Functional Interplay Between Angiotensin II and Nitric Oxide
Cyclic GMP as a Key Mediator

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Abstract—Angiotensin II (Ang II) and nitric oxide (NO) signaling pathways mutually regulate each other by multiple mechanisms. Ang II regulates the expression of NO synthase and NO production, whereas NO downregulates the Ang II type I (AT1) receptor. In addition, downstream effectors of Ang II and NO signaling pathways also interact with each other. A feedback mechanism between Ang II and NO is critical for normal vascular structure and function. Imbalance of Ang II and NO has been implicated in the pathophysiology of many vascular diseases. In this review, we focus on the diverse ways in which Ang II and NO interact and the importance of the balance between the signaling pathways activated by these mediators. (Arterioscler Thromb Vasc Biol. 2003;23:26-36.)

Key Words: nitric oxide ■ angiotensin II ■ signaling pathways

Angiotensin II (Ang II), the primary effector of the renin-angiotensin system (RAS), is a multifunctional hormone that plays an important role in vascular function. The local RAS, acting in both autocrine and paracrine fashions, is also functionally operative and important in the vasculature.1,2 The role of the RAS, particularly of Ang II, is of great interest in cardiovascular physiology and pathology because of the beneficial effects of Ang II–converting enzyme (ACE) inhibitors and Ang II receptor blockers in cardiovascular diseases (hypertension, atherosclerosis, heart failure, and stroke).1,4-6 Nitric oxide (NO) as an endogenous endothelium-derived relaxing factor has also been extensively studied. NO plays critical roles in the maintenance of vascular homeostasis. Reduction of NO production due to endothelial dysfunction is the result of many cardiovascular risk factors.

In the vasculature, Ang II and NO interact with each other (albeit indirectly) to influence each other’s functions. The interaction between Ang II and NO occurs in both the endothelial cell (EC) and vascular smooth muscle cell (VSMC). Vascular smooth muscle constricts in response to Ang II and dilates in response to NO. In addition to vascular tone, these 2 molecules antagonize each other in many vascular functions, such as cell growth, apoptosis, and inflammation.

Ang II Signaling Pathways
The actions of Ang II are primarily mediated by 2 receptors, Ang II type 1 (AT1) and type 2 (AT2). The AT1 receptor is widely present in many organs, such as the heart, kidneys, adrenal glands, and brain. The vast majority of well-known physiological and pathophysiological effects of Ang II have been shown to occur via the AT1 receptor. In the vasculature, the AT1 receptor is mainly expressed in VSMCs, where it mediates the vasoconstrictor, proliferative, and inflammatory actions of Ang II. There are AT1 receptors in the endothelium and in monocytes/macrophages as well,7,8 which are pathophysiologically important because Ang II induces the oxidized LDL receptor in the endothelium and stimulates macrophages to express tumor necrosis factor-α.8,9 The AT2 receptor is highly and ubiquitously expressed in fetal tissue, and its expression is dramatically reduced after birth.10,11 The fact that AT2 receptor expression is much higher in fetal compared with normal adult tissues has led to speculation as to its possible role in cell growth, development, and differentiation. AT2 receptor–mediated signaling pathways and function are not very well understood but in general appear to antagonize the effects of the AT1 receptor.

Functions of the AT1 receptor, a G protein–coupled receptor, have been best characterized in VSMCs. The AT1 receptor coupled to Gq leads to phospholipase C (PLC) activation and, in turn, production of inositol-1,4,5-triphosphate (IP3) and diacylglycerol, followed by Ca2+ mobilization and protein kinase C (PKC) activation. These second messengers generated through the AT1 receptor likely contribute to the vasoconstrictor function of Ang II as well as activation of downstream tyrosine and serine/threonine kinases, which contribute to the growth-promoting and cytokine-like actions of Ang II. Several key tyrosine kinases activated by the AT1 receptor have been characterized in...
VSMCs. These tyrosine kinases include (1) receptor tyrosine kinases, such as epidermal growth factor receptor and platelet-derived growth factor receptor, and (2) nonreceptor tyrosine kinases, such as c-Src, prolinc-rich tyrosine kinase 2 (PYK2), focal adhesion kinase (FAK), and Janus kinase-2.12,13 The serine/threonine kinases activated by the AT1 receptor include PKC and kinases in the mitogen-activated protein kinase (MAPK) pathways such as Raf-1, MAPK kinase, and MAPKs (extracellular signal–regulated kinase [ERK1/2], c-jun N-terminal kinase [JNK], and stress-activated protein kinase p38). For detailed information, see the 2 review articles.12-13

Ang II is a multifunctional peptide hormone that not only controls vascular tone but also promotes growth of its target cells. In cultured VSMCs, Ang II has been shown to promote hypertrophy and/or hyperplasia.14,15 In addition, cultured VSMCs migrate toward an Ang II gradient.16,17 In vivo, long-term infusion of Ang II induces VSMC proliferation in the vessel wall in normal and balloon-injured vessels.18,19 Maintaining blood pressure at control levels with hydralazine controls vascular tone but also promotes growth of its target cells. In cultured VSMCs, Ang II has been shown to promote hypertrophy and/or hyperplasia.14,15 In vivo, VSMCs migrate toward an Ang II gradient.16,17 In vivo, long-term infusion of Ang II induces VSMC proliferation in the vessel wall in normal and balloon-injured vessels.18,19 Maintaining blood pressure at control levels with hydralazine during Ang II infusion did not abolish vascular hypertrophy, indicating that the Ang II effect on vascular hypertrophy is not strongly related to hemodynamic changes.20 Taken together, these observations indicate that Ang II has a growth factor–like effect in VSMCs.

Ang II has a central role in the generation of oxidative stress in the vessel wall. Ang II has been shown to stimulate the activity of membrane-bound NAD(P)H oxidase in VSMCs21,22 and ECs.23 In vivo, Ang II inductions increase superoxide formation in the vessel wall, which is not related to the hemodynamic effects of Ang II, because norepinephrine-induced hypertension did not have a similar effect.24,25

Recently identified effects of the AT1 receptor include stimulation of inflammatory, fibrotic, and thrombotic processes, which contribute to Ang II–mediated inflammation and atherogenesis. Ang II activates transcription factor nuclear factor κB (NF-κB) in monocytes,26 VSMCs,27 and ECs.28 In ECs and VSMCs, Ang II activation of NF-κB induces cell adhesion molecules (including vascular cell adhesion molecule-1 [VCAM-1], intercellular adhesion molecule-1 [ICAM-1], and E-selectin), as well as chemokines (including monocyte chemotactattractant protein-1 [MCP-1] and interleukin-8 [IL-8]). These molecules promote adhesion, invasion, and accumulation of monocytes and T lymphocytes in atherosclerotic lesions.29 Ang II also induces expression of proinflammatory cytokines such as IL-6 in cultured VSMCs and macrophages.30,31 In atherosclerosis, IL-6 is expressed primarily by tissue macrophages in fibrous plaques.32,33 It has been proposed that IL-6 induces VSMC proliferation via induction of platelet-derived growth factor and stimulates matrix degradation via induction of matrix metalloproteases.

Tissue factor (TF) is a transmembrane glycoprotein that plays an important role in the initiation of blood coagulation.29 In vitro, Ang II increases expression of TF in cultured ECs34 and VSMCs.34 Normal vessels contain low levels of TF, but TF expression is dramatically increased in intimal macrophages and VSMCs in atherosclerotic plaque.35,36 In addition, Ang II inhibits the fibrinolytic pathway. The effects of the fibrinolytic system are mediated by plasmin, a protease generated from the inactive precursor plasminogen by the plasminogen activators, such as tissue-type plasminogen activator (t-PA) and urokinase.29 The most important inhibitor of t-PA and urokinase is plasminogen activator inhibitor type 1 (PAI-1). In vitro, Ang II induces PAI-1 mRNA and protein in cultured VSMCs and ECs.33,37,38 PAI-1 is expressed exclusively in ECs in normal vessels. PAI-1 expression is significantly increased in the neointima in atherosclerosis.39 Taken together, Ang II contributes to the initiation and progression of atherosclerosis at multiple stages, including the inflammatory process, formation of the fibrous cap, plaque rupture, and thrombosis.

The NO/cGMP Signaling Pathway

The formation of NO is catalyzed by NO synthase (NOS) from l-arginine. Three NOS isoenzymes have been identified, referred to here as neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). The cellular effects of NO can be cGMP dependent or cGMP independent.40 For the cGMP-dependent mechanism, NO activates soluble guanylyl cyclase (sGC), stimulating production of cGMP. Increased intracellular cGMP facilitates the extrusion of intracellular calcium and leads to vascular relaxation. Proliferation and migration of VSMCs are also inhibited by exogenous NO donors and endogenous NO production, which are mediated by cGMP in most cases, although this idea is still controversial.41,42 The effects of cGMP are mainly mediated through cGMP-dependent protein kinase (PKG).43,44 The effects of cGMP may also be mediated by PKG-independent interaction with other molecules in the cell, such as cGMP-gated cation channels and certain phosphodies-terases (PDEs).45 For example, increased levels of cGMP are thought to inhibit cGMP-inhibited PDE (PDE3) activity, resulting in increased levels of cAMP and activation of cAMP-dependent protein kinase (PKA). Furthermore, the cyclic nucleotide ras guanine nucleotide exchange factor can be activated by both cAMP and cGMP, activating Ras independently on PKA and PKG.46 Several cGMP-independent NO targets have been defined. For example, NO was shown to inhibit mitochondrial cytochrome oxidase,47 to activate calcium-dependent potassium channels,48 to inhibit activation of NF-κB,49 and to activate small G proteins, such as p21ras.50,51 The cGMP-independent effects of NO on the target proteins are probably mediated by the S-nitrosylation of cysteine residues in the target proteins.52,53 NO can react with O2 through a series of reaction to form peroxynitrite (ONOO-), which is a potent nitrosating agent.54 The molecular mechanisms and the physiological relevance for these cGMP-independent NO actions need to be further investigated.

NO has several critical roles in the maintenance of vascular homeostasis. In the vasculature, NO not only functions as a vasodilator but also inhibits VSMC proliferation and migration and platelet activation and adhesion, as well as leukocyte adhesion and migration.55 The importance of NO in vasomotor function has been confirmed by its ability to restore endothelial vasomotor function in dysfunctional arteries via NOS gene transfer. Genetically engineered mice lacking the eNOS gene are hypertensive owing to impaired endothelium-dependent vasodilation.55 In addition to functioning as a vasodilator, NO inhibits VSMC proliferation,56-58 migration,49 and extracellular matrix composition.60,61 all key...
components of intimal hyperplasia. In addition, NO may directly inhibit the synthesis of RNA and protein in VSMCs, independent of cGMP. Gene transfer and transgenic models have provided the strongest evidence of a role for NO in moderating VSMC proliferation. For example, mice with targeted disruption of the eNOS gene show a greater increase in wall thickness than do wild-type mice in response to external carotid artery ligation. Virus-mediated gene transfer of both eNOS and nNOS inhibits VSMC proliferation and neointima formation in balloon injury and vein graft models. Overexpression of eNOS in transgenic mice inhibits lesion formation in vascular remodeling. Moreover, eNOS function modulates atherosclerosis, because accelerated atherosclerosis has been reported in apolipoprotein E–knockout (ApoE−/−) mice that lack eNOS, whereas decreased atherosclerosis is present in ApoE−/− mice with overexpression of eNOS.

**Functional Interplays Between Ang II and NO/cGMP**

There is an impressive body of evidence regarding the functional interaction between Ang II and NO/cGMP in the vascular system. In the regulation of vascular reactivity, Ang II or NO/cGMP functions as a vasoconstrictor or vasodilator, respectively. The countervailing influences of Ang II and NO/cGMP on VSMC growth have been well documented. Ang II stimulates, whereas NO/cGMP inhibits, VSMC growth, probably through multiple mechanisms. For example, the growth arrest–specific homeobox (Gax) gene in VSMCs was downregulated by Ang II and upregulated by C-type natriuretic peptide (CNP). Simultaneous administration of Ang II and CNP revealed that CNP significantly attenuated the inhibitory action of Ang II on Gax expression. These results suggest that Gax is a common transcription factor involved in the signaling pathway of VSMC growth for Ang II and CNP. Ang II and NO/cGMP also exhibit opposing effects on cell cycle progression by differentially regulating cyclin-dependent kinase (CDK) activation, cyclin expression, and CDK inhibitor (p21 or p27) expression. In addition, VSMC apoptosis has been shown to be regulated by Ang II and NO/cGMP in a countervailing manner. Moreover, NO/cGMP also antagonizes Ang II effects in other vascular aspects. For example, the NO donor or membrane-permeant cGMP analogue 8-bromo (Br)-cGMP reduces the leukocyte-EC interactions elicited by Ang II in vivo in the rat mesenteric microcirculation. Ang II–stimulated PAI-1 expression in rat aortic VSMCs was reduced by increasing the cGMP content by atrial natriuretic peptide (ANP) and CNP, the NO donor S-nitroso-N-acetylpenicillamine, or 8-Br-cGMP. These functional interplays between Ang II and NO/cGMP are determined by the mutual regulation of Ang II and NO signaling pathways at different levels.

**NO/cGMP Regulates the Ang II Signaling Pathway**

**Effects of NO/cGMP on ACE Activity and Ang II Receptor Expression**

Several studies suggest that NO might be a direct modulator of ACE activity. For example, the NO donors 3-morpholino-sydnonimine, diethylenetriamine (DETA), and nitroprusside inhibit ACE activity. Intravenous administration of the NO precursor l-arginine to healthy volunteers reduced plasma Ang II concentration by inhibiting ACE. In contrast, long-term treatment with nitro-l-arginine methyl ester (l-NAME) to inhibit NO production increased cardiac and aortic ACE activity. NO also regulates Ang II receptors in vitro. Treatment of rat VSMCs with NO donors inhibited Ang II binding to cells without altering receptor affinity. However, treatment of the cells with cGMP analogues had no significant effect on Ang II binding, suggesting that NO regulates Ang II receptors through a cGMP-independent mechanism. In rat VSMCs, inhibition of Ang II binding by NO is due to decreased AT1 receptor mRNA expression and occurs at the transcriptional level. The downregulation of the AT1 receptor by NO may be one of the mechanisms of the antiatherogenic and antihypertensive properties of NO.

**Effects of NO/cGMP on Regulation of Intracellular Ca2+**

A critical mechanism for NO effects on VSMCs is to lower intracellular Ca2+ by increasing intracellular cGMP and activating PKG. cGMP/PKG could regulate Ca2+ in at least 5 different ways (Figure 1): (1) downregulating IP3 formation, (2) decreasing Ca2+ mobilization through the IP3 receptor, (3) promoting Ca2+ sequestration in the sarcoplasmic reticulum (SR), (4) reducing Ca2+ influx, and (5) increasing Ca2+ efflux. The molecular basis for the regulation of intracellular Ca2+ and the physiological targets of PKG appear to be complex. It is likely that, in vivo, different mechanisms operate synergistically to lower Ca2+ levels by utilizing cGMP as a second messenger.

**IP3 Formation**

Activation of PLC and IP3 formation are early key steps in Ang II increases in intracellular Ca2+ in VSMCs. G protein–activated PLC catalyzes the hydrolysis of phosphatidylinositol 4,5-biphosphates (PIP2) to generate diacylglycerol and IP3, leading to the activation of PKC and the mobilization of intracellular Ca2+. For example, superoxide-induced IP3 formation was significantly inhibited by an NO donor and potentiated by inhibition of GC and PKG in both rat VSMCs and mesenteric arteries. Superoxide-induced IP3 formation was likely due to the inhibitory effect of superoxide on cGMP formation. The mechanisms by which cGMP inhibits the generation of IP3 were examined in rat aorta segments and cultured bovine VSMCs. The results suggest that the ability of cGMP to inhibit phosphorylation of PLCβ both in vitro and in vivo at Ser26 and Ser1105. Phosphorylation of PLCβ leads to the inhibition of G protein–activated PLCβ activity. Blocking phosphorylation of PLCβ by mutation of 2 serine residues removed the inhibitory effect of PKG on the activation of the mutant PLCβ by G protein. All of these results suggest that NO and cGMP negatively regulate IP3 formation by direct inhibition of PLC activity, as well as by...
PKG in Plb phosphorylation. Furthermore, phosphorylation localized in the same cellular regions, supporting the role of the SR Ca\(^{2+}\)\(^{-}\) ATPase. Ca\(^{2+}\)\(^{-}\) influx is mediated by voltage-dependent Ca\(^{2+}\)\(^{-}\) channels (VDCCs), which normally open in response to membrane depolarization, is also directly inhibited by cGMP/PKG-dependent mechanisms. These observations suggest that PKG phosphorylates either VDCCs or a closely associated regulatory protein, thereby reducing channel opening. PKG-PKG also causes hyperpolarization of the surface membrane through activation of Ca\(^{2+}\)\(^{-}\)-sensitive potassium channels (Kca). In turn decreases the open probability of VDCCs. Ser1755 of the IP3 receptor is one of the best-known substrates of PKG. In cultured VSMCs and isolated rat aortas, PKG phosphorylates the IP3 receptor at Ser1755. This phosphorylation of the IP3 receptor reduces channel activity, leading to a decrease in Ca\(^{2+}\) concentration and smooth muscle relaxation. Recently, a protein termed the IP3 receptor-associated cGMP kinase substrate (IRAG) has been identified in many tissues, including the aorta. IRAG is associated with the IP3 receptor and PKG in microsomal smooth muscle membranes. Ca\(^{2+}\) release is inhibited after coexpression of IRAG and PKG in the presence of cGMP. These results identify IRAG as an essential NO/PKG-dependent regulator of IP3-induced Ca\(^{2+}\) release.

**Ca\(^{2+}\)\(^{-}\) Sequestration**

cGMP induces uptake of Ca\(^{2+}\) into the SR through activation of the SR Ca\(^{2+}\)\(^{-}\)-pumping ATPase (Ca\(^{2+}\)-ATPase). The activity of Ca\(^{2+}\)-ATPase in the SR is regulated by the protein phospholamban (Plb). In rat VSMCs, PKG phosphorylates Plb, which increases Ca\(^{2+}\)-ATPase activity and sequestration of Ca\(^{2+}\) into the SR, probably owing to an increase in the affinity of Ca\(^{2+}\)-ATPase for Ca\(^{2+}\). Plb and PKG are colocalized in the same cellular regions, supporting the role of PKG in Plb phosphorylation. Furthermore, phosphorylation of Plb correlates with an increase in Ca\(^{2+}\) uptake by the SR as well as vascular smooth muscle tone.

**Ca\(^{2+}\)\(^{-}\) Influx**

Voltage-dependent activation of L-type Ca\(^{2+}\) channels (VDCCs), which normally open in response to membrane depolarization, is also directly inhibited by cGMP/PKG-dependent mechanisms. These observations suggest that PKG phosphorylates either VDCCs or a closely associated regulatory protein, thereby reducing channel opening. PKG also causes hyperpolarization of the surface membrane through activation of Ca\(^{2+}\)-sensitive potassium channels (Kca). In turn decreases the open probability of VDCCs. Ser1752 of the Kca channel α-subunit is phosphorylated by PKG, and this phosphorylation is critical for the effect of cGMP/PKG on activation of Kca.

**Ca\(^{2+}\)\(^{-}\) Efflux**

Ca\(^{2+}\)\(^{-}\) efflux is mediated by activation of the membrane Ca\(^{2+}\)-pumping ATPase (mCa\(^{2+}\)-ATPase) and the Na\(^{+}/Ca\(^{2+}\)\(^{-}\)-exchanger. PKG-I, but not PKG-Iβ, stimulates the plasma membrane mCa\(^{2+}\)-ATPase without detectable phosphorylation of mCa\(^{2+}\)-ATPase. Several indirect mechanisms for the activation of mCa\(^{2+}\)-ATPase have been proposed. For example, PKG may increase the intracellular phosphatidyl inositol-4 phosphate (PI-4P) by phosphorylation and activation of phosphatidylinositol kinase. PI-4P then activates the mCa\(^{2+}\)-ATPase. Alternatively, it has been found that an intermediate protein responsible for the activation of mCa\(^{2+}\)-ATPase is phosphorylated by PKG. The driving force for extrusion of Ca\(^{2+}\) through the Na\(^{+}/Ca\(^{2+}\)\(^{-}\)-exchanger is depletion of intracellular Na\(^{+}\) via activation of Na\(^{+}/K\(^{-}\)-ATPase or hyperpolarization of the cell membrane through activation of voltage-dependent L-type Ca\(^{2+}\) channels.
K+ channels. Na+/K+-ATPase in the plasma membrane is indeed activated by cGMP through PKG.107

Effects of NO/cGMP to Desensitize the Contractile Apparatus to Ca2+
In addition to its effects to lower intracellular Ca2+ concentration, NO/cGMP also decreases the Ca2+ sensitivity of contractile proteins. For example, cGMP induces Ca2+ desensitization by altering the balance between the activities of myosin light-chain kinase (MLCK) and myosin light-chain phosphatase (MLCP) at a constant Ca2+ concentration (Figure 1). The contractile force of smooth muscle is primarily dependent on the status of MLC phosphorylation, which is regulated by the balance of MLCK and MLCP activities. MLCK is activated in a Ca2+- and calmodulin-dependent manner, and activation of MLCK leads to smooth muscle contraction. In contrast, activation of MLCP (also called PP1M) activity produces relaxation of smooth muscle, representing a mechanism for cGMP-induced Ca2+ desensitization in vasodilatation. For example, studies of isolated il- ieal108 and vascular smooth muscle109 have demonstrated that cGMP/PKG induces MLCP activity without affecting MLCK activity. MLCP is a trimer composed of a 110-kDa regulatory myosin-binding subunit (MBS), a 37-kDa catalytic subunit (PP1c), and a 20-kD protein of uncertain function (M20). PKG may increase MLCP activity by phosphorylation of the MBS subunit of MLCP.108,110 In addition, it is known that PKG-Iα is targeted to the VSMC contractile apparatus by a leucine zipper interaction with the MBS subunit of MLCP. Uncoupling of the PKG-Iα–MBS interaction prevents cGMP-dependent dephosphorylation of MLC, demonstrating that this interaction is essential to the regulation of VSMC tone.111

Effects of NO/cGMP on Ca2+-Independent Rho/Rho Kinase Signaling
Rho kinase is an important downstream effector of RhoA. The activity of RhoA is activated by guanosine exchange factors and inactivated by GTase-activating proteins. Activation of Rho kinase has been implicated in mediating many Ang II–elicited effects, such as Ang II–induced vascular contraction,112 VSMC hypertrophy,113 MCP-1 expression,114 and PAI-1 gene expression.115,116 NO is able to inactivate RhoA in a cGMP/PKG-dependent manner.117 PKG has been found to inhibit Rho kinase by phosphorylation and inactivation of RhoA, which is critical for RhoA-induced Ca2+ sensitization in VSMCs (Figure 1).117 For example, phosphorylation of the MBS regulatory subunit of MLCP by Rho kinase at Thr695 leads to inhibition of MLCP activity, inducing Ca2+ sensitization of the contractile apparatus.117–120 In contrast, PKG inactivation of RhoA/Rho kinase increases MLCP activity and inhibits vascular contraction.117,120,121 Recent studies have also shown that insulin stimulates MLCP activation in VSMCs by decreasing site-specific phosphorylation of the MBS of MLCP by way of NO/cGMP-mediated Rho/Rho kinase inactivation.120,122,123

Effects of NO/cGMP on Other Protein Kinases
PKC is an important mediator in Ang II signal transduction. Ang II stimulates PKC activation in VSMCs. However, ANP or 8-Br-cGMP significantly inhibited Ang II–stimulated PKC activity in a PKG-dependent manner. MAPKs are also well known to be activated by Ang II in VSMCs. ANP has been shown to elicit inhibitory effects on Ang II–stimulated ERK2 and p38 activities and their protein levels in cultured human VSMCs, which is PKG dependent.124 In addition, ANP stimulates MAPK phosphatase-3 protein levels, suggesting that ANP-dependent inhibition of MAPKs may also proceed by stimulating the phosphatase cascade.124

Ang II Regulates the NO/cGMP Signaling Pathway
Effects of Ang II on NO Generation
Ang II has several effects on NO generation, which are mediated by both the AT1 and AT2 receptors (Figure 2). Specifically, activation of the AT2 receptor is proposed to stimulate NO production in ECs. Gohlke et al125 have recently shown that Ang II via the AT2 receptor results in a kinin-dependent stimulation of NO production. AT1 indicates Ang II type 1 receptor; AT2, Ang II type 2 receptor; BK, bradykinin; EC-SOD, extracellular superoxide dismutase; NO, nitric oxide; O2−, superoxide anion; PDE, phosphodiesterase; and sGC, soluble guanylyl cyclase.

Figure 2. Schematic representation of the influences of Ang II on the NO signaling pathway. Solid and dashed arrows stand for stimulation and inhibition, respectively. Ang II regulates NO levels, mediated by both AT1 and AT2 receptors. Ang II, via the AT1 receptor, decreases NO bioavailability by stimulating superoxide production as well as blocking NO signal transduction. For example, Ang II reduces sGC activity/expression, induces cGMP hydrolyzing PDE expression, and reduces PKG activity. Ang II, via the AT2 receptor, results in a kinin-dependent stimulation of NO production. AT1 indicates Ang II type 1 receptor; AT2, Ang II type 2 receptor; BK, bradykinin; EC-SOD, extracellular superoxide dismutase; NO, nitric oxide; O2−, superoxide anion; PDE, phosphodiesterase; and sGC, soluble guanylyl cyclase.
increased due to the marked increase in superoxide production evoked by Ang II stimulation. Therefore, whereas endothelial NO levels (measured in cultured ECs) were increased, vascular NO levels (measured in the entire vessel) were decreased. Indeed, Ang II caused a massive induction of superoxide production throughout the entire vessel, including the endothelium, media, and adventitia.128

**Effects of Ang II on GC**

GCs are a family of enzymes that catalyze the conversion of GTP to cGMP.129 The family comprises both membrane-bound (pGC) and soluble (sGC) isoforms. NO directly stimulates sGC activity. sGC is expressed in the cytoplasm of almost all mammalian cells. This protein is a heterodimeric protein consisting of α- and β-subunits, and both subunits are required for catalytic activity. Analysis of sGC from different tissues demonstrated multiple isoforms with different subunit compositions. The most abundant subunits are α1 and β1, which are found in many tissues, including the vessel wall. Infusion of Ang II into rats significantly decreased the expression of both sGC subunits α1 and β1 in blood vessels.128 Ang II also has been shown to negatively modulate sGC enzymatic activity.130 The inhibitory effects of Ang II on sGC are likely mediated by the production of superoxide in response to Ang II. For example, much experimental data demonstrate that sGC is one of the intracellular targets of superoxide, and superoxide inhibits the catalytic activity of sGC and cGMP production, both in vitro and in vivo.130–133 Peroxynitrite, the interacting product of NO and superoxide, also inhibits sGC activity.134 It also has been reported that exogenous superoxide production increases in sGC expression but decreases in sGC activity in rat aorta rings, suggesting that superoxide causes dysfunction of vascular sGC.135

**Effects of Ang II on cGMP-Hydrolyzing PDEs**

Cyclic nucleotide PDEs play critical roles in controlling intracellular cGMP levels by converting cGMP to 5′-GMP. PDEs are a superfamily of structurally and functionally related enzymes. More than 40 different isoforms have so far been identified and grouped into 11 broad families based on their distinct kinetic properties, regulatory mechanisms, and sensitivity to selective inhibitors. Most families contain several distinct genes, and many of these genes encode multiple alternative splice variants expressed and regulated in a tissue- or cell type–specific manner. It is increasingly clear that cyclic nucleotide degradation by PDEs is not a constitutive function of the cell but is regulated by different mechanisms in different physiological and pathological conditions.

Five major families of PDEs have been identified in normal human and animal VSMCs, including Ca2+/calmodulin-stimulated PDE (PDE1), which preferentially hydrolyzes cGMP; cGMP-stimulated PDE (PDE2), preferentially hydrolyzing cAMP; cGMP-inhibited PDE (PDE3), preferentially hydrolyzing cAMP; cAMP-specific PDE (PDE4), hydrolyzing cAMP; and cGMP-specific PDE (PDE5), hydrolyzing cGMP.136 The major isoforms of the PDE1 and PDE5 families in normal aortic VSMCs in vivo are PDE1A1 and PDE5A1, respectively. In the vasculature, PDE1A1 is primarily present in SMCs.137–139 The EC50 of PDE1A1 for cGMP is 50- to 100-fold higher than for cAMP (authors’ unpublished observations), even though PDE1A1 is able to hydrolyze both cAMP and cGMP in vitro. Some investigators believe that PDE1A1 is mainly responsible for cGMP metabolism in the cell. The presence of >1 PDE capable of hydrolyzing cGMP in VSMCs (PDE1A1 and PDE5A1) suggests that different PDEs may play distinct roles in VSMCs. For example, PDE5A1 is stimulated by increased cGMP levels,140 playing an important role in the negative-feedback regulation of intracellular cGMP levels.141 PDE1A1 probably takes on a dominant role when smooth muscle is exposed to an excitatory stimulus, leading to influx of Ca2+ or an endogenous agonist that increases intracellular Ca2+.142 It is important to note that PDE1A1 has unique characteristics that make it a particularly potent PDE in the presence of vasoconstrictors. This is because it is stimulated at least 10-fold in the presence of increased intracellular Ca2+ in vitro.143

Ang II exhibits inhibitory effects on cGMP accumulation elicited by an NO donor or ANP in VSMCs,144 glomerular mesangial cells,130 and vessels,145 which is very likely to be mediated by activation of Ca2+/calmodulin-stimulated PDE. We have recently found that PDE1A1 in VSMCs is rapidly activated by Ang II, probably by way of increased Ca2+. Ang II–mediated activation of PDE1A1 contributes to the effects of Ang II on attenuation of cGMP accumulation.144 These observations suggest that PDE1A1 provides a mechanism by which Ang II antagonizes the effect of NO through attenuation of cGMP accumulation.

**Effects of Ang II on cGMP Effector PKG and PKG-Mediated Phosphorylation**

PKG represents the principal intracellular mediator of cGMP signals. cGMP induces binding-dependent activation of PKG, leading to the catalytic transfer of the γ-phosphate from ATP to a serine or threonine residue on the target protein. This phosphorylated protein then mediates the translation of the extracellular stimulus into a specific biologic function. Two different genes for PKG, PKG I and PKG II, have been identified in mammals. PKG I encodes Iα and Iβ isoforms, which arise from alternative splicing of the N-terminal region. PKG I is a cytosolic 76-kDa homodimer widely expressed in mammalian tissues, including smooth muscle. PKG Iα is the major isoform detected in the vascular system.129 PKG may serve as a target regulated by Ang II. For example, it has been reported that Ang II infusion decreases phosphorylation but not expression of vasodilator-stimulated phosphoprotein (VASP), a well-characterized substrate for PKG.128 The expression level of PKG I was not changed by Ang II.128 These results suggest that a decrease in PKG activity occurred in response to Ang II treatment.

**Importance of the Balance Between Ang II and NO**

Abnormal vascular tone and vascular remodeling are key features in cardiovascular diseases such as hypertension, atherosclerosis, restenosis, and congestive heart failure. Both NO and Ang II are important players in these pathogenetic mechanisms. We propose that the development and progression of cardiovascular diseases are probably due in part to an imbalance between Ang
Ang II and NO. For example, endothelium-dependent vascular relaxation is impaired in transgenic mice overexpressing renin or angiotensinogen. Gene transfer of eNOS effectively restores vasomotor function in Ang II–infused rabbits. In contrast, the vascular response to Ang II is enhanced in eNOS-deficient mice. Long-term blockade of NO synthesis with L-NAME caused systemic arterial hypertension, which can be prevented by ACE inhibitors as well as by AT1 receptor antagonists, suggesting involvement of the RAS. Ang II and NO imbalance often results from a loss of NO due to endothelial dysfunction and oxidative stress and/or an enhancement of the local tissue actions of Ang II.

Endothelial dysfunction is measured experimentally by the decrease in the normal vasodilatory response to agonist activation (eg, acetylcholine or bradykinin). These endothelium-dependent vasodilators work through stimulation of NO production in ECs. Impairment of NO-dependent effects could be due to attenuation of NO production, NO bioavailability, and/or NO-mediated signaling events. NO is a free radical and can undergo a chemical reaction with superoxide to generate peroxynitrite. As a result, NO produced in the presence of reactive oxygen species (ROS) exhibits fewer biologic effects because peroxynitrite no longer has the vasodilating effects of NO. Cardiovascular risk factors, such as smoking, diabetes, hyperlipidemia, and insulin resistance, can initiate endothelial dysfunction by altering the redox state in the vessel wall.

For example, cigarette smoking induces endothelial dysfunction that correlates with urinary concentrations of byproducts of ROS, such as lipid peroxidation. Hyperlipidemia is associated with increased endothelial ROS generation and oxidation of LDL. In addition, impairment of NO-mediated vascular response can also be contributed by blunted NO-downstream targets. For example, long-term infusion of Ang II causes endothelial dysfunction associated with decreases in GC expression and PKG activity in rat aorta. We have recently shown that Ang II stimulates the expression of PDE1A1 and PDE5A1 (2 cGMP-hydrolyzing PDEs) in VSMCs (authors’ unpublished observations).

Increased activity of the RAS has been shown to be closely related to EC dysfunction. Recent results from the TREND and HOPE studies demonstrate that inhibition of Ang II by ACE inhibitors restores EC function and decreases cardiovascular events in high-risk patients. Similar results have been obtained for AT1 receptor antagonists, including those from the recent LIFE study. Several studies suggest that excessive Ang II is associated with EC dysfunction by virtue of increased ROS production. Ang II activates (and increases expression of) a powerful membrane oxidase (NADH/ NADPH oxidase), which results in production of superoxide anion. The best experimental lines of evidence that link Ang II, ROS, and EC dysfunction are from animal studies in which rats were made hypertensive by infusion of either Ang II or norepinephrine. EC dysfunction was observed only with Ang II and was correlated with increased superoxide production in rat arteries. In pathophysiological states, excessive Ang II produced locally may have important autocrine and paracrine effects, even in the setting of normal or low circulating Ang II levels. For example, numerous studies show increased levels of ACE, Ang II, and its receptor in human and animal atherosclerotic lesions. In addition, recent data indicate that inflammatory cells in atherosclerotic lesions can release enzymes that generate Ang II, which can result from both local generation and uptake of plasma-derived Ang I and Ang II.

Risk factors for cardiovascular diseases such as hyperinsulinemia, hyperglycemia, and insulin resistance act synergistically with Ang II to promote cellular injury. For example, RAS and factors functionally linked to the RAS have been found to be activated in type 2 diabetes, and inhibition of the RAS with ACE inhibitors and AT1 receptor blockers is particularly effective in diabetics. Hyperglycemia enhances Ang II–induced Janus kinase/signal inducers and activators of transcription signaling in VSMCs. Ang II and high glucose levels combined have additive effects on activation of specific MAPKs and downstream transcription factors, activator protein-1 and NF-κB. The insulin-resistant state sensitizes the vasculature to Ang II and other growth factors. In addition, activation of PKC, an effector molecule of Ang II signaling, is one of the sequelae of hyperglycemia and is thought to play a role in the development of diabetic complications. Thus, multiple mechanisms appear to enhance the activity of the RAS and its effects in diabetes.

Conclusions

It has become clear that Ang II and NO signaling pathways interact with each other in many ways (Figures 1 and 2). Ang II acts at both AT1 and AT2 receptors to regulate NOS activity and NO production. NO, via its downstream effector cGMP (as well as by S-nitrosylation and other chemical effects), opposes Ang II signaling pathways at multiple levels. In this way, the multiple functions of Ang II and NO are precisely balanced. Many cardiovascular diseases are associated with an imbalance of Ang II and NO actions. Cardiovascular risk factors appear to “tip” the balance in favor of Ang II, at least in part by way of oxidative stress. However, other mechanisms may include inhibition of NO effectors, such as cGMP, by inhibiting its formation (effects on pGC and sGC) and enhancing its degradation (effects on PDEs). Therapeutic strategies capable of restoring the balance within the vessel wall should be effective in preventing cardiovascular events.

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References


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