Song WZ *et al* / Acta Pharmacol Sin 2004 Dec; 25 (12): 1626-1632

©2004, Acta Pharmacologica Sinica
Chinese Pharmacological Society
Shanghai Institute of Materia Medica
Chinese Academy of Sciences
http://www.ChinaPhar.com

Increased salt sensitivity induced by sensory denervation: role of superoxide[1]

Wei-zhong SONG, Alex F CHEN, Donna H WANG[2]

Department of Medicine and Pharmacology & Toxicology, Michigan State University, East Lansing, MI 48824, USA

KEY WORDS superoxides; tempol; afferent neurons; capsaicin; denervation; dietary sodium chloride; nitric oxide

ABSTRACT

AIM: To test the hypothesis that production of superoxide in mesenteric resistance arteries is increased and contributes to the development of hypertension induced by sensory denervation plus high salt intake. **METHODS**: Newborn Wistar rats were given capsaicin 50 mg/kg sc on the 1st and 2nd d of life. After weaning, male rats were grouped as follows and treated for 3 weeks with: capsaicin pretreatment plus normal sodium diet (0.5 %, CAP-NS), CAP plus high sodium diet (4 %, CAP-HS), control plus NS (CON-NS), or CON-HS. Both tail-cuff systolic blood pressure and mean arterial pressure (MAP) were measured in each of the groups. Western blot analysis was used for measurement of manganese superoxide dismutase (MnSOD) and endothelial nitric oxide synthase (eNOS) in the mesenteric resistance arteries. Lucigenin chemiluminescence assay was used for superoxide production in the mesenteric resistance arteries. The Griess method was used for measurement of nitrite/nitrate levels in plasma. **RESULTS**: Both tail-cuff pressure and MAP were higher in CAP-HS compared with CAP-NS, CON-HS, and CON-NS rats ($P<0.05$). Both MnSOD and eNOS in the mesenteric resistance arteries were increased in CAP-HS compared with CAP-NS, CON-HS, and CON-NS ($P<0.05$). However, nitrite/nitrate levels in plasma were not different among 4 groups. Acute iv administration of tempol, a membrane-permeable superoxide scavenger, decreased MAP in both CAP-HS and CON-HS when compared with their respective controls. However, the decreases of MAP between these two groups were not different. Chronic treatment with tempol failed to prevent the development of hypertension in CAP-HS rats. Superoxide production in the mesenteric resistance arteries was increased in CAP-HS compared with CAP-NS, CON-HS, and CON-NS ($P<0.05$). However, chronic treatment with tempol did not prevent the increase of mesenteric superoxide production in CAP-HS rats. **CONCLUSIONS**: Regardless of increased vascular MnSOD levels, salt sensitive hypertension induced by sensory degeneration is associated with increased vascular superoxide production. Although tempol is incapable of preventing the development of hypertension in sensory denervated rats fed a high salt diet, increased superoxide levels may contribute to exacerbated vascular impairment which may take longer time to develop. Given that superoxide may be produced by sources other than mitochondrion, future studies using other inhibitors (eg, inhibitors of NADPH oxidase and xanthine oxidase) may unveil the effectiveness of reducing superoxide on lowering blood pressure in this model.

[1] This study was supported by National Institutes of Health (grant HL-57853 and HL-73287) and a grant from the Michigan Economic Department Corporation.
[2] Correspondence to Prof Donna H WANG, MD.
Phn 1-517-432-0797. Fax 1-517-432-1326.
E-mail donna.wang@ht.msu.edu
Received 2004-07-13 Accepted 2004-09-28

INTRODUCTION

Vascular superoxide (O_2^-) production has been implicated to contribute to blood pressure elevation in many forms of hypertension. It has been demonstrated that the production of O_2^- are increased in the aortic rings in spontaneously hypertensive rats (SHR), and that de-

creased vasodilator response in this model can be restored by giving a superoxide dismutase[1,2]. Moreover, in many other experimental models of hypertension including hypertension induced by angiotensin II (Ang II), one-kidney-one-clip (1K1C), deoxycorticosterone acetate (DOCA)-salt, obesity, and diabetes, vascular O_2^- production is increased[3-9]. Treatment with tempol, a membrane-permeable superoxide scavenger, reduces O_2^- production and ameliorates hypertension in these models[2,10,11].

Superoxide may be involved in the pathogenesis of hypertension via several pathways. It may act as a vasoconstrictor directly or may interact with nitric oxide (NO) to reduce NO bioavailability and further increases vasoconstriction[12,13]. Moreover, peroxynitrite formed from interactions between O_2^- and NO is a more potent oxidant that causes nitrosylation of tyrosine residues in proteins and leads to DNA damage and alteration of cellular function[12,13]. In addition to the source of mitochondrion, superoxide may be produced by other sources including uncoupling of eNOS, NADPH oxidase, and xanthine oxidase, *etc*.

Recently, our laboratory developed a novel salt-sensitive hypertensive model that was sensory nerve-dependent. We found that neonatal treatment with capsaicin caused a rat to respond to salt load with a significant rise in blood pressure[14-22]. In addition, plasma renin activity and plasma aldosterone levels are insufficiently suppressed and plasma endothelin-1 (ET-1) concentrations are markedly increased in sensory denervated rats fed a high salt diet when compared with rats fed a high salt diet[19-22]. Although it is known that Ang II, aldosterone, and ET-1 are potent stimulators for O_2^- production[3,4,6,23,24], production of O_2^- and its role in increased salt sensitivity of arterial pressure in sensory denervated rats are unknown. Therefore, this study was designed to test the hypothesis that production of O_2^- in mesenteric resistance arteries was increased and contributed to the development of hypertension induced by sensory denervation plus high salt intake.

MATERIALS AND METHODS

Treatment groups Pregnant Wistar female rats (Charles River Laboratories Inc, Wilmington, Mass) were housed in the animal unit for at least 1 week before parturition. On the first and second days of life, neonatal rats received capsaicin 50 mg/kg sc as described[14]. Control rats were treated with equal volumes of vehicle solution (5 % ethanol, 5 % Tween-80 in saline). After 3 weeks, male rats were pair-fed different sodium diets and subjected to following treatments for 3 weeks: capsaicin pretreatment plus normal sodium diet (0.5 %, CAP-NS), capsaicin pretreatment plus high sodium diet (4 %, CAP-HS), capsaicin pretreatment plus high sodium diet plus tempol (1 mmol·kg^{-1}·d^{-1} by oral gavage) (CAP-HS-T), control plus normal sodium diet (CON-NS), control plus high sodium diet (CON-HS), and control plus high sodium diet plus tempol (CON-HS-T). Additional four groups were used for acute iv administration of tempol, ie, CAP-HS-T1 and CON-HS-T1 (tempol, 72 μmol/kg by iv), and CAP-HS-T2 and CON-HS-T2 (tempol, 216 μmol/kg by iv). The rat food was purchased from Harlan Teklad Diets. These doses of tempol have been shown to be effective in decreasing O_2^- levels and blood pressure in SHR and Ang II-induced hypertension[1,2,10]. The rats were anesthetized with ketamine and xylazine (80 and 4 mg/kg ip, respectively). The right jugular vein was cannulated for administration of tempol. The right carotid artery was cannulated for monitoring mean arterial pressure (MAP) with a Statham 231D pressure transducer coupled to a Gould 2400s recorder (Gould Instrument Systems, Valley View, Ohio, USA). Three hours after surgery, baseline MAP and its response to tempol were measured with the rats fully awake and unrestrained. Baseline MAP was calculated as the average of the recording over 30-min period and MAP after tempol treatment represented peak decreases. By the end of the experiments, rats were decapitated and blood (3 mL) was collected on ice in 0.01 % EDTA tubes. Mesenteric resistance arteries were dissected and used either for measurement of O_2^- levels immediately or for determination of manganese superoxide dismutase (MnSOD) and endothelial nitric oxide synthase (eNOS) levels in snap-froze samples.

Systolic blood pressure Tail-cuff systolic blood pressures were routinely obtained in all rats by use of a Narco Bio-Systems Electro-Sphygmomanometer. The pressures were measured in conscious rats every 7 d for 20 d, beginning 1 d before dietary treatment. The blood pressure value for each rat was calculated as the average of 3 separate measurements at each 15-min session and represents steady state.

Measurement of O_2^- levels by lucigenin chemiluminescence assay Measurement of O_2^- production by lucigenin chemiluminescence assay has been described previously[6,25]. Briefly, the first- to third-order

mesenteric resistance arteries were cut into 5 mm segments and incubated in chilled Krebs-HEPES buffer on ice. The buffer consisted of (in mmol/L) NaCl 119, HEPES 20, KCl 4.6, $CaCl_2$ 1.2, Na_2HPO_4 0.15, KH_2PO_4 0.4, $MgSO_4$ 1.0, $NaHCO_3$ 25.0, glucose 5.5, at pH7.4. The segments were then maintained at 37 ºC for 30 min in Krebs-HEPES buffer with diphenyleneiodonium (DPI, 0.1 mmol/L), rinsed and gently transferred to test tubes containing 1 mL Krebs-HEPES buffer and lucigenin (5 μmol/L), and allowed to equilibrate in the dark for 10 min at 37 ºC. Lucigenin chemiluminescence was recorded every 30 s for 5min with a luminometer and blood vessels were blotted to soak up excess liquid and weighed. Superoxide generation was quantified against a standard curve of superoxide generation by xanthine/xanthine oxidase. Tissue superoxide formation was expressed as counts·min^{-1}·mg^{-1} wet tissue.

Measurement of nitrite/nitrate in plasma by Griess method Nitric oxide has a very short half-life and is oxidized to form NO_2 and NO_3 *in vivo*. Measurement of NO_x (NO_2+NO_3) concentrations in plasma has been used as an indicator for NO formation *in vivo*. Concentration of nitrate/nitrite in plasma was determined by the method of Griess reaction after enzymatic conversion of nitrate to nitrite[26]. Briefly, 200 μL of plasma was incubated with 100 mmol/L HEPES 100 μL, 125 mmol/L NADPH 100 μL, and 4 mg/L nitrate reductase 20 μL. Excessive NADPH, proteins, and interfering pigments were precipitated by adding 0.4 mol/L zinc sulfate and 0.4 mol/L Na_2CO_3. After centrifugation at 12 000×*g* for 20 min at 4 ºC, 100 μL supernatant was incubated with Griess reagent (1 % sulphanilamide, 0.1 % *N*-1-naphthyl-ethylenediamine in 5 % phosphoric acid) for 10 min at room temperature in the dark. All reactions were carried out in duplicate. Absorbance was measured at 560 nm. Sodium nitrite diluted in the distilled water in the range of 0-100 mmol/L was used for calibration. Amounts of NO_x were expressed as μmol/L of plasma sample.

Measurement of MnSOD and eNOS levels by Western blot analysis Mesenteric resistance arteries were dissected and homogenized in buffer containing 10 mmol/L Tris-HCl (pH 7.6), 0.5 mmol/L $MgCl_2$ with protease inhibitors (10 mg/L leupeptin, 10 mg/L aprotinin, 1 mmol/L phenylmethylsulfonyl fluoride and 1.8 g/L iodoacetamide). Homogenates were treated as previously described[19-21]. The protein concentration was determined with a protein assay kit (Bio-Rad). Equal amounts of protein from each animal (10 mg) were electrophoresed on SDS polyacrylamide gel (12 % for MnSOD and 7.5 % for eNOS) and then transferred to a polyvinylidene fluoride membrane. Membranes were blocked in 5 % milk and reacted either with polyclonal antibody against MnSOD at 1:1000 dilution (Transduction Laboratories) or with polyclonal antibody against eNOS at 1:1000 dilution (Transduction Laboratories), followed by incubation with HRP-labeled secondary antibody (Amersham Life Science) at 1:4000 dilution. Bands were detected by the use of ECL chemiluminescence (Amersham Life Science). The intensity of the reaction was normalized by total protein content detected by Coomassie blue staining and expressed as a ratio of MnSOD or eNOS density to total protein content.

Statistical analysis Values are expressed as mean±SEM. The data were analyzed by one-way ANOVA followed by the Tukey-Kramer multiple comparison test. Differences were considered statistically significant at $P<0.05$.

RESULTS

Systolic blood pressure (A) and MAP (B) were significantly increased in CAP-HS rats when compared with CON-NS, CON-HS, and CAP-NS rats by the end of the 3-week dietary treatment. There was no statistically significant difference in systolic blood pressure or MAP between CON-NS, CON-HS, and CAP-NS rats, a data in agreement with our previous findings[14-16] (Fig 1).

MnSOD contents in mesenteric resistance arteries were significantly higher in CAP-HS rats compared with CON-NS, CON-HS, and CAP-NS rats, and there was no significant difference in MnSOD contents between the later three groups (Fig 2). Likewise, the content of eNOS, a major nitric oxide synthase in endothelial cells, was significantly increased in mesenteric resistance arteries of CAP-HS rats when compared with CON-NS, CON-HS, and CAP-NS rats (Fig 3). However, the concentration of nitrite/nitrate (NOx) in plasma, a reliable index for NO production *in vivo*, was not altered in CAP-HS (15.8±2.2 mol/L) rats when compared with CON-NS (14.5±0.8 mol/L), CON-HS (11.8±0.8 mol/L), and CAP-NS (15.4±1.0 mol/L) rats.

To assess the role of elevated O_2^- production in the development of hypertension in this model, tempol, a stable membrane-permeable O_2^- scavenger, was given acutely by iv injection and MAP responses to tempol

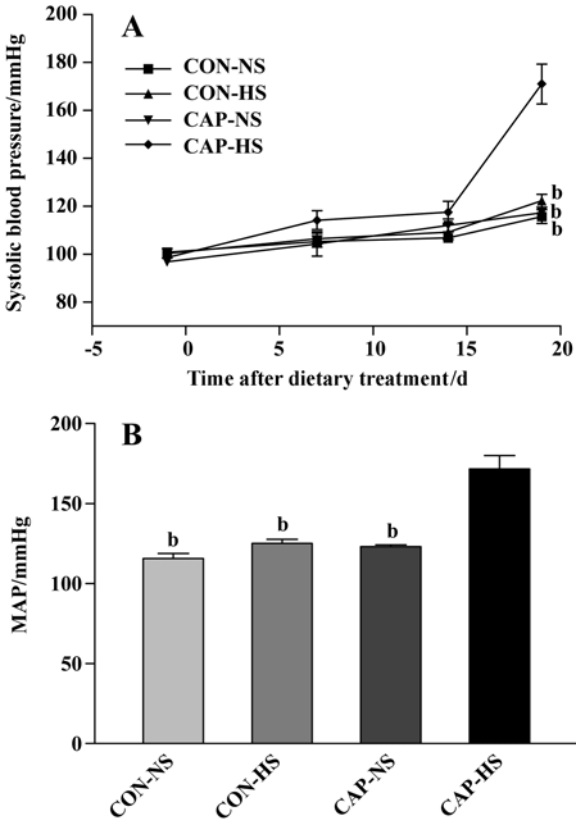

Fig 1. Systolic blood pressure (A) and mean arterial blood pressure (MAP, B) in capsaicin-pretreated rats fed a normal sodium diet (0.5 %, CAP-NS), capsaicin-pretreated rats fed a high sodium diet (4 %, CAP-HS), control rats fed a normal sodium diet (CON-NS), and control rats fed a high sodium diet (CON-HS). *n*=5-6. Mean±SEM. $^{b}P<0.05$ *vs* CAP-HS.

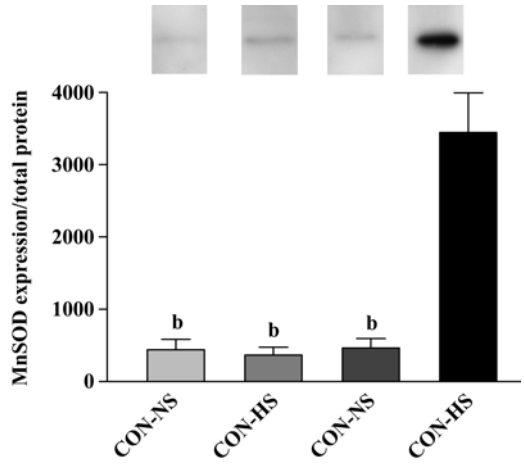

Fig 2. MnSOD contents in the mesenteric resistance arteries of rats in capsaicin pretreated rats fed a normal sodium diet (0.5 %, CAP-NS), capsaicin pretreated rats fed a high sodium diet (4 %, CAP-HS), control rats fed a normal sodium diet (CON-NS), and control rats fed a high sodium diet (CON-HS). *n*=4-5. Mean±SEM. $^{b}P<0.05$ *vs* CAP-HS.

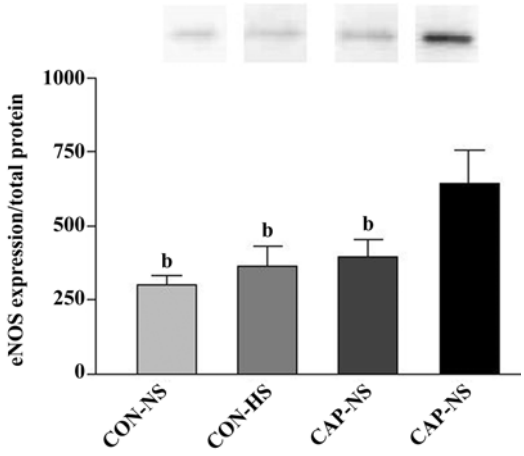

Fig 3. eNOS content in mesenteric resistance arteries of rats in capsaicin-pretreated rats fed a normal sodium diet (0.5 %, CAP-NS), capsaicin-pretreated rats fed a high sodium diet (4 %, CAP-HS), control rats fed a normal sodium diet (CON-NS), and control rats fed a high sodium diet (CON-HS). *n*= 4-5. Mean±SEM. $^{b}P<0.05$ *vs* CON-NS, CON-HS, CAP-NS.

were assessed. Baseline MAP was significantly elevated in CAP-HS rats (149±3 mmHg) compared with CON-HS rats (102±4 mmHg, $P<0.05$). Tempol at the low dose (72 mmol/kg) did not cause significant changes in MAP in either CAP-HS (145±4 mmHg) or CON-HS (97±4 mmHg) rats. Tempol at the high dose (216 mmol/kg) decreased MAP in both CAP-HS (131±4 mmHg, $P<0.05$) and CON-HS (88±3 mmHg, $P<0.05$) rats. However, these decreases in MAP were not significantly different between CAP-HS (ΔMAP, -18±2 mmHg) and CON-HS (ΔMAP, -14±2 mmHg) rats. Finally, chronic treatment with tempol by oral gavage for 3 weeks did not prevent the development of hypertension in CAP-HS rats (Fig 4).

O_2^- production in mesenteric resistance arteries was significantly increased in CAP-HS rats when compared with CON-NS, CON-HS, and CAP-NS rats, whereas there was no significant difference in mesenteric O_2^- production between the later three groups. Moreover, chronic treatment with tempol did not alter mesenteric O_2^- levels in either CAP-HS or CON-HS rats, a finding that was consistent with effect of tempol on MAP (Fig 5).

DISCUSSION

The goal of the present study was to determine whether O_2^- production in mesenteric resistance arteries was increased in sensory denervated rats fed a high salt

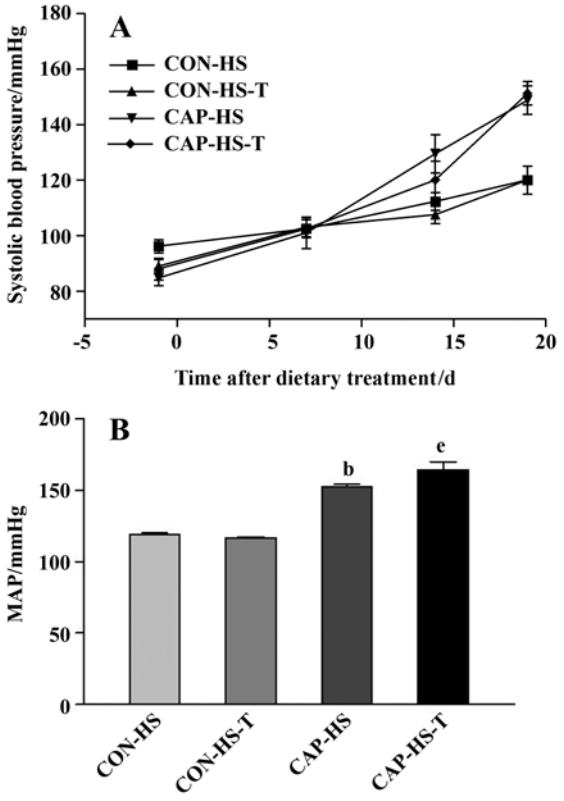

Fig 4. Systolic blood pressure (A) and mean arterial pressure (MAP, B) in capsaicin pretreated rats fed a high sodium diet (4 %, CAP-HS) and control rats fed a high sodium diet (CON-HS) with or without chronic tempol treatment for 3 weeks. T: tempol given by oral gavage at 1 mmol·kg^{-1}·d^{-1}. *n*=5-6. Mean±SEM. $^{b}P<0.05$ *vs* CON-HS. $^{e}P<0.05$ *vs* CON-HS-T.

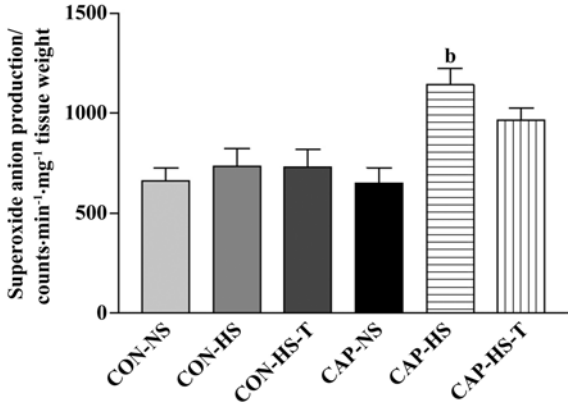

Fig 5. Superoxide anion production in mesenteric resistance arteries of rats in capsaicin-pretreated rats fed a normal sodium diet (0.5 %, CAP-NS), capsaicin-pretreated rats fed a high sodium diet (4 %, CAP-HS), capsaicin-pretreated rats fed a high sodium diet plus tempol (CAP-HS-T), control rats fed a normal sodium diet (CON-NS), control rats fed a high sodium diet (CON-HS), and control rats fed a high sodium diet plus tempol (CON-HS-T). *n*=6. Mean±SEM. $^{b}P<0.05$ *vs* CON-NS.

diet, and if so, whether increased O_2^- production contributed to increased salt sensitivity of arterial pressure in this model. We found that 1) MnSOD contents in mesenteric resistance arteries were markedly increased in sensory denervated rats fed a high salt diet compared with control rats fed a high salt diet; 2) although NO production, indicated by plasma nitrite/nitrate levels, was not altered by sensory denervation and/or high salt intake, eNOS contents in mesenteric resistance arteries were increased in sensory denervated rats fed a high salt diet compared with control rats fed a high salt diet; 3) despite the fact that acute infusion of tempol decreased blood pressure in both sensory denervated and control rats fed a high salt diet, the decreases in blood pressure were not different between the two groups and chronic treatment with tempol did not prevent or attenuate the development of hypertension in sensory denervated rats fed a high salt diet, and 4) mesenteric O_2^- production was markedly increased in sensory denervated rats fed a high salt diet compared with control rats fed a high salt diet. However, the increase in O_2^- production in the former was not sensitive to chronic tempol treatment. Taken together, these data indicate for the first time that, regardless of increased vascular MnSOD levels, salt sensitive hypertension induced by sensory denervation was associated with an elevation in vascular O_2^- production. A superoxide scavenger, tempol, failed to lower elevated O_2^- production and did not prevent the development of hypertension in this model. Several possibilities may exist and deserve further discussion.

We have previously shown that neonatal treatment with capsaicin caused permanent sensory nerve degeneration and rendered a rat salt sensitive in terms of blood pressure regulation[14-16]. Increased blood pressure in this model is accompanied by insufficiently suppressed plasma renin activity and plasma aldosterone levels in response to high salt intake[19-21]. Moreover, plasma ET-1 concentrations are markedly increased in sensory denervated rats fed a high salt diet when compared with control rats fed a high salt diet[22]. Ang II, aldosterone, and/or ET-1, either alone or in combination, may contribute to increased mesenteric O_2^- production in this model given the fact that these hormonal factors have been demonstrated to be very potent stimulators for O_2^- production in the vascular tissue[3,4,6,23,24]. Interestingly, a concomitant increase in mesenteric MnSOD

levels is found in sensory denervated rats fed a high salt diet. The increase in MnSOD contents may constitute a compensatory mechanism to prevent further increases in mesenteric O_2^- production in this model.

Superoxide may be involved in the pathogenesis of hypertension via several pathways. It may act as a vasoconstrictor directly or may interact with NO to reduce NO bioavailability and further increases vasoconstriction[12-13]. Although mesenteric O_2^- production is markedly increased in sensory denervated rats fed a high salt diet, NO production as assessed by measurement of nitrite/nitrate levels in plasma is not altered in this model. Several possibilities exist: 1) eNOS becomes uncoupled under high blood pressure to produce superoxide and counteracts the increased MnSOD activity; 2) because mesenteric eNOS levels are increased in sensory denervated rats fed a high salt diet, increased NO production induced by elevated eNOS contents may be masked by increased O_2^- production that leads to suppressed levels of NO; 3) elevated mesenteric eNOS levels are incapable of generating more NO due to the lack of sufficient substrates in sensory denervated rats fed a high salt diet; and 4) plasma nitrite/nitrate levels do not reflect NO levels in mesenteric resistance arteries.

Despite an increase in MnSOD, superoxide levels are still high, suggesting that there are sources other than mitochondrion that produce superoxide. It has been shown that tempol, a superoxide scavenger, is capable of decreasing blood pressure in several models of hypertension associated with elevated vascular O_2^- production[2,10,11]. Despite the fact that mesenteric O_2^- production is increased and may contribute to elevated blood pressure in sensory denervated rats fed a high salt diet, chronic treatment with tempol does not prevent the development of hypertension in this model. This may result from several possibilities: 1) increased mesenteric O_2^- production does not play a major role in the development of hypertension in sensory denervated rats fed a high salt diet; future examination of O_2^- production in the other vascular bed or tissues, eg, kidney, may shed some light on this issue; 2) tempol is ineffective in lowering mesenteric O_2^- production due to the overwhelmingly strong stimulation of O_2^- production by Ang II, aldosterone, and ET-1 in this model. Given that intracellular concentrations of tempol is unknown, it makes more difficult to assess the effectiveness of this drug; 3) increased mesenteric O_2^- production in this model is not due to a lack of a scavenger; and 4) other reactive oxygen species, such as hydrogen peroxide and hydroxyl radical, are increased resulting from tempol treatment and contribute to the development and maintenance of hypertension in this model[12,13]. Indeed, sources for generating superoxide, eg, NADPH oxidase, xanthine oxidase, and uncoupled eNOS may be more important and may mask the tempol's effect. The ascertaining of these possibilities waits for future *in vivo* and *in vitro* investigation of multiple oxidative stress pathways.

In conclusion, salt sensitive hypertension induced by sensory degeneration is associated with increased mesenteric O_2^- production and compensatory elevations in vascular MnSOD and eNOS levels. Although tempol does not prevent the development of hypertension in sensory denervated rats fed a high salt diet, increased superoxide levels may contribute to exacerbated vascular impairment which may take longer time to develop. Despite an increase in MnSOD, superoxide levels are still high, suggesting that there are sources other than mitochondrion that produces superoxide. Future studies using other inhibitors (*eg* inhibitors of NADPH oxidase and xanthine oxidase) on lowering blood pressure in this model, may shed some light on the pathogenesis of increased salt sensitivity of arterial pressure, and may provide information necessary for the development of novel anti-hypertensive drugs.

ACKNOWLEDGEMENTS Dr Wang is an American Heart Association Established Investigator. We would like to thank Yan HUANG for her technical assistance.

REFERENCES

1 Schnackenberg CG, Welch WJ, Wilcox CS. Normalization of blood pressure and renal vascular resistance in SHR with a membrane-permeable superoxide dismutase mimetic: role of nitric oxide. Hypertension 1998; 32: 59-64.

2 Schnackenberg CG, Wilcox CS. Two-week administration of tempol attenuates both hypertension and renal excretion of 8-Iso prostaglandin f2alpha. Hypertension 1999; 33 (1 Pt 2): 424-8.

3 Laplante MA, Wu R, El Midaoui A, De Champlain J. NAD (P)H oxidase activation by angiotensin II is dependent on p42/44 ERK-MAPK pathway activation in rat's vascular smooth muscle cells. J Hypertens 2003; 21: 927-36.

4 Landmesser U, Cai H, Dikalov S, McCann L, Hwang J, Jo H, *et al*. Role of p47 (phox) in vascular oxidative stress and hypertension caused by angiotensin II. Hypertension 2002; 40: 511-5.

5 Dobrian AD, Schriver SD, Prewitt RL. Role of angiotensin II and free radicals in blood pressure regulation in a rat model of renal hypertension. Hypertension 2001; 38: 361-6.

6 Li L, Galligan JJ, Fink GD, Chen AF. Vasopressin induces vascular superoxide via endothelin-1 in mineralocorticoid hypertension. Hypertension 2003; 41(3 Pt 2): 663-8.
7 Frisbee JC, Maier KG, Stepp DW. Oxidant stress-induced increase in myogenic activation of skeletal muscle resistance arteries in obese Zucker rats. Am J Physiol Heart Circ Physiol 2002; 283: H2160-8.
8 Frisbee JC. Impaired dilation of skeletal muscle microvessels to reduced oxygen tension in diabetic obese Zucker rats. Am J Physiol Heart Circ Physiol 2001; 281: H1568-74.
9 Schnackenberg CG, Wilcox CS. The SOD mimetic tempol restores vasodilation in afferent arterioles of experimental diabetes. Kidney Int 2001; 59: 1859-64.
10 Kawada N, Imai E, Karber A, Welch WJ, Wilcox CS. A mouse model of angiotensin II slow pressor response: role of oxidative stress. J Am Soc Nephrol 2002; 13: 2860-8.
11 Xu H, Fink GD, Galligan JJ. Nitric oxide-independent effects of tempol on sympathetic nerve activity and blood pressure in DOCA-salt rats. Am J Physiol Heart Circ Physiol 2002; 283: H885-92
12 Wilcox CS. Reactive oxygen species: roles in blood pressure and kidney function. Curr Hypertens Rep 2002; 4: 160-6.
13 Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 2000; 87: 840-4.
14 Wang DH, Li JP, Qiu JX. Salt sensitive hypertension induced by sensory denervation: introduction of a new model. Hypertension 1998; 32: 649-53.
15 Wang DH, Li JP. Antihypertensive mechanisms underlying a novel salt-sensitive hypertensive model induced by sensory denervation. Hypertension 1999; 33: 499-503.
16 Wang DH, Zhao Y. Increased salt sensitivity induced by impairment of sensory nerves: is nephropathy the cause? J Hypertens 2003; 21: 403-9.
17 Wang DH, Wu W, Lookingland KJ. Degeneration of capsaicin-sensitive sensory nerves leads to increased salt sensitivity through enhancement of sympathoexcitatory response. Hypertension 2001; 37 (2 Part 2): 440-3
18 Wu W, Zhang Y, Ballew JR, Fink G, Wang DH. Development of hypertension induced by subpressor infusion of angiotensin II: role of sensory nerves. Hypertension 2000; 36: 549-52.
19 Wang DH, Huang Y. Development of salt sensitive hypertension in a sensory denervated model: the underlying mechanisms. J Renin Angiotensin Aldosterone System 2001; 2: 125-9.
20 Huang Y, Wang DH. Role of renin-angiotensin-aldosterone system in salt-sensitive hypertension induced by sensory denervation. Am J Physiol Heart Circ Physiol 2001; 281: H2143-9.
21 Huang Y, Wang DH. Role of AT_1 and AT_2 receptor subtypes in salt-sensitive hypertension induced by sensory nerve degeneration. J Hypertens 2001; 19: 1841-6.
22 Ye DZ, Wang DH. Function and regulation of endothelin-1 and its receptors in salt sensitive hypertension induced by sensory nerve degeneration. Hypertension 2002; 39 (2 Pt 2): 673-8.
23 Virdis A, Neves MF, Amiri F, Viel E, Touyz RM, Schiffrin EL. Spironolactone improves angiotensin-induced vascular changes and oxidative stress. Hypertension 2002; 40: 504-10.
24 Bauersachs J, Heck M, Fraccarollo D, Hildemann SK, Ertl G, Wehling M, *et al*. Addition of spironolactone to angiotensin-converting enzyme inhibition in heart failure improves endothelial vasomotor dysfunction: role of vascular superoxide anion formation and endothelial nitric oxide synthase expression. J Am Coll Cardiol 2002; 39: 351-8.
25 Li L, Crockett E, Wang DH, Galligan JJ, Fink GD, Chen AF. Gene transfer of endothelial NO synthase and manganese superoxide dismutase on arterial vascular cell adhesion molecule-1 expression and superoxide production in deoxycorticosterone acetate-salt hypertension. Arterioscler Thromb Vasc Biol 2002; 22: 249-55.
26 Ma J, Nakajima T, Iida H, Iwasawa K, Terasawa K, Oonuma H, *et al*. Inhibitory effects of ursodeoxycholic acid on the induction of nitric oxide synthase in vascular smooth muscle cells. Eur J Pharmacol 2003; 464: 79-86.