Role of Endothelin and Isoprostanes in Slow Pressor Responses to Angiotensin II

Maria Clara Ortiz, Elisabeth Sanabria, Melissa C. Manriquez, Juan C. Romero, Luis A. Juncos

Abstract—We tested the hypothesis that angiotensin II (Ang II)–induced stimulations of endothelin (ET) and isoprostanes are implicated in the slow pressor responses to Ang II. We infused either vehicle (group 1) or Ang II (groups 2 to 4) intravenously at 5 ng/kg per minute via osmotic pumps for 15 days into Sprague-Dawley rats. Groups 3 and 4 received 30 mg/kg per day of either losartan (Ang II type 1 receptor blocker) or bosentan (ET_{A} and ET_{B} receptor blocker) in their drinking water. We measured systolic blood pressure (SBP) every 3 days during the infusion. Plasma levels of Ang II, ET, isoprostanes, and urinary nitrites were determined at 15 days. Vehicle infusion did not change SBP (from 138±13 to 136±2 mm Hg at day 15). Circulating Ang II, ET, and isoprostane levels were 35±9, 39±3, and 111±10 pg/mL, respectively, whereas urinary nitrites were 2.3±0.4 μg/d. Ang II increased SBP (from 133±10 to 158±8 mm Hg), plasma Ang II (179±77 pg/mL), and isoprostanes (156±19 pg/mL) without altering ET levels (38±5 pg/mL) or urinary nitrites (1.8±0.5 μg/d). Losartan prevented Ang II–induced increases in SBP and isoprostanes (SBP went from 137±5 to 120±4 mm Hg; isoprostanes were 115±15 pg/mL) while increasing urinary nitrite levels (5.2±1.1 μg/d). Losartan did not alter Ang II (141±57 pg/mL) or ET (40±4 pg/mL) levels. Bosentan also blocked Ang II–induced hypertension (from 135±4 to 139±3 mm Hg) but did not decrease isoprostanes (146±14 pg/mL). Ang II (63±11 pg/mL), ET levels (46±2 pg/mL), and urinary nitrites (2.8±0.4 μg/d) were not altered. In conclusion, our results suggest that low-dose Ang II increases isoprostanes via its Ang II type 1 receptor and causes an ET-dependent hypertension, without altering circulating ET levels. (*Hypertension. 2001;37[part 2]:505-510.*)

Key Words: blood pressure ■ free radicals ■ hypertension, arterial ■ kidney ■ losartan

The renin-angiotensin system plays an important role in the regulation of blood pressure and may be implicated in the pathogenesis of essential hypertension.1–3 Drugs that block this system (ie, ACE inhibitors and angiotensin receptor blockers) are effective in reducing blood pressure.4–6 Interestingly, these agents can lower blood pressure even when plasma levels of angiotensin II (Ang II) are normal or just slightly elevated. This observation has raised questions regarding the mechanisms by which Ang II participates in the maintenance of hypertension. One observation that may help explain this is that if a small nonpressor dose of Ang II is infused chronically, blood pressure gradually increases. This response, known as the slow pressor response to Ang II, occurs without plasma concentrations of Ang II reaching pressor levels, suggesting that blood pressure is increasing via mechanisms other than the direct vasoconstrictor action of Ang II. Yet the nature of these mechanisms remains obscure. Indeed, Ang II is known to have many other actions, which may help to explain the slow pressor responses. For instance, recent studies have shown that Ang II can stimulate the formation of other factors, such as superoxide (thus increasing oxidative stress) and endothelin (ET).10–12 Both of these factors are capable of increasing blood pressure and have been implicated in several models of hypertension.13,14 Indeed, several studies have found that antioxidants and ET receptor blockers can attenuate the hypertensive effects of higher doses of Ang II and decrease blood pressure in some models of Ang II–dependent hypertension.13–15 Thus, these studies raise the possibility that oxidant stress and ET are involved in the slow pressor responses to Ang II, but how they contribute to these responses is poorly understood. For instance, superoxide (O_{2}^{•−}) can increase blood pressure by quenching NO16,17 and/or by increasing the levels of F_{2\alpha}-isoprostanes (which are vasoconstrictors18–20) via nonenzymatic oxidation of arachidonic acid. ET may increase blood pressure via direct vasoconstriction or by also increasing oxidative stress. Thus, we examined the hypothesis that the hypertensive response to a chronic infusion of a subpressor dose of Ang II is due to enhanced oxidative stress (with subsequent generation of isoprostanes) and ET. To test this hypothesis, we infused rats with 5 ng/kg per minute of Ang II for 15 days and confirmed that it increased systolic blood pressure (SBP) and plasma isoprostanes. This infusion did not alter plasma levels of ET or nitrite/nitrates or the urinary excretion of nitrites. We then established that the Ang II type 1 (AT_{1}) receptor mediated the increase in both SBP and...
isoprostanes. Finally, we provide evidence suggesting that ET plays a role in the hypertensive response to Ang II that is independent of lowering isoprostane levels.

Methods

Surgical Procedure

All experiments were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health approved by the Institutional Animal Care and Use Committee of the Mayo Clinic. Male Sprague-Dawley rats (weight ~300 g, Harlan, Indianapolis, Ind) were anesthetized with an intramuscular injection of ketamine (100 mg/kg body wt, Fort Dodge Laboratories) and xylazine (50 mg/kg body wt, Lloyd Laboratories). Under sterile conditions, incisions were made in the midscapular region and in the ventral neck. Osmotic minipumps were implanted in a pocket created in the midscapular region, and a catheter connected to the minipumps was cannulated under the skin to the ventral neck and implanted into the external jugular vein. The animals were randomized into 1 of the 4 experimental groups and infused intravenously with vehicle, 0.9% NaCl solution (group 1, n=6), or Ang II (groups 2 to 4, n=6 each) at doses of 5 ng/kg per minute for 15 days. In addition to the Ang II infusion, groups 3 and 4 received 30 mg/kg per day of either bosentan (a blocker of both ET<sub>A</sub> and ET<sub>B</sub> receptors) or losartan (an AT<sub>1</sub> receptor blocker) in their drinking water starting on the same day that the minipumps were implanted. These doses of bosentan and losartan have previously been shown to not alter blood pressure (previous study<sup>21</sup> and M.C. Ortiz, unpublished data, 1998), and we confirmed that they blocked ET and Ang II by performing acute studies in which we gave an intravenous bolus of ET-1 (1 nmol/kg) and Ang II (1 mg/kg) and assessed the changes in mean arterial pressure (MAP). ET-1 and Ang II increased MAP by 17±2.19% and 3.22%, respectively, in the bosentan- and losartan-treated rats, indicating effective blockade of the ET<sub>A</sub>/ET<sub>B</sub> and AT<sub>1</sub> receptors.

Animal Preparation

SBP, assessed by the tail-cuff plethysmography method, and urine collections were taken during the time of infusion at days 1, 3, 6, 10, 12, and 15. The rats were handled daily and exposed to the environment eventually used for the metabolic studies (metabolic cages) and for blood pressure measurements. This training was performed for 7 days before implantation of the minipumps (day 0). At 15 days, the rats were anesthetized with ketamine (100 mg/kg body wt, BYK-Gulden) and placed on a heated table to maintain body temperature at 37°C. A tracheotomy was performed to facilitate respiration (PE-240 tubing). Polyethylene cannulas (PE-50 tubing) were placed in the right femoral vein for infusions to facilitate respiration (PE-240 tubing). Polyethylene cannulas (PE-50 tubing) were placed in the right femoral vein for infusions and in the right femoral artery for blood collection and for measuring blood pressure (Gould 8188G-402 pressure transducer and registser 8188-4400 Gould Inc.). All animals received an intravenous infusion of 0.9% saline solution containing 1.5% albumin and 1% inulin (Sigma Chemical Co) at a rate of 1.5 mL/h per 100 g body wt (Harvard Pump 22, Harvard Apparatus). Then, the left ureter was cannulated for urine collection, and the abdomen was covered with Parafilm (American National Can) to minimize evaporation. A 60-minute stabilization period was then allowed.

Experimental Protocol

After the stabilization period, urine was collected for two 15-minute clearance periods, and blood samples were drawn slowly at the midpoint of each period for hemocrit and inulin measurements. MAP was recorded continuously throughout the experiment. All pressure transducers were calibrated before each recording, with the zero reference point being the midportion of the rat. After completion of the experiment, we obtained arterial blood samples for measurement of plasma renin activity (PRA) and plasma levels of Ang II, ET, nitrates/nitrites, and isoprostanes and stored them in a −80°C freezer. The animals were euthanized by thoracotomy, and the left kidney was removed, blotted dry, and weighed. The concentration of inulin in urine and plasma samples was measured by using colorimetric methods. The glomerular filtration rate (GFR) was calculated as the clearance of inulin (urine-to-plasma ratio times urine flow). All the variables from the 2 control periods were averaged and factored per gram of kidney tissue.

Analytic Determinations

Free and total isoprostane levels in plasma were measured by using extraction and enzyme immunoassay procedures, after a modification of the methods provided in the isoprostane measurement kit from Cayman Chemical, as we previously described.<sup>22</sup> Plasma levels of Ang II were determined using an enzyme immunoassay kit purchased from Société de Pharmacologie et d’Immunologie-BIO. PRA was estimated by radioimmunoassay of the Ang II generated (Dupon, NEN Research Products) and expressed as nanograms per milliliter per hour. Total plasma nitrates/nitrites were measured by using nitrate/nitrite assay kit (Cayman Chemical). Urinary excretion of nitrites was determined by using the Griess reaction. Plasma ET was measured by using an ET radioimmunoassay kit supplied by Peninsula Laboratories. For this measurement, plasma samples (0.6 mL) were collected in EDTA, centrifuged, and then added to disposable C-18 extraction columns (PreSep R-C-18, Fisher Scientific) that had been preconditioned once with methanol and subsequently twice with 1% trifluoroacetic acid. The samples were eluted from the columns with a solution containing 1% trifluoroacetic acid (1:3) and acetonitrile (2:3), and the eluents were lyophilized and reconstituted in 0.6 mL of assay buffer. To assay, standards and samples were added to tubes containing rabbit ET antiserum and incubated for 6 hours. The ET tracer (<sup>125</sup>I-labeled peptide) was then added, and the tubes were incubated overnight at 4°C. The next day, goat anti-rabbit IgG serum was added to each tube and incubated for 5 minutes. Normal rabbit serum was then added, and the tubes were incubated for 2 hours. Finally, a precipitating solution of assay buffer/6% polyethylene glycol (PEG 8000) was added, the tubes were centrifuged, and the precipitate was counted in a γ-counter for 1 minute. The concentration of ET was calculated as picograms per milliliter of plasma.

Statistical Analysis

The results are expressed in mean±SEM, and the level of significance was considered to be P<0.05. The differences in values between groups of animals and different treatments were tested by 1-way ANOVA of repeated measurements. To examine for differences in individual groups, a Student 2-sample t test with the Bonferroni multiple comparison adjustment was used.

Results

SBP and Renal Function in Rats During Chronic Ang II Infusion

Figure 1 shows SBP in control and Ang II–treated rats. Ang II increased SBP (from 133±11 mm Hg on day −1 to 158±8 mm Hg on day 15), with a maximal SBP at day 10 (173±6 mm Hg). SBP did not increase in the control rats (138±13 mm Hg on day −1 versus 136±2 mm Hg on day 15). The administration of the ET receptor blocker, bosentan, did not alter basal blood pressure (130±14 versus 130±7 mm Hg on days −1 and 3, respectively) but inhibited Ang II–induced increases in SBP (140±4 mm Hg on day 15). On the other hand, losartan decreased basal SBP (133±7 versus 105±11 mm Hg on days −1 and 3, respectively) and blocked Ang II–induced increases in SBP (121±6 mm Hg on day 15). GFR in the control group was not different from that in the group treated with Ang II (1.7±0.2 versus 1.9±0.3 mL/min per gram, respectively). Neither bosentan nor losartan significantly altered GFR (1.2±0.4 and 1.1±0.4 mL/min per gram). Diuresis and natriuresis were also not significantly
different in any of the 4 groups at any of the time points. On day 21, diuresis was 13 ± 6, 14 ± 6, 14 ± 4, and 13 ± 3 mL/d, and natriuresis was 1.6 ± 0.2, 1.9 ± 0.1, 2.4 ± 0.4, and 2.6 ± 0.2 mEq/d in the vehicle, Ang II, bosentan, and losartan groups, respectively. On day 15, diuresis was 18 ± 2, 17 ± 1, 16 ± 1, and 16 ± 2 mL/d, and natriuresis was 2.4 ± 0.1, 2.3 ± 0.4, 3.0 ± 0.2, and 2.9 ± 0.1 mEq/d in the 4 groups, respectively.

Changes in PRA and Plasma Levels of Ang II
Chronic infusion of Ang II did not change PRA (23 ± 8 versus 23 ± 4 ng/mL per hour for Ang II versus controls, respectively). Bosentan did not alter PRA (26 ± 6 ng/mL per hour), whereas chronic blockade of AT1 receptors with losartan increased PRA to 40 ± 4 ng/mL per hour, as expected. Plasma concentration of Ang II in the controls was 35 ± 9 pg/mL. The plasma levels of Ang II were significantly increased in all 3 groups that received Ang II (Ang II 179 ± 77 pg/mL, bosentan 63 ± 11 pg/mL, and losartan 141 ± 57 pg/mL).

Plasma Levels of ET and Isoprostanes
As shown in Figure 2A, plasma levels of ET were 39 ± 3 pg/mL in the control rats and were not significantly altered by the Ang II infusion (38 ± 5 pg/mL). Moreover, neither losartan (40 ± 4 pg/mL) nor bosentan (46 ± 2 pg/mL) altered the circulating levels of ET. The plasma levels of free F2-isoprostanes in all 4 groups are depicted in Figure 2B. Ang II increased the free F2-isoprostane levels in plasma (111 ± 10 pg/mL in control rats versus 157 ± 20 pg/mL in Ang II-treated rats). Despite the efficacy of bosentan in lowering blood pressure, it did not decrease the plasma levels of F2-isoprostanes (147 ± 15 pg/mL). In contrast, treatment with losartan prevented the increase in isoprostanes (115 ± 15 pg/mL). Total isoprostanes were not different in any of the experimental groups.

Plasma Levels of Nitrates/Nitrites and 24-Hour Urinary Nitrates
Figures 3A and 3B depict the plasma levels of nitrates/nitrites and the urinary excretion rates of nitrates, respectively. Ang II did not alter the plasma concentration of nitrates/nitrites (19 ± 5 versus 14 ± 3 nmol/mL for control versus Ang II-treated rats, respectively) or the 24-hour urinary nitrite excretion (2.3 ± 0.4 versus 1.8 ± 0.5 μg/d for control versus Ang II-treated rats, respectively). Likewise, bosentan had no effect on the plasma nitrate/nitrite level, although there was a trend for it to be lower (11 ± 3 nmol/mL), nor did it alter urinary nitrite excretion (2.8 ± 0.4 μg/d). On the other hand, losartan significantly decreased the plasma nitrate/nitrite level (8 ± 2 nmol/mL) but markedly enhanced urinary nitrite excretion (5.2 ± 1.1 μg/d).

Discussion
We evaluated whether ET and oxidative stress are implicated in the hypertension induced by subpressor doses of Ang II. We found that increased plasma isoprostanes but not ET accompanied this hypertensive response. Blocking AT1 receptors with losartan prevented the rise in blood pressure and in isoprostane levels and increased urinary nitrite excretion (despite decreased plasma nitrates). Blocking the ET receptors (ETα and ETβ) with bosentan also prevented hypertension but did not decrease the plasma isoprostane levels and had no effect on plasma or urinary nitrates. Neither losartan nor bosentan altered plasma concentrations of ET.

In the present study, we investigated potential mechanisms by which subpressor doses of Ang II cause hypertension. For this investigation, we needed to find a dose of Ang II that
isoprostane levels. In fact, isoprostane levels are elevated not only during infusion of Ang II but also in conditions characterized by exaggerated renal vasoconstriction and increased Ang II levels (ie, cirrhosis). And second, isoprostanes possess vasoconstrictor activity (via specific receptors that can be blocked with thromboxane receptor antagonists[^9][^20]). However, despite these facts, we found that bosentan was very effective in reducing the blood pressure but that it did so without decreasing the isoprostane levels, suggesting that the isoprostanes by themselves are not responsible for the slow pressor responses to Ang II. Rather, they might be a cofactor or an intermediate step in the formation of another factor that ultimately causes the hypertension.

One factor that appears to be important with respect to either of these possibilities is ET. Indeed, previous studies have found that Ang II can stimulate the formation of ET and that the hypertensive effects of Ang II can be blocked or attenuated with ET receptor blockers[^13][^14]. In the present study, we also found that ET receptor blockade was very effective in preventing Ang II–induced hypertension; however, plasma ET levels were not elevated during the Ang II infusion. The reason for this discrepancy is not clear, but there are several possible explanations. First, the tissue levels of ET may have increased despite the fact that the circulating levels did not. Indeed, 80% of ET is located on the abluminal side of the endothelial cell, which may suggest a predominant release to the interstitial side of the vessel[^23] thus acting as a paracrine system. Second, it is conceivable that ET is elevated only in certain key organs in the regulation of MAP, such as the kidney. Thus, it could potentially cause hypertension through its local effects (eg, causes hypertension but does not elicit fast pressor effects of Ang II. Previous studies[^6][^8][^23] had found that infusing 5 (as calculated from the reported rat weights) to 20 ng/kg per minute of Ang II into rats caused blood pressure to increase within 1 to 7 days. Thus, we ran some acute studies infusing these doses of Ang II and found that the highest dose that did not alter MAP or RBF after 45 to 60 minutes of a continuous infusion was 5 ng/kg per minute (preliminary studies, data not shown). Thus, we used this dose to minimize any confounding fast pressor effects. As in the previous study[^6], this dose of Ang II significantly elevated SBP by the sixth day. This was not accompanied by any changes in GFR, but there was an apparent shifting of the pressure natriuresis curve to the right (there were no changes in natriuresis compared with controls despite the higher blood pressure).

We next sought to determine the mechanisms by which subpressor doses of Ang II may also be causing the hypertension. One potential mechanism that has received increasing attention is oxidant stress[^15][^21][^23]. Indeed, Ang II is known to directly stimulate the vascular production of O$_2^-$ via a membrane NADH/NADPH oxidase activation[^9]. This stimulation is seen in conditions in which circulating or local levels of Ang II are elevated (regardless of whether hypertension is present). The O$_2^-$ formed reacts with NO in a radical/radical reaction and results in peroxynitrite (OONO$^-$), one of the most potent oxidants known[^16][^17]. The peroxidation of arachidonic acid by peroxynitrite leads to the formation of F$_2$$_{23a}$-isoprostanes, which are prostaglandin-like compounds[^18][^20]. Thus, O$_2^-$ can increase blood pressure via at least 2 mechanisms: by quenching NO and by forming isoprostanes.

Isoprostanes are reportedly good markers of oxidative stress and should be increased during slow pressor responses to Ang II if there is increased oxidant stress. Ang II has been shown to increase isoprostane levels both in vitro (albeit at nanomolar concentrations[^24]) and in vivo[^22][^23]. We have previously found that an infusion of 10 ng/kg per minute of Ang II into the pig for 28 days increases free plasma isoprostanes[^22] and Reckelhoff et al[^23] have found that rats receiving 10 ng/kg per minute for 14 days also have increased free plasma isoprostane levels. However, these studies could not discern whether the elevated isoprostane levels were due to a direct effect of Ang II or to a secondary effect that was due to hypertension. In the present study, we extend our previous findings by showing that Ang II at 5 ng/kg per minute also increases isoprostane levels and that this increase is mediated by the AT$_1$ receptor, inasmuch as it could be blocked by losartan. Furthermore, the Ang II–induced increase in isoprostane levels is not likely secondary to hypertension because bosentan inhibited the hypertension, but the isoprostane levels remained elevated.

The finding that bosentan inhibited the slow pressor responses to Ang II but did not lower plasma isoprostane levels refuted one of our hypotheses. We had postulated that isoprostanes might be the major mediators of the slow pressor responses to Ang II. We based this on the following findings: First, as mentioned above, Ang II can directly increase isoprostane levels. In fact, isoprostane levels are elevated not only during infusion of Ang II but also in conditions characterized by exaggerated renal vasoconstriction and increased Ang II levels (ie, cirrhosis). And second, isoprostanes possess vasoconstrictor activity (via specific receptors that can be blocked with thromboxane receptor antagonists[^19][^20]).

Figure 3. Plasma levels of nitrates/nitrites (A) and urinary excretion of nitrates (B) in the 4 experimental groups at the end of the 15-day treatment period. *P<0.05 vs vehicle; †P<0.05 vs Ang II.

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One factor that appears to be important with respect to either of these possibilities is ET. Indeed, previous studies have found that Ang II can stimulate the formation of ET and that the hypertensive effects of Ang II can be blocked or attenuated with ET receptor blockers[^13][^14]. In the present study, we also found that ET receptor blockade was very effective in preventing Ang II–induced hypertension; however, plasma ET levels were not elevated during the Ang II infusion. The reason for this discrepancy is not clear, but there are several possible explanations. First, the tissue levels of ET may have increased despite the fact that the circulating levels did not. Indeed, 80% of ET is located on the abluminal side of the endothelial cell, which may suggest a predominant release to the interstitial side of the vessel[^23] thus acting as a paracrine system. Second, it is conceivable that ET is elevated only in certain key organs in the regulation of MAP, such as the kidney. Thus, it could potentially cause hypertension through its local effects (eg,
shifting pressure natriuresis). Third, it is possible that a threshold level for both ET and isoprostanes needs to be reached to elicit the slow pressor responses. Thus, in the control animals, ET levels are at the necessary level, but the isoprostanes are low; consequently, there is no increase in pressure. Whereas in the bosentan-treated animals, the isoprostanes levels are high, but ET activity has been blocked; thus, once again, there is no hypertensive response. A final potential explanation relates to the possibility that Ang II–induced superoxide production may lead to quenching of the available NO. Because NO is thought to be the predominant modulator of several vasoconstrictors (including Ang II and ET), decreasing NO availability leaves the vasoconstrictor factors unopposed, thus causing exaggerated vasoconstriction without increased levels of the vasoconstrictors. These possibilities require further investigation.

As mentioned before, Ang II–induced O₂⁻ production may also contribute to the Ang II–induced slow pressor responses by quenching NO. We attempted to obtain indirect measures of NO activity in each group by measuring primarily the 24-hour excretion of urinary nitrites (inasmuch as this is thought to be more representative of endogenous NO activity) and also by measuring plasma nitrates/nitrites. Despite Ang II–induced increases in oxidative stress (as measured by isoprostane levels), neither urinary nitrites nor plasma nitrates/nitrites were decreased. This suggests either that NO was not being quenched or that there is enhanced NO production that is compensating for the increased quenching of NO (thus, urinary and plasma nitrates and/or nitrites will change very little). Interestingly, when we blocked the AT₁ receptor with losartan, urinary nitrite excretion increased dramatically, suggesting that oxidant stress (as measured by isoprostanes) fell but that the enhanced NO levels remained. Whether the increase in NO is due to a direct effect of losartan or to activation of Ang II type 2 receptors remains to be determined. On the other hand, plasma nitrates/nitrites were decreased by losartan. The reason for this discrepancy is not clear but may be due to the balance between oxidant stress and NO in different compartments (i.e., vascular versus systemic; or renal NO production may predominate in the urine nitrates) or due to design issues (the plasma was obtained at the end of the experiments during anesthesia).

Unlike losartan, bosentan did not alter either plasma nitrates/nitrites or urinary nitrates. Thus, these results, taken together with the observation that bosentan did not alter isoprostane levels, suggest that ET blockade did not decrease oxidative stress or shift the balance between NO and oxidative stress in favor of NO. The reason that it was effective at preventing Ang II–induced hypertension was likely because it directly inhibits either the vasoconstriction or perhaps the renal tubular effects of ET. In this regard, it seems that the ET may become a more result rather than an instigator of the oxidant insult in this model. Indeed, isoprostanes have been reported to stimulate the synthesis of ET. ²⁶ Because of these findings, it is tempting to speculate that Ang II increases oxidant stress with the subsequent formation of isoprostanes. These, in turn, stimulate the local production of ET, which, in turn, induces an increase in blood pressure.

In summary, a chronic infusion of 5 ng/kg per minute of Ang II increases SBP (by 6 days) and raises the plasma concentrations of free isoprostanes without altering plasma or urinary nitrates and/or nitrates. Blocking the AT₁ receptor with losartan abolishes Ang II–induced increases in SBP, decreases isoprostanes, and increases urinary nitrates. Blocking the ET receptors with bosentan also prevents Ang II–induced increases in SBP, but without decreasing isoprostane levels or increasing urinary nitrates. These data suggest that Ang II (at a dose that elicits only the slow pressor response) binds to its AT₁ receptor, which leads to an increase in free isoprostane levels. The Ang II and/or isoprostanes may then enhance either the local formation or the activity of ET, which, in turn, plays a part in the slow pressor response to Ang II.

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