Effects of Salt Intake and Angiotensin II on Vascular Reactivity to Endothelin-1

JENNIFER R. BALLEW, STEPHANIE W. WATTS, and GREGORY D. FINK

Department of Pharmacology and Toxicology, Michigan State University, East Lansing, Michigan

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ABSTRACT

Hypertension produced by chronic infusion of angiotensin II (Ang II) is significantly blunted by blockade of endothelin-1 (ET-1) ETA subtype receptors. Furthermore, this model is salt-sensitive, and the antihypertensive response to ET<sub>A</sub> receptor blockade is more pronounced in animals on high salt intake. The goal of these experiments was to evaluate the effect of salt intake and Ang II on vascular reactivity to ET-1. In superior mesenteric arteries from normal male rats, studied in a standard muscle bath, incubation for 1 h with a subcontraction concentration of Ang II (10<sup>-10</sup> M) did not affect concentration-response curves to ET-1. Pressor responses in vivo to 2-h infusions of Ang II (5 ng/min) in rats maintained on normal or high salt intake were abolished by pretreatment with the ET<sub>A</sub> receptor antagonist ABT-627. The antagonist had no effect on pressor responses to phenylephrine (PE). In other experiments, rats maintained on either high or normal salt intake received continuous infusion of Ang II (5 ng/min i.v.) for 7 days, and then their superior mesenteric arteries were tested in the muscle bath. The maximum contractile response to ET-1 in arteries from Ang II-infused rats on normal salt intake was larger than in arteries from rats not receiving Ang II. Conversely, maximum responses to ET-1 in arteries from Ang II-infused rats on high salt intake were depressed compared with controls. No differences in vascular reactivity to PE were found. Thus, chronic in vivo exposure to Ang II results in specific salt-dependent changes in vascular reactivity to ET-1.

Chronic infusion of angiotensin II (Ang II) is an experimental model of “salt-sensitive” hypertension, i.e., infusion rates of Ang II that do not affect arterial pressure in animals on low or normal salt intake cause a significant rise in pressure in animals on higher salt intakes (Muirhead et al., 1975; Kanagy et al., 1990; Ando et al., 1991; Csiky and Simon, 1997). The endothelial cell-derived vasoconstrictor peptide endothelin-1 (ET-1) is now believed to play a key role in this and several other salt-sensitive forms of hypertension (Schiffrin, 1999). Ang II has been shown to increase endothelial cell synthesis and release of ET-1 both in vitro (Emori et al., 1991; Dohi et al., 1992; Imai et al., 1992; Kohno et al., 1992) and in vivo (Barton et al., 1997; Moreau et al., 1997; Lariviere et al., 1998; Ferri et al., 1999). Ang II also potentiates the acute vascular contractile response to other agonists (Herrion et al., 1992; Qui et al., 1994; Dowell et al., 1996), although this has not been demonstrated with ET-1. Chronic Ang II infusion, however, has been reported to potentiate pressor responses to chronic ET-1 infusion (Yoshida et al., 1992). Most importantly, the development of hypertension in response to Ang II was attenuated by concomitant treatment with ET-1 receptor antagonists (d’Uscio et al., 1997; Rajagopalan et al., 1997; Herizi et al., 1998). We hypothesized that ET-1 could also participate in the salt sensitivity of Ang II-induced hypertension, because administration of a selective ET<sub>A</sub> receptor antagonist to rats with Ang II-induced hypertension produced a larger and more sustained fall in arterial pressure when animals were on high versus normal salt intake (Ballew and Fink, 2000). The goal of the current investigation was to determine whether vascular reactivity to ET-1 is differentially affected by salt intake during chronic Ang II infusion.

Materials and Methods

Animals. All animal procedures were carried out in accordance with institutional guidelines established by Michigan State University. Male Sprague-Dawley rats weighing 350 to 450 g were purchased from Charles River Laboratories (Portage, MI). Upon arrival at our facility, rats were maintained according to standards approved by the Michigan State University All-University Committee on Animal Use and Care. All experimental procedures were carried out in accordance with the Guiding Principles in the Care and Use of Animals of the American Physiological Society. Rats were acclimated for at least 2 days before surgical procedures in clear plastic boxes and were allowed access to standard rat chow (Teklad 22/5 Rodent Diet W 8640, Madison, WI) and tap water ad libitum.

Isolated Tissue Bath Protocol. Rats were killed (80 mg/kg pentobarbital i.p.) and the superior mesenteric arteries were dissected into helical strips. The endothelium was left intact. Tissues

ABBREVIATIONS: Ang II, angiotensin II; ET-1, endothelin-1; PE, phenylephrine; MAP, mean arterial pressure; NO, nitric oxide.
were placed in physiological salt solution containing 130 mmol/l NaCl, 4.7 mmol/l KCl, 1.18 mmol/l KH₂PO₄, 1.17 mmol/l MgSO₄·7H₂O, 1.6 mmol/l CaCl₂·2H₂O, 14.9 mmol/l NaHCO₃, 5.5 mmol/l dextrose, and 0.03 mmol/l CaNa-EDTA. One end of the preparation was attached to a glass rod and the other to a force transducer (model FT03; Grass Instruments, Quincy, MA), and the strip was placed under optimum resting tension (600 mg, as previously determined) and allowed to equilibrate for 1 h. Muscle baths were filled with warmed (37°C), aerated (95% O₂, 5% CO₂) physiological salt solution. Changes in isometric force were recorded on a polygraph (Grass Instruments). After the hour of equilibration, arteries were challenged with a maximal concentration of the α₁-adrenergic receptor agonist phenylephrine (PE, 10 μmol/l). Tissues were then washed, and the status of the endothelium was examined by observing arterial relaxation to the endothelium-dependent agonist acetylcholine (1 μmol/l) in tissues contracted by a half-maximal concentration of PE (~10 mmol/l).

The vessels then were incubated with increasing concentrations of PE (10⁻²–10⁻⁴ M) followed by ET-1 (10⁻¹–10⁻⁷ M). The tissues were incubated with each concentration for ~5 min before the next concentration was added. In some preparations, superior mesenteric arteries were incubated with Ang II (10⁻¹⁰ M), A-192621 (an ET₄-selective receptor antagonist, 30 nM; Abbott Laboratories, Abbott Park, IL), ABT-627 (an ET₃-selective receptor antagonist, 30 nM; Abbott Laboratories), or A-182086 (an ET₄/ET₃-selective receptor antagonist, 30 nM; Abbott Laboratories), for 1 h before the production of ET-1 dose-response curves.

In Vivo Protocol. Normal male Sprague-Dawley rats were chronically instrumented for direct, daily measurements of blood pressure and heart rate via catheterization of the femoral artery and vein, as previously described (Potter et al., 1997). Rats were housed in metabolism cages. Venous catheters were attached to a syringe pump via a hydraulic swivel mounted above the cage. Sodium intake was fixed by delivering sodium chloride via continuous intravenous pump between 8:00 and 11:00 AM. The arterial catheters were connected to digital pressure monitors (Digi-Med blood pressure recording system) and allowed to equilibrate for 1 h. Rats were infused into conscious rats. Mean arterial pressure (MAP) was similarly and significantly increased in both normal and high salt groups after 2 h infusion either of Ang II (MAP) was similarly and significantly increased in both normal and high salt groups after 2 h infusion either of Ang II

To confirm that contraction of superior mesenteric arteries to ET-1 is mediated exclusively by ET₄ receptors, we tested the ability of three different ET-1 receptor antagonists to block contractions to ET-1 (Fig. 1). With A-192621, an ET₄-selective receptor antagonist, there were no differences in EC₅₀ or maximal response compared with untreated arteries (control: 1.5 ± 0.3 × 10⁻⁹ M and 133.3 ± 11%; A-192621: 1.2 ± 0.3 × 10⁻⁹ M and 128 ± 11%). With A-182086, an ET₄ receptor antagonist, there were no differences in maximal response compared with untreated arteries (control: 133.3 ± 11%; A-182086: 148 ± 10%). However, the EC₅₀ value was significantly increased (control: 1.5 ± 0.3 × 10⁻⁹ M; A-182086: 6.5 ± 0.7 × 10⁻⁹ M). Curve parameters could not be determined for ABT-627 because the curve was extremely right-shifted.

To test the possibility that Ang II rapidly augments vascular reactivity to ET-1, an ET-1 concentration-response curve was generated in vessels preincubated for 1 h with a subcontracable concentration of Ang II (10⁻¹⁰ M). As shown in Fig. 2, the maximal response to ET-1 in animals exposed to subcontractable levels of Ang II (132.4 ± 17.8% PE contraction) was not significantly different from the maximal response in control rats (155.5 ± 12.6% PE contraction). There was no difference in EC₅₀ values between the two groups.

To determine whether pressor responses during short-term exposure to Ang II in rats on high or normal salt intake is influenced by endogenous levels of ET-1 acting on ET₄ receptors, Ang II (5 ng/min, n = 5) and PE (2 μg/min, n = 5) were infused into conscious rats. Mean arterial pressure (MAP) was similarly and significantly increased in both normal and high salt groups after 2 h of infusion either of Ang II

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**Fig. 1.** Concentration-dependent contraction to endothelin-1 (ET-1) in control, A-192621 (ET₄-selective receptor antagonist, 30 nM)-incubated, ABT-627 (ET₃-selective receptor antagonist, 30 nM)-incubated, and A-182086 (ET₄/ET₃-nonselective receptor antagonist, 30 nM)-incubated superior mesenteric artery of the rat. PE (10⁻⁵ M). Values are mean ± S.E.M. n = number of animals. For A-192621, neither the EC₅₀ value nor the maximal response was shifted. For A-182086, the EC₅₀ value was significantly larger and the maximal response was not different from control. For ABT-627, curve parameters could not be calculated.

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**Results**

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**Data Analysis.** Estimates of maximum response and EC₅₀ values were obtained from each concentration response curve using a four-parameter logistic function. Results are expressed as mean ± S.E.M. For these results, mean values were compared statistically using a one-way ANOVA followed by the protected least significant difference test for post hoc comparisons. For in vivo data, within- and between-group differences were analyzed using mixed-design ANOVA. Post hoc comparisons between groups were performed by testing for simple main effects. Within group comparisons were made using the protected least significant difference test. Criterion for statistical significance was a probability level of less than 0.05.
or PE (Fig. 3). One day later, this protocol was repeated in the same rats 1 h after administration of the selective ET<sub>A</sub> receptor antagonist ABT-627 (2 mg/kg). Treatment with ABT-627 alone did not significantly change MAP. Two hours following the start of Ang II infusion in rats exposed to ABT-627, there was no significant increase in MAP (Fig. 3). PE-infused rats showed a significant increase in MAP that was similar in magnitude to that observed in the absence of ABT-627 treatment.

Chronic infusion of Ang II produced a significant elevation of MAP compared with that seen in control rats regardless of salt intake (Table 1). This increase was significantly (p = 0.0329) larger in animals on high salt intake than in those on normal salt intake. As shown in Fig. 4, maximal responses of rat superior mesenteric arteries to PE in Ang II-infused rats on normal (132.6 ± 16.9% PE contraction) and high salt intake (90.6 ± 11.5% PE contraction) were not significantly different from each other or their respective controls (100.9 ± 9.4 and 134.7 ± 27.6% PE contraction). There were no significant differences in the EC<sub>50</sub> values between the four groups in response to PE.

As shown in Fig. 4, superior mesenteric arteries from Ang II-infused rats on normal salt intake had significantly increased maximum responses to ET-1 (251.8 ± 28.6% PE contraction) compared with controls (135.3 ± 31.9% PE contraction; p = 0.0495). Conversely, superior mesenteric arteries from Ang II-infused rats on high salt intake had significantly decreased maximum response to ET-1 (123.4 ± 35.4% PE contraction) compared with controls (245.6 ± 41.7% PE contraction; p = 0.0263). There was no significant difference in maximal contraction to ET-1 between high salt and normal salt control groups (p = 0.141). There were no significant differences in the EC<sub>50</sub> values between the four groups in response to ET-1.

**Discussion**

The overall goal of our research is to define the mechanisms responsible for the salt sensitivity of Ang II-induced hypertension. We recently demonstrated that blockade of ET<sub>A</sub> receptors in rats receiving chronic intravenous infusions of Ang II causes a larger and more sustained fall in arterial pressure when the rats were on a high versus a normal salt intake (Ballew and Fink, 2000). This result led us to conclude that ET-1 could participate in the mechanism of salt sensitivity in Ang II-induced hypertension. The goal of the current studies was to test the hypothesis that the salt-sensitivity of Ang II-induced hypertension is due in part to amplification of the vascular contractile effects of ET-1. The main new finding from this work is that chronic infusion of Ang II alters mesenteric vascular reactivity to ET-1 in a salt-dependent manner: in rats on normal salt intake maximum contractile responses to ET-1 are increased, whereas in rats on high salt intake they are decreased. If these changes also occur in

<table>
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<th>Salt Intake and Ang II on Vascular Reactivity to ET-1</th>
<th>Mean arterial pressure in rats given chronic Ang II (5 ng/min) infusion</th>
<th>Data are mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preinfusion of Ang II</td>
<td>Postinfusion of Ang II</td>
<td></td>
</tr>
<tr>
<td>Normal salt control</td>
<td>105 ± 2</td>
<td>110 ± 3</td>
</tr>
<tr>
<td>Normal salt + Ang II</td>
<td>115 ± 2</td>
<td>125 ± 5*</td>
</tr>
<tr>
<td>High salt control</td>
<td>114 ± 2</td>
<td>121 ± 6</td>
</tr>
<tr>
<td>High salt + Ang II</td>
<td>115 ± 4</td>
<td>136 ± 7*</td>
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* Significantly different from preinfusion values (p < 0.05).
other major vascular beds, such as skeletal muscle and kidney, they could exert an important effect on arterial pressure regulation.

In vitro studies show that short-term exposure (30–60 min) to very low (subthreshold for direct contraction) concentrations of Ang II potentiates vascular contractile responses to other agonists (Henrion et al., 1992a). The mechanism of this effect is not fully elucidated, but probably involves activation of protein kinase C in the vascular smooth muscle cell (Henrion et al., 1992b). This action of Ang II has not been tested with ET-1 as the second agonist, but the strong similarity in signaling mechanisms used by Ang II and ET-1 in vascular smooth muscle (Tsuda et al., 1993) makes it a likely possibility. Furthermore, there is evidence from chronic (6-day) in vivo experiments in rats that the pressor actions of ET-1 are potentiated by concomitant exposure to Ang II (Yoshida et al., 1992). Another study though failed to find such an effect during rapid, bolus injections of the two agonists in the canine coronary circulation (Kiss et al., 1998). Our data do not support the idea that contractile effects of ET-1 are amplified after short-term exposure to Ang II in vitro, at least in the superior mesenteric artery of the rat. We observed no significant change in the concentration-response curve to ET-1 in arteries pre-exposed for 1 h to Ang II.

Pressor responses, and changes in hindlimb vascular resistance, to acute bolus injections of Ang II in vivo are reported to be reduced by prior blockade of ET-1 receptors, especially when low doses of Ang II are administered (Balakrishnan et al., 1996; Champion et al., 1998). This suggests that physiological amounts of ET-1 may amplify the vascular contractile response to Ang II in vivo. We performed experiments to investigate this phenomenon in rats on normal and high salt intake. Our results confirm that pressor responses to acute (2-h) exposure to low amounts of Ang II in vivo are significantly reduced by prior blockade of ET_A receptors. This effect appears to be specific for Ang II since no change in the pressor response to phenylephrine infusion was seen after administration of the ET_A antagonist. One interpretation of these results is that Ang II infusion for 2 h stimulated the release of ET-1 from arterial endothelial cells, and that this ET-1 accounted of the accompanying rise in arterial pressure. Most studies, however, have failed to find evidence for ET-1 release by Ang II in short-term infusion protocols (Klein et al., 1995; Delemarre et al., 1998), including an investigation in humans on differing levels of salt intake (Ferri et al., 1999). Thus, we interpret the results to indicate that physiological amounts of ET-1 acting at ET_A receptors amplify the pressor actions of exogenous Ang II. Our data, however, do not provide any insight into the mechanism of this interaction. It is notable though that this short-term effect was observed in rats on both normal and high salt intake, and therefore is not likely to alone explain the salt-sensitivity of chronic Ang II-induced hypertension.

In a final experiment, we evaluated vascular reactivity to ET-1 in vitro in superior mesenteric arteries from rats made hypertensive with Ang II. We found divergent results depending on whether the rats were on normal or high salt intake. Rats on high salt intake became significantly more hypertensive than rats on normal salt intake. Furthermore, maximal response of mesenteric arteries to ET-1 from these rats was significantly suppressed compared with responses in control rats on high salt intake. Similar results have been reported by other investigators using a model of chronic Ang II-induced hypertension involving infusion of higher doses of Ang II (200 ng/kg/min, subcutaneously) in rats on normal salt intake (d’Uscio et al., 1997; Rajagopalan et al., 1997). Arteries from those rats exhibited a marked increase in preproET-1 gene expression and ET-1 peptide content (Rajagopalan et al., 1997; d’Uscio et al., 1998), and there was a strong negative correlation between reactivity to ET-1 and arterial peptide concentrations (d’Uscio et al., 1998). It has been suggested that a decrease in mesenteric response to ET-1 in vitro is a consequence of ET_A receptor down-regulation caused by chronic increase in ET-1 release (Nguyen et al., 1992). Although we did not measure arterial content of ET-1 in our studies, we propose that a similar mechanism could
account for depressed maximal responses to ET-1 in superior mesenteric arteries from rats in our study on high salt intake and Ang II infusion.

Mesenteric arteries from rats receiving Ang II but normal salt intake exhibited a significantly increased maximum response to ET-1 in vitro. This is the first report of potentiation by exogenous Ang II of in vitro contractile responses to ET-1 in vascular smooth muscle, although others have reported that Ang II amplifies the bronchoconstrictor actions of ET-1 via a leukotriene-dependent pathway (Pitt and Nally, 1999). Contraction of superior mesenteric arteries to ET-1 is mediated exclusively through ET(A) receptors (Fig. 1), and Ang II has been reported to increase ET(A) receptor expression (Hatakeyama et al., 1994). Thus, one potential explanation for our results is that chronic exposure to Ang II increased ET(A) receptor number in superior mesenteric arteries in rats on normal salt intake.

This does not explain, however, why the effects of Ang II infusion on arterial contraction to ET-1 in vitro were different in rats on high versus normal salt intake. Our experiments do not provide a definitive answer. We speculate that long-term exposure to Ang II can up-regulate both ET(A) receptor number and preproET-1 gene expression. Although high salt intake alone does not stimulate vascular ET-1 formation (Ikedu et al., 1999), our data are consistent with the idea that high salt intake plus Ang II may be a more effective stimulus to preproET-1 gene expression and ET-1 synthesis than Ang II alone. One possible mechanism could involve the effects of Ang II and high salt intake on endothelial nitric oxide (NO) action. Long-term exposure to Ang II in vivo (d‘Uscio et al., 1997), and to high salt intake (Boegehold, 1995), are reported to impair NO activity in resistance arteries. There is also evidence that increased NO activity suppresses ET-1 formation in blood vessels (Boulanger and Lüscher, 1990). The combination of Ang II and high salt could produce a larger increase in vascular ET-1 synthesis than Ang II alone because of less NO-mediated inhibition. In support of this idea, we showed that administration of the NO synthase inhibitor L-nitro-arginine methyl ester, to rats on normal salt intake caused a highly significant potentiation of the chronic pressor responses to Ang II infusion in rats (Melaragno and Fink, 1996). Finally, there is evidence that the pressor (Mortensen and Fink, 1992) and vascular resistance (Grossman et al., 1990) effects of ET-1 are increased by high salt intake. Therefore, enhanced synthesis of ET-1 in blood vessels in rats on high salt intake receiving Ang II, combined with some amplification of the pressor effect of ET-1 by high salt alone, could explain the larger contribution of ET-1 to Ang II-induced hypertension under high salt conditions. Direct evidence for this theory needs to be obtained in future experiments.

In summary, Ang II was shown to cause changes in pressor and mesenteric vascular contractile effects of ET-1 that are dependent on time of exposure to Ang II, and on salt intake. During long-term infusion Ang II can increase both mesenteric vascular reactivity to ET-1 (this study) and ET-1 formation (Barton et al., 1997; Moreau et al., 1997; Lariviere et al., 1998). Each of these mechanisms may contribute to the dependence of Ang II-induced hypertension on ET(A) receptor activation, but high salt intake apparently shifts the balance toward the latter.

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References


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Send reprint requests to: Gregory D. Fink, Ph.D., B-327 Life Science Bldg., Michigan State University, East Lansing, MI 48824. E-mail: finkg@msu.edu