Enhanced oxidative stress in kidneys of salt-sensitive hypertension: role of sensory nerves

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Wang, Youping, Alex F. Chen, and Donna H. Wang. Enhanced oxidative stress in kidneys of salt-sensitive hypertension: role of sensory nerves. Am J Physiol Heart Circ Physiol 291: H3136–H3143, 2006. First published August 18, 2006; doi:10.1152/ajpheart.00529.2006.—To determine the mechanism(s) underlying enhanced oxidative stress in kidneys of salt-sensitive hypertension, neonatal Wistar rats were given vehicle or capsaicin (CAP, 50 mg/kg sc) on the first and second days of life. After being weaned, male rats were assigned into four groups and treated for 2 wk with the following: vehicle + a normal sodium diet (NS, 0.4%, CON-NS), vehicle + a high-sodium diet (HS, 4%, CON-HS), CAP + NS (CAP-NS), and CAP + HS (CAP-HS). Systolic blood pressure was significantly increased in CAP-HS but not CAP-NS or CON-HS rats (P < 0.05). Enhanced oxidative stress in the kidney in salt-sensitive hypertensive rats (6, 21–23). Scavenging of O$_2^·$ with the membrane-permeable superoxide dismutase (SOD) mimic Tempol or antioxidant therapy with vitamin E has been shown to reduce blood pressure in various models of hypertension (20, 38), especially in those with increased salt sensitivity (20, 30).

There are common traits in salt-sensitive humans and rats, which include progressive increases in blood pressure, endothelial dysfunction, renal damage, and increased O$_2^·$ release. ROS may modulate renal hemodynamics and function both directly, by causing vasoconstriction and sodium reabsorption, and indirectly, by inducing renal inflammation. Accumulating evidence has shown that an increased oxidative stress occurs in Dahl salt-sensitive rats on high sodium intake (20, 21). Moreover, antioxidant therapy with vitamin E amelioriates renal dysfunction in Dahl salt-sensitive rats (30), indicating that an increased oxidative stress may contribute to renal damage in salt-sensitive hypertension.

It is well established that the cardiovascular system and kidney are innervated by sensory nerve terminals that contain various neuropeptides, e.g., calcitonin gene-related peptide (CGRP) and substance P (42). These neuropeptides may affect various neuropeptides, e.g., calcitonin gene-related peptide (CGRP) and substance P (42). These neuropeptides may affect ROS production and the antioxidant defense system determines the degree of oxidative stress. Oxidative stress has been suggested to be involved in the pathophysiology of hypertension, renal injury, diabetes, and atherosclerosis (12, 22). Overproduction of ROS has been found in many hypertensive models, including those of angiotensin II infusion, mineralcorticoid implantation, spontaneously hypertensive rats, and Dahl salt-sensitive hypertensive rats (6, 21–23). Scavenging of O$_2^·$ with the membrane-permeable superoxide dismutase (SOD) mimic Tempol or antioxidant therapy with vitamin E has been shown to reduce blood pressure in various models of hypertension (20, 38), especially in those with increased salt sensitivity (20, 30).

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It is well established that the cardiovascular system and kidney are innervated by sensory nerve terminals that contain various neuropeptides, e.g., calcitonin gene-related peptide (CGRP) and substance P (42). These neuropeptides may affect blood pressure via modulating cardiovascular and renal function. CGRP and substance P are released from sensory nerve terminals upon activation of the transient receptor potential vanilloid type 1 (TRPV1) channels by capsaicin (CAP), a pungent ingredient of chili peppers (42). In 1- to 2-day-old neonates before sensory nerves are fully developed, CAP administered at 50 mg/kg subcutaneously leads to permanent impairment and degeneration of sensory nerves (13, 33). We have shown that sensory nerve degeneration by neonatal capsaicin treatment renders a rat sensitive to a salt load with a significant and sustained increase in blood pressure, suggesting that sensory nerves play a counterregulatory role in preventing salt-induced increases in blood pressure (33). Moreover, several studies have provided direct and indirect evidence showing that CGRP has an inhibitory effect on ROS production in tissues of deoxycorticosterone acetate-salt hypertensive rats and in neutrophils from human (3, 29).

Despite of the fact that oxidative stress is an important contributor to renal dysfunction in salt-sensitive hypertensive rats, the mechanisms responsible for enhanced production of oxidative stress in the kidney in salt-sensitive hypertensive rats remain to be elucidated. This study was designed to test the hypothesis that sensory nerves regulate intrarenal O$_2^·$ levels during high salt intake. The results should assist in defining the mechanisms underlying increased oxidative stress in the kid-

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ney in salt-sensitive hypertensive rats subjected to sensory nerve degeneration.

METHODS

Animal preparation. All experimental protocols were approved by the Institutional Animals Care and Use Committee. Pregnant Wistar female rats (Charles River Laboratories, Wilmington, MA) were housed in the animal facility 1 wk before parturition. On days 1 and 2 of life, neonatal rats received CAP (50 mg/kg) subcutaneously as described previously (34). Control rats were treated with vehicle (5% ethanol, 5% Tween 80 in normal saline). After being weaned, male rats (3 wk old) were assigned into four groups and subjected to different sodium diets for 2 wk: control + normal sodium diet (0.4%, CON-NS, n = 7), control + high-sodium diet (4%, CON-HS, n = 8), CAP + NS (CAP-NS, n = 8), and CAP + HS (CAP-HS, n = 8). The rat food was purchased from Harlan Teklad Diet.

Systolic blood pressure. Indirect tail-cuff systolic blood pressure was obtained in conscious rats with the use of a Narco Bio-Systems electrophygmonomanometer (Austin, TX). The pressure was measured 2 days before dietary treatment (day 0) and 6 and 12 days after the treatment. Systolic blood pressure for each rat was calculated as the average of three separated measurements at each session.

Collecting samples. After blood pressure measurements were completed, rats were housed individually in metabolic cages 1 day before urine collection for adaptation. Twenty-four-hour urine samples were collected for creatinine and 8-isoprostaglandin F2α (8-isoprostane) assay on day 14 after the treatment. After decapitation of the rats, kidney and plasma samples were harvested at the end of the 2-wk treatment period and stored at −80°C for biochemical assay and Western blot analysis. Fresh renal cortex and medulla were collected for O2•− assay as described below.

O2•− assay. O2•− production in renal cortical and medullary tissues was measured by lucigenin-enhanced chemiluminescence (ECL) assay as described previously (2, 7). Lucigenin (final concentration 5 μM) was used to determine the O2•− generation. The validity of 5 μM lucigenin for measuring O2•− production has been confirmed with electron-spin resonance methods (18). Rats were anesthetized with pentobarbital sodium (60 mg/kg) intraperitoneally. The kidneys were perfused with ice-cold modified Krebs-HEPES as described below. After removal and decapsulation, the renal cortex and medulla were separated on ice and cut into pieces (∼3 × 3 × 2 mm). Tissue samples were incubated at 37°C for 30 min in 1 ml modified Krebs-HEPES buffer containing (in mM) 119 NaCl, 20 HEPES, 4.6 KCl, 1.0 MgSO4, 0.15 Na2HPO4, 0.4 KH2PO4, 5 NaHCO3, 1.2 CaCl2, and 5.5 glucose (pH 7.4) in the presence of diethyldithiocarbamate (DDC, 10 μM). Kidney tissue was transferred to a polypropylene tube containing 5 μM lucigenin in 1 ml modified Krebs-HEPES buffer and incubated at 37°C for 10 min in the dark. After incubation, the tubes were placed in a luminometer chamber (TD-20/20, Turner Designs, Sunnyvale, CA), which were maintained at 37°C. The average of 10 consecutive 30-s measurements was recorded for each sample. The cell-permeant O2•− scavenger Tiron (10 mM) was added to the sample, and 20 more consecutive 30-s measurements were made with the last 8 value averaged. The samples were dried overnight and weighed. Measurements were normalized to dry tissue weight. The remaining tissue samples were incubated with 0.1 mM apocynin, a selective NAD(P)H oxidase inhibitor, to examine the potential role of NAD(P)H oxidase in O2•− production. Data were expressed as the change in the rate of luminescence per minute per milligram of dry tissue of values before and after Tiron and were converted to O2•− contents (in nmol·min·mg dry wt−1).

8-Isoprostane assay. Plasma and urine 8-isoprostane levels were analyzed by enzyme immunoassay using the kit provided by Cayman Chemical. Purification and extraction of free 8-isoprostane were performed on a column provided with the kit, and the samples were dried using a speed vacuum. The samples were reconstituted into assay buffer. For the assay, standards and samples were added in triplicate to the 96-well plate provided with the kit, followed by addition of tracer and antibody and incubation for 18 h at room temperature. After incubation, the plate was washed several times with wash buffer, followed by addition of Ellman’s reagent. After optimal development of color, the plate was read at 405 nm with a microplate reader (Molecular Devices), and values were expressed as picograms per milliliter.

SOD assay. Total Mn and Cu/Zn SOD isoform activities were measured in renal tissues using a SOD kit (Cayman Chemical), and protocols were provided by the manufacturer. Briefly, kidneys were perfused with ice-cold phosphate-buffered saline (PBS) to remove red blood cells. Renal tissues were homogenized in ice-cold 20 mM HEPES buffer (pH 7.2) containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose. The homogenized tissues were centrifuged at 1,500 g for 5 min, and the supernatant was collected for SOD assay. The samples and standards were added in duplicate to a sample plate provided with the kit. Reactions were initiated by adding xanthine oxidase to all wells, and the samples were incubated on a shaker at room temperature for 20 min. The absorbance of each standard and sample was read at 450 nm by the use of an absorbance microplate reader (Molecular Devices). SOD activity was calculated from the linear regression of the standard curve by substituting the linearized rate for each sample. One unit was defined as the amount of enzyme needed to exhibit 50% dismutation of the O2•− radical. It has been shown that the activity of extracellular space SOD (ecSOD) is very low in rat tissues (5). Therefore, it was well accepted that Cu/Zn SOD and Mn SOD activities contribute predominantly to the total activity of SOD in rats. When Mn SOD activity was measured, potassium cyanide (3 mM) was added to block Cu/Zn SOD activity.

Creatinine assay. Plasma and urine creatinine levels were determined by the use of an improved Jaffe creatinine assay kit (BioAssay System). In brief, the samples and creatinine standards were added to a plate and incubated with working reagent supplied with the kit. The plate was read at 490 nm by a plate reader after 20 min incubation.

Western blot analysis. The renal cortex and medulla were dissected out and stored at −80°C until processing. Cytosolic and membrane protein was extracted as described previously (35, 36) and was used to determine SOD and NAD(P)H oxidase subunit expression, respectively. Protein contents of samples were measured using a Bio-Rad protein assay (Bio-Rad Laboratories). Samples were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane using a semidry transfer cell. The membranes were blocked 1 h at room temperature in 5% milk washing solution as described previously (36). Subsequently, the membranes were incubated with rabbit anti-human Cu/Zn SOD polyclonal IgG (1:1,500, Santa Cruz Biotechnology), goat anti-human Mn SOD polyclonal IgG (1:500, Santa Cruz Biotechnology), rabbit anti-human p47phox polyclonal IgG (1:500, Santa Cruz Biotechnology), or mouse anti-rat gp91phox IgG (1:2,000, BD Transduction Laboratories) in blocking solution at 4°C. After being washed, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies to rabbit, goat, or mouse IgG (Santa Cruz Biotechnology) in blocking solution at room temperature for 1 h. The membranes were developed using an enhanced chemiluminescence ECL kit (Amersham Pharmacia Biotech) and exposed to films (Hyperfilm-ECL; Amersham Pharma)

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Statistical analysis. All values are means ± SE. One-way ANOVA followed by a Bonferroni’s test for multiple comparisons was used to determine the difference among groups. Comparisons between groups at each experimental time point were determined by the use of two-way ANOVA followed by a Bonferroni’s test. Linear regression analysis was used to test the potential correlation between O2•− production and creatinine clearance. Differences were considered statistically significant at P < 0.05.
RESULTS

Tail-cuff systolic blood pressure was significantly higher in the CAP-HS group compared with CON-HS, CON-NS, and CAP-NS groups (Fig. 1). On day 12 after the dietary treatment, tail-cuff systolic blood pressure was significantly increased in CAP-HS rats (158 ± 7 mmHg, P < 0.05) compared with CON-NS (98 ± 6 mmHg), CON-HS (92 ± 6 mmHg), and CAP-NS (90 ± 5 mmHg) rats. Thus neonatal treatment with CAP did not increase blood pressure in rats fed a NS diet but led to an elevation in blood pressure in rats fed a HS diet.

As shown in Fig. 2, HS intake for 2 wk significantly increased renal cortical and medullary \( \text{O}_2^\bullet^- \) production (nmol·min\(^{-1}\)·mg dry wt\(^{-1}\)) in CAP-HS rats (3.53 ± 0.46; 2.32 ± 0.29, P < 0.05) compared with CON-NS (2.37 ± 0.49; 1.45 ± 0.21), CON-HS (2.55 ± 0.43; 1.54 ± 0.39), and CAP-NS (2.35 ± 0.55; 1.43 ± 0.40) rats. Ex vivo incubation of the renal cortex and medulla with apocynin (0.1 mM), a selective NAD(P)H oxidase inhibitor, significantly reduced \( \text{O}_2^\bullet^- \) production in the renal cortex and medulla in CAP-HS rats (cortex, 3.53 ± 0.46 vs. 2.83 ± 0.38; medulla, 2.32 ± 0.29 vs. 1.73 ± 0.23, P < 0.05). In contrast, apocynin had no significant effects on \( \text{O}_2^\bullet^- \) production in CON-NS, CON-HS, and CAP-NS rats (data not shown). The results suggest that increased NAD(P)H oxidase activity is the source of increased \( \text{O}_2^\bullet^- \) production in renal tissues in CAP-HS rats.

Plasma and urine 8-isoprostane, an index of oxidative stress, are shown for vehicle or CAP-treated rats fed a NS or HS diet in Fig. 3. HS intake alone produced a significant increase in plasma 8-isoprostane levels (71.2 ± 6.5 pg/ml) and urine 8-isoprostane excretion (10.1 ± 1.1 ng/day) compared with CON-NS rats (52.5 ± 7.3 pg/ml; 5.9 ± 0.8 ng/day). Moreover, CAP treatment did not increase plasma and urine 8-isoprostane levels in rats fed a NS diet but caused an further elevation in plasma and urine 8-isoprostane levels in rats fed a HS diet (99.2 ± 4.9 pg/ml; 14.0 ± 1.4 ng/day) compared with CON-HS rats (71.2 ± 6.5 pg/ml; 10.1 ± 1.1 ng/day, P < 0.05).

Figure 4 shows that, 2 wk after initiation of dietary treatment, the renal cortical Cu/Zn SOD and Mn SOD activity (in mU/mg protein) were increased in CAP-HS rats (74.6 ± 9.6; 14.3 ± 3.5, P < 0.05) compared with CON-NS (52.7 ± 6.6; 11.6 ± 2.2), CON-HS (57.8 ± 10.8; 10.9 ± 2.1), and CAP-NS (54.8 ± 7.6; 10.4 ± 2.5) rats. In contrast, HS intake significantly increased renal medullary Cu/Zn SOD and Mn SOD activity in both vehicle (95.6 ± 10.8; 24.8 ± 3.3) and CAP (101.7 ± 11.6; 27.1 ± 4.2)-treated rats compared with vehicle (71.2 ± 7.4; 17.9 ± 3.2) and CAP (67.0 ± 7.6; 18.7 ± 3.8) (P < 0.05)-treated rats fed a NS diet. There were no differences in renal medullary Cu/Zn SOD and Mn SOD activities between CON-HS and CAP-HS rats.

Consistent with the changes in renal Cu/Zn SOD and Mn SOD activity, the renal cortical Cu/Zn SOD and Mn SOD protein expression (%β-actin arbitrary units) were increased in CAP-HS (0.39 ± 0.05; 0.10 ± 0.01, P < 0.05) compared with CON-NS (0.30 ± 0.06; 0.08 ± 0.02), CON-HS (0.33 ± 0.06; 0.07 ± 0.02), and CAP-NS (0.29 ± 0.05; 0.07 ± 0.01) rats (Fig. 5). Also, HS intake increased renal medullary Cu/Zn SOD and Mn SOD protein expression in both vehicle (0.39 ± 0.05; 0.13 ± 0.01) and CAP (0.41 ± 0.05; 0.13 ± 0.02) (P < 0.05)-treated rats compared with vehicle (0.29 ± 0.06; 0.09 ± 0.02) and CAP (0.27 ± 0.05; 0.09 ± 0.02)-treated rats fed a NS diet.

Figure 6 shows the renal protein expression of NAD(P)H oxidase subunits, including p47\(^{\text{phox}} \) and gp91\(^{\text{phox}} \). A HS loading for 2 wk increased renal cortical and medullary p47\(^{\text{phox}} \) protein expression in CAP-treated rats (0.07 ± 0.01; 0.1 ± 0.01 arbitrary units, P < 0.05) compared with CAP-NS rats (0.05 ± 0.01; 0.07 ± 0.01 arbitrary units). Similar to p47\(^{\text{phox}} \) protein expression, renal cortical and medullary gp91\(^{\text{phox}} \) protein expression was increased in CAP-HS (0.21 ± 0.03; 0.45 ± 0.06 arbitrary units, P < 0.05) compared with CAP-NS rats (0.17 ± 0.03; 0.33 ± 0.60 arbitrary units). In contrast, a HS
loading did not increase renal cortical and medullary p47phox and gp91phox protein expression in vehicle-treated rats.

Creatinine clearance, a parameter representing glomerular filtration rate (GFR), is shown in Fig. 7. On day 14 after the dietary treatment, creatinine clearance (ml/min/1.73m²/kg kidney wt⁻¹) was significantly decreased in CAP-HS (0.25 ± 0.03) compared with CON-NS (0.46 ± 0.05), CON-HS (0.53 ± 0.08), and CAP-NS rats (0.50 ± 0.03) (P < 0.05). Moreover, the O₂⁺ levels in the renal cortex significantly correlated with creatinine clearance in CAP-HS rats (r = −0.76; P < 0.001; Fig. 7B).

**DISCUSSION**

One of the main findings in this study is that increased dietary salt affects the redox state of the kidney in sensory nerve-denervated rats, which is consistent with the notion that intrarenal O₂⁺ production is increased in the model of hypertension. Increased oxidative stress in the kidney of sensory nerve-degenerated rats was also evident by further increases in salt-induced enhancement of urinary excretion of 8-isoprostane, a marker considered most ubiquitous and reliable for oxidative stress. We further demonstrated that enhanced activation and expression of NAD(P)H oxidase subunits occurred in the kidney of sensory nerve-degenerated rats fed a HS diet. Interestingly, the expression and activity of important antioxidant elements Cu/Zn SOD and Mn SOD were increased rather than decreased in the kidney in these rats. Thus enhanced production of O₂⁺ in the kidney in salt-sensitive hypertensive rats subjected to sensory nerve degeneration is likely the result of increased activity of the NAD(P)H oxidase but not due to decreased scavenging of O₂⁺ by an impaired endogenous antioxidant defense system.

In CAP-treated rats, there was a marked reduction in creatinine clearance in response to a HS diet, which was not observed in sensory nerve-intact rats fed a HS diet. Moreover, our data show that O₂⁺ levels in the kidney negatively correlate with creatinine clearance in sensory-denervated rats fed a HS diet. These findings suggest that sensory nerves and their

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**Fig. 3.** Plasma levels (A) and 24-h urine excretion (B) of 8-iso-prostaglandin F₂α (8-isoprostane) in CON or CAP-treated rats fed a NS or HS diet. Values are means ± SE; n = 6–7 rats. *P < 0.05 vs. CON-NS and CAP-NS. #P < 0.05 vs. CON-HS.

**Fig. 4.** Renal Cu/Zn SOD (A: cortex; B: medulla) and Mn SOD (C: cortex; D: medulla) activity in CON or CAP-treated rats fed a NS or HS diet. Values are means ± SE; n = 7–8 rats. *P < 0.05 vs. CON-NS and CAP-NS.
neurotransmitters provide a renoprotective effect on maintaining the basal filtration rate in the glomerulus. Several factors could have contributed to a decrease in GFR in salt-sensitive hypertension. Salt-sensitive hypertension is associated with increased production of vasoconstrictors. Our previous studies (34) show that HS intake leads to an increase in endothelin-1 (ET-1) in plasma and the renal cortex in sensory-denervated rats, which may contribute to a decrease in GFR (41). In addition, it has been shown that HS intake is associated with an increase in intrarenal angiotensin II levels (15), which may also lead to enhanced renal vasoconstriction. Moreover, increased renal O₂⁻ release may directly modulate renal microvascular function, causing vasoconstriction (27). Furthermore, the O₂⁻ anion may react with nitric oxide (NO), decreasing NO availability and resulting in the formation of prooxidant peroxynitrite. All of these processes may impair intrarenal vascular and glomerular function (24). Finally, O₂⁻ limits NO signaling in the macula densa and interferes renal oxygen usage by tubular sodium transportors, resulting in enhanced tubuloglomerular feedback (25, 39).

Cellular ROS may be derived from xanthine oxidase, cytochrome P-450 enzymes, uncoupled NO synthase, and the NAD(P)H oxidase (43). Activation of NAD(P)H oxidase has been implicated in the pathogenesis of several models of hypertension (6, 12, 22, 23). In addition to phagocytes, NAD(P)H oxidase has been characterized in vascular smooth muscle cells, endothelial cells (43), and various tissues in the kidney (6). The phagocyte NAD(P)H oxidase is a multimeric enzyme, which is composed of a membrane-associated gp91phox and p22phox with cytosolic components composed of p47phox, p67phox, and p40phox (43). The catalytic core of NAD(P)H oxidase in phagocytes is the membrane-integrated flavocytochrome b558 comprising p22phox and gp91phox, which transfers electrons from NAD(P)H to O₂ (43). The p47phox is a critical regulatory subunit required for assembly and activation of NAD(P)H oxidase (16, 43).

NAD(P)H oxidase appears to be the most likely candidate for enhanced renal O₂⁻ production in CAP-treated rats fed a HS diet, given the fact that a specific inhibitor of NAD(P)H oxidase, apocynin, significantly reduced O₂⁻ production in these rats. Moreover, the increases in renal NAD(P)H oxidase protein expression are likely to explain the observed increase in renal O₂⁻ production caused by a HS diet in CAP-treated rats. These results indicate that activation of the NAD(P)H oxidase enzyme system contributes to renal O₂⁻ production in CAP-treated rats fed a HS diet.

Several studies have shown that CGRP released from sensory nerves plays counteractive roles in the development of salt-sensitive hypertension (28, 35). The vasodilatory actions of CGRP have been reported to be mediated by pathways linked to NO (11), which suppresses oxidative stress via inhibition of NAD(P)H oxidase (8). Therefore, degeneration of CGRP-positive sensory nerves caused by capsaicin treatment may contribute to increased O₂⁻ production due to lack of CGRP release leading to attenuated inhibition of NAD(P)H oxidase during HS intake. Moreover, salt-sensitive hypertension induced by sensory nerve-degeneration has been shown to be associated with increased plasma ET-1 levels (34, 44). It is known that ET-1 activates NAD(P)H oxidase to produce O₂⁻ in endothelial cells and pulmonary smooth muscle cells (9, 37).
It is conceivable, therefore, that intact sensory nerves and/or sensory neuropeptide release suppress ROS production via inhibiting ET-1 production during HS intake and that impaired sensory nerve function leads to enhanced ROS production attributed to, at least in part, increased ET-1 production during HS intake.

An increase in renal O$_2^-$ production may occur as a result of either increased oxidase activity or decreased antioxidant levels. O$_2^-$ is dismutated to a far less reactive product, hydrogen peroxide (H$_2$O$_2$), by a family of metalloenzymes known as SOD. Thus SOD is at the front line of defense against ROS-mediated injury. Three different isoforms of SOD have been found in cells and tissues, including a manganese containing SOD, Mn SOD in the mitochondria, a copper-zinc containing SOD, Cu/Zn SOD in the cytoplasm, and an extracellular copper-zinc SOD (ecSOD) (10). Several studies have shown that SOD activity is decreased in hypertensive humans and experimental animals (1, 4, 21). For example, Dahl salt-sensitive rats fed a HS diet had lower levels of Cu/Zn SOD and Mn SOD in the renal medulla compared with Dahl salt-resistant rats fed a HS diet (21). The decreased SOD activity is also found in the kidney of SHR rats (1). However, controversial findings also exist. In hypertensive models with enhanced oxidant stress, including 5/6 nephrectomy, N$^\omega$-nitro-l-arginine methyl ester (l-NAME) administration, salt loading, and lead-induced hypertension, renal expression of Cu/Zn SOD has been found to be decreased or increased, whereas Mn SOD was decreased or unchanged (14, 26, 31, 32). Thus a general pattern

![Fig. 6. Renal p47phox (A: cortex; B: medulla) and gp91phox (C: cortex; D: medulla) protein expression in CON or CAP-treated rats fed a NS or HS diet. Values are means ± SE; n = 4–5 rats. *P < 0.05 vs. CON-HS, CON-NS and CAP-NS.](image)

![Fig. 7. Creatinine clearance (A) in CON or CAP-treated rats fed a NS or HS diet, and relationship between creatinine clearance and renal cortical superoxide level (B) in CAP-treated rats fed a HS diet. *P < 0.05 vs. CON-HS, CON-NS, and CAP-NS.](image)
of response in SOD expression in situations of oxidative stress is not apparent. Our data indicate that increased O$_2^-$ levels in the kidney of sensory denervated rats fed a HS diet are unlikely due to decreased defense mechanism of SOD, because both the activity and protein expression of these enzymes are increased in these rats.

There is a possibility that overproduction of ROS in the kidney is caused by exposure of the kidney to high blood pressure. However, it has been shown that hypertension induced by norepinephrine infusion does not result in an increase in vascular O$_2^-$ production (23). Moreover, recent studies have shown that HS intake increases oxidative stress while blood pressure of HS-treated rats remains unchanged (14, 17). These findings support concepts that increased blood pressure per se may not stimulate O$_2^-$ production, and that elevated O$_2^-$ levels in CAP-treated rats fed a HS diet may result from pathways independent of elevated blood pressure.

It has been shown that HS intake in normotensive rats causes a release of O$_2^-$. Consistent with these results, we found that a HS diet given to vehicle-treated rats increased plasma and urinary 8-isoprostane excretion. However, neither renal cortical nor medullary O$_2^-$ production increased in rats fed a HS diet compared with their controls fed a NS diet. These results are consistent with previous studies (14, 21), suggesting that the increase in urinary 8-isoprostane may be caused by extrarenal production of oxidative stress. In addition, there is evidence showing that HS intake increases NO generation and action in the juxtaglomerular apparatus of the kidney and upregulates expression of all three NO synthase isoforms in the kidney. NO is known to act as an antioxidative agent by constant elimination of O$_2^-$ from tissues including the kidney. Therefore, NO may help to maintain the minimal level of O$_2^-$ in the kidney during HS intake, providing a protective mechanism against the harmful action of O$_2^-$ in the kidney.

In our summary data, we show that HS loading for 2 wk increases oxidative stress in the kidney of sensory-denervated rats, a finding that is supported by enhanced renal cortical and medullary O$_2^-$ production, further increased plasma and urinary 8-isoprostane levels, and increased activities and expression of NAD(P)H oxidase in these rats. These data suggest that sensory nerves play an important protective role against the production of O$_2^-$ in the kidney during HS intake.

**Perspective**

Our data reveal that HS intake increases oxidative stress possibly via the activation of NAD(P)H oxidase enzyme in the kidney of sensory nerve-denervated rats, indicating that sensory nerves or their neuropeptides may modulate the activity and/or expression of the NAD(P)H system in the kidney, especially under the conditions of salt-induced stress. It has been shown that, in salt-dependent experimental animals and in humans, elevated oxidative stress is an important contributor to the development of hypertension and renal dysfunction (21, 22). Recently, several lines of evidence have demonstrated that a defect in sensory nerve function occurs in Dahl salt-sensitive rats, which contributes to increased salt sensitivity (36). Thus it is conceivable that the increased O$_2^-$ production in salt-sensitive hypertension may be attributed, at least in part, to the lack of adequate counterregulatory action of sensory nerves. It follows that manipulations that improve sensory nerve function may have therapeutic potential in the treatment of salt-sensitive hypertension and/or associated end-organ damage.

**GRANTS**

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**REFERENCES**


