Enhanced renal expression of preproendothelin mRNA during chronic angiotensin II hypertension

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Alexander, Barbara T., Kathy L. Cockrell, A. Nicole Rinewalt, Jason N. Herrington, and Joey P. Granger. Enhanced renal expression of preproendothelin mRNA during chronic angiotensin II hypertension. Am J Physiol Regulatory Integrative Comp Physiol 280: R1388–R1392, 2001.—The purpose of this study was to determine the role of endothelin in mediating the renal hemodynamic and arterial pressure changes observed during chronic ANG II–induced hypertension. ANG II (50 ng·kg⁻¹·min⁻¹) was chronically infused into the jugular vein by miniosmotic pump for 2 wk in male Sprague-Dawley rats with and without endothelin type A (ETₐ)–receptor antagonist ABT-627 (5 mg·kg⁻¹·day⁻¹) pretreatment. Arterial pressure increased in ANG II rats compared with control rats (149 ± 5 vs. 121 ± 6 mmHg, P < 0.05, respectively). Renal expression of preproendothelin mRNA was increased by ~50% in both the medulla and cortex of ANG II rats. The hypertensive effect of ANG II was completely abolished in rats pretreated with the ETₐ-receptor antagonist (114 ± 5 mmHg, P < 0.05). Glomerular filtration rate was decreased by 33% in ANG II rats, and this response was attenuated in rats pretreated with ETₐ-receptor antagonist. These data indicate that activation of the renal endothelin system by ANG II may play an important role in mediating chronic renal and hypertensive actions of ANG II.

glomerular filtration rate; mean arterial pressure; renal preproendothelin

THE RENIN-ANGIOTENSIN SYSTEM plays an important role in the regulation of arterial pressure during various physiological and pathophysiological conditions such as hypertension and chronic renal failure (8, 12, 18). ANG II is thought to influence arterial pressure through its direct renal vasoconstrictor, sodium-retaining, and mitogenic actions (8, 12, 14, 18). Recent studies have also suggested that ANG II may exert its physiological actions via interaction with autacoid factors such as endothelin (2, 3, 6, 11, 20). Consistent with this suggestion are results of several recent studies indicating that the hypertensive effects of ANG II can be markedly attenuated or completely abolished by endothelin type A (ETₐ)–receptor antagonists (2, 3, 6, 11, 20). Although these studies support an important interaction between endothelin and ANG II, the mechanisms underlying these interactions in the kidney have yet to be fully elucidated.

One potential mechanism for this interaction is that ANG II may be enhancing renal endothelin synthesis. Several lines of evidence support this concept. ANG II is a potent stimulator of endothelin release by cultured endothelial (9, 10), smooth muscle (21), and renal mesangial cells (16). Furthermore, ANG II stimulates expression of preproendothelin mRNA in cultured cells such as endothelial (5, 13) and vascular smooth muscle (21). However, evidence supporting an effect of ANG II on synthesis of endothelin in vivo is not as abundant. Barton and colleagues (3) recently reported enhanced endothelin levels in renal tissue, but not myocardial tissue, in rats with chronic ANG II hypertension. Whether ANG II stimulated synthesis of endothelin or expression of preproendothelin in the kidneys is still unclear because the increase in renal tissue endothelin levels was associated with enhanced renal uptake of endothelin-1 (3). Although these findings suggest that ANG II may directly effect endothelin expression, the effect of ANG II on the in vivo renal synthesis of endothelin is still unclear.

Previous studies have indicated that the renal actions of endothelin play a major role in mediating the chronic hypertensive effects of ANG II (2, 3, 6, 11, 20). Although a number of studies has shown that hypertensive effects of chronic ANG II infusion can be attenuated by ETₐ-receptor antagonists, the importance of endothelin in mediating the chronic renal actions of ANG II has yet to be fully elucidated. Therefore, the purpose of this study was to determine if the renal hemodynamic and arterial pressure changes observed during chronic ANG II hypertension are associated with increases in the renal expression of preproendothelin mRNA and to determine the importance of endothelin in mediating the chronic renal actions of ANG II hypertension.

METHODS

Animal preparation. All studies were performed in 250- to 300-g male Sprague-Dawley rats purchased from Harlan.
Sprague Dawley (Indianapolis, IN). Animals were housed three to a cage in a temperature-controlled room (23°C) with a 12:12-h light-dark cycle. Animals were maintained on a custom AIN-76A diet (ICN Pharmaceuticals, Aurora, OH) with 8% sodium chloride to inhibit endogenous ANG II formation. All experimental procedures executed in this study were in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Animal Care and Use Committee at the University of Mississippi Medical Center approved all experimental protocols.

**Experimental design.** All animals were placed on the high-salt diet 5 days before the start of the study. Animals were divided into four groups: control, control plus ETA-receptor antagonist, ANG II infused, and ANG II infused plus ETA-receptor antagonist. ETA-selective receptor antagonist ABT-627 was administered in the drinking water at a dose of 5 mg\(\text{kg}^{-1}\text{day}^{-1}\), and treatment was initiated 5 days before insertion of the osmotic pumps. Animals were implanted with a miniosmotic pump (model 2002, Alza Scientific, Palo Alto, CA) into the jugular vein for chronic infusion of saline with a miniosmotic pump (model 2002, Alza Scientific, Palo Alto, CA) into the jugular vein for chronic infusion of saline. Animals were implanted into the osmotic pumps. Animals were implanted three to a cage in a temperature-controlled room (23°C) with a 12:12-h light-dark cycle. Animals were maintained on a Sprague Dawley (Indianapolis, IN). Animals were housed three to a cage in a temperature-controlled room (23°C) with a 12:12-h light-dark cycle. Animals were maintained on a custom AIN-76A diet (ICN Pharmaceuticals, Aurora, OH) with 8% sodium chloride to inhibit endogenous ANG II formation. All experimental procedures executed in this study were in accordance with National Institutes of Health Guide for the Care and Use of Laboratory Animals. The Animal Care and Use Committee at the University of Mississippi Medical Center approved all experimental protocols.

**Measurement of renal function and arterial pressure in conscious rats.** Renal hemodynamics and arterial pressure were determined in conscious control (\(n = 8\)), control plus ETA-receptor antagonist (\(n = 5\)), ANG II-infused (\(n = 15\)), and ANG II-infused plus ETA-receptor antagonist (\(n = 9\)) male rats. While being anesthetized with 2% isoflurane (15), rats were surgically instrumented with catheters in the femoral vein and carotid artery (PE-50 tubing), and the bladder was cannulated (flare-tipped PE-90 tubing) for urine collection; then all catheters were tunneled to the back of the neck and exteriorized. Isotonic saline containing Gloopf 125\textsuperscript{I} (0.05 mCi-kg\(^{-1}\)-min\(^{-1}\), sodium iohalamate; Cypros, Carlsbad, CA) and hippuran 123\textsuperscript{I} (0.1 mCi-kg\(^{-1}\)-min\(^{-1}\) sodium o-iodohippurate; Synco International, Jackson, MS) was delivered at a fixed rate of 3 ml/h through the femoral vein catheter by an infusion pump. Arterial pressure was continuously monitored by a pressure transducer connected to a Grass Model 7B chart recorder (Grass Instrument). For renal function measurements, a 3-min bolus of 25% isotope was followed by a 1-h equilibration period with two subsequent 20-min clearances for each rat. Urine volume was determined gravimetrically. Glomerular filtration rate (GFR) and renal plasma flow (RPF) were calculated from concentrations of 125\textsuperscript{I} and 131\textsuperscript{I} in plasma and urine (19).

**Isolation of total cellular RNA.** Kidneys were removed from control rats (\(n = 8\)) and chronic ANG II-treated (\(n = 8\)) rats, sliced into cortical and medullary sections, quick frozen in liquid nitrogen, and stored at \(-80^\circ\text{C}\). After each kidney was ground using a liquid nitrogen-chilled mortar and pestle, total cellular RNA was isolated using the guanidine thiocyanate, acid phenol-chloroform procedure of Chomczynski and Sacchi (4) (ToTALLY RNA kit; Ambion, Austin, TX). Total RNA concentration and purity were determined spectrophotometrically using A\textsubscript{260} and A\textsubscript{260/280} ratio, respectively. Total RNA integrity was checked using 1% agarose gel electrophoresis with a 0.4 mol/l Tris-acetate and 0.001 mol/l EDTA buffer.

**Ribonuclease protection assay.** The cDNA for rat preproendothelin was linearized with Xho I (New England Biolabs, Beverly, MA). An antisense internal control template for \(\beta\)-actin (1) was obtained from Ambion. Antisense RNA probes were synthesized and labeled with \(\alpha\)-32\textsuperscript{P}UTP (DuPont NEN, Wilmington, DE) using a MAXIscript IN VITRO Transcription kit (Ambion) according to manufacturer’s instructions. Full-length probes were purified and eluted from denaturing 5% acrylamide gels. Ribonuclease protections assays (RPA) were performed with an Ambion RPA III kit as described by the manufacturer. Protected fragments were separated on denaturing 5% acrylamide gels that were dried, exposed to a Molecular Imaging screen (BioRad, Hercules, CA), and quantified using the Molecular Analyst Imager System (BioRad). An equivalent amount of total RNA (20 \(\mu\text{g}\)) was used from each rat kidney. In each individual RPA, RNA concentration was varied to confirm that the probe was in excess and that the response was linear. Where indicated, the error bars represent the standard error from at least three separate determinations per kidney. Quantitation represents a ratio of transcript levels of preproendothelin to actin.

**Statistical analysis.** All data are expressed as means \(\pm\) SE. Comparisons of control, acute ANG II, and chronic ANG II hypertensive rats were analyzed using factorial ANOVA followed by Scheffé’s test. A value of \(P < 0.05\) was considered statistically significant.

**RESULTS**

**Mean arterial pressure in chronic ANG II-treated rats.** Figure 1 illustrates arterial pressures in control and chronic ANG II-treated rats. Mean arterial pressure (MAP) averaged 121 \(\pm\) 6 mmHg in control rats. Arterial pressure in the ANG II rats averaged 152 \(\pm\) 5 mmHg, 21% above control rats, \(P < 0.05\). Pretreatment with ETA-receptor antagonist ABT-627 (+ET\(_A\)) completely attenuated the increase in arterial pressure in the ANG II rats (114 \(\pm\) 5 mmHg, ANG II + ET\(_A\), \(P < 0.05\)). Pretreatment with the ETA-receptor antagonist in control animals did not significantly alter MAP (111 \(\pm\) 12.2 mmHg, control + ET\(_A\)).

**Renal preproendothelin expression during ANG II-induced hypertension.** RPA was used to quantitate the renal levels of preproendothelin in chronic ANG II-induced hypertension. Figure 2A shows a representative RPA blot in which a significant increase in pre-
proendothelin mRNA expression in the cortex of ANG II-induced hypertensive rats was evident. Quantitation of renal proendothelin transcript levels in ANG II rats relative to control rats for both cortex and medulla is shown in Fig. 2. Preproendothelin mRNA levels in the cortex were increased in ANG II rats compared with control rats by 50% (ANG II 629.2 ± 55.2 densitometric units vs. control 319.8 ± 24.8 densitometric units, \(P < 0.05\)). Preproendothelin mRNA levels in the medulla were also increased in ANG II rats compared with control rats (ANG II 1,361.3 ± 172.2 densitometric units vs. control 533.6 ± 22.1 densitometric units, \(P < 0.05\)).

Renal hemodynamics in chronic ANG II-treated rats. The difference in renal hemodynamics observed in control and ANG II rats is shown in Figs. 3, 4, and 5. The increase in MAP observed in the ANG II rats was associated with a significant increase in renal vascular resistance (RVR) (ANG II 17.8 ± 2.5 mmHg·ml⁻¹·min⁻¹ vs. control 10.7 ± 2.0 mmHg·ml⁻¹·min⁻¹, \(P < 0.05\); Fig. 3). Pretreatment with the ETₐ-selective receptor antagonist attenuated the RVR response to chronic ANG II infusion (ANG II + ETₐ 13.6 ± 2.3 mmHg·ml⁻¹·min⁻¹; Fig. 3B). Pretreatment with the ETₐ-receptor antagonist in control animals did not significantly alter RVR (control + ETₐ 10.3 ± 1.5 mmHg·ml⁻¹·min⁻¹; Fig. 3A). GFR was significantly decreased in the ANG II rats compared with control rats (ANG II 2.1 ± 0.2 ml/min vs. control 3.2 ± 0.9 ml/min, respectively, \(P < 0.05\); Fig. 4B). Although the GFR response to ANG II was somewhat attenuated by
ETA-receptor antagonism (ANG II + ETA 2.6 ± 0.5 ml/min), GFR was not altered in control animals by pretreatment with the ETA-receptor antagonist (control + ETA 2.4 ± 0.3 ml/min; Fig. 4A). RPF tended to decrease in ANG II rats compared with control rats (ANG II 7.4 ± 0.7 ml/min vs. control 9.6 ± 2.0 ml/min, respectively; Fig. 5B). Pretreatment with the ETA-receptor antagonist did not alter the ANG II-induced RPF response in the ANG II animals (ANG II + ETA 6.8 ± 1.1 ml/min; Fig. 5B). Pretreatment with the ETA-receptor antagonist did not alter RPF in control, non-ANG II-treated animals (control + ETA 8.3 ± 0.3 ml/min; Fig. 5A).

DISCUSSION

In this study, we demonstrated that the reduction in renal hemodynamics and elevation in arterial pressure in chronic ANG II-induced hypertension in rats are associated with enhanced renal cortical and medullary expression of preproendothelin mRNA. In addition, the hypertensive effects of chronic ANG II infusion were markedly attenuated, whereas renal actions of ANG II were diminished on pretreatment with an ETA-receptor antagonist. These data indicate that activation of the renal endothelin system by ANG II may play an important role in mediating chronic renal and hypertensive actions of ANG II.

Attenuation of the chronic arterial pressure effects of ANG II by ETA-receptor antagonists in numerous studies suggests that ANG II may exert its physiological actions via an interaction with endothelin (2, 3, 6, 11, 20). In this study, we found that a 2-wk infusion of a physiological dose of ANG II in conscious chronically instrumented rats increased MAP by ~30 mmHg. The increase in arterial pressure in the ANG II hypertensive rats was associated with significant increases in renal expression of preproendothelin. As seen in this representative RPA blot in Fig. 2A, preproendothelin expression in chronic ANG II-infused rats was increased relative to preproendothelin levels in control animals. We found that renal preproendothelin expression was significantly increased in both the medulla and cortex in chronic ANG II hypertensive rats. Therefore, chronic ANG II-induced hypertension is associated with an increase in renal preproendothelin expression.

The mechanisms whereby ANG II stimulates renal endothelin synthesis in rats with chronic ANG II hypertension are uncertain, but they may include direct and indirect pathways. Direct effects of ANG II on endothelin have been reported by others, including in vitro studies, that show ANG II as a potent stimulator of endothelin release and preproendothelin expression in cultured cells (9–11, 13, 16, 21). Additionally, in vivo studies have reported enhanced endothelin-1 uptake and tissue levels in rats with chronic ANG II infusion (2, 3). Therefore, one potential mechanism is that ANG II may be enhancing renal endothelin synthesis. In our study, we demonstrate that changes in GFR and RVR in rats with chronic ANG II-induced hypertension are associated with a significant elevation in renal expression of preproendothelin mRNA. In addition to directly stimulating endothelin synthesis, ANG II may also activate renal endothelin production indirectly by causing hypertension-induced endothelial dysfunction in the kidney. The elevation in renal cortical expression of preproendothelin mRNA could be due to hemodynamic factors because the cortical circulation would be exposed to elevated hydrostatic forces during ANG II-induced hypertension. The importance of this indirect mechanism is uncertain because normalization of blood pressure with verapamil in rats with chronic ANG II hypertension did not abolish an increase in aortic tissue endothelin concentration (7). Furthermore, the elevation in renal medullary preproendothelin mRNA observed in this study is most likely unrelated to the hypertension because pressures within the medullary circulation would be expected to decrease in response to ANG II. Therefore, stimulation of renal endothelin synthesis may be pressure independent.

Although others have shown that addition of a selective ETA-receptor antagonist will attenuate or abolish
the hypertensive response in chronic ANG II-infused rats relative to control rats, the importance of the enhanced renal synthesis of endothelin in mediating the renal changes during ANG II hypertension has been unclear. Therefore, the second part of our study was to examine the role of endothelin in mediating the chronic renal and hypertensive actions of ANG II. In our study, ETA blockade by a selective ETA-receptor antagonist ABT-627 markedly attenuated the hypertensive response to chronic ANG infusion. In the control, non-ANG II-infused animals, ETA-receptor blockade did not significantly reduce MAP, thus indicating that the fall in arterial pressure in the ANG II hypertensive rats treated with the ETA-receptor antagonist is due to a blockade of the chronic actions of ANG II.

The increase in arterial pressure in the ANG II-infused rats was associated with a significant increase in RVR. Pretreatment with the ETA-selective receptor antagonist attenuated the RVR response to chronic ANG II infusion, thus indicating an important role for endothelin in mediating this chronic hemodynamic response. Although GFR was not altered in control animals by pretreatment with the ETA-selective receptor antagonist, the GFR response to ANG II was attenuated by ETA-receptor antagonism.

In summary, chronic ANG II hypertension in rats was associated with reductions in renal hemodynamics and enhanced renal expression of preproendothelin mRNA. The renal expression of preproendothelin mRNA in both the medulla and cortex was increased by ~50% in chronic ANG II hypertensive rats relative to control rats. In addition, the hypertensive effects of chronic ANG II infusion were markedly attenuated, whereas renal actions of ANG II were diminished on pretreatment with an ETA-receptor antagonist. These data indicate that activation of the renal endothelin system by ANG II may play an important role in mediating ANG II-induced renal vasoconstriction and hypertension.

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