Mechanisms of Oxidative Stress–Induced Increase in Salt Sensitivity and Development of Hypertension in Sprague–Dawley Rats

Anees Ahmad Banday, Abdul Bari Muhammad, Fatima Rizwan Fazili, Mustafa Lokhandwala

Abstract—High salt intake produces vascular changes that contribute to the development of hypertension in salt-sensitive individuals. Because reactive oxygen species play a role in the pathogenesis of cardiovascular diseases, we investigated whether oxidative stress contributes to salt-sensitive hypertension. Sprague–Dawley rats were divided in different groups and received tap water (vehicle), 30 mmol/L of L-buthionine sulfoximine ([BSO] an oxidant), high salt ([HS] 1% NaCl), and BSO plus HS without and with antioxidant tempol (1 mmol/L) in drinking water for 12 days. Compared with vehicle, BSO treatment caused oxidative stress and mild increase in blood pressure. Thoracic aortic rings from BSO–treated rats exhibited decreased response to endothelium–independent vasorelaxants. In HS–treated rats, the response to vasoactive agents, as well as blood pressure, was unaffected. Concomitant treatment of rats with BSO and HS produced a marked increase in blood pressure and a decreased response to both endothelium-dependent and endothelium-independent vasorelaxants with an increase in EC50. Incubation of aortic tissue from BSO-treated rats with sodium nitroprusside showed decreased cGMP accumulation, whereas HS rats had decreased basal NO synthase activity. Tempol decreased oxidative stress, normalized blood pressure, and restored NO signaling and responses to vasoactive compounds in BSO and BSO plus HS rats. We conclude that BSO increases oxidative stress and reduces NO signaling, whereas HS reduces NO levels by decreasing the NO synthase activity. These phenomena collectively result in reduced responsiveness to both endothelium -dependent and endothelium- independent vasorelaxants and may contribute to salt-sensitive hypertension. (Hypertension. 2007;49[part 2]:664-671.)

Key Words: acetylcholine ■ hypertension ■ oxidative stress ■ salt sensitivity ■ tempol

Endothelial cells modulate the reactivity of the underlying vascular smooth muscle cells by releasing endothelium-derived relaxing factors. Previous studies have demonstrated that elevated dietary salt intake leads to an impaired relaxation of blood vessels to endothelium-dependent relaxations induced by a variety of vasodilator agents. A possible contributor to impaired vascular relaxation to dilator stimuli in animals on a high-salt diet is an impaired function of the endothelium. Impaired endothelium-dependent dilation in vessels of animals on a high-salt diet could occur either because the acetylcholine (Ach)–mediated production of NO by the endothelium is impaired or because of the failure of NO to cause vasodilation.

NO is a major regulator of vascular tone in humans. Decreased production or bioavailability, or decreased vascular response to NO, has been implicated in the pathogenesis of human hypertension. An increase in blood pressure (BP) in response to dietary sodium (salt sensitivity) is a well-documented phenomenon in humans and is considered to be an important factor in the pathogenesis of hypertension. In animal models of salt-sensitive hypertension, the increase in BP after salt loading is characterized by reduced NO production. Similarly, in humans with salt-sensitive hypertension, salt loading was associated with decreased plasma and urinary levels of NO metabolites.

A variety of evidence suggests that reactive oxygen species contribute to impaired endothelial function in several forms of hypertension and that there is increased oxidative stress in the microvessels of spontaneously hypertensive rats and Dahl salt–sensitive hypertensive rats. A recent report by Lenda et al has suggested that reactive oxygen species can also contribute to a reduced endothelium-dependent dilation in normotensive rats on a high-salt diet. Despite the potential importance of reactive oxygen species in contributing to impaired endothelium-dependent vasodilation and reduced NO production during elevated dietary salt intake, the nature and mechanisms of the impaired vascular relaxation with the high-salt diet and the role of enhanced oxidative stress in contributing to salt-induced changes in vascular function and hypertension are not completely understood.

The aim of this study was to examine the hypothesis that oxidative stress modulates the blood pressure responses to...
salt loading in normotensive animals. We sought to determine whether oxidative stress–mediated salt sensitivity involves changes in NO production and/or NO-mediated vasodilation. The study was performed in normotensive animals so as to exclude the possibility that any observed changes were secondary to pre-existing cardiovascular disorders.

**Methods**

The BCA protein assay kit (Pierce), CGMP assay kit (R&D Systems), NO synthase assay kit (Calbiochem), RNeasy mini kit (QIAGEN), Advantage cDNA PCR kit (BD Biosciences), Glutathione (GSH) colorimetric determination kit (OXIS International Inc.), 8-Isoprostanе enzyme immunoassay kit (Chemicon International), and Complete protease inhibitor mixture (Roche Diagnostic GmbH) were used in this study. Rabbit anti-endothelial NO synthase (NOS) eNOS/neuronal NOS and anti-nitrotyrosine polyclonal antibodies were purchased from Transduction Laboratories and Upstate Biotechnology, respectively. 

**Animals**

Male Sprague–Dawley (SD) rats (Harlan) were fed normal rat chow and dived into following weight matching 8 groups: V, animals maintained on tap water (vehicle); HS, animals provided with 30 mmol/L of L-buthionine sulfoximine; T, animals receiving high salt plus L-buthionine sulfoximine; HS + T, animals receiving high salt plus L-buthionine sulfoximine with tempol; H11001, animals receiving L-buthionine sulfoximine with tempol; H11001, animals receiving high salt plus L-buthionine sulfoximine with tempol; and BSO, animals provided with 30 mmol/L of L-buthionine sulfoximine. All of the rats were allowed to equilibrate for 90 minutes before the start of the experiments. In some rings, the endothelium was mechanically removed by gentle rubbing with moistened cotton. Isometric tension change was measured with a digital force isometric transducer (Harvard Apparatus) connected to a data acquisition system (AD Instruments).

**Vascular Relaxation Studies**

Aortic rings with intact endothelium were preconstricted with 10 μmol/L of phenylephrine. Ach (0.1 nM to 10 μmol/L), ADP (1 nM to 1 μmol/L), or calcium ionophore A23187 (0.1 nM to 0.1 μmol/L) in the presence or absence of NG-nitro-L-arginine methyl ester (100 μmol/L) was added to the bath solution after the phenylephrine-induced contractions reached a plateau. To determine the endothelium-independent vasorelaxation, endothelium-denuded aortic rings were preconstricted with phenylephrine, and SNP (0.1 nM to 1 μmol/L), NG (0.1 nM to 1 μmol/L), or 8-bromo-cGMP (1 to 100 μmol/L) was added to the bath medium. The relaxation responses obtained were expressed as a percentage of the maximal relaxation evoked by papaverine (100 μmol/L). Endothelial integrity was confirmed by achieving ≥40% relaxation with 10 μmol/L Ach, whereas denudation of the endothelium was confirmed by the disappearance of the 1 μmol/L Ach-induced relaxation response.

**Assay of NOS Activity**

Endothelium intact rings were snap frozen and maintained at −80°C. Thawed samples were lysed, centrifuged for 5 minutes, and the supernatant (5 to 10 μg of protein) was used for NOS assay. NOS activity was quantified by assay of conversion of [14C]-arginine to citrulline, performed as described in the NOS Assay kit. Total NOS activity was measured in standard Ca2+-containing buffer. The activities of eNOS and neuronal NOS, but not inducible NO, are calcium dependent.

**Western Blotting**

Portions of isolated aortic samples were homogenized in lysis buffer containing 0.25 mol/L of sucrose, 50 mmol/L of dithiothreitol, 3 mmol/L of HEPES (pH 7.9), 0.5 mmol/L of EGTA, 0.4 mmol/L of PMSF, protease inhibitor mixture, and 1% Triton X-100. Samples were centrifuged, and supernatants were mixed with an equal volume of Laemmli buffer and subjected to SDS-PAGE. The proteins were transferred to polyvinylidene difluoride membranes, blocked, and incubated with corresponding antibodies. The blots were incubated with horseradish peroxidase–conjugated secondary antibodies and visualized with the enhanced chemiluminescence method. The density (arbitrary units) of the bands was quantified by Kodak Imaging software.

**Determination of cGMP Level**

Endothelium intact rings were preconstricted with phenylephrine for 5 minutes and then stimulated with SNP (0.01 mol/L) for 1 or 3 minutes, which allowed the generation of ～75% to 80% of the maximal relaxation. The tissues were snap frozen in liquid nitrogen, followed by homogenization in a glass/glass homogenizer in ice-cold 0.1 N HCl. The cGMP content was determined with an enzyme immunoassay kit according to the manufacturer’s protocol. Protein was determined by BCA protein assay kit using bichinchoninic acid for the colorimetric detection and quantitation of total protein with BSA as standard.

**Indexes (Biomarkers) of Oxidative Stress, GSH, and Nicotinamide-Adenine Dinucleotide Phosphate Oxidase Activity**

Aortic tissue carboxymethyllysine was measured by ELIZA as described by Koo and Vaziri, and malondialdehyde was determined by the method of Mihara and Uchiyama. For nicotinamide-adenine dinucleotide phosphate oxidase activity, fluorescence spectrometric assay of O2− production was performed by Satho et al. A bioxytech GSH kit from Oxis Health was used to measure glutathione (GSH). Plasma 8-isoprostane prostaglandin F2α was measured by 8-isoprostane prostaglandin F2α immunoassay kit from Cayman.

**Statistical Analysis**

Differences between means were evaluated using the unpaired t test or ANOVA with Newman–Keuls multiple test, as appropriate. P<0.05 was considered statistically significant.
Results

As shown in Table 1, the BSO, HS, and T regimen had no effect on body weight or food intake of animals. The mean blood pressure of BSO-supplemented rats showed a mild increase, whereas animals provided with both BSO and HS showed marked increase in blood pressure compared with vehicle. Treatment with tempol normalized blood pressure in both BSO and BSO plus HS–treated rats. In the vehicle and HS groups, tempol did not have any effect on blood pressure (Table 1).

Oxidative Stress

Animals treated with BSO or BSO plus HS showed a marked decrease in thoracic aorta tissue GSH levels and a significant increase in oxidative markers, such as malondialdehyde, carboxymethyl lysine, plasma 8-isoprostanate, and nitrotyrosine levels (Table 1 and Figure 1A). Incubation of tissue homogenates with dihydroethidium and nicotinamide adenine dinucleotide phosphate oxidase substrates caused increased O2•− production in BSO and BSO plus HS rats (Figure 1B). HS and tempol showed a mild but nonsignificant increase and decrease, respectively, in superoxide formation compared with vehicle (Figure 1B). Treatment of these animals with tempol normalized tissue malondialdehyde, carboxymethyl lysine, plasma 8-isoprostone, and nitrotyrosine levels, as well as nicotinamide adenine dinucleotide phosphate oxidase activity (Figure 1A and 1B and Table 1). Although tempol supplementation increased tissue GSH levels in BSO or BSO plus HS–treated rats, it remained significantly lower than vehicle or HS–treated animals (Table 1). HS or tempol did not cause any significant effect on oxidative stress in the absence of BSO (Table 1).

Endothelium-Dependent Relaxation

There was no difference in the maximum contraction of aortic rings to KCl (data not shown) or phenylephrine between the different groups (Table 2). Endothelium-dependent relaxations in response to Ach in endothelium-intact aortic rings from animals treated with HS or BSO alone showed a mild rightward shift. In animals treated with BSO plus HS, the response was significantly reduced, and concentration-dependent responsiveness to Ach showed a marked rightward shift with significant increase in EC50 (Figure 2). Similar to Ach, ADP and calcium ionophore A23187–induced relaxation curves showed significant increases in EC50 in BSO plus HS animals (Table 2). Treatment with tempol restored the ACh, ADP, and calcium ionophore A23187–dependent va-

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### TABLE 1. Effect of HS, BSO, and T on Body Weight, BP, and Oxidative Stress

<table>
<thead>
<tr>
<th>Parameter</th>
<th>V</th>
<th>BSO</th>
<th>HS</th>
<th>BSO+HS</th>
<th>T</th>
<th>BSO+T</th>
<th>HS+T</th>
<th>BSO+HS+T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>320.5±11.2</td>
<td>310.0±16.2</td>
<td>315.5±10.2</td>
<td>330.6±19.9</td>
<td>325.5±11.9</td>
<td>316.0±17.2</td>
<td>325.5±11.2</td>
<td>318±22.9</td>
</tr>
<tr>
<td>Food intake, g</td>
<td>18.5±3.5</td>
<td>19.3±3.3</td>
<td>19.1±3.4</td>
<td>17.9±3.1</td>
<td>18.5±2.9</td>
<td>19.5±3.8</td>
<td>18.8±2.8</td>
<td>18.1±2.7</td>
</tr>
<tr>
<td>BP, mm Hg</td>
<td>105±4.0</td>
<td>118.0±4.0*</td>
<td>112.0±4.0</td>
<td>142.0±8.0*</td>
<td>107.0±7.0</td>
<td>111.0±6.0</td>
<td>110.0±4.0</td>
<td>114.0±7.0</td>
</tr>
<tr>
<td>Carboxymethyllysine, optical density/mg protein</td>
<td>0.54±0.05</td>
<td>0.92±0.1*</td>
<td>0.59±0.05</td>
<td>1.0±0.1*</td>
<td>0.48±0.04</td>
<td>0.59±0.06</td>
<td>0.61±0.04</td>
<td>0.62±0.06</td>
</tr>
<tr>
<td>MDA, nmol/mg of protein</td>
<td>0.4±0.03</td>
<td>0.8±0.04*</td>
<td>0.46±0.02</td>
<td>0.9±0.05*</td>
<td>0.38±0.02</td>
<td>0.45±0.05</td>
<td>0.44±0.04</td>
<td>0.46±0.05</td>
</tr>
<tr>
<td>8-Isop-p, pg/mg creatinine</td>
<td>35.1±2.1</td>
<td>48.1±2.2*</td>
<td>40.8±1.8</td>
<td>53.9±3.02*</td>
<td>30.1±2.3</td>
<td>38.5±2.6</td>
<td>40.6±2.9</td>
<td>42.2±3.1</td>
</tr>
<tr>
<td>GSH, nmol/mg of protein</td>
<td>0.89±0.05</td>
<td>0.3±0.03*</td>
<td>0.87±0.06</td>
<td>0.27±0.05*</td>
<td>0.93±0.08</td>
<td>0.6±0.03*</td>
<td>0.92±0.05</td>
<td>0.56±0.03*</td>
</tr>
</tbody>
</table>

The animals were treated for 12 days with V, BSO (30 mmol/L), HS (1% NaCl), BSO+HS, T (1 mmol/L), BSO+T, HS+T, and BSO+HS+T. 8-Isop-p indicates 8-isoprostane plasma; MDA, malondialdehyde. Data (n=8 animals) were analyzed by ANOVA followed by posthoc Newman–Keuls multiple comparison test. P<0.05 was considered statistically significant.
*Significantly different from vehicle; †Significantly different from BSO.

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Figure 1. Protein nitrotyrosination (A) and nicotinamide adenine dinucleotide phosphate oxidase activity (B) in thoracic aortic tissues from vehicle, BSO-, HS-, and T-supplemented rats (n=6 to 8). A, top, representative nitrotyrosine band and bars represent density arbitrary units (mean±SE). B, Bars represent fluorescence normalized with protein (mean±SE). Data were analyzed by ANOVA followed by posthoc Newman–Keuls multiple comparison test. P<0.05 was considered statistically significant. *Significantly different from vehicle.
sorelaxation and normalized the EC₅₀ (Figure 2B and Table 2). These relaxations were completely inhibited by NG-nitro-L-arginine methyl ester, an NOS inhibitor (data not shown).

**Endothelium-Independent Relaxations**

There was no significant difference between the HS group and the vehicle group in endothelium-independent relaxations in response to SNP and NG, which are NO donors, in endothelium-denuded aortic rings (Figure 3A and Table 2). However, the relaxation response was reduced in animals treated with BSO or BSO plus HS. The dose–response curve showed a significant rightward shift with marked increase in EC₅₀ in these animals (Figure 3A and Table 2). On the other hand, the relaxation in response to 8-bromo-cGMP, a stable cyclic GMP molecule, was similar in all of the experimental groups (Table 2). Treatment with tempol restored the vasorelaxant response in BSO or BSO plus HS–treated rats (Figure 3B). Table 2 also shows the values of vasodilator potency of NG in various groups.

### Table 2. Effect of HS, BSO, and T on Phenylephrine, ADP, A23187, NG, and 8b-cGMP Response in Aortic Rings

<table>
<thead>
<tr>
<th>Vasoactive Compound</th>
<th>V</th>
<th>BSO</th>
<th>HS</th>
<th>BSO+HS</th>
<th>T</th>
<th>BSO+T</th>
<th>HS+T</th>
<th>BSO+HS+T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylephrine-mediated vasoconstriction</td>
<td>PE EC₅₀ [M]</td>
<td>3.03×10⁻⁸</td>
<td>4.42×10⁻⁸</td>
<td>2.75×10⁻⁸</td>
<td>1.72×10⁻⁸</td>
<td>2.40×10⁻⁸</td>
<td>4.72×10⁻⁸</td>
<td>3.33×10⁻⁸</td>
</tr>
<tr>
<td>Endothelium dependent vasodilatation by ADP and A23187</td>
<td>ADP, EC₅₀ [M]</td>
<td>9.31×10⁻⁸</td>
<td>8.39×10⁻⁸</td>
<td>7.50×10⁻⁸</td>
<td>8.82×10⁻⁸</td>
<td>9.46×10⁻⁸</td>
<td>7.82×10⁻⁸</td>
<td>8.22×10⁻⁸</td>
</tr>
<tr>
<td>A23187 EC₅₀ [M]</td>
<td>1.04×10⁻⁷</td>
<td>3.19×10⁻⁷</td>
<td>2.01×10⁻⁷</td>
<td>2.82×10⁻⁷</td>
<td>2.63×10⁻⁷</td>
<td>1.83×10⁻⁷</td>
<td>2.86×10⁻⁷</td>
<td>3.01×10⁻⁷</td>
</tr>
<tr>
<td>Endothelium independent vasodilatation by NG and 8b-cGMP</td>
<td>NG EC₅₀ [M]</td>
<td>4.12×10⁻⁸</td>
<td>3.22×10⁻⁸</td>
<td>3.92×10⁻⁸</td>
<td>5.72×10⁻⁸</td>
<td>1.38×10⁻⁷</td>
<td>5.01×10⁻⁸</td>
<td>4.73×10⁻⁸</td>
</tr>
<tr>
<td>8b-cGMP EC₅₀ [M]</td>
<td>6.21×10⁻⁹</td>
<td>4.32×10⁻⁹</td>
<td>5.36×10⁻⁹</td>
<td>6.72×10⁻⁹</td>
<td>5.31×10⁻⁹</td>
<td>6.21×10⁻⁹</td>
<td>5.06×10⁻⁹</td>
<td>4.89×10⁻⁹</td>
</tr>
</tbody>
</table>

The animals were treated for 12 days with V, BSO, HS (1% NaCl), BSO+HS, T, BSO+T, HS+T, and BSO+HS+T. Data (n=8 animals) were analyzed by ANOVA followed by posthoc Newman–Keuls multiple comparison test. *P<0.05 was considered statistically significant.

*Significantly different from vehicle.
NOS Activity and Protein Expression

The basal NOS activity was decreased in HS-treated animals compared with vehicle (Figure 4A). The concomitant treatment with BSO and HS caused further decrease in NOS activity, whereas BSO by itself showed no effect on NOS activity (Figure 4A). Tempol normalized the NOS activity in BSO plus HS–treated rats but failed to restore the NOS activity in animals provided with HS alone (Figure 4A). There was no difference in NOS activity between vehicle and tempol-treated rats (Figure 4A).

Western blot analysis showed higher eNOS protein in BSO or BSO plus HS animals (Figure 4B). Treatment with tempol normalized protein abundance in BSO and BSO plus HS rats (Figure 4B). On the other hand, HS caused a small but significant decrease in eNOS protein expression, which was not restored by tempol (Figure 4B).

SNP Induced cGMP Accumulation

In aortic rings from vehicle and HS-treated rats, SNP stimulation increased the cGMP levels. However, SNP-induced cGMP accumulation was reduced in aortas from BSO and BSO plus HS rats (Figure 5). The decrease in SNP-induced cGMP accumulation was much higher in BSO plus HS rats than in BSO animals (Figure 5). Tempol restored the SNP-induced cGMP accumulation in both BSO and BSO plus HS animals. Tempol showed no effect on SNP signaling in vehicle or HS rats (Figure 5).

Discussion

An increase in blood pressure in response to increased dietary sodium intake is considered an important contributing factor in the pathogenesis of hypertension, particularly in humans or animals predisposed to salt sensitivity.25–28 In this study, we observed that in normotensive rats, high salt intake has no effect on response to vasodilatory factors or blood pressure despite reduced NOS activity and eNOS expression. On the other hand, treatment of animals with BSO, a γ-glutamylcysteine synthetase inhibitor, reduced GSH, increased oxidative stress, nicotinamide-adenine dinucleotide phosphate oxidase activity and eNOS protein expression, reduced SNP-mediated guanylyl cyclase (GC) activation and vascular response, and caused a mild increase in blood pressure. Concomitant administration of HS and BSO led to increased oxidative stress, nicotinamide-adenine dinucleotide phosphate oxidase activity, decreased in NOS activity, and increased eNOS expression. These animals exhibited a decreased response to both endothelial-dependent and endothelial-independent vasodilators and marked elevation in blood pressure. Treatment with tempol mitigated oxidative stress and corrected eNOS expression, NOS activity, and NO signaling in BSO and BSO plus HS-treated rats. Furthermore, tempol normalized the response to vasodilatory agents and BP in these animals.
High Salt Intake, Vascular Function, and BP

Several studies have reported a moderate increase, whereas others have found no significant change in arterial BP with high salt intake in normotensive SD rats. In the present study, we found that high salt intake had no effect on either endothelium-dependent or endothelial-independent relaxations in normotensive SD rats, and there was no change in BP in the presence of decreased aortic NOS activity and expression. These relaxations were mediated mainly by NO, because they almost disappeared in the presence of NG-nitro-L-arginine methyl ester, an inhibitor of NOS. We also found that high salt intake does not affect the NO–GC coupling as evidenced by an increase in cGMP accumulation in response to SNP. Taken together, the observations that the decrease in NOS activity or expression during salt loading is not related to BP response and that vascular relaxation response is preserved imply that, in healthy subjects, other compensatory mechanisms are of primary importance in adjusting BP to salt loading.

Oxidative Stress, Vascular Function, and BP

We used SD rats to study the role of oxidative stress on vascular function and BP. Treatment of rats with BSO produced oxidative stress and decreased relaxant response to endothelial-independent agents. These animals exhibited a mild increase in BP despite normal NOS activity and intact response to endothelial-dependent vasoactive agents indicating that factors proximal to NO production also play an important role in hypertension. It is well known that NO activates the soluble isofrom of GC to form cGMP. The increased cGMP level causes vascular smooth muscle relaxation and regulates vascular tone in various vascular beds. In hypertensive animal models, altered vascular activity of the NO-cGMP pathway, such as reduced GC activity and decreased cGMP accumulation, has been reported. We also observed that BSO reduced SNP-mediated cGMP accumulation in aortic tissue, indicating reduced NO–GC signaling. Therefore, in the presence of oxidative stress, a decreased NO-mediated GC stimulation followed by a reduced cGMP level may lead to impairment of smooth muscle relaxation and a subsequent increase in BP.

We observed a significant increase in eNOS expression in BSO-treated rats, although their NOS activity was similar to control animals. There are reports indicating that superoxide radicals can interact with NO and decrease its bioavailability. Although we did not measure the NO levels in the present study, it is possible that the increase in eNOS protein synthesis could be a compensatory mechanism to maintain the normal NO levels. This is supported by the finding that treatment of BSO rats with tempol decreased oxidative stress, restored the NO-mediated relaxation and signaling, corrected eNOS expression, and normalized BP. These studies are in agreement with Vaziri et al showing that BSO caused oxidative stress and increased BP, and these changes were reversed by antioxidant supplementation.

High Salt Intake Plus Oxidative Stress, Vascular Function, and BP

Based on our findings that animals treated with high salt had decreased NOS activity and were normotensive, whereas animals provided with BSO alone exhibited a mild increase in BP despite normal endothelium-dependent vasodilation, we hypothesized that oxidative stress may be a triggering factor for salt sensitivity. In support of our view, we found impaired vasorelaxation in response to both endothelium-dependent and endothelium-independent agents accompanied by a marked increase in BP in animals provided with HS and BSO. Treatment with tempol restored the vascular function and normalized BP. There are reports indicating that oxidative stress plays an important role in salt-sensitive hypertension. Swei et al found that hypertensive Dahl S rats have significantly higher microvascular superoxide and plasma H2O2 than Dahl R rats.

Although the exact mechanisms for decreased NO production in salt sensitivity are not known, it is suggested that reduced NO activity could be a contributing factor. Our data also revealed a significant decrease in NOS activity in aortic homogenates, confirming an association between reduced NOS activity and impaired endothelium-

![Figure 6](image-url)
mediated relaxation in these animals. Similar to BSO rats, we also observed increased protein expression of eNOS in BSO plus HS rats, thus ruling out that a decrease in eNOS abundance could be a factor for reduced NOS activity in these hypertensive rats. Based on the report of Satoh et al., it is possible that increased oxidative stress caused NOS uncoupling by decreasing the cofactors that led to NOS inactivation. This is supported by our data showing that treatment of BSO plus HS rats with tempol normalized the NOS activity, as well as eNOS protein expression, thus reinforcing the role of oxidative stress in endothelium dysfunction.

In summary, the present study reveals that increased salt intake has no effect on vascular relaxation, BP, and NO signaling despite impaired NOS activity and expression in normotensive SD rats. On the other hand, oxidative stress reduces the vasorelaxation in response to NO donors, decreases the GC/cGMP signaling, and contributes to mild hypertension. However, the high salt intake in animals with oxidative stress leads to reduced vasorelaxation in response to both endothelium-dependent and -independent vasoactive compounds. These impairments are mediated by decreased NOS activity and NO-GC uncoupling, which leads to reduced levels of cGMP in smooth muscle. Finally, tempol, while reducing oxidative stress, restores the endothelium function and NO signaling and normalizes BP, providing unequivocal support for the role of oxidative stress in vascular diseases and salt sensitivity (Figure 6).

Perspectives

Our findings have important clinical relevance as they relate to the role of high salt intake in the pathogenesis of hypertension. Based on the results of the present study, it can be suggested that, in the absence of preexisting conditions, which may result in oxidative stress, an organism is able to adjust to high salt intake by triggering compensatory mechanisms, including renal compensation, and thereby maintaining BP. However, in situations associated with oxidative stress, where some of these compensatory mechanisms are impaired, the organism acquires salt sensitivity such that excess salt intake leads to the development of hypertension. Therefore, either lowering oxidative stress or preventing an increase in oxidative stress may be beneficial in affording protection against salt-induced hypertension.

Disclosures

None.

References


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