The AT₁-Type Angiotensin Receptor in Oxidative Stress and Atherogenesis

Part I: Oxidative Stress and Atherogenesis

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Angiotensin II, the principal effector of the renin angiotensin system, is a multifunctional peptide that modulates blood pressure, water and sodium homeostasis, neuronal function, and other neurohumoral systems. Initial research with angiotensin II focused on its role in the pathogenesis of hypertension; however, in the past decade, increasing evidence has accumulated to indicate that this octapeptide is involved in the development of atherosclerosis, myocardial infarction, vascular and myocardial remodeling, and congestive heart failure. The effects of angiotensin II are mediated by 2 plasma membrane receptors, referred to as the AT₁ and AT₂ subtypes. In adult tissues, the AT₂ receptor is predominantly expressed in the brain and adrenals, with lower levels expressed elsewhere. AT₂ receptor stimulation leads to vasodilatation and inhibition of vascular smooth muscle growth. During embryogenesis, the AT₁ receptor is expressed in developing arteries, and seems to play a role in curbing vascular smooth muscle growth and guiding the ultimate thickness of the vascular wall. Although interesting and important roles of the AT₂ receptor continue to be defined, most of the known functions of angiotensin II are related to AT₁ receptor activation. AT₁ receptors belong to the 7-membrane–domain superfamily of G-protein–coupled receptors and are expressed in vascular smooth muscle cells, heart, lung, brain, liver, kidney, and adrenal glands. AT₁ receptors are coupled to a variety of intracellular signaling molecules, including the phospholipases A₂, C, and D, adenylyl cyclase, voltage-dependent Ca²⁺ channels, and to a variety of kinases involved in phosphorylation cascades. Depending on the cell and organ type, stimulation of these signal transduction pathways leads to cellular contraction, hypertrophy, proliferation, and/or apoptosis. During the past few years, it has become apparent that one of the most important consequences of AT₁ receptor activation, particularly in the cardiovascular system, is the production and release of reactive oxygen species.

AT₁ Receptor and Oxidative Stress

During normal cellular metabolism, several enzyme systems reduce molecular oxygen, resulting in formation of a variety of reactive oxygen species, including superoxide (O₂⁻), hydroxyl radical (HO), hypochlorous acid (HOCl), lipid radicals, and hydrogen peroxide (H₂O₂). Reactive oxygen species likely play a critical role in the normal functioning of cells. For example, the normal growth of vascular smooth muscle cells seems to require reactive oxygen species. An excessive production of reactive oxygen species, outstripping antioxidant defense systems, has been referred to as oxidant stress and has been implicated in many pathophysiological conditions in the cardiovascular system, including cigarette smoking, hypercholesterolemia, diabetes, hypertension, and heart failure. There are several untoward events that occur as a consequence of oxidant stress. These include oxidative modifications of DNA, lipid oxidation, modification of proteins, and activation of redox sensitive genes. Redox-sensitive genes are important in cardiovascular disease because many of these, such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and monocyte chemoattractant protein-1 (MCP-1) are proinflammatory and play a critical role in the initiation and progression of atherosclerosis. Another significant consequence of reactive oxygen species is modification of enzyme function. An excellent example of this is the activation of matrix metalloproteinases (MMPs). These enzymes play a key role in allowing any cell to grow or migrate and are crucial in both vascular and myocardial remodeling.

There is a broad body of evidence that AT₁ receptor activation leads to production of reactive oxygen species in the vessel wall, in part because the AT₁ receptor is linked to activation of an NADH/NADPH oxidase in vascular cells. This oxidase system, which has similarities to the neutrophil oxidase, is a major source of reactive oxygen species in endothelial cells, vascular smooth muscle cells, and adventitial fibroblasts. Endothelial cells contain all of the subunits of the neutrophil oxidase, including gp91phox, p47phox, p67phox, p22phox, and the small GTPase rac1. In contrast, vascular smooth muscle cells contain a recently discovered protein termed NOX (for nonphagocytic oxidase), which seems to replace gp91phox as one of the membrane components. How the various subunits interact in vascular cells...
and how they produce superoxide is not well understood. It is clear, however, that the vascular NAD(P)H oxidase is responsive to hormones, metabolic factors, and mechanical forces.26,28 Besides growth factors and cytokines, angiotensin II is a principal activator of NAD(P)H oxidase expressed in vascular smooth muscle cells and fibroblasts.25,26 Angiotensin II activates the NAD(P)H oxidase via AT1 receptor activation through stimulation of intracellular signaling pathways such as arachidonic acid metabolites.29 Furthermore, angiotensin II induces a rapid translocation of the small GTPase rac1 to the cellular membrane, a prerequisite of NAD(P)H oxidase activation.30 Besides these rapid effects, angiotensin II exerts long-term alterations by enhancing the gene expression of rac1, p22phox, and NOX-1.25–27,30

The NADPH oxidase is probably not the only source of reactive oxygen species stimulated by angiotensin II. Recently, it has become apparent that endothelial nitric oxide synthase (eNOS) can produce large amounts of superoxide under certain pathophysiological conditions.31,32 One mechanism for this relates to oxidative destruction of tetrahydrobiopterin, which is a critical cofactor for eNOS function.33–35 In the absence of tetrahydrobiopterin, eNOS transfers electrons to molecular oxygen rather than to l-arginine resulting in superoxide production. Recent studies have suggested that any condition that increases superoxide production (so called “kindling radicals”) in the endothelium may ultimately lead to production of a large amount of superoxide (“bonfire radicals”) from uncoupled eNOS.36

Endogenous reactive oxygen species stimulated by activation of the AT1 receptor play critical roles as intracellular signaling molecules. Of the many reactive oxygen species generated in mammalian cells, hydrogen peroxide seems particularly important in cell signaling. Hydrogen peroxide is relatively stable and uncharged, allowing it to diffuse from one cell to the next. Numerous intracellular targets for hydrogen peroxide and other reactive oxygen species have been described, including the mitogen-activated protein kinase (MAPK) family, the cell survival kinase Akt, ras/rac, c-src, protein kinase C, and tyrosine phosphatases. Reactive oxygen species also modulate intracellular Ca2+ levels, altering numerous early signaling events. Many of these events have also been shown to be downstream of AT1 receptor activation and are known to be redox-sensitive signaling targets.37–41

Interactions Between the AT1 Receptor, the Nitric Oxide Pathway, and Atherogenesis

One of the important consequences of increased superoxide production in response to angiotensin II is inactivation of nitric oxide.17 The two radicals superoxide and nitric oxide react with one another at a rate of 6.7 × 10^9 mol/L·s·1, resulting in formation of peroxynitrite.14,15,17 Generally, the endogenous superoxide dismutases and other scavengers of superoxide keep this reaction to a minimum; however, when cellular levels of superoxide are increased, for example on angiotensin II stimulation, this reaction proceeds.42 Loss of nitric oxide via this mechanism leads to endothelial dysfunction, one of the earliest steps in the atherosclerotic process. Numerous cell culture, animal model, and clinical studies have supported the concept that AT1 receptor activation causes vascular superoxide release in vitro and in vivo leading to impaired endothelium-dependent vasodilation.25,43

In concert with these findings, inhibition of AT1 receptor activation by AT1 receptor antagonists or ACE inhibitors improves endothelial dysfunction.44–46 Loss of nitric oxide and formation of peroxynitrite promote atherosclerosis at virtually all stages of the disease.17 The earliest stages of atherosclerosis disease are associated with increased attraction and adhesion of monocytes to the endothelium.5 Inflammatory molecules such MCP-1 and VCAM-1 are critically important in this process. Importantly, expression of these is redox-sensitive and inhibited by nitric oxide.19–21,47 Angiotensin II seems to induce their production and secretion via generation of reactive oxygen species and suppression of nitric oxide.

Another phase of the atherosclerotic process is fatty streak formation, which is characterized by increased oxidation of LDL, uptake of oxidized LDL by macrophages, and foam cell formation, processes that are promoted by AT1 receptor activation.48 Of note, it has recently been demonstrated that expression of a receptor for oxidized LDL, known as the LOX receptor, is dramatically increased by AT1 receptor activation.48 In addition, oxLDL uptake may be also enhanced via angiotensin II effects on the cellular proteoglycan content.39

Plaque formation is propagated by migration and proliferation of vascular smooth muscle cells. Again, there is ample evidence suggesting that the oxidant stress induced by angiotensin II plays a major role in stimulating growth and migration of vascular smooth muscle cells.5,17

Finally, plaque rupture is the result of increased lipid deposition, inflammatory events, apoptosis, and accelerated matrix degradation within the preformed vascular lesion.5 Besides the enhanced lipid deposition, AT1 receptor activation initiates inflammatory processes such as interleukin-6 production.50 Vascular smooth muscle cell apoptosis, which seems to be a prelude to plaque rupture, is induced via angiotensin II and prevented by AT1 receptor blockade.10,11 In keeping with these observations, angiotensin II increases MMP activity, resulting in plaque degradation and ultimately rupture.24 Interestingly, recent reports have suggested that angiotensin II modulates hematopoiesis within the bone marrow. Monocyte CD11b expression is enhanced, ultimately impacting on monocyte-endothelial interactions.7 To date, it is not clear whether endothelial dysfunction during early atherogenesis resembles the pathological state of endothelial cells shortly before or during plaque rupture. Within this review, both syndromes are referred to as endothelial dysfunction.

To summarize the above information, angiotensin II, via its actions on the AT1 receptor, promotes the atherosclerotic process at virtually all stages of the disease (Figure 1). These actions of angiotensin II are at least in part mediated by reactive oxygen species.

Perspectives

Atherosclerosis is clearly caused by both a genetic predisposition to the disease and a variety of exogenous risk factors.
Whereas the epidemiological correlation between risk factors and atherosclerosis has been established, the molecular events initiated by those risk factors that ultimately lead to atherosclerosis remain poorly understood. It is apparent from the data accumulated to date, that one possible link between risk factors and the development of vascular disease is in fact AT1 receptor activation. AT1 receptor–evoked oxidative stress has been implicated in all states of atherosclerosis, starting with the development of endothelial dysfunction and ultimately leading to plaque rupture and occlusion of the diseased vessel. Besides the traditional effects of AT1 receptor activation, the interaction of the AT1 receptor with radical-producing systems such as the NAD(P)H oxidase and the eNOS enzyme is thought to be a key event in atherogenesis.

AT1 receptor antagonists or ACE inhibitors normalize oxidative stress and endothelial dysfunction and reduce the progression of atherosclerosis.14–46 ACE inhibitors are known to retard the progression of atherosclerosis and heart failure.51–53 In men suffering from coronary heart disease, ACE inhibitors improve endothelial dysfunction and reduce rate of death from cardiovascular causes.54 Recent studies in hypertensive and atherosclerotic individuals showed that AT1 receptor antagonists improved endothelial dysfunction, suggesting that these drugs, like ACE inhibitors, benefit vascular function.45,46

Thus, AT1 receptor–elicited oxidative stress has been identified as one key event of the atherosclerotic disease process. Therefore, ACE inhibitors as well as AT1 receptor antagonists are potent antiatherosclerotic drugs, which is predominately related to their antioxidative properties. However, large-scale investigations will have to show that AT1 receptor antagonists not only decrease free radicals and improve endothelial dysfunction but also inhibit atherosclerosis-associated mortality and morbidity.
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