Kidney International, Vol. 55 (1999), pp. 1327–1334

Unilateral ureteral obstruction impairs renal antioxidant enzyme activation during sodium depletion

MICHAEL KINTER, JENNIFER T. WOLSTENHOLME, BARBARA A. THORNHILL, ELIZABETH A. NEWTON, MICHAEL L. MCCORMICK, and ROBERT L. CHEVALIER

Departments of Microbiology, Pathology, and Pediatrics, University of Virginia Health Sciences Center, Charlottesville, Virginia; and Research Service, VA Medical Center, and Department of Internal Medicine, University of Iowa, Iowa City, Iowa, USA

Unilateral ureteral obstruction impairs renal antioxidant enzyme activation during sodium depletion.

Background. Obstructive nephropathy leads to progressive renal tubular atrophy and interstitial fibrosis and is associated with sodium wasting and sodium depletion. Renal damage resulting from unilateral ureteral obstruction (UUO) may be aggravated by reactive oxygen species (ROS), which are produced by a variety of processes. Ideally, deleterious effects of ROS are attenuated by antioxidant enzymes, including the superoxide dismutases, glutathione peroxidases, catalase, and glutathione-S-transferases. The general paradigm is that tissue damage occurs when ROS production is greater than the protective capacity of the antioxidant enzymes.

Methods. This study was designed to investigate the response of renal antioxidant enzymes to UUO and sodium depletion. Adult, male Sprague-Dawley rats received normal-sodium or sodium-depleted diets and were subjected to UUO or sham operation. Obstructed (UUO), intact opposite, or sham-operated kidneys were harvested after 14 days, and antioxidant enzyme activities were measured in kidney homogenates. Thiobarbituric acid reactive substances were measured in these homogenates at 3 and 14 days after UUO or sham operation as an index of ROS production.

Results. Renal interstitial area, a measure of fibrosis, was increased by UUO and was doubled in sodium-depleted animals. Sodium depletion increased manganese superoxide dismutase, glutathione peroxidases, and glutathione-S-transferase activities in sham-operated kidneys but not in UUO kidneys. Relative to intact opposite kidneys, UUO kidneys had reduced activities of catalase, manganese superoxide dismutase, and glutathione-S-transferase in normal-sodium animals and all antioxidant enzymes tested in sodium-depleted animals. Renal thiobarbituric acid reactive substances were increased by three days of UUO and were increased further by 14 days of sodium depletion.

Conclusion. In summary, sodium depletion increased several renal antioxidant enzymes, consistent with a stress response to increased ROS production. Further, UUO not only reduced antioxidant enzyme activities but also inhibited increases seen with sodium depletion. We conclude that suppression of renal antioxidant enzyme activities by UUO contributes to the progression of renal injury in obstructive nephropathy, a process exacerbated by sodium depletion.

Key words: progressive renal failure, tubular atrophy, obstructive uropathy, reactive oxygen species, superoxide dismutase, renal injury.

Received for publication October 24, 1997
and in revised form November 3, 1998
Accepted for publication November 3, 1998

Chronic unilateral ureteral obstruction (UUO) leads to progressive renal tubular atrophy and interstitial fibrosis [1]. This process involves activation of the renin-angiotensin system, expression of transforming growth factor-β1 (TGF-β1), clusterin (a glycoprotein), and the induction of tubular cell apoptosis [2]. As a consequence of tubular injury, obstructive nephropathy may lead to renal sodium wasting and sodium depletion [3].

Recent reports indicate that the release of ureteral obstruction (unilateral or bilateral) leads to enhanced renal production of reactive oxygen species (ROS) [4, 5]. Moreover, treatment with the antioxidant probucol improves renal function following the release of bilateral ureteral obstruction [4]. ROS has been implicated as a contributing factor in a variety of tissue injuries, including lung injury [6], liver injury [7], and neurological injuries [8], as well as other renal disorders, such as ischemic acute renal failure [9]. Also, ROS has been implicated as an initiator of apoptosis [10, 11].

The general paradigm for oxidant-induced tissue injury is that injury occurs when the production of ROS is greater than the antioxidant capacity of that tissue. This antioxidant capacity is determined in a large part by the activities of the antioxidant enzymes, primarily the superoxide dismutases, manganese superoxide dismutase (MnSOD) and copper-zinc superoxide dismutase (CuZnSOD), the glutathione peroxidases (GPx), and catalase, but also includes glutathione reductase and the glutathione-S-transferases (GTSs). These enzymes, along with cofactors such as glutathione, oxidized forms of nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$), and the reduced forms of nicotinamide adenine dinucleotide

(NADH) and nicotinamide adenine dinucleotide phosphate (NADPH), form an antioxidant enzyme system that can interact with a variety of ROS, detoxify those species, and prevent injury. Imbalances between the production of ROS and the activities of antioxidant enzymes leading to tissue injury can occur by either increased production of ROS, decreased antioxidant enzyme activities, or both.

The purpose of these experiments was to evaluate the renal antioxidant enzyme activities and the response of that system to UUO and sodium depletion, a common sequela of obstructive nephropathy. The experiments used a well-characterized animal model of UUO in combination with sodium depletion via a low-sodium diet. The study design produced three groups of kidneys—obstructed (UUO), intact opposite (IO), and sham operated—and two dietary regimens (normal sodium and sodium deficient), for a total of six groups. Activities of MnSOD, CuZnSOD, catalase, GPx, and glutathione-S-transferase (GST) were measured, and Western blot analyses were performed to evaluate the amounts of immunoreactive enzyme. The results show that UUO is accompanied by a significant decrease in the activities of these antioxidant enzymes, which is accompanied by a corresponding loss of the amount of enzyme. We have also found that sodium depletion generates an increase in several antioxidant enzyme activities and enzyme amounts in sham-operated kidneys that suggest a stress response to increased ROS production with sodium depletion.

METHODS

Animals and diets

Twenty male, 200 to 250 g, Sprague-Dawley rats were pair fed a salt-deficient diet (Bioserve, Frenchtown, NJ, USA) with 0.06% sodium content or normal chow with 0.43% sodium content for 14 days. Diets were begun three days prior to surgery to acclimate and normalize the animals. An additional 10 rats receiving normal chow were studied three days after being subjected to UUO or sham surgery as described later in this article.

Surgical procedures

Rats underwent either left UUO or sham surgery. Under halothane and oxygen anesthesia, a 2 cm midline incision was made, and the left ureter was exposed and, in the UUO animals, ligated with 4-0 silk suture. The incision was closed in two layers with 4-0 silk and staples. The animals were recovered in a heated environment and returned to their cages. Three or 14 days following surgery, the animals were sacrificed, and the kidneys were harvested for study.

Measurement of renal interstitial volume

Kidneys from rats subjected to 14 days of UUO were fixed in Bouin's solution, embedded in paraffin, sectioned and stained with Masson trichrome stain (hematoxylin, scarlet-acid fuchsin, and aniline blue dye). Sections were examined under $\times 40$ objective, and 10 nonoverlapping fields were scanned for each kidney of UUO and sham-operated animals. The digital images were superimposed on a grid, and the number of grid points overlapping the interstitial blue-staining collagen was recorded for each field as previously reported [12].

Thiobarbituric acid reactive substances assay

Thiobarbituric acid reactive substances (TBARS) were measured in whole kidney homogenates of rats 3 or 14 days following surgery. One-hundred microliter aliquots of the homogenates were mixed with 0.75 ml 1% phosphoric acid and 0.25 ml 0.6% thiobarbituric acid and heated at 90°C for one hour. Absorbance was read at 532 nm, and TBARS was calculated as nmol malondialdehyde equivalents normalized on a per mg protein.

Measurement of antioxidant enzyme activities

Total protein, MnSOD, CuZnSOD, catalase, glutathione peroxidase, and glutathione transferase activities were measured in whole kidney homogenates of rats subjected to 14 days of UUO or sham operation. To prepare the homogenates, the kidney was harvested at the sacrifice of the animal, cleaned of any adhering tissue, and quickly washed and weighed. The kidney was coarsely divided, washed, mixed with a five volumes of 50 mM phosphate, pH 7.8, with 1 mM diethylenediamine pentaacetic acid, and homogenized. The homogenates were stored at −20°C until analysis. At analysis, the homogenates were sonicated prior to using aliquots in the respective enzyme assays. Total protein was determined by the method of Lowry [13]. MnSOD and CuZnSOD activities were measured by the method of Spitz and Oberley [14]. Glutathione peroxidase activity was measured by the method of Lawrence and Burk [15]. Hydrogen peroxide was used as a substrate, so this measurement represents the Se-dependent component of glutathione peroxidase activity. Glutathione transferase activity was measured by the method of Simons and Vander Jagt using 1-chloro-2,4-dinitrobenzene as the substrate [16]. Catalase activity was measure spectrophotometrically by the method of Beers and Sizer [17]. All enzyme activities were calculated on a per mg protein basis. In Figures 3B and 4B, specific activities were also normalized to the respective activity in normal-sodium animals. The purpose of this normalization was to evaluate the magnitude of any increase or decrease in activity.

Western blot analysis

Aliquots of the kidney homogenates were used for Western blot analysis to determine amounts of immuno-

reactive protein for the respective antioxidant enzymes. The Western blots were performed using the method of Towbin, Staehelin and Gordon [18]. Samples of the kidney homogenates (0.5 μg) and commercially available standards (0.1 μg) were heat denatured in sodium dodecyl sulfate buffer containing β-mercaptoethanol, run in 12.5% polyacrylamide gels, and the proteins were transferred to nitrocellulose. The nitrocellulose was blocked for four hours with 4% bovine serum albumin (BSA) in 10 mM Tris-buffered saline (TBS), pH 8.0, at room temperature. The nitrocellulose blot was incubated for four hours at room temperature in the appropriate primary rabbit polyclonal antibody diluted in TBS containing 2% BSA. Rabbit polyclonal antibodies against the antioxidant enzymes were prepared as previously described and kindly provided by Dr. Larry Oberley (University of Iowa, IA, USA) [19]. The nitrocellulose was rinsed and incubated for two hours at room temperature in a 1:10 000 dilution of goat antirabbit IgG alkaline phosphatase-conjugated secondary antibody (Sigma, St. Louis, MO, USA). The bands were visualized with an alkaline phosphatase-staining system containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

Statistical analyses

Five animals were included in each study group. Statistically significant differences were determined by a one-way or two-way analysis of variance using a Tukey–Kramer multiple comparisons test or an unpaired *t*-test using a commercially available statistics program (InStat; GraphPad, San Diego, CA, USA).

RESULTS

Sodium depletion increased unilateral ureteral obstruction injury

Renal injury caused by UUO was measured by quantitating renal interstitial collagen 14 days after obstruction. Figure 1 shows the renal interstitial area in UUO and IO kidneys of normal and sodium-depleted animals. UUO injury is reflected by an increase in interstitial area in both the normal-sodium and sodium-depleted groups. As shown in this figure, sodium depletion doubled interstitial area in UUO kidneys relative to normal-sodium UUO kidneys. There was, however, no effect of sodium depletion on interstitial area in the IO kidneys. As shown in Figure 2A, renal TBARS were increased three days after UUO. Although renal TBARS 14 days after UUO were lower than that of sham-operated kidneys, sodium depletion significantly increased renal TBARS regardless of surgical treatment (Fig. 2 B, C).

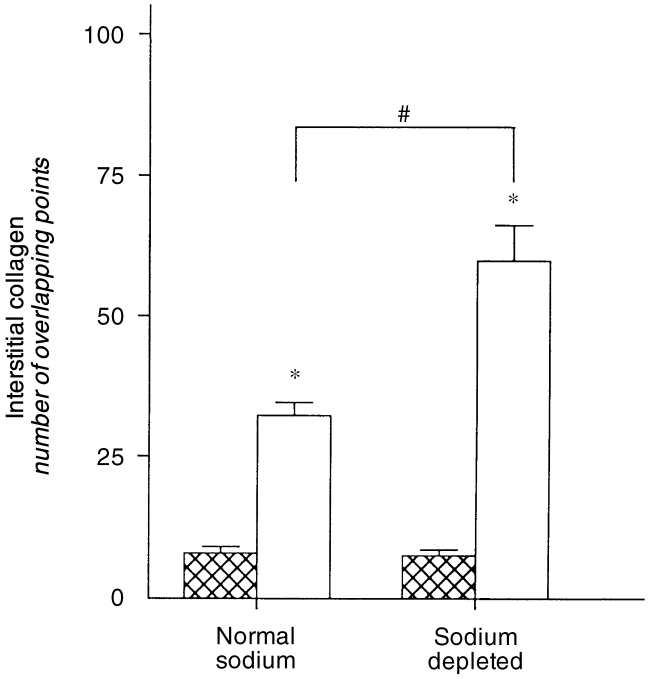

Fig. 1. Renal interstitial collagen in obstructed (UUO; □) or intact opposite (IO; ⊠) kidneys of normal sodium or sodium depleted animals. Data are means ± SEM. *$P < 0.05$, IO vs. UUO groups; #$P < 0.05$, between UUO groups.

Sodium depletion increased renal glutathione peroxidases, manganese superoxide dismutase, and glutathione-S-transferase activities in sham-operated kidneys

Figure 3A shows the activities of the antioxidant enzymes in sham-operated kidneys of normal-sodium and sodium-depleted animals. Statistically significant increases were observed in the activities of GPx, MnSOD, and GST. The magnitude of these increases, seen in Figure 3B, were 30% for GPx activity, 130% for MnSOD activity, and 140% for GST activity. There were no differences in catalase and CuZnSOD activities between sham-operated normal-sodium and sham-operated sodium-depleted kidneys.

Sodium depletion had no effect on antioxidant enzyme activities in the unilateral ureteral obstruction kidney

The antioxidant enzyme activities in UUO kidneys were compared between normal-sodium and sodium-depleted animals to determine if induction of GPx, MnSOD, and GST seen with sodium depletion in sham-operated kidney was preserved with UUO. As illustrated in Figure 4, no statistically significant differences were seen in any antioxidant enzyme activities between UUO

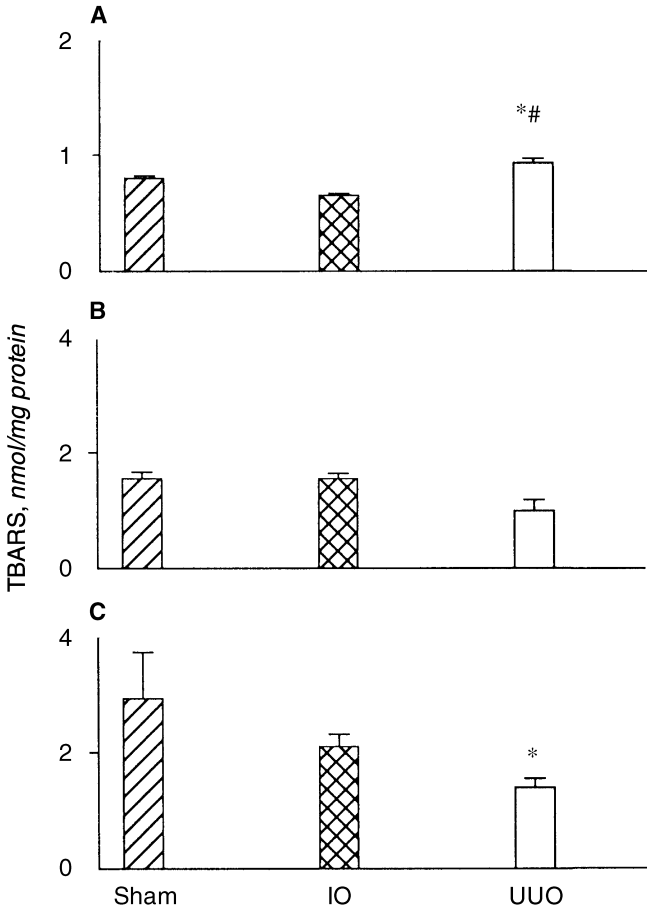

Fig. 2. Renal thiobarbituric acid reactive substances (TBARS). (*A*) Renal TBARS three days following sham operation or unilateral ureteral obstruction (UUO) in rats receiving normal sodium intake. (*B*) Renal TBARS 14 days following sham operation or UUO in rats receiving a normal sodium intake. (*C*) Renal TBARS 14 days following sham operation or UUO in sodium-depleted rats. Mean ± SEM. *$P < 0.05$ vs. sham; #$P < 0.05$ vs. intact opposite (IO) kidney. As determined by two-way analysis of variance, there was a significant overall increase in TBARS in sodium-depleted (C) compared with normal-sodium groups (B; $P < 0.02$).

normal sodium and UUO sodium-depleted kidneys. These results are in contrast to the increased activities shown in Figure 3 for sham-operated kidneys from sodium-depleted animals.

Ureteral obstruction decreased renal antioxidant enzyme activities and increased ROS. The activities of the antioxidant enzymes in UUO kidneys were compared with IO and sham-operated kidneys to determine the effects of UUO on the renal antioxidant enzyme system. In normal-sodium animals, ureteral obstruction produced significant decreases in catalase and GPx activities in UUO kidneys relative to sham-operated kidneys, whereas the activities of CuZnSOD, MnSOD, and GST remained unchanged (Fig. 5). In sodium-depleted animals (Fig. 6), ureteral obstruction decreased catalase, GPx, and MnSOD activities in UUO kidneys relative to sham-operated kidneys, with CuZnSOD and GST activities unchanged.

Antioxidant enzyme activities in UUO kidneys in both normal-sodium and sodium-depleted animals were also less than the IO kidneys. Relative to IO kidneys, UUO kidneys in normal-sodium animals have significantly reduced activities of catalase, MnSOD, and GST. In sodium-depleted animals, UUO kidneys relative to IO kidneys have significantly reduced activities of all antioxidant enzymes tested.

Figures 5 and 6 also show that in the IO kidneys, MnSOD and GST were significantly increased relative to sham-operated kidneys in the normal-sodium animals with catalase, GPx, and CuZnSOD activities unchanged. In sham-operated, depleted rats, GST was greater in IO than sham-operated kidneys, with no difference in the remaining enzymes.

Amounts of immunoreactive protein correlated with antioxidant enzyme activities

Figure 7 shows immunoblot analyses of catalase, GST, MnSOD, and CuZnSOD in kidney homogenates. No appropriate antibody was available for GPx. The relative amounts of immunoreactive protein detected in these analyses correlate to the relative activities measured in the different treatment groups. Specifically, increases are seen in the amounts of GST and MnSOD protein, but not catalase or CuZnSOD protein, in sham-operated kidneys in sodium-depleted animals relative to normal-sodium animals by comparisons of lanes 4 versus 7 in each blot. Also, reduced amounts of all four enzymes in UUO kidneys relative to IO kidneys can be seen in comparisons of lanes 2 versus 3 and 5 versus 6 in each blot.

DISCUSSION

The major findings in this study are that UUO reduces renal antioxidant enzyme activity and also prevents an induction of these enzyme activities by sodium depletion. Sodium depletion increased renal TBARS and increased the activities of GPx, MnSOD, and GST in sham-operated kidneys. The increased enzymatic activities were reflected in increased amounts of immunodetectable protein. The magnitude of the increased antioxidant activities is comparable to increases found in fibroblast cell lines made resistant to oxidant injury by treatment with hydrogen peroxide or 80% oxygen [20–23]. For example, an oxygen-resistant fibroblast cell line (designated O2R95) developed by growth of a parent fibroblast cell line (designated HA1) in 80% oxygen showed GPx, MnSOD, and GST activities that were increased 190%, 26%, and 100%, respectively, over the HA1 cells [20, 21]. A similar fibroblast cell line developed by sequential passage of the HA1 cells in concentrations of hydrogen peroxide

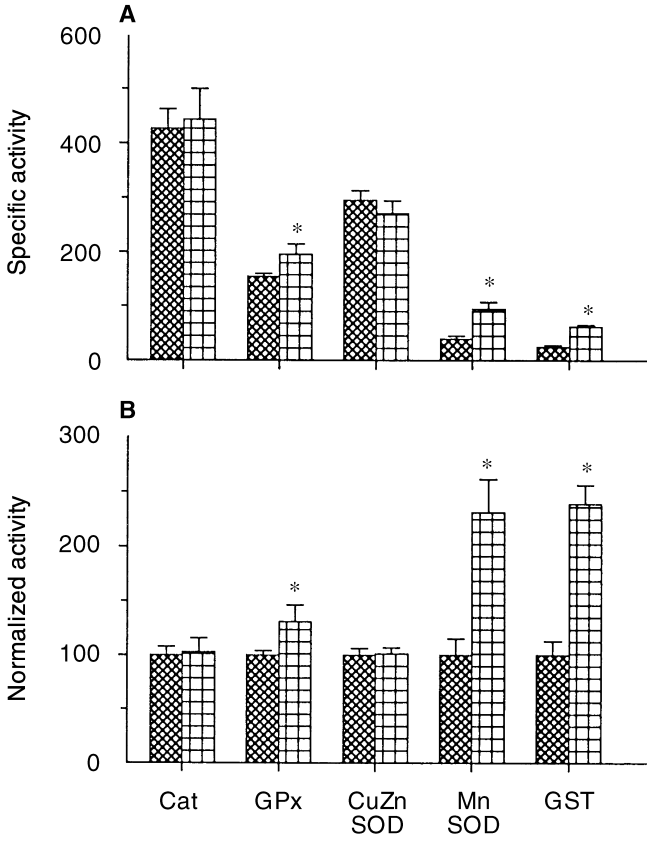

Fig. 3. Antioxidant enzyme activities in sham-operated kidneys of normal-sodium (▨) and sodium-depleted (⊞) rats as either (*A*) specific activity or (*B*) normalized activity with normalization to the respective normal-sodium activity. Data are means ± SD. *$P < 0.05$, relative to normal sodium. The units for the specific activities are mU/mg protein for glutathione peroxidase (GPx) and glutathione-S-transferase (GST), U/mg protein for MnSOD and CuZnSOD, and mK/mg protein for catalase.

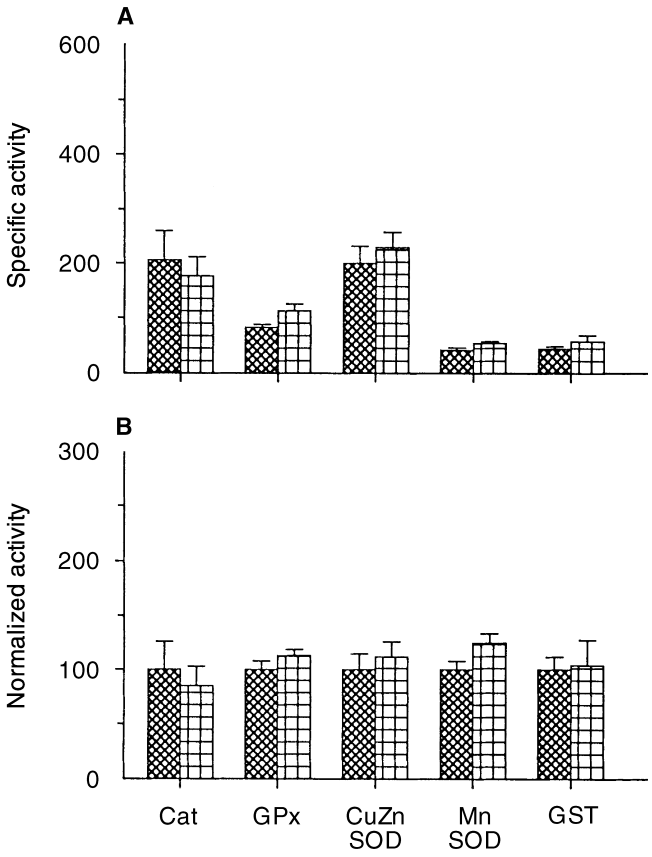

Fig. 4. Antioxidant enzyme activities in UUO kidneys of normal-sodium (▨) and sodium-depleted rats (⊞) as either (*A*) specific activity or (*B*) normalized activity with normalization to the respective normal-sodium activity. Data are means ± SD. The units for the specific activities are mU/mg protein for GPx and GST, U/mg protein for MnSOD and CuZnSOD, and mK/mg protein for catalase.

up to 400 μM (designated OC14 cells) had GPx, MnSOD, and GST activities that were increased 150%, 70%, and 290%, respectively, over the HA1 cells [20, 22, 23]. Therefore, not only are the increases in renal antioxidant enzyme activities consistent with sodium depletion being an oxidative stress that generates an oxidative stress response, but the magnitude of these increases are comparable to those seen in cell lines exposed to either 80% oxygen or high concentrations of hydrogen peroxide.

The nature of the enzyme activities that are increased is also noteworthy. Of particular interest are the increase in MnSOD activity, but not CuZnSOD, and the increase in GPx activity, but not catalase activity. These contrasts are significant because the two sets of enzymes catalyze similar reactions. Although MnSOD and CuZnSOD both catalyze the dismutation of superoxide, MnSOD is localized primarily in the mitochondria and is considered inducible, whereas CuZnSOD is localized primarily in the cytosol. As a result, one could speculate that increases in MnSOD activity, but not CuZnSOD activity, indicate the oxidative stress presented by sodium depletion is some action involving the mitochondria. Similar logic may be applied to the distinction between GPx and catalase because both enzymes catalyze the breakdown of hydroperoxides. A significant difference, however, between these two enzymes is that although both enzymes can catalyze the breakdown hydrogen peroxide, isoforms of GPx metabolize lipid hydroperoxides, whereas catalase cannot. Therefore, the increase in GPx activity, without an increase in catalase activity, may indicate a role for lipid hydroperoxides in the oxidative stress generating this response. This observation is supported by the increase in GST activity that is also seen. GST catalyzes conjugation of a variety of toxic species, including lipid hydroperoxides and aldehydes formed by oxidative degradation of lipid hydroperoxides, to glutathione.

Renal TBARS were also increased in the early period following UUO and increased markedly following 14 days of sodium depletion. Catalase and GPx activities were reduced in UUO kidneys from normal-sodium ani-

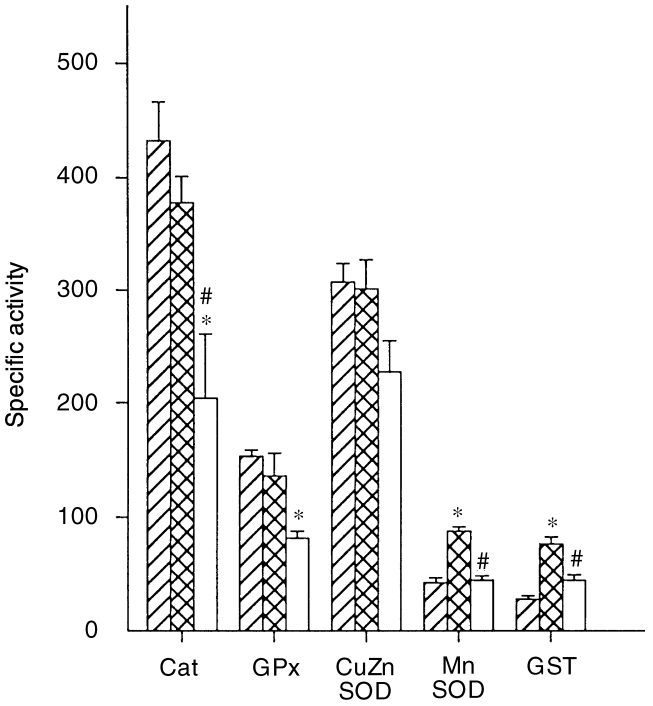

Fig. 5. Antioxidant enzyme activities in UUO (□), IO (⊠), and sham-operated (▨) kidneys of normal-sodium rats. Data are means ± SD. *$P < 0.05$ relative to sham-operated kidneys; #$P < 0.05$ relative to IO kidneys. The units for the specific activities are mU/mg protein for GPx and GST, U/mg protein for MnSOD and CuZnSOD, and mK/mg protein for catalase.

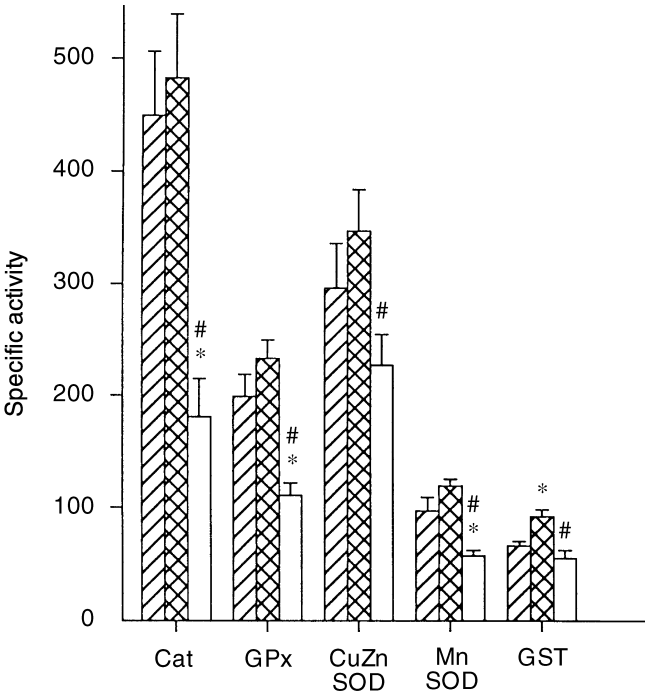

Fig. 6. Antioxidant enzyme activities in UUO (□), IO (⊠), and sham-operated (▨) kidneys of sodium-depleted rats. Data are mean ± SD. *$P < 0.05$ relative to sham-operated kidneys; #$P < 0.05$ relative to IO kidneys. The units for specific activities are mU/mg protein for GPx and GST, U/mg protein for MnSOD and CuZnSOD, and mK/mg protein for catalase.

mals, whereas catalase, GPx, and MnSOD activities were reduced in UUO kidneys from sodium-depleted animals. The loss of these enzyme activities represents a defect in the antioxidant enzyme system that would disrupt any balance between increased production of ROS, evidenced by increased TBARS, and the subsequent metabolism. Western blot data show that these losses in activity are accompanied by losses of immunoreactive enzyme. These findings are consistent with those of Ricardo et al [24], who reported significant reduction in catalase and CuZnSOD in proximal tubule suspensions from rats with four days of ipsilateral UUO. These investigators also demonstrated a 14-fold increase in levels of hydrogen peroxide in hydronephrotic kidney [24], which is consistent with our observation of increased ROS. The increased GPx, MnSOD, and GST activities observed in sham-operated kidneys from sodium-depleted animals were lost with UUO kidneys. Because the increase in antioxidant activities seen in sodium-depleted animals appears as a response to increased ROS production with sodium depletion, the loss of these activities would create a situation in which increased ROS production is not countered by appropriate increases in antioxidant enzyme activities. One could speculate, based on these data, that the imbalance created with sodium depletion by increased ROS production and decreased antioxidant enzyme activity with UUO contributes to the increased injury with UUO in the sodium depleted animals seen in Figure 1.

In IO kidneys relative to sham-operated kidneys, MnSOD and GST activities were increased in normal-sodium animals, and GST activity was increased in sodium-depleted animals. These findings are consistent with the concept of "renal counterbalance," in which the kidney contralateral to UUO undergoes adaptive changes [25]. These changes include compensatory growth, vasodilation, and alterations in the activity of the renin-angiotensin system [25]. The increased metabolic demands placed on the IO kidney presumably underlie the observed increase enzyme activities.

These results have a number of important implications. The hallmarks of chronic obstructive nephropathy are tubular atrophy and interstitial fibrosis. Tubular atrophy likely results from a progressive loss of renal tubular epithelial cells by apoptosis [2, 26, 27]. Apoptosis, in turn, may be stimulated by increased expression of TGF-β1, which is up-regulated by activation of the renin-angiotensin system [28]. Oxidant injury is also a recognized

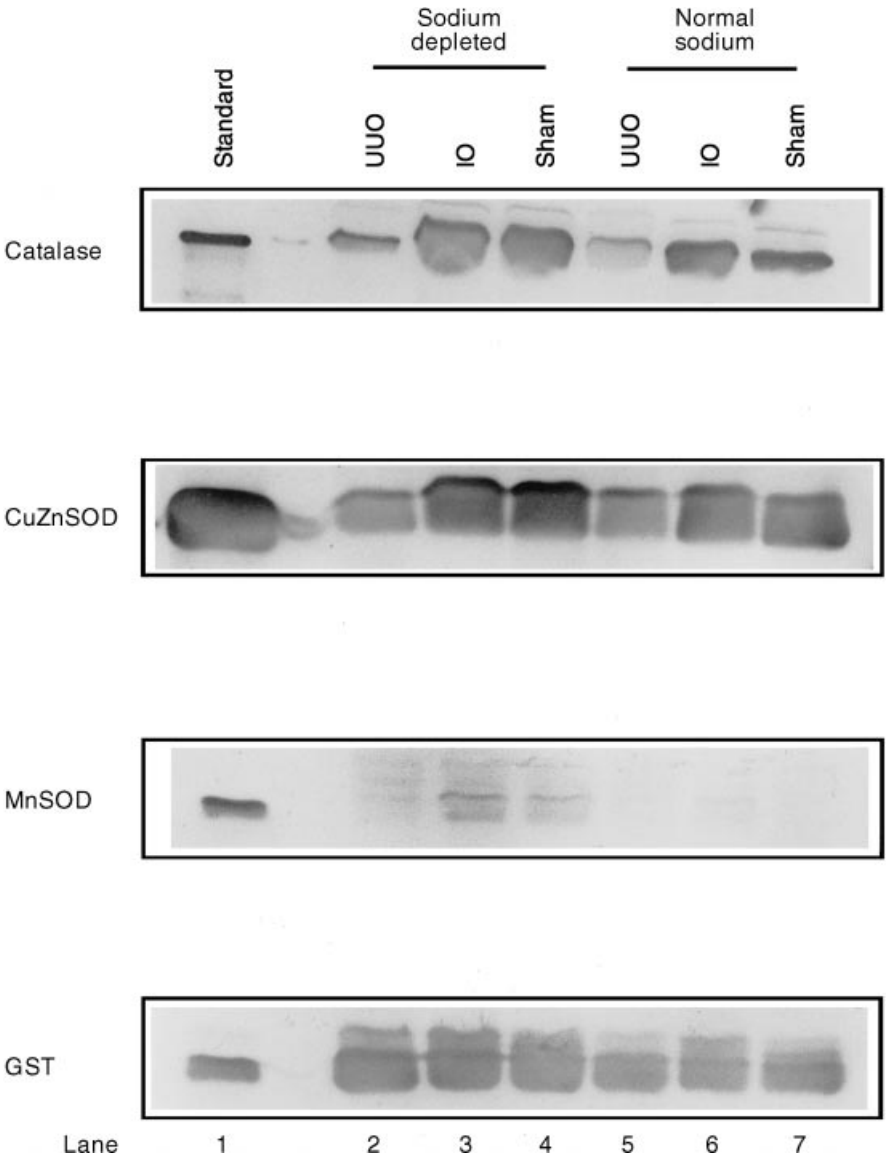

Fig. 7. Western blots of total kidney protein using polyclonal antibodies against catalase, CuZnSOD, MnSOD, and GST. For clarity, only the band comigrating with the respective standards is shown.

stimulus to apoptosis [10, 11]. In addition, the administration of antioxidants to mice with experimental pyelonephritis significantly reversed the tubular lesions and interstitial inflammation characterizing this disorder [29]. As in obstructive nephropathy, pyelonephritis has been shown recently to involve massive tubular cell apoptosis [30].

The role of sodium depletion in the stimulation of antioxidant enzymes is less clear. Volume depletion by water deprivation in rats subjected to diatrizoate (contrast medium) injection reduces antioxidant enzyme activities and renal function, an effect that can be abrogated by catalase treatment [31]. This suggests that volume depletion, rather that sodium depletion itself, may be a key factor in promoting renal oxidant injury. However, recent studies implicate oxidant injury in angiotensin-induced hypertension [32], and angiotensin-converting enzyme inhibitors can enhance renal antioxidant defenses [33]. Thus, the combined stimulation of the renin-angiotensin system by UUO and sodium depletion in this study may account for the enhanced injury caused by ROS. A similar mechanism has been implicated in experimental cyclosporine nephropathy, in which sodium depletion raises plasma renin activity, TGF-β1, matrix proteins, and interstitial fibrosis [34].

In summary, these experiments have found that sodium depletion increased several renal antioxidant enzymes, consistent with a stress response to increased ROS production. Sodium depletion also increased renal injury due to UUO. Finally, UUO not only reduced renal antioxidant enzyme activities but also inhibited the increased activities seen with sodium depletion. It

is likely that suppression of renal antioxidant enzyme activities by UUO contributes to the progression of renal injury in obstructive nephropathy.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants HL42057, DK45179, and HD28810. This work was presented in abstract form at the Pediatric Academic Societies' 1997 Annual Meeting, May 2–6, 1997, Washington, D.C., USA (*Pediatr Res* 41:280A, 1997).

Reprint requests to Dr. Robert L. Chevalier, Department of Pediatrics, University of Virginia, Health Science Center, Box 386, Charlottesville, Virginia 22908, USA.
E-mail: rlc2m@virginia.edu

APPENDIX

Abbreviations used in this article are: CuZnSOD, copper-zinc superoxide dismutase; GPx, glutathione peroxidase; GST, glutathione-S-transferase; IO, intact opposite; MnSOD, manganese superoxide dismutase; ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances; TGF-β1, transforming growth factor-β1; UUO, unilateral ureteral obstruction.

REFERENCES

1. Chevalier RL, Gomez RA: Obstructive uropathy: Pathophysiology, in *Pediatric Nephrology*, edited by Holliday M, Barratt TM, Avner ED, Baltimore, Williams and Wilkins, 1994, p 994
2. Chevalier RL: Growth factors and apoptosis in neonatal ureteral obstruction. *J Am Soc Nephrol* 7:1098–1105, 1996
3. Chevalier RL: Causes and consequences of neonatal urinary tract obstruction, in *Pediatric Nephrology in Perspective: Current Concepts in Diagnosis and Management*, edited by Strauss J, Miami, University of Miami Press, 1995, p 87
4. Modi KS, Morrissey J, Shah SV, Schreiner GF, Klahr S: Effects of probucol on renal function in rats with bilateral ureteral obstruction. *Kidney Int* 38:843–850, 1990
5. Young MR, Young IS, Johnston SR, Rowlands BJ: Lipid peroxidation assessment of free radical production following release of obstructive uropathy. *J Urol* 156:1828–1832, 1996
6. Sosenko IR, Chen Y, Price LT, Frank L: Failure of premature rabbits to increase lung antioxidant enzyme activities after hyperoxic exposure: Antioxidant enzyme gene expression and pharmacologic intervention with endotoxin and dexamethasone. *Pediatr Res* 37:469–475, 1995
7. Brown KE, Kinter MT, Oberley TD, Freeman ML, Frierson HF, Ridnour LA, Tao Y, Oberley LW, Spitz DR: Enhanced γ-glutamyl transpeptidase expression and selective loss of CuZn superoxide dismutase in hepatic iron overload. *Free Radic Biol Med* 24:545–555, 1998
8. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX: Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral. *Nature* 362:59–62, 1993
9. Paller MS, Hoidal JR, Ferris TF: Oxygen free radicals in ischemic acute renal failure in the rat. *J Clin Invest* 74:1156–1164, 1984
10. Wood KA, Youle RJ: Apoptosis and free radicals. *Ann NY Acad Sci* 738:400–407, 1994
11. Sugiyama H, Kashihara N, Makino H, Yamasaki Y, Ota Z: Reactive oxygen species induce apoptosis in cultured human mesangial cells. *J Am Soc Nephrol* 7:2357–2363, 1996
12. Chevalier RL, Goyal S, Wolstenholme JT, Thornhill BA: Obstructive nephropathy in the neonate is attenuated by epidermal growth factor. *Kidney Int* 54:38–47, 1998
13. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ: Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275, 1951
14. Spitz DR, Oberley LW: An assay for superoxide dismutase activity in mammalian tissue homogenates. *Anal Biochem* 179:8–18, 1989
15. Lawrence RA, Burk RF: Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* 45:841–846, 1976
16. Simons PC, Vander Jagt DL: Purification of glutathione-S-transferases from human liver by glutathione affinity chromatography. *Anal Biochem* 82:334–341, 1977
17. Beers RF, Sizer IW: A spectrophotometric method for measuring the breakdown of H_2O_2 by catalase. *J Biol Chem* 195:133–140, 1952
18. Towbin H, Staehelin T, Gordon J: Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc Natl Acad Sci USA* 76:4350–4354, 1979
19. Spitz DR, Elwell JH, Sun Y, Oberley LW, Oberley TD, Sullivan SJ, Roberts RJ: Oxygen toxicity in control and H_2O_2-resistant Chinese hamster fibroblasts. *Arch Biochem Biophys* 279:249–260, 1990
20. Kinter M, Roberts RJ: Glutathione consumption and glutathione peroxidase inactivation in fibroblast cell lines by 4-hydroxy-2-nonenal. *Free Radic Biol Med* 21:457–462, 1996
21. Sullivan SJ, Oberley TD, Roberts RJ, Spitz DR: A stable O_2-resistant cell line: Role of lipid peroxidation byproducts in O_2-mediated injury. *Am J Physiol* 262:L748–L756, 1992
22. Spitz DR, Adams DT, Sherman CM, Roberts RJ: Mechanisms of cellular resistance to hydrogen peroxide, hyperoxia and 4-hydroxy-2-nonenal toxicity: The significance of increased catalase activity in H_2O_2-resistant fibroblasts. *Arch Biochem Biophys* 292:221–227, 1992
23. Spitz DR, Kinter MT, Roberts RJ: Contribution of increased glutathione content to mechanisms of oxidative stress resistance in hydrogen peroxide resistant hamster fibroblasts. *J Cell Physiol* 165:600–609, 1995
24. Ricardo SD, Ding G, Eufemio M, Diamond JR: Antioxidant expression in experimental hydronephrosis: Role of mechanical stretch and growth factors. *Am J Physiol* 272:F789–F798, 1997
25. Chevalier RL: Counterbalance in functional adaptation to ureteral obstruction during development. *Pediatr Nephrol* 4:442–444, 1990
26. Gobe GC, Axelsen RA: Genesis of renal tubular atrophy in experimental hydronephrosis in the rat: Role of apoptosis. *Lab Invest* 56:273–281, 1987
27. Chevalier RL, Chung KH, Smith CD, Ficenec M, Gomez RA: Renal apoptosis and clusterin following ureteral obstruction: The role of maturation. *J Urol* 156:1474–1479, 1996
28. Chung KH, Gomez RA, Chevalier RL: Regulation of renal growth factors and clusterin by AT1 receptors during neonatal ureteral obstruction. *Am J Physiol* 268:F1117–F1123, 1995
29. Gupta A, Sharma S, Nain CK, Sharma BK, Ganguly NK: Reactive oxygen species-mediated tissue injury in experimental ascending pyelonephritis. *Kidney Int* 49:26–33, 1996
30. Serlachius E, Sundelin B, Eklof A-C, Jahnke M, Laestadius A, Aperia A: Pyelonephritis provokes growth retardation and apoptosis in infant rat renal cortex. *Kidney Int* 51:1855–1862, 1997
31. Yoshioka T, Fogo A, Beckman JK: Reduced activity of antioxidant enzymes underlies contrast media-induced renal injury in volume depletion. *Kidney Int* 41:1008–1015, 1992
32. Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, Harrison DG: Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation* 95:588–593, 1997
33. de Cavanagh EM, Fraga CG, Ferder L, Inserra F: Enalapril and captopril enhance antioxidant defenses in mouse tissues. *Am J Physiol* 272:R514–R518, 1997
34. Shihab FS, Andoh TF, Tanner AM, Bennett WM: Sodium depletion enhances fibrosis and the expression of TGF-β1 and matrix proteins in experimental chronic cyclosporine nephropathy. *Am J Kidney Dis* 30:71–18, 1997