Role of Endothelin B Receptors in Enhancing Endothelium-Dependent Nitric Oxide–Mediated Vascular Relaxation During High Salt Diet

Jena B. Giardina, GaChavis M. Green, Anna N. Rinewalt, Joey P. Granger, Raouf A. Khalil

Abstract—High salt diet is often associated with increases in blood pressure, and the state of activation of endothelium-dependent vascular relaxation pathways is critical under these conditions. Basal activation of endothelial endothelin B (ET\(_B\)) receptors by endothelin has been suggested to stimulate the release of factors that promote vascular relaxation. However, whether ET\(_B\) receptors play a role in enhancing endothelium-dependent vascular relaxation during high salt diet is unclear. In this study, we investigated whether chronic treatment with an ET\(_B\) receptor antagonist is associated with impaired endothelium-dependent vascular relaxation and enhanced vascular reactivity particularly during high salt diet. Isometric contraction was measured in aortic strips isolated from male Sprague-Dawley rats on normal sodium (NS, 1%) and high sodium diet (HS, 8%) for 7 days and untreated or treated with the ET\(_B\) receptor antagonist A-192621 (30 mg/kg per day) for 5 days. The mean arterial pressure was (in mm Hg) 122±3 in NS, 132±3 in HS, 144±2 in NS/ET\(_B\) antagonist, and 171±12 in HS/ET\(_B\) antagonist rats. In endothelium-intact strips, phenylephrine (Phe, 10\(^{-5}\) mol/L) increased active stress to 7.6±1.0×10\(^{-3}\) N/m\(^2\) in NS rats and 8.2±0.9×10\(^{-3}\) N/m\(^2\) in HS rats. Phe (10\(^{-3}\) mol/L) -induced stress was significantly greater in NS/ET\(_B\) antagonist (11.3±0.9×10\(^{-3}\) N/m\(^2\)) than NS and far greater in HS/ET\(_B\) antagonist (14.1±0.1×10\(^{-3}\) N/m\(^2\)) than HS rats. Also, Phe was more potent in NS/ET\(_B\) antagonist and HS/ET\(_B\) antagonist rats (ED\(_{50}\)=0.3×10\(^{-3}\) and 0.15×10\(^{-3}\) mol/L) than in NS and HS rats (ED\(_{50}\)=0.8×10\(^{-3}\) and 0.7×10\(^{-3}\) mol/L). Removal of the endothelium enhanced Phe-induced contraction significantly in NS and to a greater extent in HS, but not in NS/ET\(_B\) antagonist or HS/ET\(_B\) antagonist rats. In endothelium-intact strips, acetylcholine (ACh) caused relaxation of Phe contraction that was less in NS/ET\(_B\) antagonist than NS and far less in HS/ET\(_B\) antagonist than HS rats. Pretreatment of endothelium-intact strips with L-NAME (10\(^{-4}\) mol/L), to inhibit nitric oxide (NO) synthase, or with methylene blue (10\(^{-5}\) mol/L) or 1H-[1,2,4]oxadiazolo[4,3-d]quinoxalin-1-one (ODQ, 10\(^{-6}\) mol/L), to inhibit cGMP production in smooth muscle, inhibited ACh-induced relaxation and enhanced Phe-induced contraction significantly in NS and HS, slightly in NS/ET\(_B\) antagonist, but not in HS/ET\(_B\) antagonist rats. Measurement of basal and ACh-induced nitrite/nitrate production from aortic strips showed a significant reduction in NS/ET\(_B\) antagonist compared with NS, and a greater reduction in HS/ET\(_B\) antagonist compared with HS rats. Relaxation of Phe contraction with sodium nitroprusside was not significantly different among the different groups of rats. Thus, an endothelial ET\(_B\) receptor-mediated pathway of vascular relaxation involving release of NO seems to be active under basal conditions and may protect against excessive vasoconstriction and increased blood pressure particularly during high salt diet. (Hypertension. 2001;37[part 2]:516-523.)

Key Words: arterial pressure ■ endothelium ■ nitric oxide ■ vascular smooth muscle ■ contraction

High salt diet has been implicated in the pathogenesis of hypertension, particularly in salt-sensitive individuals.\(^1\)-\(^4\) and salt moderation is often recommended to protect against excessive increases in blood pressure.\(^1\),\(^3\),\(^5\),\(^6\) Studies in salt-sensitive experimental animals such as the Dahl salt-sensitive rat have shown that high salt diet is associated with significant increases in blood pressure.\(^7\),\(^8\) Also, in salt-sensitive rats, high salt diet has been shown to be associated with significant endothelial cell dysfunction and exaggerated vascular reactivity to vasoconstrictor stimuli, which may contribute, at least in part, to the increases in blood pressure.\(^5\),\(^6\) In contrast, in non–salt-sensitive (salt-resistant) individuals and experimental animals, the same high salt diet is associated with only modest increases in blood pressure.\(^7\),\(^8\) Also, when salt-resistant animal models are fed high salt diets, major endothelial cell dysfunction or alterations in vascular reactivity are not usually seen.\(^9\) These studies raised recent interest in further elucidating the mechanisms by which high salt diet mediates its detrimental effects on arterial function.

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516
which high salt diet may affect vascular function and led to the hypothesis that the state of activation of endothelium-dependent vascular relaxation pathways could play an important role in the control of vascular reactivity and arterial pressure during high salt diet.

The vascular endothelium is known to release endothelium-derived relaxing factors, and several studies have shown nitric oxide (NO) to be one of those factors. NO diffuses into the smooth muscle, where it stimulates the enzyme guanylate cyclase leading to increased cyclic guanosine monophosphate (cGMP) production and smooth muscle relaxation. Other vasodilator substances have also been suggested to be released by the endothelium, including prostacyclin and endothelium-derived hyperpolarizing factor. In addition to endothelium-derived relaxing factors, the vascular endothelium also releases contracting factors such as endothelin, one of the most potent vasoconstrictors described. The interaction of endothelin with specific endothelin A (ET\textsubscript{A}) and endothelin B (ET\textsubscript{B}) receptors in smooth muscle initiates a cascade of biochemical events leading to smooth muscle contraction. ET\textsubscript{B} receptor antagonist (A-192621) in Sprague-Dawley rats has also been shown to interact with specific ET\textsubscript{B} receptors in the endothelium. Basal activation of endothelial ET\textsubscript{B} receptors by endothelin and the ensuing release of relaxing factors such as NO, prostacyclin, and endothelium-derived hyperpolarizing factor have been suggested to promote vascular relaxation and reduce vascular reactivity, and to play a role in the control of arterial blood pressure. However, whether ET\textsubscript{B} receptors play a role in enhancing endothelium-dependent vascular relaxation and thereby protecting against excessive vasoconstriction and increased blood pressure during high salt diet is unclear.

The purpose of this study was to test the hypothesis that basal activation of the ET\textsubscript{B} receptors plays a role in enhancing endothelium-dependent vascular relaxation and, thereby, protects against excessive increases in vascular reactivity and arterial blood pressure particularly during high salt diet. To test this hypothesis, we investigated whether chronic treatment with an ET\textsubscript{B} receptor antagonist (A-192621) in Sprague-Dawley rats on normal salt diet is associated with impaired endothelium-dependent vascular relaxation and enhanced vascular reactivity, and whether the vascular effects of the ET\textsubscript{B} antagonist are enhanced in rats on high salt diet. Experiments were designed to determine (1) whether the vascular reactivity to the \textalpha\textadeg-agonist phenylephrine is enhanced in rats treated with the ET\textsubscript{B} antagonist particularly during high salt diet; (2) whether endothelium-dependent vascular relaxation is reduced in rats treated with the ET\textsubscript{B} antagonist particularly during high salt diet; and (3) whether the changes in vascular relaxation and vascular reactivity associated with the ET\textsubscript{B} antagonist treatment particularly during high salt diet involve alterations in the endothelium-dependent NO-cGMP pathway.

**Methods**

Animals: Male Sprague-Dawley rats (12 weeks of age, Harlan) were housed individually and maintained on ad libitum standard rat chow and tap water in 12 hours/12 hours light/dark cycle. After a 1 week acclimation period, the rats were divided into 4 groups, 12 rats each: untreated on normal salt (NS) diet, untreated on high salt (HS) diet, ET\textsubscript{A} antagonist-treated on NS diet, and ET\textsubscript{A} antagonist-treated on HS diet. The NS rats were fed a diet containing 1% sodium chloride. The HS groups were fed a diet containing 8% sodium chloride. The rats were kept on their respective diets for 7 days. The rats received the ET\textsubscript{A} antagonist A-192621 (Abbott), 30 mg/kg per day dissolved in 50 μL of the vehicle ethanol, as an oral gavage during the last 5 days of the dietary regimen. Control rats received the vehicle ethanol. Two days before the end of the protocol, the rats were anesthetized with isoflurane and underwent a surgical procedure for catheter implantation. A PE-50 arterial catheter was placed in the carotid artery for measurement of mean arterial pressure in conscious rats. The catheter was filled with heparin and exteriorized at the back of the neck. Rats were housed individually in metabolic cages and allowed to recover for 48 hours. All procedures were performed in accordance with the guidelines of the Animal Care and Use Committee at the University of Mississippi Medical Center.

**Measurement of Mean Arterial Pressure**

On the day of the experiment, each rat was placed in a Plexiglas restrainer. The carotid arterial catheter was connected to a Statham pressure transducer, and the mean arterial pressure in conscious rats was continuously recorded on a Grass polygraph (Model 7D, Astro-Med).

**Tissue Preparation**

On the day of the experiment, the rats were anesthetized by inhalation of isoflurane. The thoracic aorta was rapidly excised, placed in oxygenated Krebs solution, and cleaned of connective tissue. The aorta was cut transversely into 3 mm wide rings. Aortic rings were cut open into strips. For endothelium-intact aortic strips, extreme care was taken throughout the procedure to avoid injury of the endothelium. For endothelium-denuded aortic strips, the endothelium was removed by gently rubbing the vessel interior with wet filter paper.

**Isometric Contraction**

One end of the aortic strip was attached to a glass hook using a thread loop and the other end was connected to a Grass force transducer (FT03). Aortic strips were stretched to L\textsubscript{max} (1.5 the unloaded initial length, L). The strips were allowed to equilibrate for 1 hour in a water-jacketed, temperature-controlled tissue bath filled with 50 mL of Krebs solution continuously bubbled with 95% O\textsubscript{2}, 5% CO\textsubscript{2} at 37°C. The changes in isometric contraction were recorded on a Grass polygraph (Model 7D).

A control contraction was elicited by applying phenylephrine (Phe, 10\textsuperscript{-4} mol/L) to the tissue bath solution. Once the Phe contraction reached a plateau, the tissue was rinsed with Krebs solution 3 times, 10 minutes each. The whole procedure of contraction and washing was repeated two times. Increasing concentrations of Phe were applied, the contractile responses were recorded, and concentration-response curves were constructed. In other tissues, a contraction to submaximal concentration of Phe (3×10\textsuperscript{-7} mol/L) was elicited. Increasing concentrations of acetylcholine (ACH) or sodium nitroprusside were added and the extent of vascular relaxation was measured. In other experiments, the tissues were pretreated for 30 minutes with N\textsuperscript{\textgamma}-nitro-l-arginine methyl ester (L-NAME, 100 μmol/L), to inhibit NO synthase, or with methylene blue (10 μmol/L) or H[1,2,4]oxadiazolo[4,3-b]quinolin-1-one (ODQ, 1 μmol/L), to inhibit cGMP production in smooth muscle, and the effects on the Phe-induced contraction and on the ACh-induced relaxation of Phe contraction were observed.

**Nitrite/Nitrate Production**

Endothelium-intact aortic strips were placed in test tubes containing 2 mL of Krebs solution aerated with 95% O\textsubscript{2},5% CO\textsubscript{2} at 37°C and the solution was changed every 30 minutes for 1 hour. Samples for basal accumulation of nitrite formed from released NO were first taken. The Krebs solution was replaced, and the strips were stimulated with different concentrations of ACh for 5 minutes. The strips were rapidly removed, dabbed dry with tissue paper, and weighed. The incubation solutions were assayed for the stable end product of NO.
Briefly, samples of incubation solution (50 μL, in triplicate) were mixed in a 96-well microtiter plate with 100 μL of the Griess reagent. The chromophore generated by the reaction with nitrite was detected spectrophotometrically (550 nm) using a microtiter plate reader (BioTek). The concentration of nitrite was calculated using a calibration curve with known concentrations of NaNO₂.

Solutions, Drugs, and Chemicals

Normal Krebs solution contained the following (in mmol/L): NaCl, 120; KCl, 5.9; NaHCO₃, 25; NaH₂PO₄, 1.2; dextrose, 11.5; MgCl₂, 1.2; CaCl₂, 2.5 at pH to 7.4. Stock solutions of L-phenylephrine HCl, acetylcholine, sodium nitroprusside, N⁶-nitro-L-arginine methyl ester (L-NAME) and methylene blue (Sigma) were prepared in distilled water. ¹H-[1,2,4]oxadiazolo[4,3-b]quinazolin-1-one (ODQ) (Calbiochem) was dissolved in dimethyl sulfoxide (DMSO). The final concentration of DMSO in solution was ≤0.1. All other chemicals were of reagent grade or better.

Statistical Analysis

The developed force was corrected for the cross-sectional area of each individual strip and expressed as active stress (N/m²) using the equation: Stress = force/cross-sectional area, where cross-sectional area = wet weight/tissue density × length of the strip, and tissue density = 1.055 g/cm³. Data were analyzed and expressed as the mean ± SEM. Data were compared using ANOVA with multiple classification criteria [rat type (NS versus HS, NS versus NS/ETB antagonist, HS versus HS/ETB antagonist), condition of endothelium (intact versus denuded), and treatment (untreated versus pretreated with L-NAME or methylene blue)] followed by Bonferroni’s post test to compare selected groups or Dunnet’s post test to compare all groups with the NS salt group. Differences were considered statistically significant if P < 0.05.

Results

On the day of the experiment, the mean arterial pressure was 122 ± 3 mm Hg in NS and 132 ± 3 mm Hg in HS rats. The arterial pressure was significantly (P < 0.05) greater in NS/ETB antagonist (144 ± 2 mm Hg) than NS, and far greater in HS/ETB antagonist (171 ± 12 mm Hg) than HS rats.

In endothelium-intact aortic strips of all groups of rats, phenylephrine (Phe) caused concentration-dependent increases in active stress (Figure 1A). The maximal Phe (10⁻⁵ mol/L) stress in NS rats (7.6 ± 1.0 × 10⁻³ N/m²) was not significantly different from that in HS rats (8.2 ± 0.9 × 10⁻³ N/m²). The maximal Phe stress was significantly greater in NS/ETB antagonist (11.3 ± 0.9 × 10⁻³ N/m²) than NS and far greater in HS/ETB antagonist (14.1 ± 1.2 × 10⁻³ N/m²) than HS rats (Figure 1A). When the Phe response was presented as a percent of maximum Phe contraction, the ED₅₀ for Phe in NS rats...
(0.8±0.02×10^{-7} \text{ mol/L}) was not significantly different from that in HS rats (0.7±0.02×10^{-7} \text{ mol/L}) (Figure 1B). Phe was more potent in producing contraction in NS/ETB antagonist (ED_{50}=0.3±0.01×10^{-7} \text{ mol/L}) than NS and far more potent in HS/ETB antagonist (ED_{50}=0.15±0.01×10^{-7} \text{ mol/L}) than HS (Figure 1B). Removal of the endothelium significantly enhanced the maximal Phe-induced contraction in NS rats (Figure 2A) and to a greater extent in HS rats (Figure 2B). In contrast, removal of the endothelium did not significantly affect the Phe-induced stress in NS/ETB antagonist (Figure 2A) or HS/ETB antagonist rats (Figure 2B). When the Phe response was presented as a percentage of maximum Phe contraction, Phe was significantly more potent in causing contraction in endothelium-denuded (ED_{50}=0.4±0.02×10^{-7} \text{ mol/L}) than in endothelium-intact strips of NS rats (ED_{50}=0.8±0.02×10^{-7} \text{ mol/L}) (Figure 2C). Phe was far more potent in causing contraction in endothelium-denuded (ED_{50}=0.2±0.01×10^{-7} \text{ mol/L}) than in endothelium-intact strips of HS rats (ED_{50}=0.7±0.02×10^{-7} \text{ mol/L}). In contrast, the potency of Phe was not significantly different between endothelium-denuded and endothelium-intact strips of NS/ETB antagonist rats (Figure 2C) or between endothelium-denuded and endothelium-intact strips of HS/ETB antagonist rats (Figure 2D).

In endothelium-intact strips, pretreatment with L-NAME (100 \mu\text{mol/L}) for 30 minutes, to inhibit nitric oxide (NO) synthase, significantly enhanced Phe-induced stress in NS rats to a maximum of 10.6±1.1×10^{3} \text{ N/m}^2 (Figure 3A) and to a greater extent in HS rats (12.9±1.1×10^{3} \text{ N/m}^2) (Figure 3B). Also, in methylene blue–pretreated strips of NS rats, Phe was far more potent in causing contraction (ED_{50}=0.42±0.02×10^{-7} \text{ mol/L}) than in untreated strips of NS rats (Figure 3C). Phe was far more potent in causing contraction in methylene blue–treated strips of HS rats (ED_{50}=0.15±0.03×10^{-7} \text{ mol/L}) than in untreated strips of HS rats (Figure 3D).

In contrast, L-NAME–pretreated strips of NS/ETB antagonist or HS/ETB antagonist rats, the maximal Phe-induced stress (Figure 3A and 3B) and the ED_{50} of Phe (Figure 3C and 3D) were not significantly different from that in untreated strips of NS/ETB antagonist or HS/ETB antagonist rats. Similarly, in endothelium-intact strips, pretreatment with methylene blue (10 \mu\text{mol/L}) for 30 minutes, to inhibit cGMP production in smooth muscle, enhanced Phe-induced stress in NS rats to a maximum of 9.9±0.7×10^{3} \text{ N/m}^2 (Figure 3A) and to a greater extent in HS rats (13.6±1.4×10^{3} \text{ N/m}^2) (Figure 3B). Also, in methylene blue–pretreated strips of NS rats, Phe was more potent in causing contraction (ED_{50}=0.42±0.02×10^{-7} \text{ mol/L}) than in untreated strips of NS rats (Figure 3C). Phe was far more potent in causing contraction in methylene blue–treated strips of HS rats (ED_{50}=0.15±0.03×10^{-7} \text{ mol/L}) than in untreated strips of HS rats (Figure 3D). In contrast, in methylene blue–pretreated strips of NS/ETB antagonist or HS/ETB antagonist rats, the maximal Phe-induced stress (Figure 3A and 3B) and the ED_{50} of Phe (Figure 3C and 3D) were far less in HS/ETB antagonist than HS rats, respectively. Similar enhancements of Phe contraction were observed in aortic strips pretreated with ODQ (10^{-7} \text{ mol/L}), a more specific inhibitor of guanylate cyclase, for 30 minutes.

In endothelium-intact aortic strips of all groups of rats, ACh caused concentration-dependent relaxation of Phe (3×10^{-7} \text{ mol/L}) contraction (Figure 4A). The ACh-induced relaxation of Phe contraction was not significantly different between NS and HS rats, but was significantly less in NS/ETB antagonist than NS and far less in HS/ETB antagonist than HS rats (Figure 4A). Because the aortic strips of NS/ETB antagonist and HS/ETB antagonist rats showed greater vascular reactivity compared with NS and HS rats, control experiments were performed on strips of NS/ETB antagonist and HS/ETB antagonist rats in which the initial Phe concentration...
was lowered to 1×10⁻⁷ mol/L to produce a submaximal contraction that is roughly equal in magnitude to the contraction observed in strips of NS and HS rats precontracted with 3×10⁻⁷ mol/L Phe. These experiments showed that the ED₅₀ of ACh in aortic strips of NS/ETB antagonist precontracted with 1×10⁻⁷ mol/L Phe was not significantly different from that in strips precontracted with 3×10⁻⁷ mol/L Phe. Pretreatment of endothelium-intact strips with L-NAME (10⁻⁴ mol/L), to inhibit NO synthase (Figure 4B), or methylene blue (10⁻⁵ mol/L), to inhibit cGMP production in smooth muscle (Figure 4C), inhibited ACh-induced relaxation significantly in NS and HS, and slightly in NS/ETB antagonist, but not in HS/ETB antagonist rats. Similar inhibitions of ACh-induced relaxation were observed in aortic strips pretreated with ODQ (10⁻⁶ mol/L), a more specific inhibitor of guanylate cyclase, for 30 minutes. Removal of the endothelium completely inhibited the ACh-induced relaxation of Phe contraction in all groups of rats.

In endothelium-intact strips, the basal nitrite/nitrate production was 47.3±8.5 pmol/mg tissue weight in NS rats and was significantly greater in HS rats (76.2±7.1 pmol/mg tissue weight) (Figure 5). The basal nitrite/nitrate showed significant reduction in NS/ETB antagonist rats compared with NS rats, and far greater reduction in HS/ETB antagonist rats compared with HS rats (Figure 5). Measurement of ACh-induced nitrite/nitrate production showed no significant difference between NS and HS rats, but a significant reduction in NS/ETB antagonist compared with NS and a greater reduction in HS/ETB antagonist compared with HS rats (Figure 5).

In endothelium-denuded aortic strips of all groups of rats, sodium nitroprusside, an exogenous NO donor and a standard guanylate cyclase activator, caused concentration-dependent relaxation of Phe (3×10⁻⁷ mol/L) contraction. However, no significant differences in the magnitude of sodium nitroprusside-induced relaxation of Phe contraction were observed between aortic strips of NS, HS, NS/ETB antagonist or HS/ETB antagonist rats (Figure 6).

**Discussion**

The main findings of the present study are as follows: (1) the mean arterial pressure is significantly elevated in ETB antagonist–treated rats particularly those on high salt diet, (2) vascular reactivity is greater in ETB antagonist–treated rats particularly when fed high salt diet, (3) endothelium-dependent vascular
relaxation is significantly reduced in ET<sub>B</sub> antagonist–treated rats particularly with high salt diet, and (4) the reduction in vascular relaxation and enhanced vascular reactivity in ET<sub>B</sub> antagonist–treated rats particularly those on high salt diet involves a NO-cGMP–dependent pathway.

The present study showed that treatment with an ET<sub>B</sub> antagonist in Sprague-Dawley rats on normal salt diet caused significant elevation of blood pressure, suggesting a role for the ET<sub>B</sub> receptors in the control of blood pressure. It has been suggested that, under basal conditions, activation of endothelial ET<sub>B</sub> receptors by endothelin increases the release of relaxing factors from endothelial cell leading to smooth muscle relaxation. If the endothelial ET<sub>B</sub> receptors are active under basal conditions, one would predict that blockade of endothelial ET<sub>B</sub> receptors would decrease endothelium-dependent relaxation, leading to increased vasoconstriction and, thereby, increased blood pressure. The present observation that the arterial pressure is significantly elevated in rats treated with ET<sub>B</sub> antagonist suggests that the endothelial ET<sub>B</sub> receptors are active under basal conditions in rats on normal salt diet.

The present results in Sprague-Dawley rats showed that high salt diet alone, in the absence of ET<sub>B</sub> antagonist, caused only a modest increase in blood pressure. This finding is in agreement with other studies, which have shown that feeding Dahl salt-resistant rats a high salt diet is associated with modest elevation in blood pressure. On the other hand, when high salt diet was combined with ET<sub>B</sub> antagonist, significant elevations in blood pressure were observed even to levels greater than those observed in rats on normal salt diet combined with ET<sub>B</sub> antagonist. These results are in agreement with reports that salt-sensitive hypertension develops in ET<sub>B</sub> receptor-deficient rats and suggest that the ET<sub>B</sub> receptors are possibly hyperactivated in the presence of high salt diet.

We found that the vascular reactivity to the α-adrenergic agonist Phe is enhanced during treatment with ET<sub>B</sub> antagonist, and further enhanced when the ET<sub>B</sub> antagonist–treated rats on high salt diet. These results are consistent with reports that ET<sub>B</sub> receptor antagonism enhances contraction in isolated rabbit pulmonary artery. In search for the possible mechanisms involved in the observed enhanced vascular reactivity in the ET<sub>B</sub> antagonist–treated rats, we found that removal of the endothelium significantly enhanced the Phe-induced contraction in NS and HS rats, but had minimal effects in NS and HS rats treated with ET<sub>B</sub> antagonist. Also, the ACh-induced relaxation was reduced in ET<sub>B</sub> antagonist–treated rats particularly those on high salt diet. These results provide evidence that an endothelium-dependent relaxation pathway involving endothelial ET<sub>B</sub> receptors is active in NS rats and is even hyperactive during high salt diet.

The vascular endothelium is known to release relaxing factors, and several studies have suggested NO to be a major endothelium-derived factor that causes smooth muscle relaxation. The reduced ACh-induced relaxation in ET<sub>B</sub> antagonist–treated rats could be due to either a decrease in the synthesis and/or release of NO from endothelial cells or may reflect a change in the sensitivity of vascular smooth muscle to relaxation by NO. The sensitivity of vascular smooth muscle to relaxation by NO could be evaluated by its sensitivity to relaxation by exogenous NO donors such as sodium nitroprusside. The observation that relaxation of endothelium-denuded vascular strips by sodium nitroprusside was not significantly different between ET<sub>B</sub> antagonist–treated rats and untreated rats provided evidence that the endothelium-independent mechanisms of vascular relaxation and the sensitivity of vascular smooth muscle to relaxation by NO are not impaired in ET<sub>B</sub> antagonist–treated rats and, thereby, suggest that the impaired ACh-induced relaxation in ET<sub>B</sub> antagonist–treated rats is most likely due to a decrease in the synthesis and/or release of NO from endothelial cells.

To further investigate the possible role of NO synthesis and release in the proposed impaired endothelium-dependent relaxation pathway in the ET<sub>B</sub> antagonist–treated rats we found that pretreatment of the vascular strips with L-NAME, which is known to block NO synthesis, significantly inhibited vascular relaxation by ACh and significantly enhanced the vascular reactivity to Phe in NS and HS rats, but had minimal effect in ET<sub>B</sub> antagonist–treated NS and HS rats. These results provide evidence that NO synthesis by endothelial cells is significantly impaired during treatment with the ET<sub>B</sub> antagonist particularly with high salt diet. This evidence is further supported by the observation that both the basal and the ACh-induced nitrite/nitrate production were significantly reduced in aortic strips of rats treated with the ET<sub>B</sub> antagonist, particularly when fed high salt diet.

The NO produced by endothelial cells is known to promote vascular relaxation by activating guanylate cyclase and increasing cGMP production in smooth muscle. We found that methylene blue, which is known to inhibit guanylate cyclase and to decrease cGMP production in smooth muscle, and ODQ, a more specific inhibitor of guanylate cyclase, significantly inhibited the endothelium-dependent vascular relaxation by ACh significantly and enhanced the vascular reactivity to Phe in endothelium-intact strips of NS and HS rats, but not in ET<sub>B</sub> antagonist–treated NS and HS rats. These results further support the hypothesis that NO production or release by endothelial cells and, thereby, the activity of the NO-cGMP pathway in smooth muscle is reduced in ET<sub>B</sub> antagonist–treated rats particularly those on high salt diet.

It is important to note that the vascular endothelium releases other vasodilator substances in addition to NO, such as prostacyclin and endothelium-derived hyperpolarizing factor. This finding may explain why, in the aortic strips of ET<sub>B</sub> antagonist–treated rats, some relaxation to ACh was still observed and was not completely inhibited by L-NAME or methylene blue. The small magnitude of the remaining ACh-induced relaxation in L-NAME– or methylene blue–treated aortic strips may be related to the fact that the relative contribution of NO and prostanoids to vascular relaxation is tissue-specific, with the contribution of prostanoids being more significant in resistance vessels. On the other hand, the complete absence of ACh-induced relaxation in endothelium-denuded strips of ET<sub>B</sub> antagonist–treated rats still supports the contention that the ACh-induced relaxation is endothelium-dependent.

The present study showed that chronic high salt diet, particularly during chronic treatment with ET<sub>B</sub> antagonist, is
associated with increases in vascular reactivity and reduced vascular relaxation of isolated vascular strips. Because the isolated strips were not acutely exposed to high salt or ET\(_A\) antagonist, it is possible that, under these conditions, some activation of the endothelial ET\(_B\) receptors by locally released endothelin would occur and would stimulate the release of endothelium-derived vasodilators. If this is the case, then the observed enhancement of vascular reactivity and reduction of vascular relaxation in isolated strips of HS/ET\(_B\) antagonist rats are probably underestimated.

Finally, although the present results with the ET\(_B\) antagonist are consistent with a role of endothelial ET\(_B\) receptors in the regulation of vascular function and arterial pressure, these results should be interpreted with caution. The role of the ET\(_B\) receptors in vascular homeostasis is rather complex, because the receptor has both pressor and depressor effects in vivo. The pressor effects are mediated by ET\(_{B1}\) receptors in vascular smooth muscle, whereas the depressor effects are mediated by ET\(_{B2}\) receptors in endothelial cells.\(^{23,26,27}\) Because the compound A-192621 does not discriminate between the ET\(_B\) receptor subtypes, the ET\(_B\) antagonistic effects observed in the present study may represent the combined contribution of ET\(_{B1}\) receptors in endothelial cells and smooth muscle. Also, because blocking the ET\(_B\) receptor in smooth muscle is predicted to reduce vascular reactivity and arterial pressure, then the endothelial ET\(_B\) receptor component of the observed ET\(_B\) antagonist effects on vascular reactivity and arterial pressure is probably underestimated. The development of compounds with specific antagonistic effects to ET\(_{B1}\) and ET\(_{B2}\) receptors would help further delineate the contribution of each receptor subtype to vascular function and arterial pressure and should represent important areas for future investigation. Additionally, ET\(_B\) receptors have been suggested to play a role as endothelin clearance receptors.\(^{33}\) Blockade of ET\(_B\) receptors may, therefore, influence vascular reactivity and arterial pressure by increasing endogenous endothelin levels and activating ET\(_B\) receptors. This proposition is supported by reports that the increased susceptibility to deoxycorticosterone acetate (DOCA) -salt–induced hypertension and vascular and renal injuries in ET\(_B\) receptor-deficient rats was reduced during chronic treatment with an ET\(_A\) receptor antagonist.\(^{34}\) However, other reports have shown that ET\(_A\) receptor antagonism did not significantly alter blood pressure in ET\(_B\) receptor–deficient rats maintained on a high sodium diet,\(^{35}\) thus making the quantitative importance of the ET\(_A\) receptors in mediating the hypertension rather unclear. Furthermore, because activation of ET\(_B\) receptors has been shown to inhibit tubular sodium reabsorption,\(^{36}\) blockade of ET\(_B\) receptors could influence arterial pressure through a direct renal mechanism. In conclusion, an endothelial ET\(_B\) receptor-mediated pathway of vascular relaxation involving release of NO is active under basal conditions and may protect against excessive vasoconstriction and increased blood pressure particularly during high salt diet.

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References


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