During the last few years, there has been an exponential rise in understanding functions and signal transduction mechanisms for angiotensin II (Ang II) type 2 receptors (AT2s). These studies are particularly relevant in view of the pivotal role of upregulation of AT2 in mediating tissue remodeling in many cardiovascular diseases, including vascular injury, atherosclerosis, cardiac hypertrophy, myocardial infarction, and congestive heart failure. Furthermore, Ang II type 1 receptor (AT1) antagonists, commonly used for treatment of hypertension and congestive heart failure, increase plasma levels of Ang II and upregulate AT2 expression. Under these conditions, the increase in AT2 is unopposed by AT1, Thus, understanding the role of AT2 in cardiovascular remodeling as well as the consequences of AT2 stimulation or inhibition during medical therapy is clinically important. AT2s only partially share the signaling mechanisms with AT1s and, in fact, counteract the signaling mechanisms activated by AT1s (Figure). This negative nature of AT2 signaling has made the elucidation of its function more difficult than that of AT1. However, recent studies on the cardiovascular functions of AT2 seem to have reached a consensus: AT2s exert growth inhibitory effects either by suppressing cell proliferation and hypertrophy or by stimulating apoptosis.1,2,6 These actions alone may not explain the diverse cardiovascular phenomena attributed to AT2, resulting in unanswered questions. Why are AT2s abundant in growing fetal tissues? Why are AT2s abundant in tissues undergoing remodeling? Recently, an elegant genetic study has provided an answer to some of these questions, showing that an important function of AT2 in the fetal kidney is to stimulate apoptosis. Targeted deletion of AT2 causes malformation of the kidney and urinary tract similar to that observed in human CAKUT (congenital anomalies of the kidney and urinary tract), a malformation caused by delayed apoptosis of undifferentiated mesenchymal cells. Although execution of apoptosis is certainly an important function of AT2 during postnatal (pathological) cardiovascular remodeling, some studies have pointed out that AT2s may also be involved in inflammatory and cell growth processes.8–11

In this issue of Circulation Research, Ruiz-Ortega et al12 show that Ang II activates nuclear factor-κB (NF-κB) through both AT1 and AT2 in vascular smooth muscle cells. NF-κB is a ubiquitous transcription factor of particular importance in inflammatory responses.13 Many stimuli relevant to cardiovascular diseases, including proinflammatory cytokines (interleukin [IL]-1β and tumor necrosis factor-α [TNF-α]), signals elicited by ischemic stress (nitric oxide [NO] and reactive oxygen species), and mechanical forces, have been shown to activate NF-κB. Activation of NF-κB leads to coordinated increases in the expression of many genes whose products mediate inflammatory responses, including cytokines, chemokines, and adhesion molecules.13 Although activation of NF-κB by AT1 has been demonstrated previously,14 the study by Ruiz-Ortega et al12 is the first to show the linkage between AT2 and NF-κB and thus potentially clarifies the mechanism of many presently unexplained cardiovascular phenomena known to be mediated by AT2.

Functional Roles of the AT2/NF-κB Pathway
As Ruiz-Ortega et al12 point out, many inflammatory cytokines (TNF-α, IL-6, and IL-8), chemokines (monocyte chemotactant protein-1 [MCP-1]), cell adhesion molecules (vascular cell adhesion molecule-1 and intercellular adhesion molecule-1), and other molecules (tissue factor) relevant for cardiovascular remodeling are regulated by NF-κB. Other important candidates regulated by the AT2/NF-κB pathway include inducible NO synthase15 and cyclooxygenase-2,16 which mediate NO and prostaglandin and thromboxane production, respectively, in inflammatory diseases. NO synthesis and subsequent production of cGMP are stimulated by AT2 in many organs.17 Induction of cyclooxygenase-2 and activation of NF-κB have been observed in fibrotic scars in the myocardium of failing human hearts.18 Remarkably, AT2s in human hearts are predominantly localized in fibroblasts present in the interstitial region, suggesting that AT2 may be responsible for progression of inflammation and interstitial fibrosis during cardiac remodeling. Stimulation of AT2 generally inhibits growth of vascular smooth muscle cells4 and cardiac myocytes,5 but, paradoxically, it may also be involved in cell growth. For example, AT2 blockade inhibits medial smooth muscle hypertrophy and fibrosis in the thoracic aorta of Ang II–infused rats7 and spontaneously hypertensive rats.19 Because these growth stimulatory effects are seen only in vivo (and have not been reported in pure smooth muscle cell cultures), upregulation of cytokines and cell adhesion molecules by the AT2/NF-κB pathway, attraction of inflammatory cells, and additional amplification of trophic (paracrine) factors in vivo may be responsible for these AT2-mediated cell growth responses. Recently, a preliminary study suggested that pressure overload–induced cardiac hypertrophy is completely suppressed in mice with targeted

The opinions expressed in this editorial are not necessarily those of the editors of or the American Heart Association.
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Cytokine Actions of Angiotensin II

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AT₁ and AT₂ have distinct downstream targets, which counteract each other and mediate opposite cellular functions. AT₁ and AT₂ also have shared downstream targets, such as arachidonic acid and NF-κB. Whether the shared downstream targets mediate the same cellular functions is unknown. PKC indicates protein kinase C; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; MKP-1, mitogen-activated protein kinase phosphatase-1; PP2A, serine/threonine phosphatase 2A; and SHP-1, Src homology 2 protein containing tyrosine phosphatase-1.

deletion of AT₂.¹¹ Although this observation seems contradictory to known acute antihypertrophic effects of AT₂,⁵ the AT₁/NF-κB pathway may chronically regulate expression of cytokines, thereby promoting the tropic environment for hypertrophy and remodeling. Alternatively, stretch-induced secretion of Ang II and subsequent amplification of inflammatory cytokines through the AT₂/NF-κB pathway may mediate pressure overload–induced cardiac hypertrophy. In fact, macrophage infiltration has been demonstrated in the myocardium subjected to mechanical overload.¹⁹ Expression of AT₂ is upregulated by IL-1β, insulin, and insulin-like growth factor and downregulated by glucocorticoids.²⁰ All these stimuli exert directionally similar effects on NF-κB and AT₂. This raises the possibility that the NF-κB site found in the AT₂ promoter may mediate transcription of AT₂. Products of genes that are regulated by NF-κB in many cases cause activation of NF-κB, and this positive-feedback loop can amplify and perpetuate local inflammatory responses.¹³ This suggests that the AT₂/NF-κB pathway could be a component of such an amplification loop, which eventually enhances expression of both cytokines and AT₂. Increased production of angiotensinogen by NF-κB may additionally enhance the amplification loop.²¹ As noted above, stimulation of AT₂ promotes apoptosis, whereas activation of NF-κB generally promotes cell survival. Although this seems contradictory, NF-κB can promote apoptosis by increasing expression of Fas and Fas ligand in fibroblasts²² or decreasing bcl-2 expression in aortic endothelial cells.²³ This cell-type specific action of NF-κB may in part explain why AT₂ does not promote apoptosis in some cell types.²⁴

**Signaling Mechanism of NF-κB Activation by Ang II**

Another interesting aspect of the work by Ruiz-Ortega et al¹² is that both AT₁ and AT₂ are able to activate NF-κB. AT₂ activates protein phosphatases, including SHP-1, PP2A, and MKP-1, thereby inactivating tyrosine kinases and mitogen-activated protein kinases stimulated by AT₁.² It is interesting that NF-κB activation is one of the few examples reported thus far in which AT₁ and AT₂ share the signaling mechanism at least in part (Figure). Although both AT₁ and AT₂ stimulation cause degradation of inhibitor κB (IkB),²³ an essential step for nuclear translocation of NF-κB, the mechanism leading to IkB degradation by AT₁ and AT₂ stimulation remains to be clarified. Degradation of IkB depends on phosphorylation of the two N-terminus serines by IkB kinase, and the phosphorylated IkB undergoes polyubiquitination and proteasome degradation.²⁴ Degradation of IkB can be also mediated by phosphorylation of IkB at tyrosine 42 or by unknown mechanisms involving protein tyrosine phosphatases.²⁵,²⁶ Therefore, it will be interesting to determine whether NF-κB activation by AT₁ and AT₂ is mediated by either IkB kinase–dependent mechanisms or other mechanisms in which tyrosine kinases or phosphatases (directly or indirectly) modulate IkB degradation. Ruiz-Ortega et al²² suggest that oxygen radicals and ceramide are common mediators of IkB-κB activation by AT₁ and AT₂. However, how AT₂ stimulates production of oxygen radicals and ceramide in cardiovasculatcell types remains to be elucidated.

**Unresolved Questions**

Several important questions remain unanswered. First, in cultured smooth muscle cells, Ang II–induced increases in MCP-1 and angiotensinogen, well-known targets of NF-κB, were mediated predominantly by AT₁, despite both AT₁ and AT₂ stimulation activating NF-κB in this cell type.¹² Because AT₁ is the predominant Ang II receptor subtype in cultured aortic vascular smooth muscle cells, the possibility remains that these NF-κB targets may be regulated more potently by AT₁ when expression of AT₂ is upregulated in pathological conditions. It is also possible that AT₁ and AT₂ regulate different molecules through NF-κB. For example, the AT₁/NF-κB pathway may regulate predominantly proinflammatory cytokines, whereas the AT₂/NF-κB pathway may have different targets. The mechanisms by which AT₁ and AT₂ regulate distinct NF-κB targets remain to be clarified. Because NF-κB is known to function in concert with other transcription factors such as AP-1 and C/EBP, these cofactors may be regulated differentially by AT₁ and AT₂.

Another important question is whether the AT₁/NF-κB pathway always exerts a beneficial action in cardiovascular diseases.¹ The answer is not simple, considering that NF-κB activation can be both beneficial and detrimental. For example, NF-κB activation is essential for the development of the cardioprotective effects of preconditioning.²⁷ On the other hand, many studies have shown that inhibition of NF-κB can also have a salutary effect on cardiovascular diseases. For example, in vivo transfer of NF-κB decoy oligonucleotides reduces the extent of myocardial infarction and reperfusion injury.²⁸,²⁹ Inhibition of NF-κB reduces Ang II–induced organ damage in the heart and kidney by preventing inflammatory mediators.³⁰ In the vasculature, inhibition of NF-κB suppresses development of atherosclerotic lesions by preventing inflammation,³¹ smooth muscle cell proliferation,³² and dysregulation of apoptosis.³³ In a recent review, Matsubara¹ discussed the potential advantages of shunting Ang II toward...
AT2 (during AT1 blockade) in the treatment of cardiovascular diseases. This hypothesis is well supported by the results of a recent study showing that losartan was associated with a lower mortality rate than captopril in the treatment of older heart failure patients.\textsuperscript{54} It has been shown that cardioprotective effects of AT1 antagonists in a rat model of heart failure are prevented by AT1 blockade.\textsuperscript{35} It will be extremely important to identify the targets of the AT2/NF-kB pathway in each disease condition and evaluate in which disease states stimulation of AT2 could be beneficial.

Acknowledgments

The author thanks Dr Stephen F. Vatner for critical reading of this manuscript.

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Cytokine Actions of Angiotensin II
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*Circ Res.* 2000;86:1187-1189
doi: 10.1161/01.RES.86.12.1187

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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