular lumen obstruction [11].

Lipid peroxidation was measured after 60 minutes of ischemia plus 15 minutes of reperfusion, a time of maximum lipid peroxidation [11, 12] by determining the renal cortical content of the lipid peroxidation product malondialdehyde (MDA). MDA was measured by the method of Ohkawa, Ohishi and Yagi [22]. After ischemia plus reflow kidneys were rapidly removed and placed in iced phosphate buffered saline. Sections of renal cortex were suspended in a total volume of 3 ml 100 mM KCl plus 0.003 M EDTA and homogenized with a Polytron (Brinkman Instruments, Westbury, New York, USA) at setting 8 for 15 seconds. Homogenates were then centrifuged at 600 g for 10 minutes. Two hundred microliters of supernate were added to 0.2 ml 8.1% sodium dodecyl sulfate, 1.5 ml 20% acetic acid (pH 3.5), 1.5 ml 0.8% thiobarbituric acid and 6.6 ml water. This solution was heated to 95°C for 60 minutes. After addition of 1.0 ml water and 5.0 ml of an n-butanol/pyridine mixture (15:1 vol/vol) the mixture was vigorously shaken and centrifuged at 2000 g for 15 minutes. The absorbance of the upper organic

layer at 532 nm was determined in a spectrophotometer. Absorbance of tissue samples was compared to results obtained using malonaldehyde tetraethylacetal standards (Sigma Chemical Co., St. Louis, Missouri, USA). MDA values were expressed per mg protein. All determinations of MDA were performed in duplicate. The intra-assay coefficient of variation in previous studies was 4.2%. Because the inter-assay coefficient of variation was as high as 25%, on each day a comparable number of control and experimental animals were studied.

Effect of renal GSH on susceptibility to renal ischemic injury

To increase renal GSH content animals underwent right nephrectomy and were infused with GSH (Sigma), 1 mmol/kg in 0.5 ml 0.9% saline intravenously, one hour before renal ischemia. Control animals received saline. This protocol has previously been determined to increase renal GSH by 42%[1] [16]. To decrease renal GSH rats were given diethylmaleate (DEM) prior to ischemia. DEM forms thioether conjugates with GSH catalyzed by glutathione-S-transferase. The conjugates are then degraded and excreted [23]. DEM (Sigma) was given in a dose of 0.8 ml/kg intraperitoneally two hours before renal ischemia. In a separate group of animals the ability of DEM to decrease renal GSH was determined.

Renal cortical GSH content was measured by the method of DeLucia et al [24]. The kidneys were perfused in situ with iced 0.1 M phosphate buffered saline for eight minutes to remove intravascular erythrocytes. The kidneys were then removed and placed in iced phosphate buffer. Sections of renal cortex were homogenized in 2 ml 0.25 M potassium phosphate buffer with a Polytron. Homogenates were deproteinized by immersion in a beaker of boiling water for five minutes, then cooled in an ice bath and treated with several drops of 10% trichloroacetic acid until the supernate was clear. Particulate debris was removed by centrifugation at 3000 g for 10 minutes. Reduced glutathione was measured by adding 0.3 ml supernate to a quartz cuvette containing 0.6 ml 0.25 M potassium phosphate buffer (pH 6.8), 50 μl 1% bovine serum albumin and 15 μl glyoxalase I (Sigma). The contents were mixed and absorbance measured at 240 nm. Then, 10 μl of 0.1 M methylglyoxal (Sigma) was added and absorbance recorded after a plateau was reached (generally less than 8 min). The concentration of glutathione was then calculated from the increase in absorbance by the reaction product S-lactyl glutathione using an extinction coefficient of 3.37 $mM^{-1}\ cm^{-1}$. All measurements were performed in duplicate. Glutathione content was expressed per mg homogenate protein using a Lowry protein assay and bovine serum albumin standards. Using glutathione (Sigma) standards intra-assay coefficient of variation was 6.8% and inter-assay coefficient of variation was 4.3%.

Effect of renal work on renal GSH

Renal work was altered by decreasing the filtered load of sodium and/or sodium absorption, either by inducing renal artery stenosis or by infusing a combination of natriuretic agents. Renal artery stenosis was produced by placing a 0.3 mm silver clip on the left renal artery. In one group of rats the effect of renal artery stenosis for 24 hours on renal GSH was compared in stenotic (left) and intact (right) kidneys. The effect of 24 hours of renal stenosis on GFR was determined in another group of animals measuring inulin clearance in both kidneys using bilateral ureteral catheters.

An alternative method of reducing renal transport work we used was to infuse rats with a combination of pharmacologic agents which inhibit sodium reabsorption. The effect of decreased sodium reabsorption on renal GSH was then determined. A modification of the protocol described by Middendorf and Grantham using lower doses of drugs was employed [25]. Animals were given an intravenous bolus of vanadate (0.25 mg in 1 ml saline) followed 30 minutes later by a continuous infusion of diuretics (vanadate 0.23 mg/ml, furosemide 1.5 mg/ml, amiloride 0.6 mg/ml, acetazolamide 2.5 mg/ml and hydrochlorothiazide 1.9 mg/ml) given at the rate of 0.64 ml/hr. All drugs were obtained from Sigma. To maintain fluid and sodium balance rats also received an intravenous bolus of saline equal to 1% body weight at the time of surgery followed by a continuous infusion of saline at 6.8 ml/hr. This final protocol was arrived at following several pilot studies in an attempt to develop a regimen which would produce a substantial, stable natriuresis without hemodynamic instability and during which time replacement fluids would minimize fluid and electrolyte imbalances.

In addition to determining the effect of pharmacologic inhibition of sodium reabsorption on renal GSH the effect of the above protocol on mean arterial pressure, hematocrit, GFR, renal plasma flow, renal blood flow, and fractional excretion of sodium was measured in a separate group of animals. The renal venous concentration of inulin was measured in plasma samples obtained through a curved 23 gauge needle secured in the renal vein with cyanoacrylate. Renal plasma and blood flow were then calculated from the renal extraction of inulin using standard formulae during three separate 10 minute periods 40, 70, and 100 minutes after starting the constant infusion.

Effect of decreased renal work on susceptibility to postischemic renal injury

Renal artery stenosis was produced as described above and used to test the effect of a decrease in filtered load of sodium and absolute sodium reabsorption on postischemic renal injury. Silver clips were removed from the left artery after 24 hours and both renal arteries were completely occluded for 60 minutes. The effect of ischemia on inulin clearance and renal histology after 24 hours of reperfusion was determined. The effect of ischemia plus 15 minutes of reperfusion on lipid peroxidation in both kidneys was also determined.

Statistical analysis

All data are reported as mean ± standard error. Statistical analyses employed Student's *t*-test for unpaired samples, or for nonparametric data the Wilcoxon rank sum test. For analysis of the effects of renal artery stenosis, left and right kidney data were compared by the *t*-test for paired samples.

Results

Effect of renal GSH on susceptibility to renal ischemic injury

When renal GSH was increased by infusion of GSH there was considerable protection against postischemic renal dysfunction.

[1] The values for renal GSH in our previous publication, reference 16, were mistakenly reported as values 10^3 times greater than their actual value.

Table 1. Effect of natriuretic agents on renal function

	Baseline	Period 1	Period 2	Period 3
C_{In} *ml/min*	(1.0 ± 0.08)[a]	1.03 ± 0.09	1.05 ± 0.09	1.05 ± 0.11
Filtration fraction	(0.30 ± 0.04)	0.39 ± 0.08	0.36 ± 0.06	0.31 ± 0.04
Renal plasma flow *ml/min*	(3.2 ± 0.4)	2.99 ± 0.34	3.06 ± 0.55	3.42 ± 0.60
Renal blood flow *ml/min*	(5.9 ± 0.7)	5.52 ± 0.82	5.58 ± 0.96	6.13 ± 1.06
Hematocrit %	44.2 ± 1.4	44.4 ± 1.4	44.4 ± 1.3	43.7 ± 1.2
FE_{Na} %	(0.05 ± 0.01)	3.5 ± 0.8	3.7 ± 0.5	4.6 ± 0.6

[a] Baseline values not obtained. Values in parentheses represent usual values for this laboratory.

Inulin clearance 24 hours after ischemia was 0.15 ± 0.02 ml/min in saline-infused control animals ($N = 7$) and 0.43 ± 0.08 ml/min in GSH-infused animals ($N = 8$; $P < 0.01$). GSH-mediated protection of tubular function after ischemia was suggested by a trend to lower FE_{Na} in treated animals: 4.7 ± 1.1% in control animals and 3.0 ± 0.7% in GSH infused animals. GSH infusion also lessened postischemic lipid peroxidation. Renal MDA was 0.791 ± 0.079 nmol/mg protein in control kidneys ($N = 6$) and 0.557 ± 0.03 nmol/mg protein in kidneys of rats given GSH ($N = 5$; $P < 0.05$).

Conversely, when renal GSH was decreased by administration of DEM there was potentiation of renal ischemic injury. DEM decreased renal GSH to 0.117 ± 0.025 nmol/mg protein ($N = 5$) from 0.556 ± 0.098 in control kidneys ($N = 5$; $P < 0.005$). Twenty-four hours after ischemia C_{In} was 0.05 ± 0.02 ml/min in DEM-treated animals ($N = 5$) and 0.28 ± 0.06 ml/min in control animals ($N = 5$; $P < 0.005$). In addition DEM treatment resulted in increased lipid peroxidation after ischemia. Renal MDA was 0.535 ± 0.038 nmol/mg protein in control kidneys ($N = 6$) and 0.892 ± 0.109 nmol/protein in kidneys of rats given DEM ($N = 6$; $P < 0.02$).

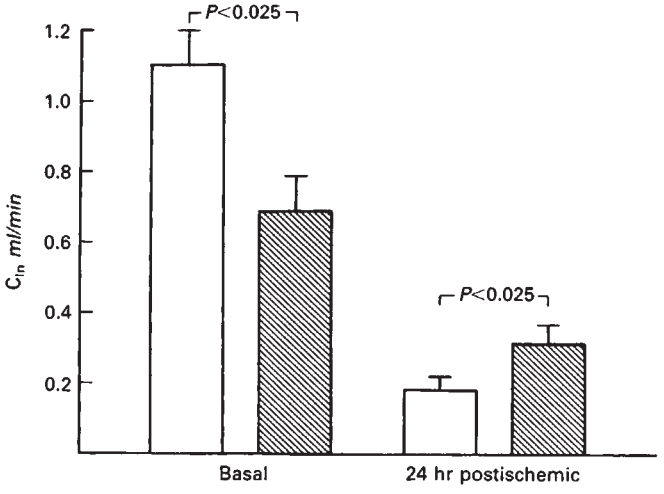

Fig. 2. *Effect of renal artery stenosis for 24 hours on inulin clearance (C_{In}) before and 24 hours after renal ischemia.* Symbols are: □ right, ▨ left (stenotic).

Effect of renal work on renal GSH

Renal artery stenosis produced a 37% decrease in GFR. Inulin clearance was 1.07 ± 0.12 ml/min in intact right kidneys and 0.69 ± 0.11 ml/min in stenotic left kidneys ($N = 5$; $P < 0.025$). Associated with the decrease in filtered load of sodium in the stenotic kidneys was an increase in renal GSH. Twenty-four hours of renal artery stenosis also resulted in an increase in renal GSH. GSH was 0.435 ± 0.089 nmol/mg protein in intact right kidneys and 0.804 ± 0.239 nmol/mg protein in stenotic left kidneys ($N = 10$; $P < 0.05$).

The combined infusion of five natriuretic agents produced dramatic natriuresis without hemodynamic instability (Table 1). There were no significant changes in GFR, renal plasma flow, hematocrit or mean arterial pressure (data not shown) during the observation period and all values were within the normal range for our lab. FE_{Na} was increased to 4.1 ± 0.3% during this period. Pharmacologic inhibition of sodium reabsorption also had significant effects on renal GSH. Kidneys from rats infused with saline (3 ml/hr) for two hours had a GSH level of 0.833 ± 0.084 nmol/mg protein ($N = 7$) whereas kidneys from rats infused with the combination of natriuretic agents for two hours had a GSH level of 1.669 ± 0.418 nmol/mg protein ($N = 7$; $P < 0.01$).

Effect of decreased renal work on susceptibility to postischemic renal injury

Although renal stenosis caused a decrease in C_{In} in the basal state, C_{In} after ischemia was higher in previously stenotic kidneys compared with intact kidneys (Fig. 2). Twenty-four hours after renal ischemia C_{In} was 0.31 ± 0.06 ml/min in previously stenotic kidneys, but only 0.18 ± 0.05 ml/min in intact kidneys ($N = 8$; $P < 0.025$). Therefore, GFR fell by 84% in intact kidneys, but only 55% in previously stenotic kidneys.

Kidneys which had previously been stenotic also underwent less lipid peroxidation after ischemia and reperfusion. Renal MDA content before ischemia was similar in intact right kidneys (0.391 ± 0.057 nmol/mg protein) and 24 hour stenotic left kidneys (0.401 ± 0.073 nmol/mg protein; $N = 8$; NS). However, after ischemia and reperfusion right kidneys showed a significant increase in MDA to 0.860 ± 0.143 nmol/mg protein ($N = 10$; $P < 0.05$) whereas previously stenotic left kidneys showed no significant increase in MDA (0.476 ± 0.049 nmol/mg prot; $N = 10$). Postischemic MDA was therefore significantly less in previously stenotic kidneys compared with intact kidneys ($P < 0.025$).

When six pairs of kidneys were examined histologically 24 hours after renal ischemia, injury was found to be less in previously stenotic kidneys. Coded slides were reviewed in

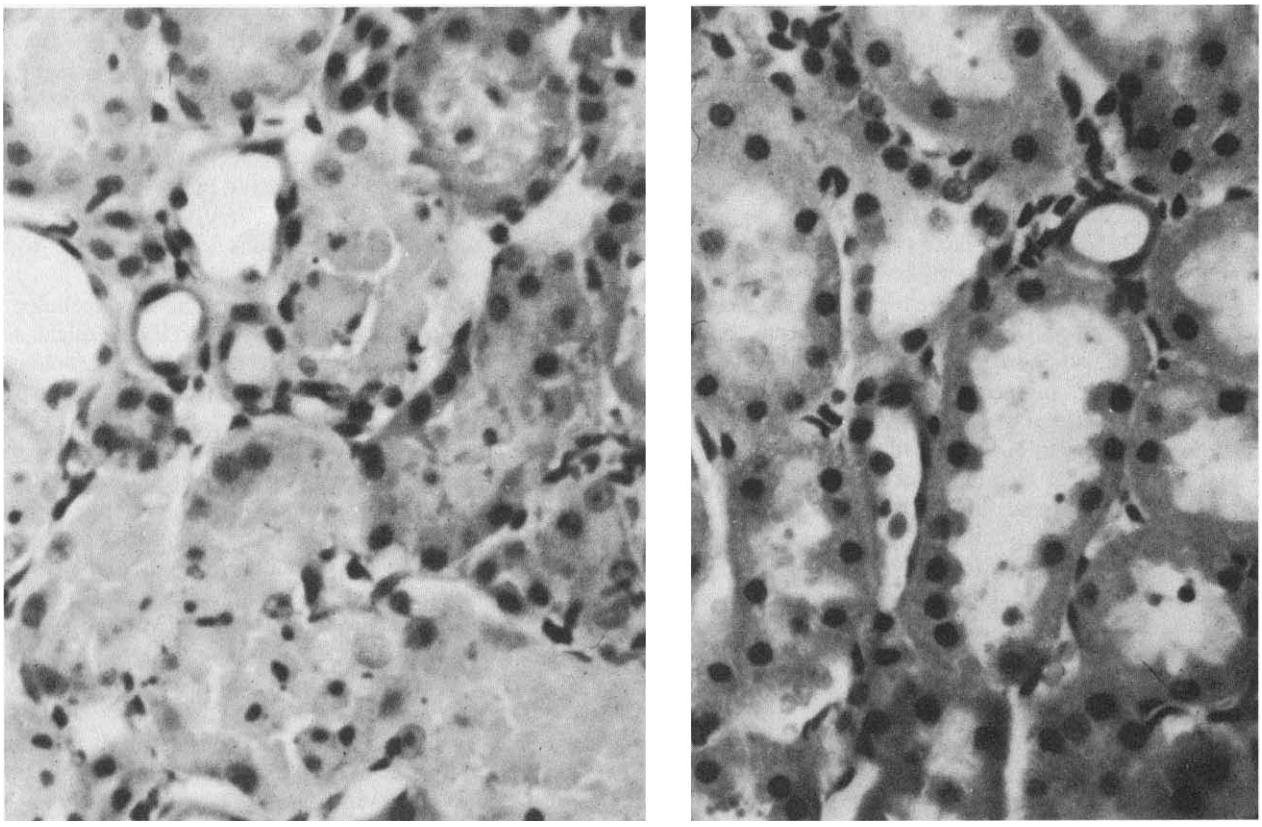

Fig. 3. *Photomicrograph of a pair of kidneys 24 hours after bilateral renal ischemia.* Previously intact kidney (left panel) shows more necrosis, cell membrane loss and lumenal obstruction than previously stenotic kidney (right panel).

pairs and the kidney with more extensive injury picked. When the code was later broken, it was found that in all six cases the intact right kidney was judged to have been more severely injured in terms of necrosis and membrane injury ($P < 0.05$ by Wilcoxon signed rank test). Figure 3 shows one such pair of kidneys.

Discussion

These studies suggest that renal GSH is an important free radical scavenger after renal ischemia and that the renal content of reduced glutathione is a determinant of susceptibility to postischemic renal injury. Renal dysfunction was more severely impaired after ischemia in animals with lower renal GSH after treatment with DEM and less severely impaired after ischemia in animals with higher levels of renal GSH following GSH infusion. Since GSH has cellular effects in addition to its antioxidant actions and since DEM may inhibit several intracellular enzymes as well as decrease renal GSH, these individual studies should be interpreted with caution. Kidneys subjected to partial renal artery occlusion also had higher renal GSH levels and sustained less postischemic injury than contralateral intact kidneys. In the latter situation, although intrarenal hemodynamics were obviously different between intact and stenotic kidneys, the volume status, nutritional status and hormonal status of the animal would affect both kidneys and cannot have been responsible for the differential sensitivity to renal ischemia in the two kidneys.

GSH also modulates other forms of oxidant injury. Cellular GSH depletion potentiates hydrogen peroxide-induced injury of cultured endothelial cells [23], and postischemic liver injury [26]. In preliminary studies of isolated renal tubules DEM increased injury after oxidative stress due to tert-butylhydroperoxide [27]. However, GSH depletion in the absence of oxidant stress may not be particularly harmful. Cultured hepatocytes remain viable after GSH depletion [28]. In the isolated perfused kidney, although decreased sodium reabsorption and a renal concentrating defect develop after GSH depletion, glomerular filtration rate is not affected [29]. However, as shown in the present study when the oxidant stress of renal ischemia is superimposed upon renal GSH depletion, injury is exacerbated.

These studies also shed light on the mechanism of control of renal GSH. Glutathione in the plasma is effectively removed by the kidney by both glomerular filtration with subsequent apical resorption and by peritubular transport [30, 31]. Although filtered GSH is probably hydrolyzed to its constituent amino acids (glutamate, cysteine, and glycine) it is resynthesized within the kidney [30, 31]. Renal GSH stores can thus be increased by delivering more GSH to the kidney as occurred after intravenous infusion of GSH.

In the kidney approximately 80% of the oxygen consumed is to support reabsorption of filtered sodium [32–34]. It was therefore possible to examine the link between filtered load of sodium, oxidant stress and renal GSH. Renal O_2 consumption is linearly related to renal blood flow. Renal O_2 consumption thus

decreases when blood flow is restricted by aortic occlusion [32]. Renal artery stenosis would be expected to similarly decrease renal O_2 consumption since the filtered load of sodium was decreased by approximately 37% compared to intact kidneys. Numerous other studies have shown a linear correlation between sodium reabsorption and renal O_2 consumption [32–34] and between Na-K ATPase activity and O_2 consumption [35]. Whole kidney O_2 consumption also decreased in dogs given hydrochlorothiazide to inhibit sodium reabsorption [36]. In the present study since sodium reabsorption was pharmacologically inhibited in the proximal nephron (predominantly by vanadate) and loop of Henle as well as in the distal nephron, renal O_2 consumption would also decrease.

Renal GSH content can apparently be altered by changing renal oxidant stress. When renal work (and, therefore, renal oxygen consumption) was lessened either by decreasing the filtered load of sodium as in renal artery stenosis or by pharmacologically-inhibiting renal sodium reabsorption throughout the nephron in the face of a constant filtered load of sodium, renal GSH levels rose. We hypothesize that renal GSH rose because the decrease in renal O_2 consumption which would accompany the decrease in renal sodium reabsorption resulted in the formation of fewer oxygen free radicals. Less GSH was consumed to protect the cell against these free radicals and GSH levels therefore rose.

When renal GSH content increased by the above mechanism, kidneys were relatively protected against free radical-mediated postischemic injury. We cannot be sure that GSH must remain intact in the kidney to provide this clearly demonstrated protection. Weinberg and coworkers have recently suggested that it is the glycine component of GSH which protects against hypoxic tubule cell injury in isolated renal tubules [37]. Nevertheless, in vivo protection against postischemic injury correlates with the level of intact GSH. After renal artery stenosis, for example, renal GSH was incresed, and after renal ischemia there was less renal dysfunction (higher C_{In} at 24 hr), less histologic injury, and, importantly, less free radical-mediated lipid peroxidation. We did not test the effect of pharmacologic inhibition of sodium reabsorption on susceptibility to renal ischemia because we felt it would be impossible to adequately increase solute excretion and urine flow in control kidneys without changing renal sodium resorption and O_2 consumption. Since increased solute excretion has been amply demonstrated to protect against ischemic renal injury [38], without an appropriate control group it would be impossible to separate the beneficial effects of increased solute excretion from the potential beneficial effects of decreased renal work on renal injury in natriuretic agent-infused animals. However, in a previous study we found a beneficial effect of hypothyroidism on susceptibility to renal ischemic injury. In hypothyroidism, as in renal artery stenosis, the filtered load of sodium was decreased (presumably renal O_2 consumption was also decreased), renal GSH was elevated, and hypothyroid animals were less susceptible to renal dysfunction and free radical-mediated lipid peroxidation after ischemia [16]. When hypothyroid animals were also given DEM to lower renal GSH to euthyroid levels (0.506 ± 0.158 vs. 0.541 ± 0.034 nmol/mg protein), the protective effect of hypothyroidism against postischemic renal dysfunction was lost. Twenty-four hours after ischemia C_{In} in euthyroid animals ($N = 8$) was 0.33 ± 0.08 ml/min compared to 0.31 ± 0.1 in DEM-treated hypothyroid animals ($N = 6$, NS; unpublished data). When mean data from the present study and our previous study [16] are examined, the correlation between preischemic renal GSH and postischemic glomerular filtration rate is apparent. Plotting the change in GSH (x-axis) versus the change in postischemic C_{In} (y-axis) (in terms of percent change from the control to experimental group) gave the following relation by linear regression: $y = 35.1 \times + 1.27$; $N = 6$; $r = 0.75$; $P < 0.05$. Taken together these observations are consistent with the hypothesis shown in Figure 1.

The relationship between O_2 consumption, cellular GSH stores and susceptibility to postischemic injury might also be relevant to other tissues. Oxygen free radical participation in postischemic injury has been demonstrated in the small intestine, myocardium, brain, liver, and skeletal muscle, as well as the kidney [10]. Although the specific role of GSH in protecting against postischemic injury has not yet been thoroughly investigated, there is no reason to doubt its importance since in all organs GSH is an important cellular antioxidant and hydroxyl radical scavenger as well as cofactor for glutathione peroxidase [20]. Therefore, GSH can directly interact with free radicals and can metabolize lipid hydroperoxides and hydrogen peroxide by glutathione peroxidase. It remains to be directly tested whether an increase in myocardial O_2 consumption, for example, would lead to a decrease in myocardial GSH stores.

These studies suggest additional means to produce protection against free radical-mediated injury following renal ischemia. Previous studies have shown beneficial effects of allopurinol to inhibit superoxide radical formation, superoxide dismutase to enhance superoxide elimination, catalase to enhance hydrogen peroxide elimination and a variety of hydroxyl radical scavengers (that is, dimethylthiourea, dimethylsulfoxide) to attenuate injury [11–15]. Enhancement of renal GSH stores is equally efficacious and can be produced by changing renal work as well as by infusing exogenous GSH. Additional studies will be necessary to determine whether any part of the beneficial effect of low dietary protein intake to decrease ischemic renal injury [39], or whether any part of the deleterious effect of amino acid infusion [40] or high body temperature [41] can be explained by changes in free radical generation by a similar manner.

In summary, renal GSH is an important free radical scavenger during postischemic reperfusion. Alterations in renal work produced reciprocal changes in renal GSH. In renal artery stenosis, a decrease in renal work led to an increase in renal GSH and protection against free radical-mediated injury following ischemia.

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