Endothelin-Induced Increases in Ca\textsuperscript{2+} Entry Mechanisms of Vascular Contraction Are Enhanced During High-Salt Diet

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Abstract—High-salt diet is often associated with increases in arterial pressure, and a role for endothelin (ET)-1 in salt-sensitive hypertension has been suggested; however, the vascular mechanisms involved are unclear. We investigated whether ET increases the sensitivity of the mechanisms of vascular contraction to changes in dietary salt intake. Active stress and \textsuperscript{45}Ca\textsuperscript{2+} influx were measured in endothelium-denuded aortic strips of male Sprague-Dawley rats not treated or chronically infused intravenously with ET (5 pmol/kg per minute) and fed either normal-sodium diet (NS, 1%) or high-sodium diet (HS, 8%) for 9 days. Phenylephrine (Phe) caused increases in active stress that were similar in NS and HS, but were greater in NS/ET (maximum, 10.5±0.7) than in NS (maximum, 7.4±0.9) rats, and further enhanced in HS/ET (maximum, 14.4±1.1) compared with HS rats (maximum, 8.0±0.8×10\textsuperscript{4}N/m\textsuperscript{2}). Phe was more potent in causing contraction in NS/ET than in NS rats and in HS/ET than in HS rats. In Ca\textsuperscript{2+}-free (2 mmol/L EGTA) Krebs, stimulation of intracellular Ca\textsuperscript{2+} release by Phe (10\textsuperscript{-5} mol/L) or caffeine (25 mmol/L) caused a transient contraction that was not significantly different in all groups of rats. In contrast, membrane depolarization by high-KCl solution, which stimulates Ca\textsuperscript{2+} entry from the extracellular space, caused greater contraction in ET-infused rats, particularly those on HS diet. Phe (10\textsuperscript{-5} mol/L) caused an increase in \textsuperscript{45}Ca\textsuperscript{2+} influx that was greater in NS/ET (27.9±1.7) than in NS (20.1±1.8) rats and further enhanced in HS/ET (35.2±1.8) compared with HS rats (21.8±1.9 μmol/kg/min). The Phe-induced \textsuperscript{45}Ca\textsuperscript{2+} influx-stress relation was not different between NS and HS rats, but was enhanced in ET-infused rats particularly those on HS. The enhancement of the \textsuperscript{45}Ca\textsuperscript{2+} influx–active stress relation in ET-infused rats was not observed in vascular strips treated with the protein kinase C inhibitor GF109203X or calphostin C (10\textsuperscript{-6} mol/L). Thus, low-dose infusion of ET, particularly during HS, is associated with increased vascular reactivity that involves Ca\textsuperscript{2+} entry from the extracellular space, but not Ca\textsuperscript{2+} release from the intracellular stores. The ET-induced enhancement of the Ca\textsuperscript{2+} influx–stress relation particularly during HS suggests activation of other mechanisms in addition to Ca\textsuperscript{2+} entry, possibly involving protein kinase C. The results suggest that ET increases the sensitivity of the mechanisms of vascular smooth muscle contraction to high dietary salt intake and may, in part, explain the possible role of ET in salt-sensitive hypertension. (Hypertension. 2003;41[part 2]:787-793.)

Key Words: arterial pressure ♦ endothelium ♦ vascular smooth muscle ♦ calcium ♦ contraction

High-salt diet (HS) has been implicated in the pathogenesis of hypertension, particularly in salt-sensitive individuals,\textsuperscript{1-4} and salt moderation is often recommended to protect against excessive increases in blood pressure.\textsuperscript{1,2,5,6} Studies in salt-sensitive experimental animals such as the Dahl salt-sensitive rat have shown that HS is associated with significant increases in blood pressure.\textsuperscript{7,8} Also, in salt-sensitive rats, HS is associated with exaggerated vascular reactivity to vasoconstrictor stimuli, which may contribute, at least in part, to the increases in blood pressure.\textsuperscript{9-12}

Although the mechanisms of salt-sensitive hypertension have not been clearly identified, several studies point to a possible role of endothelin (ET).\textsuperscript{7,13-17} ET has been shown to interact with specific ET\textsubscript{A} and ET\textsubscript{B} receptors in tissues involved in the regulation of arterial pressure, particularly the kidney and the vasculature.\textsuperscript{18-24} Studies with specific ET\textsubscript{A} and ET\textsubscript{B} antagonists have suggested a possible role of ET as a mediator of angiotensin II–induced hypertension in rats, particularly during HS.\textsuperscript{14,15,17} However, whether ET directly affects the control mechanisms of arterial pressure is less clear. Recent studies have suggested a prominent role of ET in the control of renal plasma flow and renal sodium excretion and, thereby, the renal control mechanisms of arterial pressure.\textsuperscript{13,16,17,25} However, the role of ET in the vascular control mechanisms of arterial pressure has not been clearly established.

ET is one of the most potent vasoconstrictors described.\textsuperscript{26} The interaction of ET with specific ET\textsubscript{A} and ET\textsubscript{B} receptors in
smooth muscle initiates a cascade of biochemical pathways, leading to smooth muscle contraction.\textsuperscript{18–24} One major pathway of vascular smooth muscle contraction is increases in intracellular Ca\textsuperscript{2+}, owing to Ca\textsuperscript{2+} release from the intracellular stores and Ca\textsuperscript{2+} entry from the extracellular space.\textsuperscript{27} Although studies in humans suggest possible elevation of plasma ET levels in salt-sensitive hypertension,\textsuperscript{28} and studies in the rat suggest increased ET production during HS,\textsuperscript{17} the effects of HS, irrespective of hypertension, and the effects of ET on the mechanisms of vascular contraction, particularly during HS, have not been clearly elucidated.

The purpose of this study was to test the hypothesis that ET increases the sensitivity of the mechanisms of vascular contraction to changes in dietary salt intake. To test this hypothesis, we investigated whether chronic ET infusion in Sprague-Dawley rats on normal salt diet (NS) is associated with enhanced vascular reactivity, and whether the vascular effects associated with ET infusion are enhanced in rats on HS. Experiments were designed to determine (1) whether the vascular reactivity to the \( \alpha \)-adrenergic agonist phenylephrine (Phe) is enhanced during chronic infusion of ET, particularly during HS, and (2) whether ET infusion particularly during HS is associated with changes in the Ca\textsuperscript{2+} mobilization mechanisms of vascular smooth muscle contraction, ie, Ca\textsuperscript{2+} release from the intracellular stores and Ca\textsuperscript{2+} entry from the extracellular space.

Methods

Animals

Male Sprague-Dawley rats (12 weeks of age, Harlan, Indianapolis, Ind) were housed individually and maintained on ad libitum standard rat chow and tap water in 12-hour/12-hour light/dark cycle. After a 1-week acclimation period, the rats were divided into 4 groups, with 12 rats each: not treated on NS, not treated on HS, ET-treated on NS, and ET-treated on HS (NS/ET), and ET-treated on HS (HS/ET). The NS rats were fed a diet containing 1% sodium chloride. The TS groups were fed a diet containing 8% sodium chloride. The rats were kept on their respective diets for 9 days. The ET-treated rats were chronically infused intravenously with ET (Alexis Laboratory, 5 mmol/kg per minute) by using osmotic minipumps (Alzet) during the last 5 days of the dietary regimen. Control rats were infused with saline. 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, and the rats were 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 pressure transducer (Cobe model CDX II, Sema), and the mean arterial pressure was measured in conscious rats and continuously recorded on a Grass polygraph (model 7D, Astro-Med). The arterial pressure was recorded every 5 minutes over a period of 2 hours. A total of 24 measurements were averaged to indicate the arterial pressure value for each rat.

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. The vascular endothelium is known to release endothelium-derived relaxing factors that could depress vascular smooth muscle contraction.\textsuperscript{29–31} Also, the interaction of ET with endothelial E\( \text{T} \) receptors has been shown to promote vascular relaxation by increasing the release of relaxing factors such as NO.\textsuperscript{20,23–24} To avoid the contribution of endothelium-derived relaxing factors to the vascular response, the endothelium was removed by gently rubbing the vessel interior with wet filter paper. Removal of the endothelium was routinely verified by the absence of acetylcholine (10\textsuperscript{-3} mol/L)-induced vasorelaxation in vascular strips precontracted with Phe (3 \times 10\textsuperscript{-7} mol/L).

Isometric Contraction

One end of the aortic strip was attached to a glass hook by 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 30 mL 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).

Three different agonists were used. The \( \alpha \)-adrenergic agonist Phe was used to stimulate both Ca\textsuperscript{2+} release from the intracellular Ca\textsuperscript{2+} stores and Ca\textsuperscript{2+} entry from the extracellular space.\textsuperscript{27,32} Caffeine was used to activate the Ca\textsuperscript{2+}-induced Ca\textsuperscript{2+} release mechanism from the intracellular stores.\textsuperscript{33} High-KCl solution was used to activate the Ca\textsuperscript{2+} entry mechanism from the extracellular space.\textsuperscript{27,32}

Two control high-KCl (96 mmol/L) contractions followed by rinsing with Krebs solution 3 \times 10 minutes were first performed. Increasing concentrations of Phe or KCl were applied, and concentration-contraction curves were constructed. In other experiments, the vascular strips were incubated in nominally 0 mmol/L Ca\textsuperscript{2+} Krebs for 10 minutes, then stimulated with Phe (10\textsuperscript{-5} mol/L), then increasing concentrations of extracellular Ca\textsuperscript{2+} (0.1, 0.3, 0.6, 1.0, and 2.5 mmol/L) were added to the bathing solution, and the changes in Phe contraction were measured. The relation between extracellular Ca\textsuperscript{2+} and the Phe-induced contraction was constructed both in the absence and presence of the protein kinase C (PKC) inhibitors GF109203X and calphostin C. In another set of experiments, the vascular strips were incubated in Ca\textsuperscript{2+}-free (2 mmol/L EGTA) Krebs for 5 minutes and then stimulated with Phe (10\textsuperscript{-5} mol/L) or caffeine (25 mmol/L) to stimulate Ca\textsuperscript{2+} release from the intracellular stores, and the resulting transient contraction was measured.

\( ^{45} \text{Ca}^{2+} \) Influx

Vascular strips were incubated in Krebs solution containing specific extracellular Ca\textsuperscript{2+} then stimulated with Phe (10\textsuperscript{-5} mol/L) for 10 minutes. The tissues were transferred to the respective radioactive \( ^{45} \text{Ca}^{2+} \) labeled solution (specific activity, 2 \muCi/mL; ICN) for 90 sec. Preliminary experiments have shown that the relationship between Ca\textsuperscript{2+} uptake versus time is linear during 15–30, 60, and 90-second exposures to the \( ^{45} \text{Ca}^{2+} \) label. The tissues were transferred to ice-cold Ca\textsuperscript{2+}-free (2 mmol/L EGTA) Krebs for 45 minutes to quench extracellular \( ^{45} \text{Ca}^{2+} \) label. The vascular strips were weighed and placed in 2 mL hypotonic (5 mmol/L) EDTA for 24 hours at 4°C to disrupt the cell membranes and release the intracellular content of \( ^{45} \text{Ca}^{2+} \). The next day, 4 mL of Ecolite scintillation cocktail was added, and the samples were counted in a scintillation counter (Beckman LS 6500).\textsuperscript{23,34}

Solutions and Chemicals

Normal Krebs contained the following (in mmol/L): NaCl 120, KCl 5.9, NaHCO\textsubscript{3} 25, NaH\textsubscript{2}PO\textsubscript{4} 1.2, Dextrose 11.5, MgCl\textsubscript{2} 1.2, and CaCl\textsubscript{2} 2.5 at pH 7.4. For nominally 0 mmol/L Ca\textsuperscript{2+} Krebs, CaCl\textsubscript{2} was omitted. For Ca\textsuperscript{2+}-free Krebs, CaCl\textsubscript{2} was omitted and replaced with 2 mmol/L EGTA. The high-KCl depolarizing solution was prepared as Krebs but with equimolar substitution of NaCl with KCl. L-Phenylephrine, acetylcholine, and caffeine were prepared in distilled water. GF109203X and calphostin C (Kamiya) were dissolved
in 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) by using the following 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/ET versus NS, HS/ET versus HS], and tissue treatment [nontreated versus pretreated with GF109203X or calphostin C]) followed by the Bonferroni post test to compare selected groups or the Dunnet post test to compare all groups to the NS group. Differences were considered statistically significant if *P* < 0.05.

**Results**

On the day of the experiment, the mean arterial pressure was 114 ± 3 mm Hg in NS rats and 120 ± 4 mm Hg in HS rats. The arterial pressure appeared to be greater in NS/ET (119 ± 3 mm Hg) than in NS rats; however, the difference did not reach significant levels (*P* = 0.251). The arterial pressure measurements showed tendency to higher levels in ET/HS (127 ± 3 mm Hg) compared with HS rats, but this tendency did not materialize into a statistically significant difference (*P* = 0.175).

In vascular strips of all groups of rats, Phe caused concentration-dependent increases in active stress (Figure 1). The maximal Phe (10⁻⁵ mol/L)-induced stress in NS rats (7.4 ± 0.9 × 10⁴ N/m²) was not significantly different (*P* = 0.623) from that in HS rats (8.0 ± 0.8 × 10⁴ N/m²). The maximal Phe stress was significantly greater (*P* = 0.013) in NS/ET (10.5 ± 0.7 × 10⁴ N/m²) than in NS rats and far greater in HS/ET (14.4 ± 1.1 × 10⁴ N/m²) than in HS rats (*P* < 0.01) (Figure 1A). When the Phe response was presented as a percentage of maximum Phe contraction, the ED₅₀ for Phe in NS rats (0.8 ± 0.21 × 10⁻⁷ mol/L) was not significantly different (*P* = 0.721) from that in HS rats (0.7 ± 0.18 × 10⁻⁷ mol/L) (Figure 1B). Phe was more potent in producing contraction in NS/ET (ED₅₀ = 0.3 ± 0.09 × 10⁻⁷ mol/L) than in NS rats (*P* < 0.040) and far more potent in HS/ET (ED₅₀ = 0.13 ± 0.07 × 10⁻⁷ mol/L) than in HS rats (*P* = 0.007) (Figure 1B).

To investigate whether the differences in Phe contraction among the different groups of rats reflect changes in Ca²⁺ release from the intracellular stores, Phe- and caffeine-induced contraction in Ca²⁺-free (2 mmol/L EGTA) Krebs were measured. In Ca²⁺-free Krebs, Phe (10⁻⁵ mol/L) and caffeine (25 mmol/L) caused a transient increase in contraction in vascular strips of NS rats, which was not significantly different from that in HS, NS/ET, or HS/ET rats (Figure 2A and 2B).

Membrane depolarization by high KCl is known to stimulate Ca²⁺ entry from the extracellular space. Increasing concentrations of KCl caused concentration-dependent increases in active stress (Figure 3). The maximal KCl-induced active stress was not significantly different between NS and HS rats, but significantly greater in NS/ET than in NS rats and further enhanced in HS/ET than in HS rats (Figure 3). To further investigate the role of Ca²⁺ entry, the Phe (10⁻⁵ mol/L)-induced active stress and ⁴⁰Ca²⁺ influx were measured.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Phe-induced contraction in vascular strips of NS, HS, NS/ET, and HS/ET rats. Endothelium-denuded aortic strips were incubated in normal Krebs (2.5 mmol/L Ca²⁺) then stimulated with increasing concentrations of Phe. The Phe contraction was measured and presented as active stress (A) or as a percentage of maximum Phe contraction (B). Data points represent the mean ± SEM of measurements in 24 aortic strips from 12 rats of each group.

![Figure 2](http://hyper.ahajournals.org/)

**Figure 2.** Phe and caffeine induced contraction in vascular strips of NS, HS, NS/ET, and HS/ET rats. Aortic strips were incubated in Ca²⁺-free (2 mmol/L EGTA) Krebs for 5 minutes then stimulated with Phe (10⁻⁵ mol/L; A) or caffeine (25 mmol/L; B). Data points represent the mean ± SEM of measurements in 12 vascular strips from 6 rats of each group.
at increasing concentrations of extracellular Ca\textsuperscript{2+}. In all groups of rats, increasing concentrations of extracellular Ca\textsuperscript{2+} were associated with increases in Phe-induced stress and \[^{45}\text{Ca}^{2+}\] influx (Figure 4). At all extracellular Ca\textsuperscript{2+} tested, the Phe-induced active stress was not significantly different between NS and HS rats. At extracellular Ca\textsuperscript{2+} \(\geq 0.1\) mmol/L, the Phe-induced active stress was significantly greater in NS/ET than in NS rats and further enhanced in HS/ET than in HS rats (Figure 4A). At all extracellular Ca\textsuperscript{2+} tested, the Phe-induced \[^{45}\text{Ca}^{2+}\] influx was slightly, but not significantly enhanced in NS rats than in NS rats. At extracellular Ca\textsuperscript{2+} \(\geq 0.1\) mmol/L, the Phe-induced \[^{45}\text{Ca}^{2+}\] influx was significantly greater in NS/ET than in NS rats and further enhanced in HS/ET than in HS rats (Figure 4B).

The data from Figures 4A and 4B were used to construct the Ca\textsuperscript{2+} influx–active stress relationship in ET-infused rats on NS and HS (Figure 5). If the increases in the Phe-induced active stress associated with chronic ET infusion, particularly during HS, involved changes only in the Ca\textsuperscript{2+} entry mechanism, then the Ca\textsuperscript{2+} influx/stress relationship in ET-infused rats would not be different from, but rather an extension of, that in noninfused rats. As shown in Figure 5A, at different levels of Ca\textsuperscript{2+} influx the Phe-induced active stress was not significantly different between NS and HS rats. In contrast, at Ca\textsuperscript{2+} influx levels \(\geq 7\) \(\mu\)mol/kg per minute, the Phe-induced active stress was significantly greater in NS/ET than in NS rats and further enhanced in HS/ET than in HS rats. In tissues pretreated with the PKC inhibitor GF109203X (10\textsuperscript{-6} mol/L) for 30 minutes, the Phe-induced \[^{45}\text{Ca}^{2+}\] influx was still enhanced in ET-infused rats, particularly those on HS. However, in tissues pretreated with GF109203X, at different levels of Ca\textsuperscript{2+} influx, no enhancement of Phe contraction could be observed in ET-infused rats (Figure 5B). Similar results were observed in tissues pretreated with the PKC inhibitor calphostin C (10\textsuperscript{-6} mol/L) for 30 minutes (data not shown).

**Discussion**

The main findings of the present study are as follows: (1) the vascular reactivity is greater in ET-infused rats particularly when fed HS; (2) the increased vascular reactivity during ET infusion is associated with increases in Ca\textsuperscript{2+} entry from the extracellular space, but not Ca\textsuperscript{2+} release from the intracellular stores; (3) the Phe Ca\textsuperscript{2+} influx-stress relationship is enhanced in ET-infused rats.
in ET-infused rats particularly during HS; and (4) the enhancement of the Ca²⁺ influx-stress relationship observed in ET-infused rats is abolished in tissues pretreated with PKC inhibitors.

We found that the vascular reactivity to the α-adrenergic agonist Phe is enhanced during chronic ET infusion in rats, and further enhanced when the ET-infused rats were fed HS. The increased vascular reactivity to Phe in ET-infused rats can be explained, in part, by an increase in the sensitivity to Phe at the α-adrenergic receptor level. This is supported by the present observation that Phe was more potent, and that the Phe EC₅₀ was significantly smaller in ET-infused compared with noninfused rats. However, the enhanced vascular reactivity could also be owing to stimulation of signaling mechanisms downstream from α-adrenergic receptor activation.

It is generally accepted that activation of α-adrenergic receptors by agonists such as Phe causes activation of phospholipase C and increases the hydrolysis of phosphatidylinositol 4,5-bisphosphate into inositol 1, 4, 5-trisphosphate (IP₃) and diacylglycerol. IP₃ stimulates Ca²⁺ release from intracellular stores and diacylglycerol stimulates PKC. In addition, α-adrenergic agonists enhance Ca²⁺ entry through the plasma membrane Ca²⁺ channels.

We found that the transient Phe- and caffeine-induced contractions in Ca²⁺-free solution, which are often used as a measure of IP₃-induced Ca²⁺ release and Ca²⁺-induced Ca²⁺ release from the intracellular Ca²⁺ stores, respectively, were not significantly different in the ET-infused and noninfused rats on NS and HS, suggesting that the enhanced vascular reactivity during ET infusion is not owing to changes in Ca²⁺ release from the intracellular stores. On the other hand, the enhanced Phe-induced Ca²⁺ influx in ET-infused rats suggests enhancement of Ca²⁺ entry from the extracellular space. To examine the possible Ca²⁺ entry pathway involved, we investigated whether ET infusion is associated with changes in the vascular reactivity to high KCl. High KCl is known to cause membrane depolarization and to stimulate Ca²⁺ entry through voltage-gated Ca²⁺ channels. The observation that the KCl-induced contraction was enhanced in ET-treated compared with untreated rats, particularly during HS, provides further evidence that Ca²⁺ entry from the extracellular space is enhanced under these conditions. The cause of the increased Ca²⁺ entry into vascular smooth muscle during ET infusion is not clear at the present time, but may be related to direct effect of ET on the vasculature and possible ET-induced upregulation of the Ca²⁺ channels. This is supported by reports that ET increases the number of Ca²⁺ channels expressed in vascular smooth muscle. The greater enhancement of Ca²⁺ entry in ET-infused rats on HS may be related to possible increases in Ca²⁺ channel activity with HS. This is supported by reports that the L-type Ca²⁺ channels in resistance arteries of Dahl salt-sensitive rats become more available for opening during dietary salt-loading. However, activation of the reverse mode of the Na⁺/Ca²⁺ exchanger in response to sodium loading, and thereby stimulation of Ca²⁺ entry into vascular smooth muscle cells, cannot be excluded under these conditions.

To investigate whether other mechanisms in addition to Ca²⁺ entry contribute to the increased vascular reactivity during ET infusion, we compared the relationship between Phe-induced Ca²⁺ entry and active stress in rats treated with ET and fed NS or HS. If the increase in vascular reactivity during ET infusion is merely caused by increases in Ca²⁺ entry through plasma membrane Ca²⁺ channels, then one would expect the Phe-induced Ca²⁺ entry-active stress relationship in ET-treated rats to be an extension of that in untreated rats. The Ca²⁺ entry-stress relation did not appear to be different between NS and HS rats. On the other hand, the Phe-induced Ca²⁺ entry/stress relation was significantly enhanced in HS/ET rats compared with NS rats and further enhanced in HS/ET rats compared with HS rats. These data suggest that other vascular contraction mechanisms in addition to Ca²⁺ entry through plasma membrane Ca²⁺ channels are enhanced during ET infusion. These additional mechanisms may include (1) disruption of superficially located Ca²⁺ buffering systems, thus allowing more Ca²⁺ to be available for the myofilaments to cause contraction; and (2) increase in the myofilament force sensitivity to Ca²⁺, perhaps through activation of PKC. This is supported by reports that ET stimulates diacylglycerol production and increases PKC activity in vascular smooth muscle. The possible role of PKC in the enhanced vascular reactivity in ET-infused rats is further supported by the present observation that the enhancement of the 45Ca²⁺ influx-active stress relation was abolished in tissues pretreated with 2 mechanistically distinct PKC inhibitors, ie, GF109203X and calphostin C.

The present study showed that infusion of ET in NS rats caused modest, but not significant, elevation of arterial pressure. ET infusion in HS rats was associated with slightly greater elevations of arterial pressure, but these elevations were still not significant. Although the present ex vivo experiments suggest ET-induced enhancement of the mechanisms of vascular contraction particularly during HS, the relation between the effects of ET infusion on vascular contraction and its effects on the arterial pressure in vivo should be interpreted with caution. The role of the ET receptors in vascular homeostasis is rather complex because the receptor has both pressor and depressor effects in vivo. The pressor effects are mediated by ET₆ and ET₉ receptors in vascular smooth muscle, whereas the depressor effects are mediated by ET₅ receptors in endothelial cells. Because ET does not discriminate between the ET₆ receptor subtypes, the effects of ET infusion on the arterial pressure may represent the combined contribution of ET₆ receptors in endothelial cells and smooth muscle. Because the present experiments were performed in endothelium-denuded vascular strips, the present observations more likely represent the effect of ET on ET₆ receptors of vascular smooth muscle. However, in the in vivo conditions, the activation of the ET₆ receptor in endothelial cells is predicted to reduce the vascular reactivity and thereby counterbalance ET-induced enhancement of the mechanisms of vascular smooth muscle contraction, with a net result of modest changes in vascular resistance and arterial pressure. Therefore, the role of the ET₆ receptor subtypes in vasoconstriction and vasodilation of not only the aorta but also other vascular beds should be further examined in future studies. Also, in addition to the vascular control mechanisms, the arterial pressure is regulated by other
control mechanisms, including renal, neuronal, and hormonal mechanisms. Although the ET-induced enhancement of the mechanisms of vascular reactivity is expected to increase the vascular resistance and arterial pressure, ET infusion particularly during HS may cause additional effects on the other control mechanisms of arterial pressure. This is supported by reports that ET receptors have been identified in the kidney, and that HS may differentially affect ET levels in the renal cortex and medulla and may significantly decrease ET content within the renal inner medulla.17

Perspectives

The present study has shown that low-dose infusion of ET, particularly during HS, is associated with increased vascular reactivity that involves Ca\(^{2+}\) entry from the extracellular space, but not Ca\(^{2+}\) release from the intracellular stores. The ET-induced enhancement of the Ca\(^{2+}\) influx-stress relation, particularly during HS, suggests activation of other mechanisms in addition to Ca\(^{2+}\) entry, possibly involving PKC. The results suggest that ET increases the sensitivity of the mechanisms of vascular smooth muscle contraction to high dietary salt intake and may, in part, explain the possible role of ET in the vascular mechanisms of salt-sensitive hypertension.

However, several points need to be highlighted regarding the above interpretations. First, HS has been shown to increase the production of endogenous ET.17 If this is the case, one would expect the vascular reactivity to be greater in HS than in NS rats. The present study showed that HS alone, which increases endogenous ET, was associated with a slight, but not significant, increase in vascular reactivity. On the other hand, infusion of exogenous ET was associated with significant increases in vascular reactivity. Whether the vascular effects of exogenous ET are different from endogenous ET remains to be investigated. Also, the source of exogenous ET could influence the results. This may explain why no significant increase in arterial pressure was observed in the present study, whereas other studies have shown that chronic ET infusion could cause salt-sensitive hypertension.45 Limitations of studying the vascular reactivity in the aorta should also be considered and should highlight the importance of studying the vascular effects of HS in the small and more relevant resistance arteries. Finally, the present study demonstrated an enhancement of PhE-induced vascular contraction during chronic infusion of ET and HS. However, we cannot generalize that the enhanced vascular contraction during ET infusion and HS may apply to other vasoconstrictor agonists and should represent important areas for future investigations.

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References

38. Drimal J, Koprdova V. Endothelin-1 significantly increased number of specific high-affinity 1,4-dihydropyridine (DHP) binding sites photolabelled on vascular smooth muscle cells with [3H]-azidopine. *Physiol Res*. 1996;45:51–58.
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