Subpressor Doses of Angiotensin II Increase Plasma F$_2$-Isoprostanes in Rats

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Abstract—The present study was performed to determine whether physiologically relevant doses of angiotensin II (Ang II), which do not affect renal hemodynamics but do cause slow response hypertension, result in oxidative stress as measured by production of vasoconstrictor F$_2$-isoprostane, a prostaglandin-like non–cyclooxygenase-produced arachidonic acid metabolite that is the end product of lipid peroxidation. Rats were instrumented with abdominal aortic and left femoral venous catheters, and before and throughout Ang II (or saline) infusion, all rats received enalapril (250 mg/L). Four days after the initiation of enalapril, rats were infused with Ang II (10 ng · kg$^{-1}$ · min$^{-1}$, n=6) or saline (n=6) for 14 days. Mean arterial pressure was measured 24 hours per day, and on day 12, glomerular filtration rate and renal plasma flow were measured. Mean arterial pressure in control rats averaged 85±1 mm Hg, and with Ang II infusion, mean arterial pressure increased slowly and reached a plateau on day 3, averaging 117±2 mm Hg (P<0.0001 compared with enalapril alone). Glomerular filtration rate and renal plasma flow were not affected by Ang II. Free F$_2$-isoprostanes in plasma increased by 54% with Ang II (P<0.01), and the production of F$_2$-isoprostanes esterified in plasma lipids tended to be higher with Ang II also but did not reach significance (P=0.1). These studies suggest that low doses of Ang II are capable of producing oxidative stress in animals. Whether oxidative stress plays a causative role in Ang II–mediated slow-response hypertension or is secondary to the hypertension is not clear from these data and will require further study. (Hypertension. 2000;35[part 2]:476-479.)

Key Words: stress, oxidative ■ blood pressure ■ glomerular filtration rate ■ renal blood flow ■ angiotensin-converting enzyme

Small nonpressor doses of angiotensin II (Ang II) are capable of producing hypertension when given chronically for days. However, the mechanism(s) responsible for the hypertension is not clear. In recent studies, Rajagopalan et al infused rats with pharmacological doses of Ang II (0.7 mg · kg$^{-1}$ · d$^{-1}$ SC by minipump) for 5 days and found that blood pressure increased by 50% on day 5. These investigators found that superoxide levels were increased by 2-fold in aortic segments from Ang II–treated rats. In contrast, norepinephrine infusion, which increased blood pressure to levels similar to those found with Ang II, had no effect on superoxide levels in vascular tissue, thus proving that the increase in superoxide associated with Ang II was not merely a secondary response to the higher blood pressure. In addition, these investigators found that the increased superoxide levels could be normalized with losartan, the Ang II receptor antagonist, or with liposomes containing superoxide dismutase. These data clearly indicated that large pharmacological doses of Ang II, but not norepinephrine, were capable of causing oxidative stress, despite similar increases in blood pressure with Ang II and norepinephrine. Superoxide is also capable of inactivating nitric oxide (NO), which, in turn, could lead to increased vascular tone and contribute to elevated blood pressure. It is not clear whether similar effects on oxidative stress could also be elicited by low doses of Ang II.

In addition to the quenching of NO by superoxide, some products of oxidative stress are known to exert vasoconstrictive actions. One such group of compounds includes the isoprostanes, which are prostaglandin-like compounds produced by nonenzymatic free radical–induced peroxidation of arachidonic acid. One of the F-ring isoprostanes (8-iso-prostaglandin F$_{2a}$) has been shown to be a very potent renal vasoconstrictor, mainly by increasing afferent resistance, and, when infused intrarenally at high doses, has also been shown to raise blood pressure. We have previously found that aging is associated with a 50% reduction in glomerular filtration rate (GFR) and with an increase in F$_2$-isoprostanes in the kidneys of rats. Chronic treatment of rats, while aging, with the antioxidant vitamin E reduced the levels of F$_2$-
isoprostanes and improved GFR significantly.\textsuperscript{8} Taken together, these data suggest that isoprostanes, which are produced in situations of oxidative stress, can exert effects on both renal and systemic hemodynamics.

The present study was performed to determine whether subpressor doses of Ang II, which increase blood pressure slowly, also lead to oxidative stress as measured by increases in the production of F\textsubscript{2}-isoprostanes in the plasma and kidney. If so, a reduction in the vasodilator action of NO and an increase in vasoconstrictor F\textsubscript{2}-isoprostanes may explain the development of the slow pressor response to Ang II.

**Methods**

**Rats**

Male Sprague-Dawley rats (n=12) were obtained from Harlan Sprague Dawley (Indianapolis, Ind) at 3 to 4 months of age. After their arrival, the rats were maintained on standard rat chow (Teklad, Harlan Sprague Dawley) and tap water and exposed to a 12-hour light/12-hour dark cycle until the day of catheter placement. The protocols complied with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Mississippi Medical Center.

**Chemicals**

Unless otherwise stated, chemicals, including Ang II and enalapril, were obtained from Sigma Chemical Co.

**Catheter Placement**

Chronic catheters were placed as we have previously described.\textsuperscript{9} Briefly, rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and placed on a heating pad. A midline abdominal incision was made, and the abdominal aorta was separated from the inferior vena cava, and a catheter (V/4 medical vinyl tubing, Scientific Commodities, Inc) was placed in the abdominal aorta below the level of the kidneys. A catheter (V/3, Scientific Commodities, Inc) was also placed in the left femoral vein. Both catheters were advanced subcutaneously along the back and exteriorized at the nape of the neck through a buttontuck to the skin and connected to a spring. Rats were placed in individual metabolism cages, and the spring was connected to a 2-channel hydraulic swivel (Instech) above the cage. The femoral catheter was connected via the swivel to an infusion pump (Harvard Apparatus) at a rate of 0.75 mL/h. The arterial catheter was connected via the swivel to a transducer (Argon) connected to an analog-to-digital converter for 24-hour blood pressure recording. Rats were provided with normal sodium intake (2.2 mEq/d) via the combination of diet and saline infusion.

**Protocol for Ang II Infusion**

Rats were divided into 2 groups: group 1 received only enalapril throughout the experiment to suppress endogenous Ang II formation; group 2 received enalapril and Ang II during the experimental period. As shown in Figure 1, 7 days were allowed for recovery from surgery before the recording of blood pressure. On day 3 of recovery from surgery, all rats were given enalapril (250 mg/L) in their drinking water, resulting in a dose of 1.15 mg · kg\textsuperscript{-1} · h\textsuperscript{-1}, which continued until the end of the experiment. In preliminary studies, this dose of enalapril was effective in blocking the pressor response to a 50 ng bolus of angiotensin I in 4 rats. Beginning on day 8 after surgery, rats were infused with Ang II (10 ng · kg\textsuperscript{-1} · min\textsuperscript{-1}) in saline or were given saline alone (vehicle) for 14 days. On day 12 of Ang II infusion, GFR and estimated renal plasma flow (RPF) were measured, as we have previously described,\textsuperscript{9} by a 24-hour infusion of [\textsuperscript{135}I]iothalamate (0.05 mCi · kg\textsuperscript{-1} · min\textsuperscript{-1}, Iso-Tex Diagnostics) and 1% p-aminohippurate at a rate of 0.75 mL/h. After 18 hours of infusion, blood samples were drawn for calculation of GFR and RPF.

**Measurement of Serum F\textsubscript{2}-Isoprostanes**

F\textsubscript{2}-isoprostanes are initially formed esterified in phospholipids and then released. For this reason, we measure not only free F\textsubscript{2}-isoprostanes in plasma but also levels of F\textsubscript{2}-isoprostanes esterified in plasma lipids. F\textsubscript{2}-isoprostanes, free in plasma and esterified in plasma lipids, were measured by a highly accurate stable isotope dilution gas chromatography–negative ion chemical ionization mass spectrometric assay as previously described.\textsuperscript{10}

**Statistical Analyses**

Differences in data between Ang II–infused and control groups were analyzed by Student t test, and significance was defined as P<0.05.

**Results**

At the time of catheter placement, there was no difference in body weights between rats destined to receive saline vehicle and those destined to receive Ang II infusion (control, 386±5 g; Ang II, 377±5 g). As shown in Figure 2, in rats receiving enalapril treatment alone, blood pressure averaged 86±1 mm Hg throughout the study period. Enalapril treatment and Ang II infusion resulted in a small increase, to 100±2 mm Hg, on day 1, which increased to 118±8 mm Hg on day 2, and reached a peak on day 3 of 123±9 mm Hg. Blood pressure with Ang II averaged 118±2 mm Hg throughout the 14 days of infusion (Figure 2). On day 12 of Ang II infusion, renal hemodynamics were measured in all rats. Ang II had no effect on either GFR or RPF (GFR 3.13±0.10 [control] and 2.95±0.13 [Ang II] mL · min\textsuperscript{-1} · kg\textsuperscript{-1}, RPF 27.71±1.28 [control] and 26.84±2.01 [Ang II] mL · min\textsuperscript{-1} · kg\textsuperscript{-1}).

On day 14 of Ang II infusion at the time of euthanasia, body and kidney weights of rats were not different between control rats and rats receiving Ang II infusion (body weights 390±8 [control] and 397±6 [Ang II] g; kidney weights 2.34±0.07 [control] and 2.45±0.03 [Ang II] g). As shown in

![Figure 1. Schematic diagram of protocol to determine the effect of chronic (14-day) Ang II infusion in rats. Male rats, aged 3 to 4 months (n=6, control rats; n=6, Ang II–infused rats), were implanted with chronic catheters on day 1. The rats were placed into individual metabolism cages and allowed to recover from surgery for 7 days before the start of Ang II infusion. On day 4 of the recovery period, rats began receiving enalapril (250 mg/L) in drinking water. Rats received Ang II (10 ng · kg\textsuperscript{-1} · min\textsuperscript{-1}) for 14 days by intravenous infusion. On day 12, renal hemodynamics were measured. After 14 days of Ang II infusion, plasma was taken for determination of F\textsubscript{2}-isoprostanes. ERPF indicates estimated RPF.](image-url)
P-elevated (0.1). Receiving saline vehicle, but the levels were not significantly increased in rats that received Ang II compared with rats.

**Figure 3.** Effect of chronic Ang II on plasma F₂-isoprostanes. Rats received either enalapril alone or enalapril and Ang II for 14 days. At the end of the study, plasma was obtained for measurement of free F₂-isoprostanes (A) or F₂-isoprostanes esterified in plasma lipids (B). **P**<0.01 vs control.

Discussion

In the present study, we attempted to determine whether chronic low doses of Ang II are capable of causing oxidative stress in rats. We found that in the presence of converting enzyme inhibition, which blocked endogenous Ang II formation, the infusion of low doses of Ang II, which did not cause changes in renal hemodynamics, increased blood pressure by ≈40% and also resulted in a 50% increase in free plasma F₂-isoprostanes, the most sensitive and most reliable measure to date of oxidative stress. The data show that nonpressor doses of Ang II are capable of producing oxidative stress in rats. Furthermore, it is possible that the reduction of NO as a consequence of increased superoxide in combination with increases in vasoconstrictor isoprostanes may contribute to the induction of the slow pressor response to Ang II.

This phenomenon of slow-response hypertension to Ang II was first described in rabbits by Dickinson et al. Later in dogs by McCubbin et al but is now known to occur in all species in which it has been tried. The slow pressor response to Ang II requires 5 to 10 hours to develop and reaches a maximum increase in blood pressure after 3 to 5 days. It has been shown that this response develops without significant changes in basal levels of circulating Ang II. The mechanism(s) responsible for the hypertension is not clear, but many systems (eg, thromboxanes and the sympathetic nervous system) have been hypothesized to play a role. However, the time delay required to generate the elevated blood pressure with small doses of Ang II does suggest the necessity for activation of additional vasoconstrictor systems, which may then trigger an autocatalytic reaction to potentiate the vasoconstrictor properties of Ang II.

Superoxide, which was shown by Rajagopalan et al to be increased with large infusions of Ang II, is known to interact with NO to produce peroxynitrite, one of the most potent oxidative compounds known. The reaction rate of NO and superoxide is more rapid than the reaction rates of superoxide and its scavenger, superoxide dismutase. A reduction in NO due to preferential superoxide scavenging could induce vasoconstriction and favor the development of hypertension. Although peroxynitrite itself is a vasodilator, Villa et al have demonstrated that tachyphylaxis occurs at peroxynitrite concentrations of 3 μmol/L, which is subthreshold as a vasodilator in coronary circulation, and not only prevents further response to its own vasodilator actions but also causes long-lasting impairment of the response to other vasodilators. In support of this notion, Benkusky et al have found that the development of tachyphylaxis to peroxynitrite attenuates the hemodynamic effect produced by systemic administration of acetylcholine and prostacyclin. Therefore, not only will quenching of NO by superoxide increase the vascular tone, but the increase in peroxynitrite could also potentiate this effect by causing tachyphylaxis to residual NO.

In addition to the observations of Rajagopalan et al in which Ang II increased superoxide levels in vascular tissue in vivo, Ang II has also been shown to increase oxidative stress in porcine vascular smooth muscle cells in vitro, as measured by increased F₂-isoprostanes. Natarajan et al found that free F₂-isoprostanes released into the media were increased by almost 200% in response to Ang II at doses as low as 10⁻⁹ mol/L for 24 to 36 hours. Cell-associated esterified F₂-isoprostane was also increased with Ang II. As mentioned previously, F₂-isoprostanes have been shown to be highly vasoconstrictive. Takahashi et al injected Ang II and its scavenger, superoxide dismutase. The reaction rate of NO and superoxide is more rapid than the reaction rates of superoxide and its scavenger, superoxide dismutase. A reduction in NO due to preferential superoxide scavenging could induce vasoconstriction and favor the development of hypertension. Although peroxynitrite itself is a vasodilator, Villa et al have demonstrated that tachyphylaxis occurs at peroxynitrite concentrations of 3 μmol/L, which is subthreshold as a vasodilator in coronary circulation, and not only prevents further response to its own vasodilator actions but also causes long-lasting impairment of the response to other vasodilators. In support of this notion, Benkusky et al have found that the development of tachyphylaxis to peroxynitrite attenuates the hemodynamic effect produced by systemic administration of acetylcholine and prostacyclin. Therefore, not only will quenching of NO by superoxide increase the vascular tone, but the increase in peroxynitrite could also potentiate this effect by causing tachyphylaxis to residual NO.

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mainly by increases in afferent resistance when micropuncture studies were performed.\textsuperscript{7} The F\textsubscript{2} isoprostane dose of 5 ng \textcdot kg\textsuperscript{-1} \textcdot min\textsuperscript{-1} for 30 minutes resulted in a plasma isoprostane level of 1 ng/mL.\textsuperscript{7} However, in the present study, F\textsubscript{2} isoprostane levels increased to only 86 pg/mL with Ang II infusion, and these lower levels may explain why there was no effect on GFR and RPF despite the increase in F\textsubscript{2} isoprostanes with Ang II infusion. It should be mentioned that Ang II and F\textsubscript{2} isoprostanes have also been shown to stimulate the production of endothelin.\textsuperscript{20–22} Hence, it is important to conduct further studies to determine the relative role that each of these constrictors plays in the pressor response to low doses of Ang II.

In summary, we have found that chronic infusion of physiological doses of Ang II stimulates oxidative stress, as measured by the increase in vasoconstrictor F\textsubscript{2} isoprostanes in serum and peroxynitrite in the kidney. Whether the mechanism(s) responsible for the slow pressor response to Ang II is mediated by any of the vasoconstrictor effects associated with oxidative stress remains to be determined and must be studied further.

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References

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