Hypertension is very prevalent in chronic kidney disease (CKD) and contributes strongly to its progression. Sympathetic activation has been demonstrated to play an important role in the pathogenesis of hypertension associated with CKD. Clinical studies have demonstrated that plasma catecholamines were elevated and muscle sympathetic nerve activity was enhanced in CKD patients, whose blood pressure decreased pronouncedly with adrenergic inhibition by clonidine. Furthermore, moxonidine, a central sympatholytic agent, was shown to reduce urinary albumin excretion associated with huge depressor effects in patients with essential hypertension with microalbuminuria. In fact, renal denervation ameliorated hypertension in various animal models including ours. Recently, catheter-based renal denervation safely reduced blood pressure in patients with treatment-resistant hypertension. The renal nerves consist of afferent and efferent signals. Although both of them are important in the pathophysiology of hypertension, the renal afferent nerve has been suggested to contribute to sympathetic activation and the resultant hypertension in the diseased kidney. Afferent renal denervation prevented the development of hypertension in 5/6 nephrectomized rats. Renal injury may activate afferent pathways connecting with brain regions involved in the control of blood pressure. Afferent renal nerves play an essential and obligatory role in the normal renal adaptive response to oral salt loading under physiological conditions. On the other hand, in the pathophysiological conditions, the impairment of the renorenal reflex leads to salt-induced arterial pressure elevation. However, what happens in the brain to cause sympathoexcitation in CKD patients has not been fully explained.

In our previous report, salt loading enhanced a rise in arterial pressure that was induced by central sympathoexcitation, possibly through the overproduction of brain reactive oxygen species. Antioxidant treatment with chronic intracerebroventricular Tempol decreased sympathetic nerve activity and arterial pressure, which, in turn, led to a decrease in renal damage. Similar effects were elicited by treatment with oral moxonidine, the central sympatholytic agent. In conclusion, sympathoexcitation by brain oxidative stress may mediate arterial pressure elevation in salt-induced chronic kidney disease. (Hypertension. 2012; 59:00-00.)

Key Words: hypertension ■ oxidative stress ■ brain ■ salt ■ sympathetic nervous system ■ chronic kidney disease
oxygen species (ROS), in an animal model of increased oxidative stress. We have also shown that increased oxidative stress in the brain elevated arterial pressure, possibly through central sympathoexcitation, in a salt-sensitive hypertension model, Dahl salt-sensitive rats\(^1\) and in an obesity-induced hypertension model, high-fat diet-fed rats\(^2\), in both of which systemic ROS overproduction has been reported.\(^3\)\(^,\)\(^4\)\(^,\) Simil- similar, increased oxidative stress has also been reported in CKD.\(^2\)\(^,\)\(^2\)\(^,\)\(^4\)\(^,\)\(^2\)^ Actual- y, salt-sensitive hypertension\(^5\) and obesity\(^6\) are associated with abnormal salt handling in the kidney and an increased risk of the development of CKD. Recently, central sympathetic activation has been reported to be medi- ated by brain ROS in the phenol renal injury model of hypertension\(^7\) and in the 2-kidney, 1-clip hypertension model.\(^8\) Thus, brain ROS overproduction might be a char- acteristic of hypertension associated with renal dysfunction. Therefore, we hypothesized that central sympathetic activa- tion, possibly because of the overproduction of oxidative stress in the brain, could contribute to arterial pressure elevation in salt-induced CKD.

In the present study, to clarify our hypothesis, we exam- ined the following points by using young, salt-loaded, unine- phrectomized Sprague-Dawley rats\(^2\)\(^,\)\(^3\)\(^,\)\(^3\)\(^,\)\(^5\)\(^,\)\(^6\) to a salt-induced CKD model. We evaluated the responses of renal sympathetic nerve activity (RSNA) and arterial pressure to acute intrace- rebroventricular (ICV) administration of an antioxidant agent, Tempol, and the level of ROS in the hypothalamus. In our previous reports,\(^1\)\(^7\)\(^–\)\(^1\)\(^9\),\(^3\)\(^5\) we showed the effects of acute ICV Tempol but did not show the effects of chronic ICV Tempol. Thus, our previous data only suggested a pathophysiological role of brain ROS but did not show the usefulness of central antioxidant treatment. Therefore, we elucidated whether chronic ICV Tempol could recover sympathetic nerve activity and arterial pressure.

### Methods

#### Animals

Three-week-old male Sprague-Dawley rats (Tokyo Laboratory Ani- mals Science, Tokyo, Japan) were randomly assigned to a unine- phrectomy or sham operation and then subjected to either a normal- salt (0.5%) or high-salt (8.0%) diet for 4 weeks. In addition, the salt-loaded uninephrectomized rats were treated with moxonidine (1.5 mg/kg per day orally),\(^2\)\(^5\)\(^,\)\(^3\)\(^0\) a central sympatholytic agent, for 4 weeks, to investigate the role of sympathetic nerve activity in arterial pressure elevation in salt-induced CKD. Moreover, to investigate the role of brain ROS, ICV administration of Tempol was continued for 4 weeks in the salt-loaded uninephrectomized rats. In this way, 6 different experimental groups were created: sham-operated rats raised with a normal (sham) or high-salt diet (sham + HS), uninephrectomized rats raised with a normal (Unx) or high-salt diet (Unx + HS),\(^2\)\(^5\)\(^,\)\(^3\)\(^0\) moxonidine-treated Unx + HS rats (Unx + HS + Mox), and chronic ICV Tempol-treated Unx + HS rats (Unx + HS + ICV temp).

Either a unilateral nephrectomy or a sham operation was per- formed on 3-week-old Sprague-Dawley rats anesthetized with sodium pentobarbital (40 mg/kg IP) in the same way as that described previously.\(^3\)\(^2\) For the continuous and ICV administration of Tempol in 3-week-old rats anesthetized with sodium pentobarbital (40 mg/kg IP), after uninephrectomy, cannulae were led to the cranium using small screws and dental cement and were connected by silastic tubing to osmotic minipumps (0.5 μL/h, ALZET Osmotic Pump, model 2002) implanted subcutaneously at the back of the neck. The cannulae and osmotic minipumps were replaced by new ones after 2 weeks of salt loading. Tempol (25 μg in 0.5 μL/h)\(^3\) dissolved in artificial cerebrospinal fluid (ACSF; in millimoles per liter: NaCl, 123.00; CaCl₂, 0.86; KCl, 3.00; MgCl₂, 0.89; NaHCO₃, 25.00; NaH₂PO₄, 0.50; Na₂HPO₄, 0.25) was infused into the lateral cerebral ventricles of the Unx + HS rats by the osmotic minipumps for 4 weeks. The accuracy of the ICV injection was confirmed by injecting Evans blue dye after the animals were euthanized. Moreover, ICV vehicle (ACSF) infusion was performed to confirm the effect of ICV infusion itself in the other Unx + HS rats. To rule out the peripheral effect of Tempol, the same dose of Tempol (25 μg in 0.5 μL/h) dissolved in saline was also infused intraperitoneally by the osmotic minipumps for 4 weeks in additional Unx + HS rats.

All of the rats were housed in a room maintained at 23°C to 25°C with a 12-hour light/dark cycle and were given food and water ad libitum. All of the experimental procedures were approved by the ethics committees on animal research of the Faculty of Medicine, University of Tokyo.

#### Evaluation of Blood Pressure and Systemic Sympathetic Nerve Activity

Systolic blood pressure (SBP) was measured by the tail-cuff method (P-98A; Softron, Tokyo, Japan). In some unrestrained conscious rats, the arterial pressure was directly measured. The femoral artery and vein were cannulated under ether anesthesia. Mean arterial pressure (MAP) was recorded in the conscious state after waiting for 3 hours for recovery from anesthesia, as described previously.\(^1\)\(^7\)\(^–\)\(^1\)\(^9\) After the baseline MAP measurement, the response of the MAP to a gangli- onic blockade was examined by injecting IV 30 mg/kg of body weight of hexamethonium hydrochloride, to evaluate systemic symp- pathetic nerve activity. The maximal decrease in the MAP was considered as an index of sympathetic activity.\(^3\)

#### Acute ICV Administration of Tempol

In urethane-anesthetized (1 g/kg) and artificially ventilated rats, the MAP, heart rate (HR), and RSNA were recorded, as mentioned in our previous reports.\(^1\)\(^7\)\(^–\)\(^1\)\(^9\),\(^3\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\) The distal ends of real nerves were cut to measure their efferent discharge. For detailed methods about how to record RSNA, please see the online Data Supplement (available at http://hyper.ahajournals.org). After recording the basal MAP, HR, and RSNA during a 30-minute stabilization period, Tempol (20 μmol in 10 μL) dissolved in ACSF or vehicle (ACSF) was infused into the lateral ventricle for 10 minutes, and changes in the parameters were recorded. The dose of Tempol was decreased as described previously.\(^1\)\(^7\)\(^–\)\(^1\)\(^9\)\(^,\)\(^3\)\(^5\)\(^,\)\(^6\)\(^,\)\(^7\)\(^,\)\(^8\) The accuracy of the ICV injection was confirmed by injecting Evans blue dye after each experiment.

#### Measurement of NADPH-Induced Superoxide Production in the Isolated Hypothalamus

The production of superoxide anions induced by NADPH (final concen- tration: 100 μmol/L) was measured by bis-N-methylacridinium (lucigenin) chemiluminescence in the isolated hypothalamus, where several nuclei critically involved in cardiovascular regulation are known to be located,\(^3\)\(^6\) as described previously.\(^1\)\(^8\)\(^,\)\(^1\)\(^9\)\(^,\)\(^3\)\(^7\)

#### Urinary Protein Excretion and Renal Histology

We measured urinary protein excretion using the pyrogallol red method after 4 weeks of dietary treatment. For morphological evaluations, paraffinized kidney sections (3-μm thickness) were stained with periodic acid-Schiff reagents and analyzed semiquanti- tatively for glomerulosclerosis and tubulointerstitial injury, as de- scribed previously.\(^2\)\(^2\)\(^,\)\(^2\)\(^3\) For the detailed method of how to calculate the glomerular and tubulointerstitial scores, please see the online Data Supplement. All of the morphometric measurements were per- formed by 2 examiners (H.K. and H.S.), who were blinded to the treatment protocol.

#### Statistical Analysis

All of the values were presented as mean ± SEM. In the acute ICV administration experiments (Figures 2 and 3), the baseline value was defined as the mean value over a 1-minute stabilization period before
the administration of drugs, and the peak value was defined as a mean value for 10 seconds around the maximum response. The magnitude of the changes was expressed as the percentage of change between the peak and the baseline values. In addition, to evaluate the magnitude and time course responses to hexamethonium (Figure S1, available in the online Data Supplement) and ICV Tempol (Figure S2), the area under the curve was calculated (please see the online Data Supplement). Dunnett test was used for comparisons between the Unx+HS rats and the other groups of rats, and it was performed with JMP 9.0.0 (SAS Institute, Cary, NC) computer software. P values of P<0.05 were considered to indicate statistical significance.

Results

Effects of Salt Loading and Uninephrectomy on Brain ROS-Mediated Sympathetic Nerve Activity, Blood Pressure, and Renal Function in Young Sprague-Dawley Rats

Response of MAP to Ganglionic Blockade With Hexamethonium Hydrochloride

The MAP reduction induced by hexamethonium was significantly greater in the Unx+HS rats than in the other 3 groups of rats (Unx+HS: −102.1±10.0 versus sham: −40.5±3.5, sham+HS: −44.3±1.5, Unx: −45.8±3.3 mm Hg; P<0.001, respectively; Figure 1A), which suggests that sympathetic activity increased significantly in the Unx+HS rats. Area under the curve elicited similar results (Figure S1).

Blood Pressure

The SBP was significantly elevated in the Unx+HS rats compared with the other 3 groups of rats (Unx+HS: 196.6±10.3, N=12 versus sham: 142.7±2.5, N=11, sham+HS: 145.0±2.2, N=12, Unx: 143.2±3.4 mm Hg, N=13; P<0.001, respectively). MAP was also higher in the Unx+HS rats than in the other 3 groups of rats, as indicated by direct measurements during the conscious state (Unx+HS: 166.6±14.4 versus sham: 96.1±1.7, sham+HS: 93.5±1.0, Unx: 96.5±1.8 mm Hg; P<0.001, respectively; Figure 1B).

Effects of Acute ICV Tempol on RSNA, MAP, and HR

Integrated RSNA, MAP, and HR started decreasing a few minutes after ICV Tempol administration and reached their lowest values within ~15 minutes (RSNA and MAP; Figure 2). The reduction in RSNA was significantly greater in the Unx+HS rats (Unx+HS: −13.0±2.6% versus sham: −2.9±0.7%; P=0.004, sham+HS: −1.8±1.3%; P=0.001, Unx: −5.2±1.7%; P=0.024; Figure 3A). Likewise, the reduction in MAP was significantly greater in the Unx+HS rats than in the other 3 groups of rats (Unx+HS: −28.9±2.4% versus sham: −5.7±1.7%, sham+HS: −7.0±2.3%, Unx: −7.3±2.6%; P<0.001, respectively; Figure 3B). In a similar fashion, the reduction in HR was significantly greater in the Unx+HS rats (Unx+HS: −13.6±2.9% versus sham: −1.3±1.6%; P=0.003, sham+HS: −0.5±0.6%; P=0.001, Unx: −1.5±2.4%; P=0.003; Figure 3C). Area under the curve elicited similar results (Figure S2).

Measurement of NADPH-Induced Superoxide Production in the Isolated Hypothalamus

NADPH-induced superoxide production significantly increased in the isolated hypothalamus of the Unx+HS rats compared with the other 3 groups of rats (Unx+HS: 2.5±0.2×10^6 versus sham: 1.7±0.3×10^6 relative light units [RLU]/10 min per gram; P=0.009, sham+HS: 1.7±0.2×10^6 RLU/10 min per gram; P=0.010, Unx: 1.7±0.1×10^6 RLU/10 min per gram; P=0.007; Figure 4).

Urinary Protein Excretion and Renal Histology

Compared with the other 3 groups of rats, the Unx+HS rats had significantly higher urinary protein excretion levels (Unx+HS: 162.7±39.0 versus sham: 11.1±1.0, sham+HS: 8.9±1.0, Unx: 10.9±0.8 mg/d; P<0.001, respectively; Figure 5A), more pronounced global glomerulosclerosis (Unx+HS: 1.8±0.2 versus sham: 0.2±0.0, sham+HS: 0.2±0.0, Unx: 0.1±0.0; P<0.001, respectively; Figure 5B and 5C), and greater tubulointerstitial damage (Unx+HS: 1.6±0.1 versus sham: 0.3±0.1, sham+HS: 0.5±0.2, Unx: 0.5±0.1; P<0.001, respectively; Figure 5B and 5C).

Effect of Chronic Oral Moxonidine on Sympathetic Nerve Activity, Blood Pressure, and Renal Function in Salt-Induced CKD Rats

Chronic oral moxonidine resulted in sympathoinhibitory, depressor, and renoprotective effects in the Unx+HS rats, the
salt-induced CKD model. The MAP reduction induced by hexamethonium was inhibited (−75.5±1.7 mm Hg; P=0.012; Figure 1A) in the Unx+HS+Mox rats. The blood pressure decreased significantly (SBP: 150.7±4.5 mm Hg, N=7; P<0.001, MAP: 123.8±5.1 mm Hg; P=0.002, Figure 1B). The Unx+HS+Mox rats also showed decreased proteinuria (10.4±2.3 mg/d; P<0.001; Figure 5A) and improved glomerular (0.2±0.0; P<0.001; Figure 5C) and tubulointerstitial scores (0.3±0.1; P<0.001; Figure 5C) compared with the untreated Unx+HS rats. Hypothalamic NADPH-induced superoxide production decreased significantly in the Unx+HS+ICV temp rats (1.5±0.1×10⁶ RLU/10 min per gram; N=4; P=0.042), compared with the untreated Unx+HS rats. Therefore, we confirmed the superoxide-scavenging effects of chronic ICV Tempol. Central antioxidant treatment with chronic ICV Tempol indeed showed sympathoinhibitory, depressor, and renoprotective effects as follows. The MAP reduction induced by hexamethonium was inhibited (−45.8±4.5 mm Hg; P<0.001; Figure 1A) in the Unx+HS+ICV temp rats. The blood pressure decreased significantly (SBP: 159.2±7.2 mm Hg, N=5; P=0.003, MAP; 113.4±3.2 mm Hg; P<0.001; Figure 1B). The Unx+HS+ICV temp rats also showed decreased proteinuria (13.7±1.9 mg/d; P<0.001; Figure 5A) and improved glomerular (0.3±0.0; P<0.001; Figure 5B and 5C) and tubulointerstitial scores (0.5±0.1; P<0.001; Figure 5B and 5C), compared with the untreated Unx+HS rats.

Effect of Chronic ICV Tempol on Sympathetic Nerve Activity, Blood Pressure, and Renal Function in Salt-Induced CKD Rats

As mentioned above, a central sympatholytic agent, moxonidine, showed depressor and renoprotective effects, which suggests that central sympathoexcitation is involved in the arterial pressure elevation and the resultant progression of CKD in the Unx+HS rats. Subsequently, the effect of treatment with chronic ICV Tempol on sympathetic nerve activity, blood pressure, and renal function was evaluated in the Unx+HS rats to determine whether sympathoexcitation by continuously increased brain oxidative stress mediates arterial pressure elevation and renal injury. Hypothalamic NADPH-induced superoxide production decreased significantly in the Unx+HS+ICV temp rats (1.5±0.1×10⁶ RLU/10 min per gram; N=4; P=0.042), compared with the untreated Unx+HS rats. Therefore, we confirmed the superoxide-scavenging effects of chronic ICV Tempol. Central antioxidant treatment with chronic ICV Tempol indeed showed sympathoinhibitory, depressor, and renoprotective effects as follows. The MAP reduction induced by hexamethonium was inhibited (−45.8±4.5 mm Hg; P<0.001; Figure 1A) in the Unx+HS+ICV temp rats. The blood pressure decreased significantly (SBP: 159.2±7.2 mm Hg, N=5; P=0.003, MAP; 113.4±3.2 mm Hg; P<0.001; Figure 1B). The Unx+HS+ICV temp rats also showed decreased proteinuria (13.7±1.9 mg/d; P<0.001; Figure 5A) and improved glomerular (0.3±0.0; P<0.001; Figure 5B and 5C) and tubulointerstitial scores (0.5±0.1; P<0.001; Figure 5B and 5C), compared with the untreated Unx+HS rats.

Chronic ICV ACSF did not affect blood pressure (SBP: 199.6±5.6 mm Hg; N=5) or proteinuria (134.3±4.7 mg/d;
N=4). Moreover, the peripheral chronic administration of the same dose of Tempol as the ICV infusion did not change the blood pressure (SBP: 205.4±10.7 mm Hg; N=5) or proteinuria (203.4±81.6 mg/d; N=4) in the Unx+HS rats. Thus, the above-mentioned effects of chronic ICV Tempol were not attributed to the effect of the ICV infusion itself or to Tempol leakage into the peripheral vessels.

**Discussion**

In the present study, uninephrectomy and salt loading from a young age in rats resulted in arterial pressure elevation and the progression of renal damage and were associated with sympathoexcitation. Importantly, we have demonstrated 2 major findings suggesting brain ROS-mediated sympathoexcitation and the resultant blood pressure elevation in the above-mentioned salt-induced CKD rats. First, the acute ICV antioxidant infusion experiment suggests that brain ROS stimulates sympathetic nerve activity, leading to hypertension in the salt-induced CKD rats, in a manner similar to the hypertension models in our previous studies17–19; reductions in the salt-induced CKD rats, in a manner similar to the above-mentioned salt-induced CKD rats. First, the acute ICV antioxidant infusion experiment suggests that brain ROS contribute to the effect of the ICV infusion itself or to Tempol leakage into the peripheral vessels.

Figure 4. Reduced NADPH-dependent superoxide anion production assessed by lucigenin chemiluminescence in the isolated hypothalamic sections from sham, sham+HS, Unx, and Unx+HS rats. The chemiluminescence value was significantly higher in Unx+HS rats than in the control rats (sham, sham+HS, and Unx). Data are represented as mean±SEM. RLU indicates relative light units. Abbreviations for rats are as follows: sham-operated rats raised with a normal (sham) or high-salt diet (sham+HS), uninephrectomized rats raised with a normal (Unx) or high-salt diet (Unx+HS).

Figure 5. A. Twenty-four-hour urinary protein excretion in sham, sham+HS, Unx, Unx+HS, Unx+HS+Mox, and Unx+HS+ICV temp rats. Significantly higher proteinuria was elicited in Unx+HS rats than in the control rats (sham, sham+HS, and Unx) or the Unx+HS+Mox and Unx+HS+ICV temp rats. B. Representative photomicrographs of the glomerular (top) and tubulointerstitial area (bottom) of the kidney in Unx, Unx+HS, and Unx+HS+ICV temp rats after staining with the periodic acid-Schiff (PAS) stain. C. The glomerular (top) and tubulointerstitial scores (bottom) in sham, sham+HS, Unx, Unx+HS, Unx+HS+Mox, and Unx+HS+ICV temp rats. Significantly greater glomerular and interstitial damage was elicited in Unx+HS rats; this damage was reversed to almost normal levels by oral moxonidine and ICV Tempol administration. Data are represented as mean±SEM. Abbreviations for rats are as follows: sham-operated rats raised with a normal (sham) or high-salt diet (sham+HS), uninephrectomized rats raised with a normal (Unx) or high-salt diet (Unx+HS), moxonidine-treated Unx+HS rats (Unx+HS+Mox), and chronic ICV Tempol-treated Unx+HS rats (Unx+HS+ICV temp).
microinjection of Tempol into the dorsomedial hypothalamus or the rostral ventrolateral medulla (RVLM) attenuated sympathoexcitatory responses to emotional stress in rabbits. ICV infusion of antioxidants, including in the present study, elicited a sympathoinhibitory effect, suggesting that oxidative stress directly stimulates central sympathetic nerve activity. In stroke-prone spontaneously hypertensive rats, the level of ROS was higher in the RVLM, and manganese superoxide dismutase overexpression in the RVLM decreased sympathetic nerve activity. Thus, brain ROS overproduction may induce central activation of the sympathetic nervous system.

In the present study, we showed that sympathetic inhibition by central antioxidant treatment with chronic ICV Tempol normalized arterial pressure, which, in turn, led to a reduction in renal damage. Thus, it is suggested that sympathoexcitation by brain oxidative stress mediates arterial pressure elevation in some types of hypertension that are associated with salt retention and renal injury, both of which are associated with salt-sensitive and obesity-induced hypertension and CKD. In fact, chronic ICV Tempol significantly decreased brain ROS in the Unx+HS rats (Figure 4). In addition, because continuous oral administration of moxonidine improved hypertension and alleviated renal damage despite unchanged levels of brain ROS, sympathetic inhibition did not reduce brain ROS levels; instead, brain ROS would appear to be an upstream element governing central sympathoexcitation in salt-induced CKD rats. Because several investigators have reported that the peripheral administration of Tempol decreases blood pressure and ameliorates renal function in rats with hypertension and CKD, some-one may speculate that leakage of Tempol into the peripheral vasculature causes depressor and renoprotective effects in the Unx+HS rats. However, the data from the present study do not support this speculation, because the peripheral administration of Tempol at the same dose as ICV-administered Tempol did not exert any hypotensive or renoprotective effects. The dose of Tempol used in the present study was apparently lower than that used in reports that showed the depressor and renoprotective effects of peripherally administered Tempol. Thus, the central antioxidant effect must play a major role in the depressor effect via sympathetic inhibition by central ICV Tempol.

In CKD, renal injury may activate afferent pathways, which project to the central cardiovascular region in the brain and regulate arterial pressure through the efferent sympathetic nerve. Our findings are compatible with the possibility that the renal afferent nerve can increase brain oxidative stress, leading to arterial pressure elevation through sympathoexcitation. These findings support other reports showing brain ROS overproduction in the phenol renal injury model of hypertension and the 2-kidney, 1-clip hypertension model. ICV administration can act in the hypothalamic area, which is supposed to contain several nuclei critically involved in the cardiovascular regulation system, such as the subfornical organ, paraventricular hypothalamic nuclei, and organum vasculosum of the stria terminalis. Other regions of the brain, such as the RVLM, have also been recognized as key areas that can mediate oxidative stress-induced sympathoexcitation. In contrast, previous studies that used ICV infusion of antioxidants, including those in our laboratory, suggest that the hypothalamus is a critical area for ROS generation and the maintenance of sympathetic control of the cardiovascular system. These results are compatible with the present data, although the region-related functions of the brain are complicated and remain unknown.

Although the present study has demonstrated that central oxidative stress-induced sympathoexcitation could mediate hypertension, the potential mechanisms underlying the increase in brain oxidative stress in CKD remain unclear. Several studies have shown that a centrally administered angiotensin II has a sympathoexcitatory effects, possibly by generating oxidative stress. Furthermore, the brain renin-angiotensin system was upregulated in rats with chronic renal failure. Therefore, the renin-angiotensin system in the brain may stimulate the generation of central oxidative stress associated with renal dysfunction. Moreover, brain aldosterone and mineralocorticoid receptors, which are stimulated by the renin-angiotensin system, can also be involved in central oxidative stress-induced sympathoexcitation. Aldosterone acts centrally to increase brain oxidative stress. Furthermore, hypothalamic aldosterone levels are increased by salt loading. ICV aldosterone synthase inhibitor, as well as a mineralocorticoid receptor blocker, can prevent salt-induced hypertension in Dahl salt-sensitive rats. The blockade of central mineralocorticoid receptors also improved salt-induced left-ventricular systolic dysfunction through attenuation of sympathoexcitation in pressure-overload model mice. Thus, aldosterone and mineralocorticoid receptors may contribute to the overproduction of central oxidative stress overproduction, resulting in sympathoexcitation-induced hypertension. On the other hand, any abnormality in NO synthase or the superoxide-scavenging system, such as extracellular superoxide dismutase in the central nervous system, might contribute to the upregulation of brain oxidative stress.

Although we focused on brain ROS as a mechanism of sympathoexcitation in salt-induced CKD in the present study, central mechanisms other than ROS in the brain could play important roles in the sympathetic regulation of arterial pressure. For example, a simple reduction in dietary salt intake revealed a tonic sustaining effect of angiotensin II in the RVLM, leading to an increase in RSNA. Further study is needed to clarify the precise mechanisms underlying sympathoexcitation in hypertension associated with CKD.

In the present study, sympathoexcitation, in addition to hypertension and renal dysfunction, was confirmed in the Unx+HS rats, and the reductions in RSNA, MAP, and HR levels elicited by acute ICV administration of the antioxidant Tempol and hypothalamic NADPH-induced ROS were significantly greater in the Unx+HS rats than in the control rats. Furthermore, chronic ICV Tempol normalized sympathetic nerve activity and then normalized arterial pressure, which, in turn, led to a reduction in renal damage. In conclusion, these findings suggest that, in salt-induced CKD, sympathoexcitation by increased oxidative stress in the brain may mediate arterial pressure elevation.
Perspectives
Hypertension associated with CKD, a highly predisposing condition for cardiovascular disease, requires appropriate management. However, the detailed pathological mechanisms have not been fully elucidated. In CKD subjects, high salt intake increases blood pressure and causes a greater degree of renal damage than in CKD-free subjects. In the present study, we have demonstrated that sympathoexcitation via oxidative stress in the brain may mediate arterial pressure elevation in salt-induced CKD rats. Salt-sensitive and obesity-induced hypertension, in which the brain ROS increases blood pressure through sympathoexcitation, also tends to be associated with CKD. Therefore, our series of findings suggest that sympathoexcitation via brain oxidative stress could be a common mechanism for the rise in blood pressure associated with salt-sensitive and obesity-induced hypertension and CKD. Sympathoexcitation has been reported to lead to cardiovascular events, in addition to the development of renal dysfunction and hypertension, in CKD patients, and it is directly related to a patient’s prognosis.

Based on our new insights, a novel strategy, such as the administration of a blood-brain barrier–permeable antioxidant agent with a sympathoinhibitory effect, may be useful for preventing and managing not only hypertension but also cardiovascular events in CKD patients.

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References


Sympathoexcitation by Brain Oxidative Stress Mediates Arterial Pressure Elevation in Salt-Induced Chronic Kidney Disease

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Sympathoexcitation by Brain Oxidative Stress Mediates Arterial Pressure Elevation in Salt-induced Chronic Kidney Disease

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Short title: Sympathoexcitation via Brain ROS in CKD

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Methods

**Acute ICV Administration of Tempol**

*How to record renal sympathetic nerve activity (RSNA)*

The left renal nerve was approached retroperitoneally through a left flank incision and prepared for recording from near the renal artery. To record the efferent discharge of the renal nerve, the central portion of the cut end of the nerve was placed on bipolar silver hook electrodes connected to an amplifier (AVB-8, Nihon Kohden) and the discharge was displayed on an oscilloscope (VC-11A, Nihon Kohden). The lower and higher cutoff frequencies of the recording system were 100 and 3000 Hz, respectively. The nerve was immersed in warm paraffin oil to prevent drying. RSNA was obtained as multifiber discharge, full-wave rectified, and integrated over a 10-second interval (EI-601G, Nihon Kohden). The instrumental noise level was recorded after cutting the renal nerve at the end of the experiment and was subtracted from all of the experimental values of renal nerve discharge. RSNA data were fed into a personal computer after analog-to-digital conversion (Power Lab, ADInstruments, Castle Hill, NSW, Australia), together with data on arterial pressure, heart rate and timing pulses of drug administration, and analyzed by signal analysis software (Chart, ADInstruments).

**Urinary Protein Excretion and Renal Histology**

*How to calculate the glomerular and tubulointerstitial scores*

The degree of glomerulosclerosis (×20 objective) was determined on the basis of the disappearance of cellular elements from the tuft, capillary loop collapse and folding of the glomerular basement membrane with the accumulation of amorphous material. Depending on the percentage of glomeruli involved, the sections were graded as 0 (0%), I (1–25%), II (26–50%), III (51–75%) and IV (76–100%). The glomerulosclerosis score was calculated as follow: 
\[ \frac{(1 \times \%\text{Grade I}) + (2 \times \%\text{Grade II}) + (3 \times \%\text{Grade III}) + (4 \times \% \text{Grade IV})}{\text{number of glomeruli}} \]

For each animal, between 70 and 100 glomeruli were examined. Tubulointerstitial injury was defined as tubular cast formation, sloughing of tubular epithelial cells, tubular atrophy or thickening of the tubular basement membrane. For each kidney, 30 cortical fields (×10 objective) were scored as 0 (0%), I (1–25%), II (26–50%), III (51–75%) and IV (76–100%). The areas of injured tubulointerstitium were measured digitally by using an image analysis program (ImageJ).
Figure S1: Appendix of Figure 1A. The calculated data as the area under the curve (AUC) from the time of injection for 300 seconds with decrease in mean arterial pressure (MAP) (units of mmHg) x time from the injection (seconds) to take into account the response of MAP to intravenous hexamethonium. The AUC of the MAP induced by hexamethonium was significantly greater in Unx+HS rats than in the control rats (Sham, Sham+HS and Unx) or the Unx+HS+Mox and Unx+HS+ICV temp rats. Sham: the sham-operated and normal salt-loaded group, Sham+HS: the sham-operated and high salt-loaded group, Unx: the uninephrectomized and normal salt-loaded group, Unx+HS: the uninephrectomized and high salt-loaded group, Unx+HS+Mox: the Unx+HS group treated with oral moxonidone, Unx+HS+ICV temp: the Unx+HS group treated with chronic intracerebroventricular (ICV) tempol. Data are represented as mean±SEM.
Figure S2: Appendix of Figure 3. The calculated data as AUC from the time of injection for 1200 seconds with decrease in renal sympathetic nerve activity (RSNA), MAP or heart rate (HR) (units of percent) x time from the injection (seconds) to take into account the responses of RSNA, MAP and HR to ICV tempol. ICV tempol significantly decreased RSNA, MAP and HR in Unx+HS rats compared with the control rats (Sham, Sham+HS and Unx). See abbreviations to the legend of Figures S1. Data are represented as mean±SEM.