Reduced Angiotensin II and Oxidative Stress Contribute to Impaired Vasodilation in Dahl Salt-Sensitive Rats on Low-Salt Diet

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Abstract—This study investigated the role of impaired angiotensin II (Ang II) modulation in contributing to reduced vascular relaxation in isolated middle cerebral arteries (MCA) (100 to 200 μm in diameter) of normotensive Dahl salt-sensitive (SS) rats maintained on low salt (LS) diet (0.4% NaCl) for 9 to 10 weeks. MCA from SS rats on LS diet (n=6 to 9) constricted in response to reduction of perfusate and superfusate PO2 to 35 to 40 mm Hg or acetylcholine (ACh). Vasodilator responses to reduced PO2 and ACh were restored in SS.13BN consomic rats that are 98% genetically identical to SS rats, but exhibit normal regulation of their renin-angiotensin system (RAS). This restored dilation could be prevented by feeding SS.13BN rats high-salt (HS) diet (4% NaCl) for 3 days to suppress Ang II. A continuous intravenous infusion of a subpressor dose (3 ng/kg per minute) of Ang II for 3 days restored vasodilator responses to ACh and reduced PO2 in SS.13BN rats on HS diet and in SS rats on LS diet. Superoxide scavenging with tempol (100 μmol/L) restored vasodilator responses to ACh and reduced PO2 in MCA of SS rats on LS diet, but did not affect vasodilator responses in MCA of SS.13BN rats on LS diet. These data indicate that exposure to chronically low Ang II levels leads to impaired vascular relaxation in SS rats, even when the animals are on LS diet and normotensive. This impaired relaxation appears to be mediated by increased levels of oxidative stress in the arteries. (Hypertension. 2005; 45[part 2]:687-691.)

Key Words: angiotensin II • endothelium • muscle, smooth, vascular • oxygen • vasodilation

Dahl salt-sensitive (SS) rats exhibit impaired regulation of their plasma renin activity1–3 and are presumably exposed to chronically low levels of angiotensin II (Ang II). SS.13BN consomic rats are produced by substituting chromosome 13 of the Brown Norway rat containing a normally functioning renin gene into the genetic background of the SS rat. SS.13BN rats are 98% genetically identical to the SS rat4 but are able to regulate their renin-angiotensin system (RAS) normally and do not show the extreme hypertension exhibited by SS rats when they are fed high-salt (HS) diet.2

A recent study demonstrated a striking impairment of vascular relaxation mechanisms in middle cerebral arteries (MCA) of SS rats, even when the rats are on a low-salt (LS) diet and are normotensive.5 Impaired vascular relaxation in SS rats on LS diet appeared to be caused by chronic exposure to low Ang II levels, because normal vasodilator responses were restored in SS.13BN consomic rats that show normal regulation of the RAS.5 This restored dilation in SS.13BN rats could be eliminated by feeding the animals HS diet to suppress plasma Ang II or by blocking the AT1 receptor with losartan when the rats were on LS diet.5 The potential link between impaired vascular relaxation and low Ang II levels in SS rats on LS diet is consistent with existing studies demonstrating that Ang II suppression with HS diet leads to striking impairments of vascular reactivity in normotensive Sprague-Dawley rats that appear to be caused by loss of Ang II interaction with its AT1 receptor.6,7

Recent studies by Lenda et al8–10 suggest that impaired vascular relaxation in normotensive rats on HS diet may be caused by increased oxidative stress, possibly as a result of downregulation of antioxidant enzymes. The latter observation suggests that exposure to chronically low Ang II levels, as seen during exposure to HS diet, may lead to increased oxidative stress that may contribute to impaired vascular relaxation in SS rats, even when the animals are on LS diet and are normotensive.

The goal of this study was to directly test the role of reduced Ang II levels in contributing to impaired vasodilation of MCA in SS rats on LS diet and in SS.13BN rats on HS diet by continuously infusing a low (subpressor) dose of Ang II to restore normal plasma Ang II levels. The role of increased oxidative stress in contributing to impaired vascular relaxation was tested by evaluating vasodilator responses to reduced PO2 and acetylcholine (ACh) in the presence or absence of the superoxide scavenger tempol.

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Methods

Experimental Groups
Male SS (SS/J/Hsd/Mewi) and consomic SS.13B6 rats were fed a LS (0.4% NaCl) (Dyets, Inc, Bethlehem, Pa) diet with water ad libitum. Another group of SS.13B6 rats was switched to HS diet (4% NaCl) for 3 days with water ad libitum to suppress Ang II. The effect of chronic intravenous infusion of a low-dose of Ang II was also tested in SS rats fed LS diet and SS.13B6 rats fed HS diet for 1 week. Rats in the latter groups were instrumented with indwelling arterial and venous catheters for measurement of mean arterial pressure and intravenous infusion of Ang II. After recovery on LS diet and the change to HS diet in the SS.13B6 rats, isotonic saline (1 mL/h) was infused intravenously for 3 days as a control period for mean arterial pressure monitoring. After the control measurements, intravenous infusion of Ang II (3 ng/kg per minute) (Ang II, human acetate; Sigma Aldrich, St. Louis, Mo) was begun and mean arterial pressure was monitored daily in the conscious animals. Three days later, the animals were anesthetized and MCA were isolated for the cannulated vessel studies. Rats used in these experiments were 10 to 12 weeks old at the time of the experiment, and each group included 6 to 10 rats. The Medical College of Wisconsin IACUC approved all procedures used in this study.

Isolated Vessel Studies
On the day of the study, rats were anesthetized with a low dose of pentobarbital (30 mg/kg, intraperitoneal; Abbot Laboratories, North Chicago, Ill), because of increased sensitivity of SS and SS.13B6 rats to anesthetic. MCA were isolated and cannulated using procedures described earlier. Intravascular pressure was maintained at 80 mm Hg and the vessels were perfused and superfused with physiological salt solution (PSS) equilibrated with a 21% O2, 5% CO2, 74% N2 gas mixture. The PSS used in these experiments had the following constituents (mmol/L): 119 NaCl, 4.7 KCl, 1.17 MgSO4, 1.6 CaCl2, 1.18 NaH2PO4, 24 NaHCO3, 0.026 EDTA, and 5.5 dextrose. Vessels that did not show active tone at rest, as indicated by a large dilation in response to Ca2+-free PSS, were not used in the study.

After the control equilibration period, responses of MCA to the endothelium-dependent dilator ACh (1 μmol/L), and to reduction of perfusate and superfusate PO2 to 40 to 45 mm Hg (produced by equilibrating the PSS with a 0% O2, 5% CO2, and 95% N2 gas mixture) were determined by measuring arterial diameter via video microscopy. In the initial experiments, responses to ACh and reduced PO2 were tested in MCA from SS rats on LS diet with or without Ang II infusion) or in SS.13B6 rats maintained on HS diet (with or without Ang II infusion). In a second series of studies, responses to ACh and reduced PO2 were determined in arteries of SS and SS.13B6 rats on LS diet before and after addition of the superoxide scavenger tempol (100 μmol/L) to the perfusion and superfusion solutions. Maximum diameter was determined by measuring the diameter increase during maximal relaxation of the MCA with Ca2+-free relaxing solution containing the following constituents (mmol/L): 92.0 NaCl, 4.7 KCl, 1.17 MgSO4, 20.0 MgCl2, 1.18 NaH2PO4, 24.0 NaHCO3, 0.026 EDTA, 2.0 EGTA, and 5.5 dextrose.

Statistical Analysis
Data were summarized as mean±SEM. Changes in vessel diameter in response to reduced PO2 or a single dose of an agonist (relative to the resting control value) were assessed by a paired Student t test. Differences between 2 means were assessed using an unpaired Student t test and differences among multiple group means were assessed using ANOVA with a Student-Newman-Keuls test post hoc. P<0.05 was considered to be statistically significant.

Results
Arterial Blood Pressure During Ang II Infusion
Figure 1 summarizes mean arterial pressure (MAP) measurements in conscious chronically instrumented rats receiving an intravenous infusion with a low dose of Ang II (3 ng/mL per minute) for 3 days. Relative to pre-infusion control values, Ang II infusion did not cause any significant changes in MAP in the SS rats on LS diet or in SS.13B6 rats fed HS diet for 1 week.

Effect of Ang II Infusion on Responses to ACh and Reduced PO2
Figure 2 summarizes the responses to ACh (Figure 2A) and reduced PO2 (Figure 2B) in MCA of SS rats on LS diet with and without low-dose Ang II infusion. Vessels from SS rats
on LS diet constricted in response to ACh and reduced PO2, whereas MCA of SS rats on LS diet and receiving low-dose Ang II infusion dilated in response to ACh and reduced PO2.

Figure 3 summarizes the effect of Ang II infusion on the responses to ACh (Figure 3A) and reduced PO2 (Figure 3B) in MCA from SS.13BN rats maintained on a HS diet for 1 week. HS diet represents response of MCA from SS.13BN controls on HS diet alone and solid bar represents responses of MCA from SS.13BN rats on HS and receiving a continuous intravenous infusion of a low dose of angiotensin II (3 ng/kg per minute). Data are expressed as mean change in diameter (μm)±SEM for 6 rats per group. *Significant difference (P<0.05) from HS value in the absence of Ang II infusion.

Effect of Tempol on Responses to ACh and Reduced PO2 in MCA from SS rats and SS.13BN Rats on LS Diet

Figures 4 and 5 summarize the effect of tempol on the responses to ACh (Figure 4A) and reduced PO2 (Figure 4B) in MCA from SS.13BN rats on HS diet alone and solid bar represents responses of MCA from SS.13BN rats on HS and receiving a continuous intravenous infusion of a low dose of angiotensin II (3 ng/kg per minute). Data are expressed as mean change in diameter (μm)±SEM for 6 rats per group. *Significant difference (P<0.05) from HS value in the absence of Ang II infusion.

Effect of Tempol on Responses to ACh and Reduced PO2 in MCA from SS rats and SS.13BN Rats on LS Diet

Figures 4 and 5 summarize the effect of tempol on the responses to ACh and reduced PO2 in MCA from SS rats and consomic SS.13BN rats on a LS diet. MCA from SS rats exhibited a paradoxical constriction in response to both ACh and reduced PO2. Similar to previous reports in Sprague-Dawley rats,6,7,13 ACh and reduced PO2 caused dilation of MCA from SS.13BN rats receiving low-dose Ang II infusion while on HS diet.

Effect of Tempol on Responses to ACh and Reduced PO2 in MCA from SS rats and SS.13BN Rats on LS Diet

Figures 4 and 5 summarize the effect of tempol on the responses to ACh and reduced PO2 in MCA from SS rats and consomic SS.13BN rats on a LS diet. MCA from SS rats exhibited a paradoxical constriction in response to both ACh and reduced PO2. Similar to previous reports in Sprague-Dawley rats,6,7,13 ACh and reduced PO2 caused dilation of MCA from SS.13BN rats receiving low-dose Ang II infusion while on HS diet.

Discussion

Recent studies suggest that differences in the response of resistance arteries to vasodilator stimuli in SS and SS.13BN rats on LS diet may reflect differences in the regulation of their RAS. For example, SS rats exhibit a striking impairment of arterial dilation even when the rats are on LS diet and normotensive.5 However, vasodilator responses are restored in SS.13BN consomic rats on a LS diet,5 which regulate their RAS normally. Restored vascular relaxation in response to ACh and reduced PO2 in SS.13BN rats is also eliminated by AT, receptor blockade or by feeding the rats HS diet to suppress their Ang II.5 In the present study, increasing plasma Ang II by low-dose Ang II infusion restored vascular relaxation in SS rats on LS diet (Figure 2) and reversed the inhibitory effect of HS diet on vascular relaxation in SS.13BN rats (Figure 3). This effect is in agreement with previous findings that Ang II infusion to prevent salt induced suppression of Ang II levels restores normal vasodilator responses in Sprague-Dawley rats on HS diet.6,7,13 These observations provide further evidence in support of the hypotheses that lower levels of Ang II contribute to impaired vascular relaxation in MCA of normotensive SS rats maintained on a LS diet and that restoration of normal plasma Ang II regulation plays an important role in maintaining vascular relaxation mechanisms in MCA of SS.13BN rats on LS diet.

Increased oxidative stress has been proposed to play an important role in different experimental animal models.21 Increased oxidative stress also may contribute to impaired vascular relaxation in the microcirculation of SS hypertensive rats on HS diet compared with normotensive controls on LS diet or
Dahl salt-resistant rats. The latter observations may be relevant to human hypertension in light of a recent report that increased oxidative stress is associated with impaired endothelium-dependent dilation in response to ACh in humans with renovascular hypertension. In the present experiments, we found that superoxide scavenging with tempol (100 μmol/L) converts the vasoconstrictor response to ACh (Figure 4) and reduced PO2 (Figure 5) into dilations in MCA of SS rats on LS diet. However, tempol did not affect dilation in response to ACh (Figure 4) or reduced PO2 (Figure 5) in MCA from SS rats on HS diet. Because a major phenotypic difference between these strains of rats involves regulation of the RAS, it is attractive to hypothesize that normalization of Ang II reduces oxidative stress in the SS, thereby helping to maintain normal vascular relaxation.

In vitro and in vivo studies have demonstrated that high levels of Ang II generate oxidative stress in the vessel wall by stimulating the activity of membrane-bound NAD(P)H oxidase in the vascular smooth muscle cells. This elevated superoxide production appears to contribute, at least in part, to impaired vascular relaxation in response to ACh and the nitric oxide (NO) donor nitroglycerin. However, recent evidence suggests that increased oxidative stress also may contribute to impaired ACh-induced relaxation of arterioles in normotensive rats on HS diet (in which Ang II levels would be suppressed). Collectively, these observations suggest that increased oxidative stress can contribute to impaired vascular relaxation by reducing NO bioavailability in normotensive animals on HS diet. This effect is most likely mediated via increased destruction of NO by interaction with oxygen radicals. HS diet also appears to cause endothelial nitric oxide synthase uncoupling, which produces superoxide instead of NO in aortas challenged with methacholine. Thus, increased levels of oxidative stress may contribute to impaired vascular relaxation under conditions in which Ang II is actually reduced, rather than elevated, eg, in animals on HS diet or in SS rats on LS diet.

One possible mechanism by which elevated oxidative stress could develop in SS rats is by downregulation of antioxidant defense mechanisms. In contrast to findings demonstrating that large elevations in Ang II levels induce hypertension and increase oxidative stress, Fukai et al. recently reported that Ang II increases extracellular superoxide dismutase (ecSOD) activity, ecSOD mRNA, and ecSOD protein expression in mouse aorta and increases ecSOD mRNA in human aortic smooth muscle cells. Other studies indicate that reduced Cu/Zn SOD activity contributes to impaired ACh-induced dilation in arterioles of normotensive rats on HS diet and that Cu/Zn SOD and Mn SOD expression are reduced in the kidney of SS rats fed HS diet. Overall, those findings suggest that exposure to chronically low Ang II levels during HS diet (or in SS rats on LS diet) could lead to increased oxidative stress because of downregulation of antioxidant enzymes such as superoxide dismutase.

**Perspectives**

This study provides evidence supporting the hypothesis that Ang II is required to maintain normal vascular relaxation mechanisms, and that exposure to low levels of Ang II leads to impaired vasodilator responses in cerebral resistance arteries. Although it is well-known that elevated levels of Ang II increase superoxide formation in blood vessels, the present findings suggest that reduced Ang II levels can also lead to increased oxidative stress and impaired vascular relaxation in SS rats, a widely used model of salt-sensitive hypertension in humans. These findings could provide important insight into early alterations of vascular function that may occur before the onset of elevated blood pressure in low-renin forms of hypertension or in other conditions characterized by reduced Ang II levels, eg, elevated dietary salt intake.

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