Mechanisms and Cardiovascular Damage in Hypertension

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Abstract—Angiotensin (Ang) II is considered a regulatory hormone stimulating vascular smooth muscle cell constriction, aldosterone release from the adrenal gland, and sodium reabsorption in the renal tubule. Furthermore, Ang II may be formed and act locally as a chemokine, inducing tyrosine phosphorylation, cell growth, hypertrophy, and differentiation. In addition, evidence has recently accrued showing that Ang II is important in stimulating the production of reactive oxygen species and the activation of ancient inflammatory mechanisms. The transcription factor nuclear factor κ-B is pivotal to these processes. Nuclear factor κ-B activation stimulates the expression of a gene menagerie important to chemoattraction, surface adhesion molecule expression, coagulation, and inflammation. Anti-inflammatory interventions may have therapeutic utility. (Hypertension. 2001;37[part 2]:594-598.)

Key Words: angiotensin ■ hypertension, experimental ■ animals, transgenic ■ aldosterone

As the growing role of “oxidative stress” in the pathogenesis of hypertension becomes more appreciated, our impression of hypertension as a rather indolent, solely hemodynamic process is being revised. Reactive oxygen species are the end result of univalent reductions in oxygen, resulting in the production of superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and water (H$_2$O). Reactive oxygen species influence both normal and abnormal cellular processes, including cellular growth, hypertrophy, remodeling, lipid oxidation, modulation of vascular tone, and inflammation.  Reactive oxygen species can also act as intracellular signaling molecules in vascular cells controlling growth, survival, and apoptosis.  The potential targets of reactive oxygen species in endothelial and vascular smooth muscle cells are extracellular signal–related kinases, stress-activated protein kinases, caspases, and nuclear factor-κ-B (NF-κB). NF-κB activation has been associated with vascular inflammation and cell survival.

An important source of reactive-oxygen species are the NADH/NADPH oxidases, which are regulated in part by angiotensin (Ang) II and certain cytokines.  Touyz and Schiffrin recently showed that in vascular smooth muscle cells from human peripheral resistance arteries, Ang II increased H$_2$O$_2$ generation through phospholipase D–dependent, NADH/NADPH oxidase–sensitive pathways. A role for reactive oxygen species in Ang II–mediated transcription factor activation has been established. Mitogen activated protein (MAP) kinase activation and the stimulation of the transcription factor activator protein-1 (AP-1) have recently been delineated.  Furthermore, activation of NF-κB by Ang II is also well described, and the hypothesis that Ang II is involved in inflammatory cell recruitment has been tested.  NF-κB is a pivotal transcription factor in chronic immune responses and inflammatory diseases.

NF-κB is activated by numerous stimuli including cytokines, protein kinase C activators, viruses, immune stimuli, and above all, reactive oxygen species. The activation of NF-κB involves the phosphorylation and subsequent proteolytic degradation of the inhibitory protein I-κB by specific I-κB kinases. The free NF-κB, a heterodimer consisting of 2 proteins, p50 and p65, passes into the nucleus, where it binds to κB sites in the promoter region of numerous genes involved in inflammation. Many of these genes code for cytokines, chemokines, enzymes, proteins involved in coagulation, receptors, proteinases, and adhesion molecules. These molecules contribute to the alterations in structure and mechanical properties responsible for the remodeling of resistance arteries in hypertension.

Ang II and Inflammation

A schematic view of how Ang II operates to initiate inflammation is shown in Figure 1. NF-κB and AP-1, when activated, can initiate the transcription of various inflammation–relevant genes coding for surface adhesion molecules, chemokines, cytokines, coagulation factors, and matrix proteins. The first report associating NF-κB and the renin-angiotensin system demonstrated an activation of the angiotensinogen gene by NF-κB rather than the other way around. Brasier et al showed that the angiotensinogen gene was transcriptionally activated in hepatocytes during the acute–phase response; the mechanism involved the activation of NF-κB. This body of evidence suggests that NF-κB transcription factor complex exerts influences on angiotensinogen gene regulation and thereby the renin-angiotensin system. Additional evidence for this hypothesis stems from the study of Jamaluddin et al. These investigators found that...
protein kinase C is important in the Ang II–induced NF-κB activation.

Hernandez-Presa et al. showed that Ang II is responsible for directing neointimal monocyte infiltration by promoting NF-κB activation and monocyte chemoattractant protein (MCP)-1 expression in a model of accelerated atherosclerosis in rabbits. Their data provided direct evidence that Ang II can initiate an inflammatory response in the vessel wall through activation of NF-κB. The response was ameliorated by ACE inhibitors, supporting a therapeutic benefit of such drugs in patients with atherosclerosis. The authors then observed that ACE inhibitor treatment reduced NF-κB and dependent proinflammatory factor activation but not collagen I expression. The results of the recent Heart Outcomes Prevention Evaluation (HOPE) study, in which patients with cardiovascular risk factors but without heart failure were treated by ACE inhibitors, support the notion that ACE inhibitors exert important protective effects independent of blood pressure reduction.

Morrissey and Klahr observed that NF-κB subunits p50, p52, c-rel, p65 (RelA), and RelB were all activated in a model of ureteral obstruction. The model causes inflammation extending to the renal cortex. NF-κB activation was prominently expressed in renal tubular cells. ACE inhibitor treatment caused a substantial amelioration of the NF-κB activation, implying an anti-inflammatory effect of the drugs. Ruiz-Ortega et al. showed similar findings in a model of immune-complex glomerulonephritis. ACE inhibitor treatment ameliorated the nephritis. In separate studies of mesangial cells, the authors found that Ang II increased MCP-1 mRNA expression. NF-κB was activated in both the nephritis model and the Ang II–stimulated mesangial cells. These observations were recently extended by Ruiz-Ortega et al. who found that the Ang II degradation product Ang III also activated NF-κB in renal and mononuclear cells. MCP-1 was concomitantly expressed. The authors also observed an activation of AP-1 in their experiments. Recently, Ruiz-Ortega et al. observed that Ang II activates NF-κB through both the AT1 and AT2 receptors in vascular smooth muscle cells. Also relevant to Ang II–induced inflammation are recent observations concerning MCP-1, an NF-κB–regulated chemokine, and the chemokine receptor (CCR)2. Bush et al. used CCR2-deficient mice treated with Ang II to show that the mice had markedly decreased macrophage infiltration in the vessel walls and reduced vascular hypertrophy. These observations underscore the role of inflammatory mechanisms in vascular remodeling.

Novel Ang II signaling was recently described by Day et al. They used a transfection analysis to show that Ang II increased reporter gene activity driven by fragments of the platelet-derived growth factor (PDGF)-A promoter bearing recognition elements for the transcription factor early growth response (Egr)-1. They then showed that Ang II induces Egr-1 expression at the level of transcription. Ang II induced extracellular signal–regulated kinase (p42/44 ERK) activity, as did phorbol ester. The specific MEK1/2 inhibitor PD98059 suppressed both PDGF-A and Egr-1 endogenous and promoter-dependent expression inducible by Ang II. The AT1 receptor (AT1) antagonist losartan inhibited Ang II induction of p42/44 ERK as well as Egr-1 and PDGF-A, whereas neither an AT2 receptor antagonist nor wortmannin, an inhibitor of phosphatidylinositol 3-kinase, had any effect. Ang II induction of Egr-1 and PDGF-A was blocked by the nitric oxide (NO) donor SIN-1. The investigators then showed that this pathway was blocked by overexpression of NO synthase. Their findings demonstrate that Ang II activation of the PDGF-A promoter is mediated through the MEK/ERK/Egr-1 pathway and AT1 receptor and that this process is antagonized by NO.

The expression of Ang II and interleukin (IL)-6 in human coronary artery plaques suggests potential implications for inflammation and plaque instability. NF-κB is involved in these processes. Han et al. observed that Ang II induced IL-6 transcription in vascular smooth muscle cells through activation of NF-κB. This effect could be blocked completely by inhibiting the proteolysis of IκBa. Further evidence for the notion that Ang II induces NF-κB in vascular smooth muscle cells was presented by Kranzhofer et al. These investigators found that human vascular smooth muscle cells stimulated with Ang II released IL-6 dose dependently. The effect was eliminated by ACE inhibitor administration. NF-κB was activated by Ang II stimulation and inhibited by ACE inhibition and by a relatively specific inhibitor pyrrolidine dithiocarbamate. Pueyo et al. found that Ang II stimulates endothelial cells to express vascular cell adhesion molecule (VCAM)-1 through NF-κB activation induced by oxidative stress. Thus, endothelial cells, vascular smooth muscle cells, and infiltrating mononuclear cells can all be stimulated by Ang II activation of NF-κB and genes related to this transcription factor.
Recently, Kitamoto et al. have described increased NF-κB activity in the vascular wall as a consequence of NO inhibition with L-NAME. They found that NF-κB participates in early vascular inflammation and subsequent medial thickening in the coronary arteries of the rat. They used a novel decoy technique to block NF-κB activation and were thus able to separate inflammatory from fibrotic effects. In their working hypothesis, the authors speculated that Ang II was involved in the transmission of the inflammatory reaction in their model. Further information regarding inflammation and fibrosis stems from novel transgenic mice models. Tharaux et al. were able to separate the signaling pathways involved in Ang II–induced activation of the collagen I gene. They used mice harboring the luciferase gene under the control of the collagen I-a2 chain promoter. They showed that Ang II induced a rapid increase in MAPK/ERK activity that was involved in the expression of the tissue factor gene and subsequent local production of tissue factor. Because Ang II can induce endothelin gene expression, we also tested the hypothesis that part of the disease process might arise over endothelin-related mechanisms. The nonspecific endothelin receptor blocker bosentan interfered with both NF-κB and AP-1 activation and sharply reduced end-organ damage independent of blood pressure.

An appealing class of compounds that might modulate Ang II–related effects are the LDL-lowering 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase inhibitors, termed the “statins.” The rationale for using statins to ameliorate Ang II–induced vascular injury comes from various sources. In cell culture experiments that are clearly independent of any LDL-dependent effects, HMG-CoA reductase inhibition was effective in blocking PDGF and Ang II–mediated induction of c-Jun and c-Fos, components of AP-1. Vascular smooth muscle cells were also exposed to phorbol ester in the presence of the HMG-CoA reductase inhibitor lovastatin in these studies. Phorbol ester induction of AP-1 activation was inhibited, suggesting that protein kinase C (PKC) signaling is also influenced by HMG-CoA reductase inhibition. The protection was blocked by the concomitant addition of mevalonate, farnesylpyrophosphate, and geranylgeranylpyrophosphate, suggesting that the mechanisms involved indeed inhibition of mevalonate synthesis by lovastatin. In a rat study of aortic banding, simvastatin was successful in reducing left ventricular hypertrophy almost to the same degree as an ACE inhibitor. Hydroxyproline deposition, tissue ACE activity, and vascular Ang II content were reduced. Furthermore, clinical data suggest that statins may modulate the renin-angiotensin-aldosterone system. Nickeng et al. recently showed that hypercholesterolemic men have greater hypertensive responses to infused Ang II and high AT1-receptor expression compared with normocholesterolemic men. Statin treatment rapidly reversed the exaggerated response to Ang II infusion and led to a downregulation of AT1 receptors. Findings such as these prompted us to

**Double-Transgenic Rat Model**

Our group has studied a double-transgenic rat model outlined in Figure 2. The rats harbor both the human renin and human angiotensinogen genes. The rat and human renin-angiotensin systems do not interact, so the hypertension and vascular thickening in the coronary arteries of the rat. They used a novel decoy technique to block NF-κB activation and were thus able to separate inflammatory from fibrotic effects. In their working hypothesis, the authors speculated that Ang II was involved in the transmission of the inflammatory reaction in their model. Further information regarding inflammation and fibrosis stems from novel transgenic mice models. Tharaux et al. were able to separate the signaling pathways involved in Ang II–induced activation of the collagen I gene. They used mice harboring the luciferase gene under the control of the collagen I-a2 chain promoter. They showed that Ang II induced a rapid increase in MAPK/ERK activity that was involved in the expression of the tissue factor gene and subsequent local production of tissue factor. Because Ang II can induce endothelin gene expression, we also tested the hypothesis that part of the disease process might arise over endothelin-related mechanisms. The nonspecific endothelin receptor blocker bosentan interfered with both NF-κB and AP-1 activation and sharply reduced end-organ damage independent of blood pressure.
test whether or not we could ameliorate Ang II–induced end-organ damage in our model.

We found that HMG-CoA reductase inhibition with cerivastatin improved survival, decreased blood pressure, reduced proteinuria, improved renal function, reduced cardiac hypertrophy, and reduced myocardial fibrosis. To obtain insight into cellular mechanisms, we observed that the activation of NF-κB and AP-1 was attenuated. As a result, surface adhesion molecule expression, inflammatory infiltration, tissue factor production, matrix protein production, and cellular proliferation were all attenuated. We briefly studied some possible signal transduction pathways that might be important to the process. We presented evidence that cerivastatin interfered with the ERK and MAP kinase signaling pathway as well as PKC signaling. Both pathways could have a bearing on NF-kB–related and AP-1–related effects.

The mechanisms are unknown but may involve G proteins involved in receptor-coupled signal transduction, particularly Rho. The Rho proteins belong to the Ras superfamily. The Ras proteins alternate between an inactivated GDP-bound form and activated GTP-bound form, allowing them to act as molecular switches for growth and differentiation signals. Prenylation is a process involving the binding of hydrophobic isoprenoid groups consisting of farnesyl or geranylgeranyl pyrophosphates to membrane proteins that are able to supply prenyl groups. The prenylation is conducted by prenyl transferases. The hydrophobic prenyl groups are necessary to anchor the Ras superfamily proteins to intracellular membranes so that they can be translocated to the plasma membrane. The final cell-membrane fixation is necessary for Ras proteins to participate in their specific interactions. Statins decrease the production of mevalonate, geranyl pyrophosphate, and farnesylpyrophosphate and subsequent products on the way to construction of the cholesterol molecule. Thus, statins could act, independent of circulating LDL, by intracellularly interfering with Ras superfamily protein function. Ikeda et al recently showed that statins decrease matrix metalloproteinase-1 expression through inhibition of Rho. Thus, in a brief period of time, we have accrued a new view on Ang II. From conventional signaling pathways, our attention was directed toward signal transduction involving specific tyrosine kinases, inducing not only vasoconstriction but also proto-oncogene expression, protein synthesis, hypertrophy, and growth. More recently, our attention has been directed further beyond these effects to inflammatory reactions involving NF-κB activation and related gene expression. The mechanisms are not known for certain but probably initially involve the generation of reactive oxygen species. The subsequent NF-κB activation probably involves participation of endothelin-initiated signaling and perhaps NF-AT3 activation. Quite possibly, other compounds can also modulate Ang II–induced inflammatory responses.

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