Kidney International, Vol. 62 (2002), pp. 914–921

VASCULAR BIOLOGY – HEMODYNAMICS – HYPERTENSION

Association of renal injury with nitric oxide deficiency in aged SHR: Prevention by hypertension control with AT_1 blockade

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Association of renal injury with nitric oxide deficiency in aged SHR: Prevention by hypertension control with AT_1 blockade.

Background. Aged spontaneously hypertensive rats (SHR) develop end-stage renal disease resembling that of uncontrolled essential hypertension in humans. Nitric oxide (NO) and angiotensin II (Ang II) play an important role in the regulation of blood pressure and the growth of vascular smooth muscle and renal mesangial cells. The relationship between renal NO system, Ang II activity and renal injury in aged SHR is not fully understood.

Methods. The 8-week-old SHR were randomized into losartan-treated (30 mg/kg/day for 55 weeks) and vehicle treated groups. The age-matched Wistar-Kyoto rats (WKY) served as controls. Renal histology and tissue expressions of endothelial and inducible NO synthases (eNOS and iNOS) and nitrotyrosine were examined at 63-weeks of age.

Results. Compared to the WKY group, untreated SHR showed severe hypertension, proteinuria, renal insufficiency, a twofold decrease in renal tissue eNOS and iNOS expressions and massive nitrotyrosine accumulation. This was associated with severe glomerulosclerosis, tubular atrophy and interstitial fibrosis. Losartan therapy normalized blood pressure, prevented proteinuria and renal insufficiency, abrogated the fall in renal eNOS and iNOS protein contents, mitigated renal nitrotyrosine accumulation, and prevented the histological abnormalities found in the untreated SHR.

Conclusions. Aged SHR exhibit severe renal lesions with acquired NO deficiency that are prevented by hypertension control with AT_1 blockade. These findings point to the possible role of NO deficiency in the pathogenesis of renal lesions in aged SHR.

Effective antihypertensive therapy has dramatically reduced the morbidity and mortality from cerebrovascular incidents and ischemic heart disease. However, there has been an unrelenting increase in the numbers of hypertensive patients progressing to end-stage renal disease (ESRD) [1, 2]. In fact, hypertension is the second most common cause of chronic renal failure in the developed world and represents the primary risk factor for progression of renal disease [3, 4]. The spontaneously hypertensive rats (SHR) have been an excellent animal model for genetic hypertension that bears a resemblance to that of essential hypertension in humans [5]. The adult SHR develop severe hypertension with relatively normal cardiac and renal functions. However, with aging SHR exhibit marked proteinuria and severe glomerulosclerosis [5–8]. Inhibition of angiotensin converting enzyme (ACEi) has been shown to limit glomerular injury in aged SHR, indicating the crucial role of renin-angiotensin system in this model [8].

Nitric oxide (NO) plays a major role in regulation of vascular resistance and arterial blood pressure [9]. In addition, NO can inhibit the growth of vascular smooth muscle, mesangial cells and reduce production of extracellular matrix [10, 11]. NO is synthesized from L-arginine by a family of enzymes known as NO synthase (NOS), which include constitutive and inducible isoforms [9]. We [12] and others [13–17] have recently demonstrated an up-regulated NOS expression in young SHR both before and after the onset of hypertension. However, available data on the L-arginine:NO system in the aged SHR are very scant. In their previous study, Cuevas et al showed a dramatic decline in the percentage of endothelial cells with detectable endothelial NOS (eNOS) on histochemical examination of thoracic aorta in aged SHR [18]. Recently, Chou et al demonstrated a decreased eNOS accompanied by increased inducible NOS (iNOS) protein expression in aorta of aged SHR [19]. However, the NO system in renal tissue was not investigated in those studies.

Several recent studies have provided evidence for enhanced reactive oxygen species (ROS) activity in young SHR [20–22]. Oxidative stress can potentially contribute to generation and maintenance of hypertension via inactivation of NO, among others [20, 21]. In this regard, interaction of NO with ROS leads to generation of highly

Key words: spontaneously hypertensive rats, blood pressure, nitric oxide synthase, nitrotyrosine, essential hypertension, losartan, end-stage renal disease.

Received for publication January 31, 2002
and in revised form March 26, 2002
Accepted for publication April 10, 2002

reactive and cytotoxic byproducts, such as peroxynitrite, which can, in turn, react with free tyrosine and tyrosine residues in protein molecules to produce nitrotyrosine [23]. In fact, nitrotyrosine accumulation in the thoracic aorta has been shown in adult stoke-prone SHR [22]. However, information on aged SHR is lacking.

The present study was undertaken to investigate the relationship between renal NOS and nitrotyrosine expressions and renal injury in aged SHR. In addition, it is known that both angiotensin II (Ang II) and NO are synthesized and released in the kidney, and the antagonistic interaction of these two agents is an important determinant of histopathological events in the kidney [5, 24]. Therefore, we sought to investigate the effects of long-term Ang II subtype 1 (AT_1) receptor blockade with losartan, on renal histology and the NO system.

METHODS

Animal models

Eight-week-old male SHR and Wistar-Kyoto rats (WKY) were purchased from Harlan Sprague, Inc. (Indianapolis, IN, USA). The animals were housed in a climate-controlled and light-regulated room (12 hour light/12 hour dark cycle). Food and water were available ad libitum. The SHR were randomly divided into losartan treated (30 mg/kg/day) and vehicle treated groups. Age-matched WKY rats served as the controls. The selection of losartan dosage was based on earlier studies demonstrating optimal blood pressure control with the chosen dosage in this model [25]. The animals were carefully maintained and monitored for 55 weeks. Tail arterial blood pressure was determined using a tail sphygmomanometer (Harvard Apparatus, Natick, MA, USA) every month. At the conclusion of the study, animals (63-weeks-old) were placed in metabolic cages for a 24-hour urine collection. The urine samples were collected in sterilized containers that were chilled over ice and stored at −70°C until assayed. The animals were then sacrificed by exsanguination using cardiac puncture between the hours of 9 AM and 11 AM. The plasma and kidney were harvested immediately and appropriately stored until processed.

Histologic examination

Six animals in each group were exsanguinated for morphological studies. Immediately after exsanguination, the kidneys were removed and promptly sectioned and separately post-fixed in 10% buffered formalin. The fixed tissue was embedded in paraffin and sectioned. The sections were stained with Masson's trichrome, periodic acid-Schiff (PAS), and hematoxylin and eosin (H&E).

Glomerular and arteriolar injuries were scored semiquantitatively as described previously [8]. For the evaluation of glomerular injury score, approximately 50 subcapsular and 50 juxtamedullary glomeruli from each specimen were examined using the sections stained with PAS. Each glomerulus was graded as follows: grade 0 = normal glomerulus by light microscopy; grade 1 = involvement of up to one third of the glomerular area; grade 2 = involvement of one to two thirds of the glomerulus; and grade 3 = two thirds to global sclerosis. Each score was then calculated according to the formula: Glomerular Injury Score = [(1 × number of grade 1 glomeruli) + (2 × number of grade 2 glomeruli) + (3 × number of grade 3 glomeruli)] × 100/(number of glomeruli observed).

To evaluate the arteriolar injury score, 40 to 50 afferent arterioles were examined from each specimen and were graded as follows: grade 0 = no arteriolar changes; grade 1 = hyalinosis of the arteriolar wall up to 50% of its circumference; grade 2 = 50 to 100% hyalinosis of the wall circumference but without luminal narrowing; and grade 3 = complete hyalinosis of the wall with luminal encroachment. Each score was then calculated according to the formula: Arteriolar Injury Score = [(1 × number of grade 1 arterioles) + (2 × number of grade 2 arterioles) + (3 × number of grade 3 arterioles)] × 100/(number of afferent arterioles observed). The total injury score was then calculated by adding the glomerular and arteriolar injury scores of each rat.

Measurements of plasma and urine NO_2^-/NO_3^- (NOx)

The amount of total nitrate and nitrite in the test samples was determined as described earlier [26–29] using the purge system of a Sievers Instruments Inc., (Boulder, CO, USA).

Immunoperoxidase studies

Immunohistochemical staining for eNOS, iNOS and nitrotyrosine was performed using a standardized streptavidin-biotin-peroxidase method on formalin-fixed paraffin embedded renal tissues as described earlier [27–29]. Except for the incubation with the primary antibodies, all incubations were at room temperature and were separated by washes with phosphate-buffered saline (PBS). Microwave heat-induced antigen retrieval in citrate buffer, pH 6.0, was required for optimal staining with all antibodies. After deparaffinization, sections were treated with 3% hydrogen peroxide to block endogenous peroxidase activity. After preincubation with 10% normal horse or swine serum for 20 minutes, sections were incubated with primary antibodies overnight at 4°C, followed sequentially with biotinylated horse anti-mouse (Vector Laboratories, Burlingame, CA, USA) or biotinylated swine anti-rabbit (Dako Corporation, Carpinteria, CA, USA) antibodies for 20 minutes, and streptavidin-peroxidase complex (Dako) for 30 minutes. The working concentrations for the mouse anti-eNOS monoclonal antibody (Transduction Laboratories, Lexington, KY, USA), rabbit anti-iNOS antibody (Transduction) and rabbit anti-nitrotyro-

Table 1. Blood pressure, urinary protein excretion and creatinine clearance in the study groups

	Blood pressure *mm Hg*	Urinary protein *mg/24 h*	C_{Cr} *mL/min*
SHR	220 ± 5[a]	75 ± 2.0[a]	1.7 ± 0.2[a]
SHR+L	132 ± 5	37 ± 2.3	3.3 ± 0.2
WKY	130 ± 3	34 ± 1.8	3.5 ± 0.1

Data are mean ± SE. $N = 6$ in each group.
[a] $P < 0.05$ vs. other groups

sine antibody (Upstate Biotechnology, Inc., Lake Placid, NY, USA) were 0.1 μg/mL, 0.05 μg /mL and 5.9 μg /mL, respectively. For negative controls a monoclonal mouse IgG1 (Bethyl Laboratories Inc., Montgomery, TX, USA) or normal rabbit serum was used at equivalent concentrations. Diaminobenzidine (Sigma Chemical, St. Louis, MO, USA) was used as the chromogen and hematoxylin was used for the nuclear counterstain.

Tissue preparation and Western blot analysis

Rats were sacrificed by decapitation, and one kidney from each rat was immediately excised, cleaned with PBS, frozen in liquid nitrogen and stored at −75°C. Tissue was homogenized in five volumes of lysis buffer (1% SDS, 1.0 mmol/L sodium vanadate, 10 mmol/L Tris-HCl, pH 7.4) containing 10 μmol/L pepstatin, 13 μmol/L leupeptin, and 1 μmol/L phenylmethylsulfonyl fluoride (PMSF). Homogenates were centrifuged at 12,000 × *g* for five minutes at 4°C to remove tissue debris without precipitating plasma membrane fragments. Protein concentration was determined by using a Bio-Rad kit (Bio-Rad Laboratories, Hercules, CA, USA).

Western blot analysis was carried out to determine the endothelial (eNOS) and inducible (iNOS) nitric oxide synthase protein mass as previously described [26, 27, 29]. Anti-eNOS monoclonal antibody, peroxidase-conjugated goat anti-mouse IgG antibody, anti-iNOS human endothelial positive control, and mouse macrophage positive control were supplied by Transduction Laboratories. Briefly, kidney tissue preparations were size-fractionated on 4 to 12% Tris-glycine gel (Novex, San Diego, CA, USA) at 120 V for three hours. In preliminary experiments we found that the given protein concentrations were within the linear range of detection for our Western blot technique. After electrophoresis, proteins were transferred onto a hybond-ECL membrane (Amersham Life Science Inc., Arlington Heights, IL, USA) at 400 mA for two hours using the Novex transfer system. The membrane was prehybridized in 10 mL of buffer A (10 mmol/L Tris hydrochloride, pH 7.5, 100 mmol/L NaCl, 0.1% Tween 20 and 10% nonfat milk powder) for one hour and then hybridized for an additional one hour period in the same buffer containing 10 μL of the given anti-NOS monoclonal antibody (1:1000). The membrane was then washed for 30 minutes in a shaking bath, changing the wash buffer (buffer A without nonfat milk) every five minutes prior to one hour of incubation in buffer A plus goat anti-mouse IgG-HRP (horse radish peroxidase) at the final titer of 1:1000. Experiments were carried out at room temperature. The washes were repeated before the membrane was developed with a light emitting non-radioactive method using ECL reagent (Amersham Inc., Arlington Heights, IL, USA). The membrane was then subjected to autoluminography for one to five minutes. The autoluminographs were scanned with a laser densitometer (Model PD1211; Molecular Dynamics, Sunnyvale, CA, USA) to determine the relative optical densities of the bands. In all instances, the membranes were stained with Ponceau stain, which verified the uniformity of protein load and transfer efficiency across the test samples.

Data presentation and analysis

Data are presented as means ± SE. Analysis of variance (ANOVA) was used for data analysis. $P \leq 0.05$ was considered statistically significant.

RESULTS

General data

Data are shown in Table 1. The blood pressure value in the untreated SHR at age 63 weeks was significantly higher than that found in the age-matched WKY group. As expected, the aged untreated SHR showed a significant rise in urinary protein excretion and a significant decline in creatinine clearance. Losartan therapy controlled hypertension, prevented proteinuria and renal insufficiency in aged SHR.

Urinary NOx excretion

Figure 1 shows that the urinary excretion of NOx was significantly lower in untreated SHR than in the WKY group. AT_1 receptor blockade normalized the urinary NOx excretion rate in aged SHR.

eNOS and iNOS proteins

The untreated SHR group showed a significant decrease in renal tissue eNOS and iNOS protein expressions when compared with age-matched WKY rats (Fig. 2). Losartan therapy abrogated the fall in eNOS and iNOS protein contents in SHR.

Renal morphology, glomerular and arteriolar injury scores

The morphological appearance of glomeruli, arterioles and tubulointerstitium remained normal in the WKY group (Fig. 3A) compared with the untreated SHR (Fig. 3B). Kidneys in the untreated SHR disclosed segmental and global glomerulosclerosis, and there was focal tubular atrophy, interstitial fibrosis and patchy lymphocytic infiltrate.

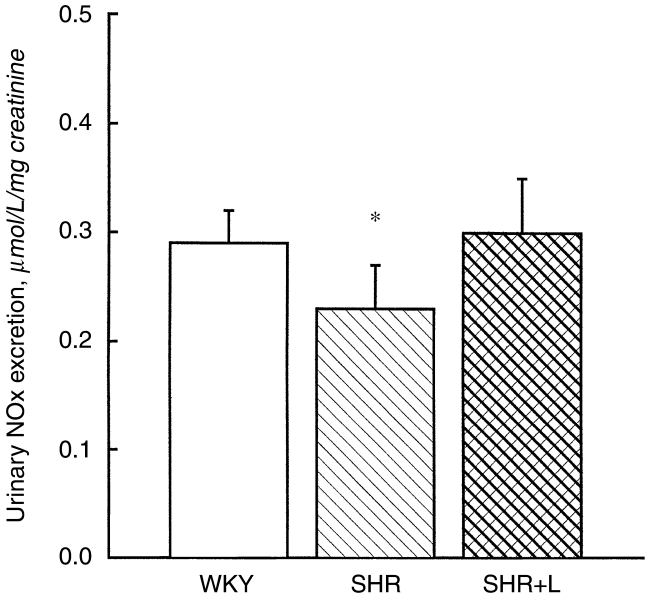

Fig. 1. Urinary excretion of NO metabolites, nitrates and nitrites (NOx), in Wistar-Kyoto rats (WKY), untreated spontaneously hypertensive rats (SHR), and losartan-treated SHR (SHR-L). Values are mean ± SEM. $N = 6$ in each group. $^*P < 0.05$ vs. other groups.

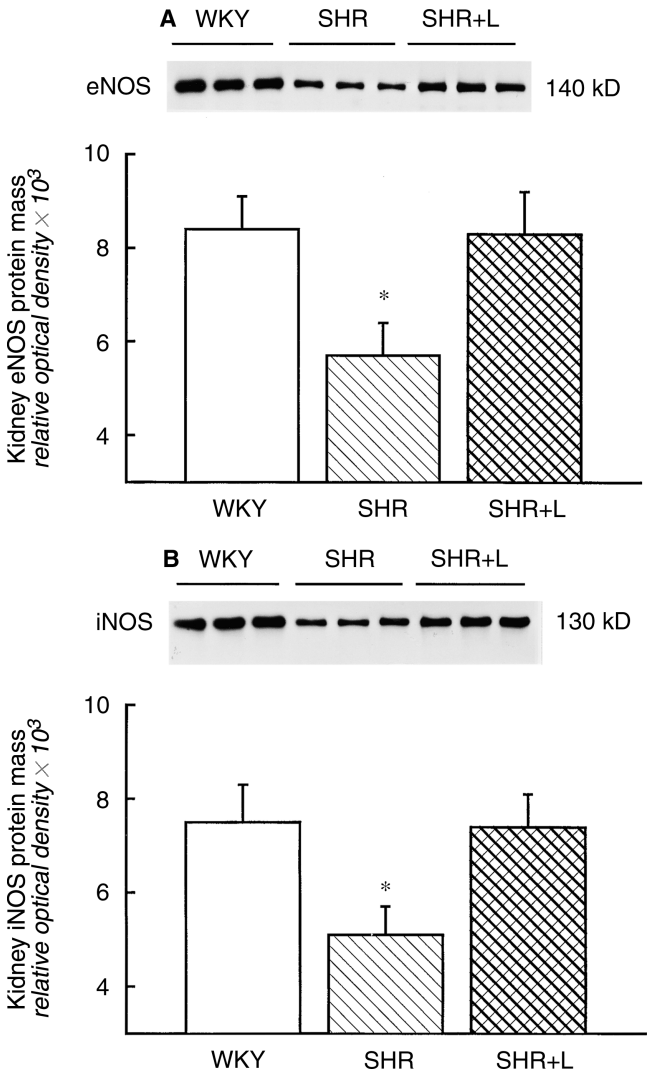

Fig. 2. Representative Western blot and the corresponding group data depicting renal eNOS (*A*) and iNOS (*B*) protein abundance in Wistar-Kyoto rats (WKY), untreated spontaneously hypertensive rats (SHR), and losartan-treated SHR (SHR-L). $N = 6$ in each group. $^*P < 0.05$ vs. other groups.

Some tubules were dilated with proteinaceous casts. The small arteries showed marked intimal fibrosis and muscular hypertrophy. The afferent arterioles demonstrated hyalinosis with luminal encroachment. AT_1 receptor blockade prevented these lesions in aged SHR (Fig. 3C).

The glomerular and arteriolar injury scores are shown in Table 2. The glomerular damage of the subcapsular region was mild and the arteriolar and glomerular changes were more severe in the juxtamedullary area.

Immunohistologic studies for NOS

Immunostaining of eNOS was similar between the rats in WKY (Fig. 4A) and losartan treated SHR (Fig. 4C) groups. There was strong positive staining of endothelial and occasional smooth muscle cells in the small arteries and arterioles. Many proximal tubules, collecting ducts, medullary thick ascending limb and vasa recta bundles as well as scattered glomerular cells were also positive. The rats in the untreated SHR group revealed eNOS distributions similar to those in the other two groups (Fig. 4B). However, there was less intensity of endothelial staining in the small arteries and arterioles. In addition, the damaged renal tubules and sclerotic glomeruli displayed marked attenuation or lack of eNOS signals. Figure 4D represents a negative control for the immunohistochemical stains.

Animals in all the three groups showed similar distributions and intensities for iNOS. The signals were localized in the scattered glomerular cells, proximal tubules, collecting ducts, scattered endothelial and smooth muscle cells of small arteries and peritubular capillaries. However, the amounts of iNOS signal were much less or negligible in the injured tubules and sclerotic glomeruli in the untreated SHR group.

Immunoperoxidase studies for nitrotyrosine

In WKY (Fig. 5A) and losartan-treated SHR (Fig. 5C), moderate nitrotyrosine expression was seen in the proximal and distal tubules of the renal cortex. The S3 segments of the proximal tubules and collecting ducts in the inner stripe of the outer medulla (ISOM) and in the inner medulla (IM) were moderately positive for nitrotyrosine. Mild-to-moderate smooth muscle staining in some of the arterial and arteriolar walls was observed. In addition, there was scattered and weak staining in

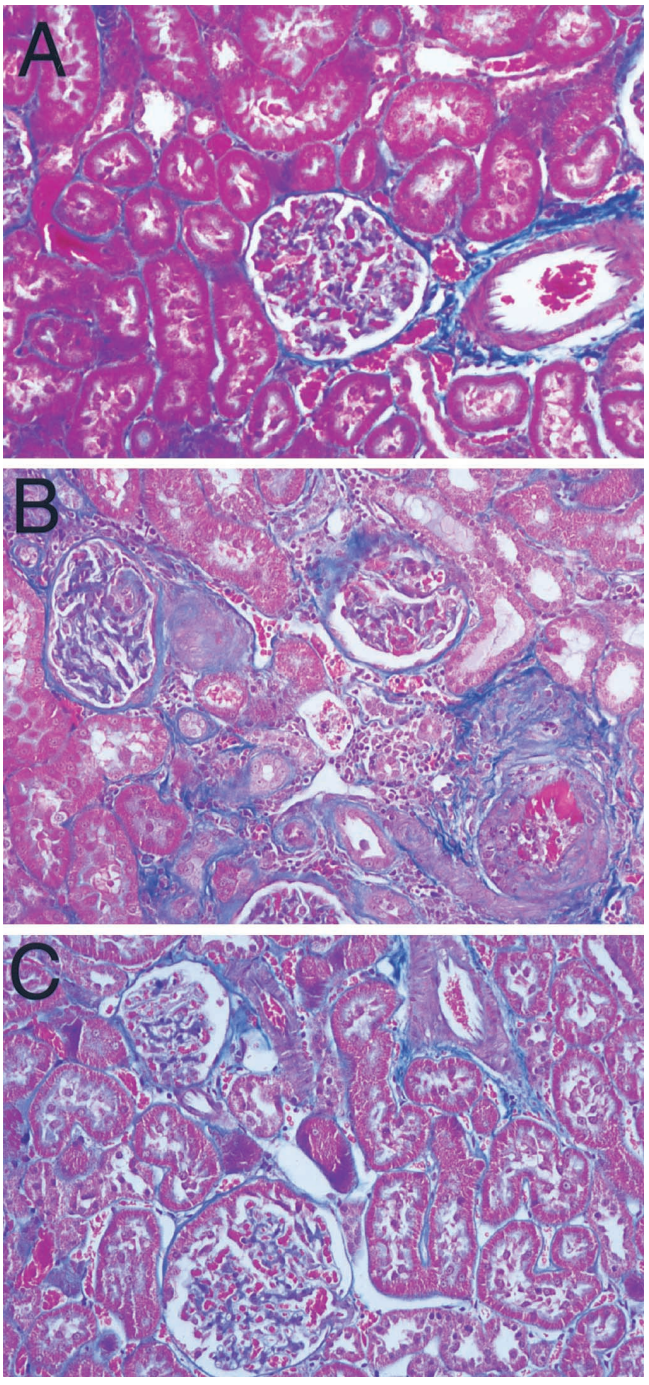

Fig. 3. Light microphotographs show juxtamedullary lesions in 63-week-old Wistar-Kyoto rats (WKY, *A*), untreated spontaneously hypertensive rats (SHR, *B*) and losartan-treated SHR (SHR-L, *C*). Normotensive WKY reveal no significant pathologic findings, while the untreated SHR demonstrate severe nephrosclerosis, focal tubular atrophy and interstitial fibrosis with patchy lymphocytic infiltrates. An interlobular artery discloses marked hyalinosis and narrowed lumen. The losartan treated SHR display few pathologic findings.

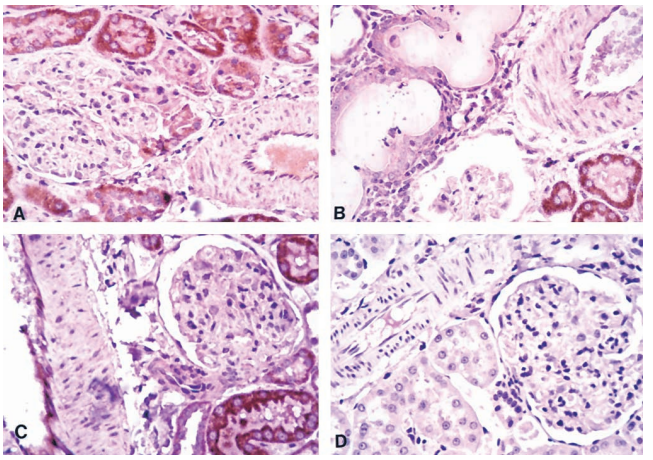

Fig. 4. Tissue expression of endothelial nitric oxide synthase (eNOS) in the kidneys of 63-week-old Wistar-Kyoto rats (WKY, *A*), untreated spontaneously hypertensive rats (SHR, *B*) and losartan-treated SHR (SHR-L, *C*). (A) Note strong endothelial and weak/patchy smooth muscle eNOS positivity in a small artery. The proximal epithelial cells are strongly positive. Positive eNOS stain also is seen in occasional glomerular cells. (B) In contrast, eNOS expression of the arterial endothelial cells is less intense in the SHR. Note the negligible eNOS signals in the damaged renal tubules and sclerotic glomerulus. (C) The SHR-L group show similar eNOS distributions and intensities when compared with WKY group. (*D*) Negative control for the immunohistochemical stains.

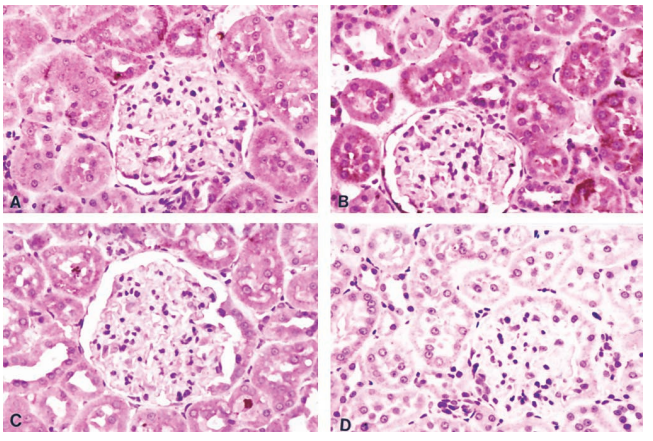

Fig. 5. Tissue expression of nitrotyrosine in the kidney of 63-week-old Wistar-Kyoto rats (WKY, *A*), untreated spontaneously hypertensive rats (SHR, *B*) and losartan-treated SHR (SHR-L, *C*). (A) Focal mild-to-moderate positivities are shown in scattered tubules. (B) Diffuse strong positivities are seen in the cortical tubules. The glomerulus reveals positive staining in occasional cells. (C) The SHR-L group shows a similar nitrotyrosine distribution and intensity when compared with WKY group. (*D*) Negative control for the immunohistochemical stains.

glomerular visceral epithelial and endothelial cells. The glomerular mesangial cells were essentially negative.

In contrast, the untreated SHR (Fig. 5B) showed diffuse strong nitrotyrosine expression in the renal cortex in virtually all of the proximal and distal tubules. The S3 segments of the proximal tubules and the collecting ducts in the ISOM and IM were strongly positive. The smooth muscle cells of the arteries and the arterioles and the endothelial cells demonstrated moderate staining. The glomeruli revealed weak positive staining in scat-

Table 2. Glomerular and arteriolar injury scores

Scores	WKY	SHR	SHR-L
Glomerular injury score (GIS)			
Subcapsular lesion	2.9 ± 0.8	3.6 ± 0.6	3.1 ± 0.4
Juxtamedullary lesion	4.3 ± 0.9	68.8 ± 9.2[a]	6.2 ± 1.6
Arteriolar injury score (AIS)	8.0 ± 1.5	78.5 ± 10.6[a]	7.7 ± 2.0
Total (GIS+AIS)	16.4 ± 3.7	150.9 ± 20.0[a]	17.2 ± 2.1

Abbreviations are: WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; SHR-L, SHR treated with losartan. Data are mean ± SE. $N = 6$ in each group.

[a] $P < 0.05$ vs. other groups

tered visceral epithelial cells, mesangial cells and occasional endothelial cells. Figure 5D represents the negative control for immunohistochemical stains.

Correlations

Total renal injury scores were inversely related to the urinary NOx excretion rate ($r = -0.69$, $P < 0.05$). Likewise, renal injury scores were negatively related to renal tissue eNOS ($r = -0.88$, $P < 0.05$) and iNOS ($r = -0.89$, $P < 0.05$) protein contents.

DISCUSSION

Data on the status of the NO system in SHR are contradictory and very sparse in aged SHR; in young SHR, both increased [12–17] and decreased [30–33] L-arginine/NO pathway activities have been reported by different investigators. We recently demonstrated an enhanced total body NO production and increased vascular and renal NOS protein expression in young SHR both before and after the onset of hypertension [12, 21]. Based on these observations, we believe that development of hypertension is not due to a primary impairment of NO production in SHR. The present study shows a markedly reduced urinary NOx excretion in aged SHR when compared with age-matched WKY rats. This reduction in urinary NOx excretion in aged SHR may be due to depressed NO production. In this regard, there was a markedly reduced renal eNOS and iNOS protein content in untreated aged SHR. The decline in eNOS protein corresponded to reduced eNOS immunoreactivity in endothelial cells of small arteries and damaged tubules and glomeruli. The decline in iNOS protein content was largely due to the loss of renal parenchyma in SHR. It should be noted that the results of the present study do not contradict our earlier study in young SHR. Instead, the data most likely represent different points in the natural course of progressive hypertension and vasculopathy in this model. In fact, we obtained serial measurements of urinary NOx excretion and found the values to be markedly increased early in the course and begin to steadily decline after approximately six months of age to the levels below that in the normal controls (data not shown). Thus, during the advanced phase of hypertension with progressive vasculopathy, endothelial dysfunction and loss of renal parenchyma, NOS expression and NO production was diminished, leading to true NO deficiency in aged SHR. The resulting NO deficiency in turn can contribute to the worsening of hypertension and associated end-organ damage, thus forming a vicious cycle.

The reduction in urinary NOx excretion also could be due to increased NO sequestration in aged SHR. This supposition was supported by detection of massive and diffuse tissue accumulation of nitrotyrosine, which is the footprint of NO/ROS interaction, in the aged SHR. Interaction of ROS, particularly superoxide with NO, leads to production of peroxynitrite, which is a highly cytotoxic reactive compound. In turn, peroxynitrite reacts with DNA, lipid and protein molecules. For instance, peroxynitrite reacts with the tyrosine residues in various proteins producing nitrotyrosine. Alternatively, ROS can initially activate tyrosine residues to produce tyrosyl radicals that then are able to oxidize NO to produce nitrotyrosine. In addition, nitrotyrosine can be formed from the interaction of tyrosine with other reactive species [34]. However, the contribution of the latter reactions to total tissue nitrotyrosine abundance is limited and, as such, nitrotyrosine abundance is largely a function of ROS interaction with NO [35]. Accordingly, the observed accumulation of nitrotyrosine in the aged SHR was necessarily indicative of inactivation and sequestration of NO by ROS. Hence, in addition to a reduced NO production in aging SHR, increased ROS-mediated NO inactivation further reduced the NO bioavailability. Notably, the enhanced ROS-mediated NO inactivation and protein nitration shown here in animals with genetic hypertension has been shown by our group to occur in several models of acquired hypertension, including hypertension induced by chronic lead exposure and chronic renal insufficiency [36, 37].

Recent animal studies suggest that in hypertension, NOS activity is linked with end-organ disease [17, 24]. For instance, increased NOS activity in young SHR was accompanied by minimal glomerular and tubulointerstitial disease as well as minimal urinary protein excretion. However, the age-matched hypertensive Dahl salt-sensitive rats (DSSR) exhibiting reduced renal NOS activity had severe glomerular injury, marked tubulointerstitial disease and heavy proteinuria [17, 24]. Occurrence of severe nephropathy in the young DSSR but not the age-matched SHR, which had hypertension of comparable severity and duration but divergent NOS activity, points to the possible renal protective action of NO. In our present study, aged SHR exhibited significant renal damage characterized by glomerulosclerosis, focal tubular atrophy, interstitial fibrosis and marked arteriolopathy. These morphological changes were accompanied by a significant fall in urinary NO metabolite (that is, total

nitrate and nitrite) excretion, markedly depressed eNOS and iNOS protein expression, and massive nitrotyrosine accumulation in the kidney. Thus, it appears that the compensatory up-regulation of NO system in the young SHR retarded renal injury. However, persistent hypertension eventually resulted in a fall of NO production capacity that, coupled with ROS-mediated NO inactivation, led to progressive nephropathy in aged SHR. Administration of the AT_1 receptor blocker losartan preserved renal eNOS and iNOS protein expression, and mitigated nitrotyrosine accumulation in aged SHR. In addition, losartan therapy prevented renal injury in aged SHR. It is of note that NADPH oxidase is thought to be a major source of ROS in the vascular tissue [38] and that this enzyme is up-regulated by both Ang II and cyclical stretch [39, 40]. Consequently, the use of AT_1 receptor antagonist most likely serves a dual function, namely, a reduction in blood pressure through inhibition of Ang II as well as alleviation of oxidative stress via reduction in superoxide production. Thus, blockade of Ang II action and amelioration of hypertension (hence, cyclical stretch) by an AT_1 blocker can attenuate oxidative stress, ROS-NO interaction, and tyrosine nitration, as was seen in the losartan-treated aging SHR. In addition, we also clearly demonstrated a negative correlation between urinary NOx excretion and the severity of renal injuries in the study groups. Likewise, renal eNOS and iNOS protein contents were negatively related to renal injury scores. Taken together, this evidence supports the notion that renal injuries in aged SHR are associated with and may be mediated, at least in part, by NO deficiency in the renal microenvironment.

The mechanisms of renal injury associated with NO deficiency are not entirely clear and a number of factors may be involved. For instance, in SHR reduced regional NO availability can further aggravate glomerular hypertension, which has been incriminated in the pathogenesis of glomerular sclerotic injury in a variety of glomerular diseases [41]. In addition, the diminished inhibitory action of NO on mesangial cell proliferation and matrix production [10, 11] may promote mesangial cell proliferation and matrix accumulation, and lead to glomerulosclerosis. In this regard, chronic NOS inhibition has been shown to cause glomerular, tubular and interstitial injury [42]. Finally, as the major endogenous antagonist of the vascular action of Ang II and other vasoconstrictors such as endothelin, a reduction in NO availability may lead to an enhanced Ang II (as well as endothelin)-mediated vasoconstriction, mesangial cell proliferation and matrix accumulation [24]. These unopposed actions of Ang II may play a major role in the occurrence of hypertensive nephropathy. Indeed, we have clearly demonstrated that AT_1 blockade completely prevented NO deficiency and renal injuries associated with aging in SHR.

It is known that aging is characterized by decreased endothelium-dependent relaxation mediated by NO in humans [43]. In addition, Cuevas et al have shown a dramatic decline in the percentage of endothelial cells with detectable eNOS on histochemical examination of thoracic aorta in aged SHR [18]. In contrast, age-matched normotensive WKY studied by these investigators showed sufficient immunostainable eNOS of thoracic aorta endothelial cells. Similar results were found by Chou et al in aged SHR [19]. However, these previous studies did not investigate the NOS expression in the kidney. To our knowledge, ours is the first study to demonstrate a marked decline in renal eNOS and iNOS protein expression in aged SHR.

In conclusion, aged SHR exhibited severe renal lesions with a marked reduction in urinary NOx excretion, significant decline in renal NOS protein abundance, and massive nitrotyrosine accumulation when compared with age-matched WKY rats. AT_1 blockade prevented renal injuries, preserved renal tissue NOS protein expressions, mitigated nitrotyrosine accumulation, and normalized urinary NOx excretion in aged SHR. These findings point to the possible contribution of acquired NO deficiency to the pathogenesis of hypertensive nephropathy with aging in this model.

ACKNOWLEDGMENTS

The authors thank Ms. B. Wortham and Ms. C. Rodriguez for excellent secretarial assistance. J. Zhang is currently employed by Affiliated Hospital of Jining Medical College, Jining, Shandong, China.

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