Angiotensin II and Catecholamines Increase Plasma Levels of 8-Epi-Prostaglandin F_{2\alpha} With Different Pressor Dependencies in Rats

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Abstract—We investigated the extent of oxidative stress evoked in the hypertensive rat by measuring plasma levels of 8-epi-prostaglandin F_{2\alpha} (8-epi-PGF_{2\alpha}), a marker of in vivo oxidative stress. Administration of angiotensin (Ang) II and norepinephrine at doses of 0.7 and 2.8 mg · kg^{-1} · d^{-1}, respectively, resulted in similar significant elevations in plasma levels of 8-epi-PGF_{2\alpha}. A 7-day infusion of Ang II at a nonpressor dose, but not norepinephrine at a nonpressor dose, also increased plasma levels of 8-epi-PGF_{2\alpha}. The norepinephrine-induced increase in 8-epi-PGF_{2\alpha} levels could be completely normalized by 3 different classes of antihypertensive drugs: prazosin, an \( \alpha \)-adrenergic receptor blocker; hydralazine, a nonspecific vasodilator; and losartan, a specific angiotensin type I (AT_{1}) receptor antagonist. This finding suggests that the norepinephrine-induced increase is a pressor-dependent event. In contrast, among these antihypertensive drugs, only losartan was effective in inhibiting the Ang II–induced increase in plasma 8-epi-PGF_{2\alpha}, suggesting that Ang II increases plasma levels of 8-epi-PGF_{2\alpha} in both a pressor-independent and an AT_{1} receptor–dependent manner. In summary, continuous infusion of both Ang II and norepinephrine potently increases plasma levels of 8-epi-PGF_{2\alpha}, and thus in vivo oxidative stress. Ang II and norepinephrine seem to induce this increase in 8-epi-PGF_{2\alpha} via mechanisms with different pressor dependencies. (Hypertension. 2002;39:149-154.)

Key Words: angiotensin II ■ AT_{1} receptor ■ oxidative stress ■ isoprostanes ■ catecholamine

The production of reactive oxygen species (ROS) is thought to increase in animal models of hypertension and in hypertensive human subjects. Because oxidative stresses may have a role in the pathogenesis of cardiovascular complications in hypertension, assessment of in vivo oxidative stress may provide useful information for determining the optimal therapeutic strategies to minimize oxidant-induced tissue injury in hypertensive patients. Free F_{2}-isoprostanes are synthesized from arachidonic acid in vivo, mostly independently of the cyclooxygenase (COX) pathway and instead through free radical–catalyzed peroxidation. Recent studies have demonstrated that 8-epi-prostaglandin F_{2\alpha} (8-epi-PGF_{2\alpha}) is one of the most abundant F_{2}-isoprostanes and, among all the F_{2}-isoprostanes, a reliable index of in vivo oxidative stress in F_{2}-isoprostanes.

Angiotensin (Ang) II is thought to increase the production of ROS by activating vascular NAD(P)H-oxidase, whereas the vasopressing agent catecholamine does not have this property. In addition, it has been shown that blockade of the AT_{1} receptor acts protectively against oxidant-induced tissue injury in the nonhypertensive animal models. These data suggest that the renin-angiotensin system plays a crucial role in the production of oxygen radicals and/or in oxidative tissue injury. In support of this concept are recent findings that long-term administration of Ang II results in an increase in plasma levels of 8-epi-PGF_{2\alpha}, and thus in oxidative stress in some animal models. These observations are consistent with the idea that Ang II possesses prooxidative properties. We have questioned whether Ang II and catecholamines evoke different degrees of in vivo oxidative stress when administered to animals. In the present study, we assessed the degree of in vivo oxidative stress in hypertensive rats subjected to long-term administration of either Ang II or catecholamines by measuring plasma levels of 8-epi-PGF_{2\alpha}. These 2 hypertensive drugs potently induced in vivo oxidative stress to a similar extent but showed different pressor dependencies.

Methods

Animal Models

For the rat hypertension model, an osmotic minipump (Alzet model 2001, Alza) was implanted into each male Sprague-Dawley rat (weight, 300 to 350 g) as described previously. Briefly, Val^\text{5}-Ang II (Sigma) and norepinephrine (NE; Sigma) were continuously infused via the osmotic minipump at doses of up to 0.7 and 2.8 mg · kg^{-1} · d^{-1}, respectively, for up to 7 days. Systolic blood pressure...
and heart rate were measured in conscious rats by tail-cuff plethysmography (Ueda Seisakusyo). In some experiments, prazosin (5 mg · kg⁻¹ · d⁻¹), hydralazine (15 mg · kg⁻¹ · d⁻¹), or losartan (a kind gift from Dupont/Merck; 25 mg · kg⁻¹ · d⁻¹) was given in the drinking water beginning 2 days before pump implantation throughout Ang II infusion. To confirm that plasma 8-epi-PGF₂α is synthesized via the COX-independent mechanism, some rats were given daily intraperitoneal injections of the COX inhibitor ibuprofen at a dose of 20 mg · kg⁻¹ · d⁻¹ for 3 consecutive days. For rats undergoing either Ang II or NE infusion, ibuprofen was injected from day 4 to day 6 of infusion of these drugs.

Instrumentation and Conditions for Electrospray Ionization Mass Spectrometry
Free 8-epi-PGF₂α was measured by liquid chromatography–electrospray ionization–mass spectrometry as described elsewhere. Briefly, a high-performance liquid chromatography system (model HP11000, Hewlett Packard) with a symmetry C₈ column (3.9×150 mm, 5 μm; Waters) was used. We used 0.1% CH₃COOH and acetonitrile as the mobile phases, and isocratic elution was performed with a CH₃COOH/acetonitrile ratio of 7/3 and a flow rate of 0.35 mL/min. A 4-sector–MSStation 700 tandem mass spectrometer (JEOL) equipped with an electrospray ionization source was used in the negative ion-selected, ion-monitoring mode. The quasimolecular ions (deprotonated ions) m/z 353.24 and m/z 357.26 for 8-epi-PGF₂α, and the internal standard, respectively, were monitored for 500 milliseconds each in the selected ion-monitoring mode at a mass spectral resolution of 1500. The lower limits of quantification of plasma and urinary 8-epi-PGF₂α were 20 and 100 pg/mL, respectively.

Statistical Analysis
Data are expressed as mean±SEM. ANOVA, followed by a multiple comparison test, was used for comparing the data and incorporated the statistical analysis software Statistica (version 5.1J, StatSoft Inc). A value of P<0.05 was considered statistically significant.

Results

Effect of COX Inhibitor on the Biosynthesis of Plasma and Urine 8-Epi-PGF₂α
Initially, we examined the COX dependency of 8-epi-PGF₂α levels in the plasma and urine. Untreated rats or rats treated with hypertensive drugs were given the COX inhibitor ibuprofen intraperitoneally. Although ibuprofen treatment did not significantly change the plasma levels of 8-epi-PGF₂α in control rats or rats receiving Ang II or NE (Figure 1A), it significantly decreased urine levels of 8-epi-PGF₂α in control rats and rats receiving Ang II (Figure 1B). Thus, the urine 8-epi-PGF₂α level does not seem to be a marker for in vivo oxidative stress in rats, as has been suggested previously.

Time Course of Plasma 8-Epi-PGF₂α Regulation After Ang II and NE Infusion
Similar trends in both blood pressure and heart rate were observed after administration of Ang II and NE (Figure 2A through 2D). Infusion of NE for 3 days slightly but significantly increased plasma levels of 8-epi-PGF₂α (118±3% of control, P<0.05), whereas infusion of Ang II for 3 days did not increase plasma levels of 8-epi-PGF₂α despite its hypertensive effect, which was comparable to that of NE (Figure 2B). In contrast, at day 7 of infusion, Ang II and NE increased plasma levels of 8-epi-PGF₂α to a similar extent (Ang II, 144±2% of control level; NE, 147±7% of control level) (Figure 2E and 2F).

Dose-Response Relationship Between Ang II and NE Infusion and Plasma 8-Epi-PGF₂α Levels
To examine the dose-response relationship between Ang II or NE and plasma levels of 8-epi-PGF₂α, 4 different doses of Ang II (0.12, 0.25, 0.5, and 0.7 mg · kg⁻¹ · d⁻¹) or NE (0.5, 1.0, 1.4, and 2.8 mg · kg⁻¹ · d⁻¹) were infused into rats for 7 days (Figure 2A through 2D). Doses of 0.12 and 0.25 mg · kg⁻¹ · d⁻¹ Ang II or 0.5 mg · kg⁻¹ · d⁻¹ NE did not increase blood pressure; therefore, these doses were considered to be nonpressor doses (Figure 3A and 3C). Infusion of Ang II at any of 4 doses, even the nonpressor doses, significantly increased the plasma levels 8-epi-PGF₂α (Figure 3E). In contrast, only pressor doses of NE increased plasma levels of 8-epi-PGF₂α (Figure 3F).

Effects of Antihypertensive Drugs on the Vasopressor-Induced Increase in Plasma 8-Epi-PGF₂α
Next, we examined the effects of antihypertensive drugs on the increases in plasma 8-epi-PGF₂α levels induced by administration of Ang II or NE for 7 days. Prazosin, a peripheral

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

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

![Figure 3](http://hyper.ahajournals.org/)
to increases plasma 8-epi-PGF$_2$ via a pressor-dependent mechanism. In contrast, because plasma levels of 8-epi-PGF$_{2\alpha}$ were increased by both nonpressor and pressor doses of Ang II and because prazosin, which completely blocked the hypertensive effects of Ang II, failed to inhibit the Ang II–induced increase in plasma 8-epi-PGF$_{2\alpha}$, Ang II seems to induce this increase via a pressor-independent mechanism.

It has been reported recently that long-term infusion of Ang II into rats increases vascular superoxide production via the activation of NAD(P)H oxidase. The finding that indexes of oxidative stress are not increased in the vascular tissue or in the kidney of an Ang II–independent model of hypertension suggests that in hypertension, the renin-angiotensin system has a critical role in the development of oxidative stress. Furthermore, the scavenging superoxide anion blocks hypertensive effects induced by Ang II but not by catecholamines, indicating that increased production of ROS is central to the development of hypertension in Ang II–infused animals. Thus, the renin-angiotensin system and/or an increased amount of circulating Ang II seems to be requisite for the development of oxidative stress in the hypertensive animal models.

More recently, and in contrast, Somers et al. have reported that vascular superoxide production is increased by chronic hypertension in the deoxycorticosterone acetate-salt–sensitive hypertensive rat in the absence of elevated Ang II levels. This seems to suggest, therefore, that both hypertension per
and activation of the Ang II–AT₁ receptor axis can increase in vivo ROS production, although the relative contributions of hemodynamic stress and the octapeptide to the production of ROS in vivo remain unclear. In addition, caution should be taken in interpreting the results of some of these studies, because the quantities of ROS produced were sometimes measured in ex vivo conditions and/or in the presence of excessive amounts of electron donors such as NADH and NADPH. Because the addition of excessive amounts of electron donor is not necessary, measurement of plasma levels of 8-epi-PGF₂α will provide a more physiological assessment of in vivo oxidative stress.

We found that continuous infusion of either 0.7 mg · kg⁻¹ · d⁻¹ Ang II or 2.8 mg · kg⁻¹ · d⁻¹ NE for 7 days resulted in similar elevation of plasma levels of 8-epi-PGF₂α, as well as in similar changes in hemodynamic variables. These similarities are rather unexpected, but they do not discount an important role of Ang II in conditions in which it is elevated; e.g., Ang II can increase the production of ROS in cultured cells, which may be physiologically relevant to the regulation of growth. It should be noted, however, that administration of catecholamines generated in vivo oxidative stress to an extent comparable to that induced by Ang II administration.

We also investigated whether different mechanisms are used by Ang II and NE during the induction of plasma 8-epi-PGF₂α. Administration of Ang II (0.7 mg · kg⁻¹ · d⁻¹) and NE (2.8 mg · kg⁻¹ · d⁻¹) for 3 days resulted in similar changes in hemodynamic variables; however, plasma levels of 8-epi-PGF₂α were elevated only in rats treated with NE. In addition, plasma 8-epi-PGF₂α levels were increased by 7-day infusions of nonpressor doses of Ang II but not by 7-day infusions of the nonpressor dose of NE. Thus, Ang II and NE may increase plasma 8-epi-PGF₂α by mechanisms that differ in their pressor dependency. This concept of different pressor dependencies is further supported by the finding that hydralazine completely blocked the NE-induced increase in plasma 8-epi-PGF₂α, whereas it only partially blocked the Ang II–induced increase. If NE infusion does not increase superoxide production from aortic tissue, which tissue is responsible for the increased ROS production in response to NE infusion and thus to elevation of blood pressure? Nowicki et al have recently shown that arterioles have increased expression of NADPH oxidase components compared with
Figure 6. Effect of losartan on changes in hemodynamic variables and on biosynthesis of plasma 8-epi-PGF$_{2\alpha}$. Ang II (0.7 mg · kg$^{-1}$ · d$^{-1}$) and NE (92.8 mg · kg$^{-1}$ · d$^{-1}$) were continuously infused into rats for 7 days. Losartan was given to rats in drinking water at a dose of 25 mg · kg$^{-1}$ · d$^{-1}$. A and B, Effect of losartan on mean blood pressure (BP). C and D, Effect of losartan on heart rate. E and F, Effect of losartan on plasma levels of 8-epi-PGF$_{2\alpha}$. †P<0.05, ‡P<0.01 vs sham-operated control rats. Numbers in parentheses indicate the number of animals studied.

Effect of losartan on mean blood pressure (BP). C and D, Ang II (0.7 mg · kg$^{-1}$ · d$^{-1}$) and NE (2.8 mg · kg$^{-1}$ · d$^{-1}$) for 7 days resulted in an increase in plasma levels of 8-epi-PGF$_{2\alpha}$, an in vivo marker of oxidative stress. Ang II increased plasma 8-epi-PGF$_{2\alpha}$ by a mechanism that was both pressor independent and AT$_1$ receptor dependent, whereas NE increased plasma 8-epi-PGF$_{2\alpha}$ by a mechanism that was pressor dependent. The results of the present study will provide useful information for determining the optimal therapeutic strategy to minimize oxidant-induced tissue injury in the treatment of hypertension.

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