Endothelin, Angiotensin, and Oxidative Stress in Hypertension

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Endothelin (ET)-1 is a potent vasoconstrictor and mitogen, and because of these properties, it is thought to play a role in the development of hypertension. The vascular endothelium is a major source of ET-1 production, although a variety of other cell types also have been shown to synthesize and release ET-1. ET-1 is believed to act in a paracrine manner on ETA and ETB receptors on smooth muscle, which mediate contraction, cell proliferation, and hypertrophy. Activation of ETB receptors on endothelial cells stimulates the production of prostacyclin and nitric oxide to induce vasorelaxation and inhibition of sodium transport in renal tubules. Given these properties, considerable attention has been paid to the mechanisms of ET-1 action as it relates to the renal control of blood pressure and the pathogenesis of salt-dependent hypertension. Renal ET synthesis is increased in experimental animals maintained on a high-salt diet and ETB receptor antagonists lower arterial pressure primarily in salt-dependent models of hypertension.

For the past 30 to 40 years, the actions of angiotensin (Ang) II have been arguably the most widely investigated factor in hypertension research. Although physiology textbooks agree on the major actions of Ang II, eg, vasoconstriction and release of aldosterone, recent attention has focused on its ability to stimulate the synthesis of ET-1, as well as reactive oxygen species. There are many reactive oxygen species such as superoxide, hydroxyl radical, and hydrogen peroxide that are produced by all cell types and can have profound effects on the vascular system to impact blood pressure regulation. Most recent attention has been paid to the role of superoxide. There are many enzymatic sources of superoxide including NADPH oxidase, xanthine oxidase, nitric oxide synthase, and cytochrome P450. The focus of the current review, however, is be on the interaction between the 2 peptide systems, ET-1 and Ang II, as they relate to oxidative stress.

ET-1 in Ang II Hypertension

Several lines of evidence support the hypothesis that Ang II stimulates the production and release of ET. First, >10 years ago, several laboratories reported that Ang II increases ET release and the expression of preproendothelin-1 mRNA by cultured endothelial cells. Second, in vivo studies have shown that rats with chronic Ang II hypertension have elevated preproendothelin mRNA and ET-1 peptide expression levels in renal tissue. Furthermore, the hypertension and changes in endothelial function associated with chronic Ang II infusion can be attenuated by selective ETB receptor antagonists and nonselective antagonists. Whereas these studies suggest a relation between Ang II and ET in the long-term regulation of arterial pressure, Riggleman et al have also shown that ETB receptor blockade prevents the renal vasoconstrictor actions of low doses of acutely administered Ang II and the natriuretic and diuretic actions of higher doses of Ang II in the rat. More recently, Montanari et al had reported that an ETB selective antagonist inhibits the acute renal hemodynamic response to Ang II in humans. This same group also provided evidence for ETB-dependent increases in renal vascular resistance in human subjects during AT1 receptor blockade. Together, these data indicate that regulation of the renal ET system by Ang II may be an important factor in mediating the renal and hypertensive effects of Ang II via stimulation of the ETB receptor. A similar relationship between the renin-angiotensin system and ET in postmenopausal hypertension has recently been reviewed elsewhere.

ET-1 and Oxidative Stress

The clinical significance of oxidative stress and its role in hypertension was recently reviewed by Touyz, and so the current discussion is limited to the interaction between ET-1, Ang II, and superoxide.

Studies from Ortiz et al have shown that the slow pressor response to Ang II is associated with increases in ET, as well as isoprostanes, a marker of lipid oxidation and an index of oxidative stress. These effects could be prevented with bosentan, suggesting a role for ET in mediating the increase in oxidative stress in this model. They went on to demonstrate that antioxidant treatment with the superoxide dismutase mimetic, tempol, or the combination of vitamins C and E reduced Ang II-induced changes in ET expression. Acute administration of tempol also has antihypertensive effects in rats chronically infused with Ang II. Long-term treatment with tempol will lower arterial pressure in several models of hypertension associated with increases in ET production, including chronic Ang II, DOCA-salt, and Dahl salt-sensitive rats.

Many in vitro studies have established that the source of superoxide production stimulated by Ang II is nicotinamide
adrenaline dinucleotide phosphate (NADPH) oxidase. This mechanism was established in vivo by observations that mice lacking the p47phox subunit of NADPH oxidase have a significantly attenuated hypertensive response to chronic Ang II infusion. In addition, mice lacking the gp91phox subunit have an attenuated hemodynamic response to acute Ang II administration. However, there appears to be a difference in the involvement of reactive oxygen species in blood pressure response to acute versus chronic Ang II. In normal rats, tempol appears to have no effect on the pressor or renal hemodynamic response to acute Ang II infusion although it is able to reduce increases in mitogen activated protein (MAP) kinase activation and decreases in GFR.

The differences in the effects of antioxidant treatment on Ang II induced increases in blood pressure after acute versus chronic Ang II administration raises the question as to whether chronic increases in circulating mineralocorticoids rather than a direct effect of Ang II per se may represent a more relevant stimulus for ET-induced increases in reactive oxygen species and increases in arterial pressure. Chronic treatment of DOCA-salt hypertensive rats with either tempol or the NADPH oxidase inhibitor, apocynin, as well as ET receptor antagonists, reduce arterial pressure in this model. Similarly, ETα receptor blockade during chronic aldosterone-induced hypertension in the rat attenuates hypertension, oxidative stress, and NADPH oxidase activity.

Although Ang II has a direct action to increase ET-1 release from endothelial cells, the mechanisms responsible for increased ET-1 in mineralocorticoid-treated rats clearly must be different given the low renin activity in these models. Vasopressin appears to contribute to increased ET production in response to mineralocorticoid treatment, at least within the vascular system. Blockade of vasopressin V1 receptors inhibits both hypertension and increased ET production in DOCA-salt treated rats. In addition, rats deficient in vasopressin are relatively protected from DOCA-salt induced hypertension. Li et al have shown that increased superoxide production in the carotid arteries of DOCA-salt rats can be inhibited by ETα receptor blockade and that vasopressin stimulates vascular superoxide through an ET-dependent pathway. ET also increases superoxide in this model through activation of NADPH oxidase.

Although ETα receptor antagonists can reduce oxidative stress in various models of hypertension, the question remains whether this is caused by inhibition of ET induced increases in oxidative stress or an indirect result of reducing arterial pressure. In vitro evidence suggests that ET-1 induced vasoconstriction may be due to an increase in superoxide anion formation in pulmonary artery smooth muscle cells, whereas ET-1 significantly increases superoxide production through an ETβ receptor pathway. Furthermore, ET-1 can stimulate superoxide anion formation in rat aortic rings. Sedeek et al reported that the superoxide dismutase mimetic, tempol, inhibited the development of hypertension produced by infusion of ET-1 for 9 days. It is important to note that Wang and Wang were unable to observe ET-1-induced hypertension in an identical preparation, i.e., the same dose of ET-1 in rats on a normal salt diet. The reasons for the disparate findings are not clear. A number of years ago, Mortensen and Fink observed that chronic infusion of ET-1 results in increased arterial pressure that is exacerbated by a high-salt diet. High salt is clearly one of the major stimuli of ET-1 production in the kidney. There are functional differences in ET actions in animals on a normal or high-salt diet consistent with a role for intrarenal ET in promoting salt excretion.

Whereas ET-1 can increase oxidative stress, there are a number of reports that reactive oxygen species can increase ET-1 production in cultured endothelial cells and vascular smooth muscle cells. Hydrogen peroxide and superoxide increase ET-1 synthesis, and 8-iso prostaglandin F2α, a product formed by free radical catalyzed lipid peroxidation, also have similar effects. The relevance of these observations is unclear given that Saito et al observed contrasting findings that hydrogen peroxide actually decreases ET-1 mRNA and protein synthesis in endothelial cells. It remains possible that increased production of oxygen-derived free radicals could provide a mechanism for Ang II-induced ET-1 synthesis, although this possibility has yet to be investigated.

**ETβ Receptors in Hypertension**

The kidney contains an abundant quantity of the machinery necessary for the paracrine and autocrine actions of ET. In addition to vascular endothelium, ET-1 and ETβ receptors are found in especially abundant quantities within the inner medullary collecting duct and, to a lesser degree, the medullary thick ascending limb. The actions of ET-1 to promote salt and water excretion appear to involve both tubular and hemodynamic mechanisms. The vasodilator action of ET-1 in the renal medulla is more evident in rats maintained on a high-salt diet consistent with a role in promoting salt and water excretion.

Acute administration of ETα receptor antagonists have little effect on renal and cardiovascular function in healthy normotensive animals, suggesting that ET does not exert a tonic influence on vascular resistance via the ETα receptor. Information regarding the role of ETβ receptors in regulating fluid volume balance has been obtained from studies using ETβ receptor antagonists or rats and mice deficient in the ETβ receptor gene. Gariepy et al have reported that rats deficient in a functional ETβ receptor become hypertensive when given a high-salt diet. A high-salt diet also induces hypertension in collecting duct-specific ET-1 knockout mice. Our laboratory has shown that the hypertension produced by chronic ETβ receptor blockade is exacerbated by a high-salt diet. We also have observed that ETβ receptors in the renal medulla are upregulated in response to DOCA-salt treatment. These findings support the hypothesis that the predominant role of ETβ receptors in pressure regulation is through the control of sodium excretion. These studies have led to a prevailing theory that the renal ET system, including ETβ receptors, are physiological regulators of renal salt and water excretion. However, the degree to which the actions of ET and the ETβ receptor are through increases in medullary blood flow or direct inhibition of tubular transport is not entirely clear.

Hypertension associated with ETβ receptor-deficient rats placed on a high-salt diet is accompanied by increased oxidative stress. This increase in oxidative stress can be attributed to the resulting elevations in ET levels because ETα receptor blockade can prevent the increase in blood pressure and oxidative stress in this model. Similarly, chronic ET-1 infusion is associated with
elevations in reactive oxygen species, although reports disagree as to the extent to which oxidative stress contributes to the associated hypertension.36,52 Whereas hypertension associated with chronic ET<sub>B</sub> receptor blockade can be inhibited by ET<sub>A</sub> receptor antagonists, evidence for a role of oxidative stress, or specifically superoxide, is not straightforward. Williams et al recently observed that the superoxide dismutase mimetic, tempol, attenuated the hypertension associated with ET<sub>B</sub> receptor blockade in rats on a high-salt diet, but only during the initial days of treatment.53 Chronic tempol was associated with a progressive and large increase in urinary hydrogen peroxide levels, which could negate the effects of reduced superoxide. Makino et al have shown that long-term increases in renal hydrogen peroxide levels will produce hypertension.54 These results may provide a partial clue as to why antioxidant treatments have been disappointing in the treatment of cardiovascular disease.

Despite mixed results using the antioxidant tempol, there is clear evidence that ET stimulates superoxide production through an NADPH oxidase-dependent manner in DOCA-salt hypertensive rats.55,56 Because ET<sub>A</sub> receptor blockade and superoxide scavenging can attenuate the hypertension associated with DOCA-salt treatment, it is reasonable to hypothesize that ET-dependent hypertension could be inhibited by disruption of NADPH oxidase subunit assembly with the inhibitor, apocynin. Elmarakby et al have recently demonstrated that apocynin inhibits the increase in oxidative stress associated with chronic ET-1 infusion, yet the resulting hypertension was unaffected.52

**ET-1 in Vascular Remodeling**

It is well-established that Ang II contributes to vascular wall remodeling associated with hypertension and vascular disease.57 However, it appears as though the actions of both Ang II and/or aldosterone on cardiac and vascular remodeling are mediated, at least in part, by ET. As mentioned, ET<sub>A</sub> or mixed ET<sub>A</sub>/ET<sub>B</sub> receptor antagonists can prevent the hypertension produced by chronic infusion of exogenous Ang II; however, surprisingly, little has been done to examine the impact of ET receptor blockade on vascular remodeling in this model. In an acute setting, Fakhouri et al have shown that Ang II activates collagen type I gene in the renal cortex and aorta that can be blocked by the nonselective antagonist, bosentan.58 Bosentan was unable to prevent hypertension in the TGRen2 model of elevated endogenous Ang II; yet changes in cardiac hypertrophy and fibrosis were severely reduced.59 In rats transgenic for both the human renin and human angiotensinogen genes, bosentan inhibited the activation of both nuclear factor-kappa B and transcription factor activator protein in the kidney and heart.60 Furthermore, inhibition of the endothelin-converting enzyme reduced mortality and ameliorated cardiac damage in this model.60 In animal models of mineralocorticoid-induced hypertension, changes in vascular wall structure and expression of extracellular matrix proteins can be prevented by ET receptor blockade.27,32,61,62

**Perspectives**

Although considerable attention has been paid to the mechanisms of ET action in the control of blood pressure and the pathogenesis of hypertension, it is clear that there is much to be deciphered regarding the relationship between ET, oxidative stress, and the associated hypertension. A more complete understanding of the physiological role of ET in the control of renal tubular and hemodynamic function is needed before we can understand the role of this peptide in the pathogenesis of hypertension. Severe limitations of the tools used to study oxidative stress have slowed and perhaps even confused the state of our understanding regarding the relationship between reactive oxygen species and the vasoactive peptides, Ang II and ET.

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**References**


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