Nitric oxide and cardiovascular and renal effects
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Abstract
Nitric oxide (NO) has multiple protective effects for regulating the cardiovascular and renal systems. The major functions include endothelium-dependent relaxation, anti-inflammatory effects, as well as antihypertrophic and antithrombotic activities. Many of the activities mediated by NO are systematically antagonized by angiotensin-II (Ang II), a vasoconstrictor peptide. Studies described in the review below have demonstrated that the balance between NO and Ang II activities rather than the absolute concentration of each molecule determines their effects on the physiology and pathophysiology of the cardiovascular and renal systems. NO donors have been used for years as therapeutic agents for a range of cardiovascular conditions including angina, myocardial infarction and for the reduction of arterial stiffness. An understanding of the mechanisms underlying the effects of these medications will enable the development of novel therapies to balance the effects of NO in the cardiovascular system.

Key words: Nitric oxide, angiotensin II, hypertension, atherosclerosis, kidney, NO donors

Introduction
Nitric oxide (NO) has multiple protective effects for regulating the cardiovascular and renal systems, including endothelium-dependent relaxation, anti-inflammatory effects, antithrombotic effects, and antihypertrophic activity (Figure 1).1 Many of these actions are systematically antagonized by the action of angiotensin-II (Ang II). Ang II is a vasoconstrictor peptide responsible for hypertension, decreased regional blood flow, impaired renal function, atherosclerosis, and cardiac hypertrophy. NO primarily mediates vascular relaxation through stimulation of soluble guanylyl cyclase (sGC), leading to an increased production of cGMP which activates cGMP-dependent protein kinases, phosphodiesterases and ion channels.2 NO can also act in a non-cGMP-dependent process, leading to reversible, covalent modification of proteins, for example by S-nitrosylation.3 NO interacts with superoxide (O2−) to generate peroxynitrite (ONOO−), which disrupts protein structures and mitochondria.4 The balance between NO and Ang II activities rather than the absolute concentration of either molecule determines the effects on physiology and pathophysiology in multiple organ systems including the cardiovascular and renal systems.5

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Fig. 1. NO generated from the endothelium has protective effects on a variety of different cardiovascular processes (modified with permission from reference 1).
NO has protective effects in the healthy endothelium

There is a complex feedback mechanism that regulates the balance of NO and Ang II (Figure 2). This balance is required for homeostasis in a number of organs and tissues including the cardiovascular and renal systems. In the cardiovascular system, NO and Ang II interact in the endothelium where both molecules undergo the final stages of synthesis. NO is synthesized by endothelial nitric oxide synthase (eNOS), a constitutive calcium- and calmodulin-dependent enzyme that is upregulated in response to mechanical stimuli including shear stress and cyclic strain. Ang II is derived from angiotensin I (Ang I) by the angiotensin-converting enzyme (ACE) which is also expressed in endothelial cells. NO can also downregulate the synthesis of ACE in the endothelium, thereby impacting the balance between NO and Ang II. The Ang II receptor AT₁ mediates the vasoconstrictive effects of Ang II as well as Ang II-induced growth in cardiovascular and renal tissues. NO also downregulates the level of the AT₁ receptor in vascular smooth muscle cells which results in the decrease of Ang II activity. This feedback loop helps explain how NO can mediate vasodilation and decreased systemic blood pressure.

Ang II can stimulate mitochondrial dysfunction via a protein kinase C (PKC)-dependent pathway, resulting in the generation of reactive oxygen species (ROS). The PKC-dependent pathway then activates NADPH oxidase, leading to the formation of peroxynitrite. Doughan et al. used bovine aortic endothelial cells to study the effects of Ang II on mitochondrial ROS and demonstrated that an increase in Ang II significantly increases mitochondrial hydrogen peroxide production. The increase was blocked by incubation with the NOS inhibitor Nω-nitro-L-arginine-methyl ester (L-NAME) and uric acid, a peroxynitrite scavenger, implicating peroxynitrite as a mediator of Ang II-induced mitochondrial dysfunction. The authors proposed a model in which Ang II stimulates NADPH oxidase via a PKC-dependent pathway, resulting in increased ROS production. Superoxide then reacts with NO to generate peroxynitrite, which damages respiratory complexes, leads to mitochondrial dysfunction, and increases hydrogen peroxide concentrations. The increased hydrogen peroxide levels will further activate NADPH oxidase, resulting in increased superoxide, decreased NO availability, and ultimately, endothelial dysfunction.

The balance between Ang II and NO also impacts the activity of membrane-bound NADPH oxidase, a major source of ROS in cardiovascular pathophysiology. Stimulation of Ang II activates a PKC-dependent pathway via AT₁, which upregulates NADPH oxidases, resulting in an increase of superoxide which promotes eNOS uncoupling; uncoupled NO preferentially generates superoxide instead of NO.

NO prevents upregulation of endothelin-1 (ET-1), a small potent vasoconstrictor that exhibits mitogenic activity in vascular smooth muscle cells, via the ET-1A receptor, suggesting NO may have a role in the pathogenesis of disease, including atherosclerosis and hypertension. Incubation of smooth muscle cells with the NO donors S-nitroso-N-acetylpenicillamine (SNAP) and sodium nitroprusside (SNP) resulted in attenuated ET-1-enhanced phosphorylation of ERK1/2, PKB, and Pyk2. The increase in NO inhibited the mitogenic and hypertrophic effects of ET-1 signaling. In contrast, inhibition of NO production by incubation with the NOS inhibitor L-NAME increased ET-1 enhanced phosphorylation. ET-1 also modulates both eNOS expression and NO production, affecting the release of NO.

In pathologic conditions, long-term disruptions in NO synthesis result in an increase in ACE production, which in turn increases the effects of Ang II in the rat aorta. Long-term inhibition of NO synthesis using the NOS inhibitor L-NAME increased ACE activity in the endothelium and resulted in oxidative stress which was visualized by increased aortic superoxide levels. Since treatment with antioxidant drugs did not affect the L-NAME-induced increase in systolic arterial pressure but did prevent increases in vascular superoxide production and ACE activity, oxidative stress was implicated in the pathogenesis of vascular ACE activation.
in rats. These data suggest that ACE is upregulated by redox-sensitive mechanisms resulting from reduced levels of bioactive NO, demonstrating that NO levels may regulate vascular function in concert with local Ang II activities and/or oxidative stress.35

HYPERTENSION

The physiological response to increasing vascular shear stress and cyclic strain which accompanies high blood pressure should be the upregulation of eNOS expression and the reduction of oxidative stress. However, in both humans and animal models, this is not always the case. Animal studies have helped to elucidate the mechanisms by which the balance between NO and Ang II is maintained. Experiments in genetic models of hypertension demonstrated that spontaneously hypertensive rats (SHR) have increased eNOS expression, causing increased NO production whereas Dahl-salt-sensitive (DS) animals on high-salt diets have decreased eNOS mass, resulting in decreased NO production. Additional experiments in age-matched animals with similar levels of hypertension showed that animals with the ability to upregulate eNOS levels (SHR) have significantly less end-organ damage than those who cannot upregulate eNOS activity (DS).28,29 Hypertensive SHR and DS rats manifest increased vascular production of superoxide. When SHR animals, which have the ability to upregulate eNOS, were exposed to increased levels of superoxide dismutase (SOD) by adenoviral infection, there was a decrease in blood pressure, suggesting that removal of superoxide increases the bioavailability of NO, resulting in an antihypertensive effect.30

Since an increase in the concentration of superoxide is in part responsible for the hypertensive effects in SHR rats, the next question was whether functional Ang II upregulation is responsible for the increase in superoxide seen in DS rats.31 When hypertensive DS animals were fed angiotensin receptor blocker (ARB), there was a significant decrease in superoxide production (visualized by fluorescence microscopy), normalization of NO-mediated vascular relaxation but no decrease in blood pressure compared to hypertensive animals. Therefore, reduction of oxidative stress by blocking Ang II receptor normalizes endothelial function, independent of blood pressure. This suggests that most of the superoxide generation results from an increase in Ang II activity, again illustrating the importance of the balance between NO and Ang II. These studies have important therapeutic implications regarding the role of Ang blockades in cardiovascular disease.

Role of NO and Ang II in atherosclerosis

Maintaining the balance between NO and Ang II is also important for preventing atherosclerosis. NO has a protective effect and can inhibit the initiation and progression of atherosclerotic plaque as well as act as an anti-inflammatory mediator, independent of its vasodilatory effects. Specifically NO down-regulates the adhesion proteins VCAM-1 and P-selectin, inhibiting the ability of leukocytes to adhere to the endothelial wall. NO also inhibits platelet aggregation by blocking fibrinogen binding with activated platelets which blocks thrombin receptor-activating phosphoinositide 3-kinase activity.32 All three isoforms of NOS are expressed in atherosclerotic plaques.35 When mice null for eNOS were fed a Western-style diet they had significantly more atherosclerotic lesions than those with wildtype levels of eNOS,36 illustrating the protective effect of NO on the formation of atherosclerotic lesions. Further experiments demonstrated that animals lacking the receptor for prostacyclin also have an increased rate of atherosclerotic progression.37 NO also has antiproliferative effects in vascular smooth muscle cells and can downregulate both AT1 receptors and the ACE enzyme. Finally, NO inhibits the expression of the proatherogenic molecules MCP-1 and LOX-1.

In contrast, ROS generated by Ang II-dependent activation of NADPH oxidase contributes to the pathogenesis of atherosclerosis by leading to endothelial dysfunction and upregulating proatherogenic molecules, including MCP-1 and LOX-2.38

The renin-angiotensin system is also associated with inflammation and oxidative stress during atherosclerosis. Mice that are null for the AT2 receptor have increased atherosclerotic disease progression compared to control animals.39 Later experiments demonstrated that LDL-receptor knockout mice overexpressing the AT2 receptor showed slower progression of atherosclerosis than LDL-null animals alone. In the LDL-null animals, NADPH oxidase is upregulated and eNOS expression is decreased but these changes are not present when AT1 is overexpressed. These results demonstrate that AT2 can regulate atherosclerosis, perhaps by modulating oxidative stress and the balance between NO and Ang II activities.40

Role of NO and Ang II in the kidney

The balance between Ang II and NO is critical for normal kidney function and is responsible for renal vascular resistance and filtration rate. NO mediates renal vascular tone and blood pressure, glomerular and medullary hemodynamics, and extracellular fluid volume. Experiments in hypertensive DS rats demonstrated that eNOS activity was decreased in the renal medulla when compared to normotensive rats. The DS animals developed glomerular injury and severe tubulointerstitial disease and renal hypertrophy whereas hypertensive SHR rats had increased eNOS activity and showed no increase in tubulointerstitial disease and minimal renal hypertrophy. These experiments suggest that a decrease in eNOS activity is a maladaptive response that contributes to the maintenance of hypertension and progression of renal injury.41 Finally, genetic evidence also supports a protective effect of NO in renal function. End stage renal disease is significantly more common in subjects with a specific polymorphism in the eNOS gene compared to healthy controls (P = 0.002)42 again illustrating the protective effects of NO on renal pathophysiology.

In contrast to the protective effects of NO, Ang II decreases renal blood flow and the glomerular filtration rate and constricts afferent and efferent arterioles in a dose-dependent manner. Studies show that in eNOS-null mice, there is an increase in Ang II sensitivity43 and that NO released from NOS activation contributes to control of afferent arterioles.44 Finally, experiments show that the enhanced Ang II-dependent NADPH oxidase activation is likely caused by production of ROS.45 Increased ROS can decrease NO bioavailability and result in increased endothelial dysfunction by shifting the balance of activity of both NO and Ang II.

ANG II RECEPTORS IN THE KIDNEY

The Ang II receptor AT1 mediates constrictive effects in renal resistance arteries46 and experiments suggest that AT1 receptors mediate vasoconstriction in vessels control-
ling medullary blood flow, which are relatively insensitive to Ang II. AT₂ receptors, however, appear to oppose AT₁ function. Experiments with a specific AT₂ antagonist, PD123319, increased basal medullary blood flow, suggesting that AT₂ activity opposes AT₁ vasoconstriction. AT₁ receptors also mediate NO release and play an important role in sympathetic neurotransmission. In AT₂⁻/⁻ receptor deficient mice, there is an increased expression of AT₁ receptors and a concomitant increase in NO found in the arterioles, showing the effect AT₁ has on NO concentrations.

Finally, ROS play an important role in the pathophysiology of the kidney. Long-term Ang II exposure leads to increased arterial pressure and renal vascular resistance in rats. Welch, et al. found there was also an increase in NADPH oxidase activity in the renal cortex and reduced SOD mRNA, resulting in an increased hydrogen peroxide concentration. The authors used Tempol, a SOD mimetic to block the effects of Ang II, suggesting a pathophysiological role for superoxide in this animal model.

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