The renin–angiotensin system (RAS) plays a major role in regulating the cardiovascular system, and disorders of the RAS contribute largely to the pathophysiology of hypertension, renal diseases, and chronic heart failure. Two subtypes of angiotensin II (Ang II) receptors have been defined on the basis of their differential pharmacological and biochemical properties: Ang II type 1 receptors (AT₁), which are involved in most of the well-known physiological effects of Ang II, and Ang II type 2 receptors (AT₂), which have a less well-defined role but appear capable of counterbalancing some of the effects of AT₁ stimulation.

The 2 receptors, both of which belong to the superfamily of G-protein–coupled receptors, are believed to have different signaling pathways and different functions. AT₁ transactivates growth pathways and mediates major Ang II effects such as vasoconstriction, increased cardiac contractility, renal tubular sodium reabsorption, cell proliferation, vascular and cardiac hypertrophy, inflammatory responses, and oxidative stress. AT₂ is believed to induce essentially opposite effects, including vasodilation and antityrosogen and antihypertrophic effects, and to play a significant role in blood pressure (BP) regulation.

The 2 major pharmacological inhibitors of the RAS, which are now important elements in the treatment of hypertension and cardiovascular disease, are ACE inhibitors and angiotensin receptor blockers (ARBs). These 2 classes of drugs have different effects on the RAS: suppression of Ang II production by ACE inhibitors reduces activation of both Ang II receptor subtypes, whereas ARBs preferentially block AT₁ and leave AT₂ unopposed. Long-term administration of ARBs results in a several-fold increase in plasma Ang and thus a possible overstimulation of AT₂. It is generally accepted that the effects of stimulation of AT₁ on the cardiovascular system are beneficial and that no harm would result from increased activation of these receptors; indeed, activation of AT₁ is believed to contribute to the benefits of blocking AT₁. However, it was difficult to distinguish exactly which of the beneficial effects observed with ARBs arise from a fall in BP and which are due to activation of AT₂.

Recent work has attempted to unravel the receptor type–dependent effects by using selective AT₂ antagonists or agonists or mice that have been genetically modified so that they either lack the gene coding for AT₂ or overexpress it. The results of this work often appear to be conflicting, but some of the evidence suggests that activation of AT₂ could, in certain contexts, exert growth stimulatory and proinflammatory effects that result in parallel, rather than opposite, effects to AT₁ stimulation. This article will attempt to briefly review the evidence on the cardiovascular role of AT₂ and to discuss possible implications of overstimulation of these receptors in long-term ARB therapy.

Location and Expression of AT₂ Relative to AT₁ in Normal and Pathological States

In humans, AT₁ is widely expressed at relatively constant levels in adults and is located in numerous tissues, including the blood vessels, heart, kidneys, adrenal glands, and liver. In contrast, AT₂ is mainly present during fetal development (and is believed to have an essential role in physiological vascular development) but decreases rapidly after birth. In adults, expression of AT₂ under normal conditions is largely restricted to the adrenals, kidneys, uterus, ovary, heart, and specialized nuclei in the brain, but it is upregulated in various pathological conditions associated with tissue remodeling or inflammation, including hypertension, heart failure, postmyocardial infarction, ischemia, and diabetes. Multiple hormonal, cytokine, and metabolic factors are involved in upregulating AT₂ densities on cell surfaces. Conversely, downregulation of AT₂ may be brought about by glucocorticoids and growth factors, and perhaps also by AT₁. The latter possibility is suggested by the finding in experimental studies that AT₂-dependent NO production can only be observed after AT₁ blockade by losartan.

Thus, although AT₂ is present in the vasculature in adults, its distribution is not homogeneous and is subject to changes according to age, vessel type, and the presence of patholog-
ical states. This raises the important question of their function in the cardiovascular system relative to that of AT₁.

The 2 receptor subtypes also appear to have different signaling pathways. However, the finding that activation of AT₂ may, in some tissues, result in parallel rather than opposite effects to AT₁ activation (see below) suggests that AT₁ and AT₂ may share, at least in part, some common signaling pathways.

**Role of AT₂ in Controlling Vascular Tone and Arterial BP**

There is abundant support for the concept that AT₂ has a role in controlling vascular tone by mediating vasodilation and counterbalancing the AT₁-mediated vasoconstrictor effect of Ang II. Rats subjected to a high-salt diet were found to exhibit a higher rise in BP in response to Ang II infusion when the selective AT₂ blocker PD123319 was coadministered. In support of this finding, studies in AT₂-knockout mice (ie, mice lacking AT₂) have shown that these animals have a higher BP and increased sensitivity to the pressor action of Ang II than wild-type mice. Conversely, transgenic mice overexpressing AT₂ in the vasculature failed to show a pressor response to Ang II infusion.

The role of AT₂ in mediating control of vasomotor tone has been investigated in experimental studies in various vascular territories, including the mesenteric and kidney circulations and the uterus. These studies have indicated that AT₂ plays a protective counterregulatory role against the pressor and antinatriuretic actions of Ang II.

In the presence of AT₁ blockade, therefore, overstimulation of AT₂ is likely to have a beneficial role in controlling BP in hypertension, particularly as it has been suggested that dysregulation of AT₂-mediated vascular tone may play a role in the pathogenesis of this disease. This concept is supported by a recent study in isolated rat mesenteric resistance arteries that showed that AT₂-mediated vasorelaxation, induced either by Ang II or the AT₂ agonist CGP42112, is preserved after long-term treatment with the ARB candesartan cilexetil.

**Role of AT₂ in Control of Cardiovascular Structure**

In addition to its role in the regulation of arterial pressure, Ang II is known to mediate effects on cell growth and apoptosis and to have pro-oxidative and proinflammatory effects. Indeed, Ang II has been shown in both human and animal models to be involved in the development of cardiomyocyte hypertrophy and cardiac fibrosis and the modulation of cardiac fibroblast growth and collagen synthesis. In addition, a role in stimulating apoptosis, which is known to be a contributing cause of vascular wall remodeling and cardiomyocyte loss in ischemia-reperfusion injury and myocardial infarction, has also been demonstrated in human and animal models. These findings point to a major role of Ang II in the processes of cardiovascular remodeling and cardiac hypertrophy in association with hypertension and have led to studies of the role of the 2 subtypes of Ang II receptors in mediating this response.

**Involvement of AT₂ in Cardiovascular Hypertrophy–Associated Changes**

Hypertrophy of cardiac myocytes is an adaptive response in the damaged heart. Initially, hypertrophy acts as a compensatory mechanism to preserve cardiac function, but when sustained, it becomes a major risk factor for congestive heart failure and sudden cardiac death.

Until recently, most in vitro and in vivo studies of the roles of AT₁ and AT₂ indicated that AT₁ mediates the growth-promoting, fibrotic, and hypertrophic effects of Ang II on cardiovascular tissues and that AT₂ exerts counterbalancing suppressant effects. Recently, however, a number of reports have suggested a possible different role for AT₂, ie, that its activation may mediate a growth-promoting response in cardiovascular tissues and that these effects may parallel rather than oppose those evoked by AT₁ stimulation. For example, in 1996, we reported that in normotensive Wistar rats receiving hypertensive doses of Ang II, chronic blockade of AT₂ with PD123319 had no effect on arterial pressure but antagonized the effect of Ang II on arterial hypertrophy and fibrosis, which suggests that the in vivo vasotrophic effects of Ang II may be mediated at least in part via AT₂.

In contrast, chronic blockade of AT₁ with losartan lowered BP but led to smooth muscle cell hypertrophy and hyperplasia. This study and several others that suggest either prohypertrophic or antihypertrophic effects of AT₂ are summarized in the Table.

The fact that growth-promoting effects of AT₂ were not observed in earlier in vitro studies is not surprising, because AT₂ disappears rapidly in ordinary cell culture conditions. However, the in vivo experiments shown in the Table appear to show that AT₂ both suppresses and promotes vascular cell growth, hypertrophy, and fibrosis, and these conflicting results are more difficult to explain. One possible explanation, as suggested by Inagami and Senbonmatsu, lies in the fact that the strain of AT₂-knockout mice used by Akishita et al differed from that used by Senbonmatsu et al. In addition, the various studies examined cardiovascular responses under different conditions. Clearly, ligands, receptors, and transducers often play different roles depending on the particular environment and conditions and are not intrinsically "good" or "bad."

**Involvement of AT₂ in Apoptosis of Vascular Smooth Muscle Cells and Cardiomyocytes**

Inappropriate apoptosis contributes to the pathogenesis of a number of cardiac diseases and is recognized as an important factor in cardiovascular remodeling. Apoptosis may also play a role in microvascular rarefaction, which has been shown to occur in hypertension, and may contribute to the development of hypertension, as suggested in recent experiments in mice with a defective endothelial NO synthase gene. On the other hand, cell death by apoptosis is an important mechanism of cell population control in organ development and normal tissue homeostasis.

With apoptosis, as with hypertrophy, the results from studies using different antagonists or receptor-knockout mice have proved confusing. Stimulation of both AT₁ and AT₂ by Ang II has been shown to enhance apoptosis in aortic smooth
Recent Studies of the Effects of AT2 on Cardiovascular Remodeling

<table>
<thead>
<tr>
<th>Model</th>
<th>Intervention and Result</th>
<th>Conclusion</th>
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<tr>
<td>Studies suggesting that AT2 plays a role in inducing hypertrophy</td>
<td>1. Infusion of Ang II: BP increased relative to controls. SMC hypertrophy and hyperplasia, media thickness, and aortic elastin and collagen content all increased. 2. Infusion of Ang II + AT1 blockade with losartan: BP was lowered relative to controls. SMC hypertrophy and hyperplasia, media thickness, and aortic elastin and collagen content all increased. 3. Infusion of Ang II + AT2 blockade with PD123319: BP increased relative to controls. There was no SMC hypertrophy or hyperplasia and no increase in media thickness or aortic elastin or collagen content.</td>
<td>AT2 must be involved in mediating Ang II–induced arterial hypertrophy and fibrosis.</td>
</tr>
<tr>
<td>Normotensive rats7</td>
<td></td>
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<tr>
<td>Rats25</td>
<td>1. Infusion of Ang II: BP increased, and mesenteric SMC growth and vascular hypertrophy occurred. 2. Infusion of Ang II + AT2 blockade with valsartan: Hypertension was prevented, and the vascular changes were attenuated. 3. Infusion of Ang II + AT2 blockade with PD123319: BP increased, but the vascular changes were attenuated.</td>
<td>Both AT1 and AT2 mediate the trophic and proliferative effects of Ang II on mesenteric vasculature.</td>
</tr>
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<td>AT2-/- mice26</td>
<td>Aortic banding induced marked LVH in WT mice but no LVH in AT2-/- mice. Cardiac contractile function was unaffected in the AT2-/- mice.</td>
<td>AT2 mediates cardiac hypertrophy in response to increased BP.</td>
</tr>
<tr>
<td>AT2-/- mice27</td>
<td>Ang II elevated BP to comparable levels in AT2-/- and WT mice. WT mice developed prominent hypertrophy, fibrosis, and impaired diastolic relaxation, but AT2-/- mice had no hypertrophy or impairment of relaxation and only negligible fibrosis.</td>
<td>AT2 plays a significant role in cardiomyocyte hypertrophy and fibrosis in Ang II–induced hypertension.</td>
</tr>
<tr>
<td>Studies suggesting that AT1 inhibits hypertrophic remodeling</td>
<td>Placement of a nonconstricting cuff around the femoral artery (to stimulate vascular inflammation) led to greater arterial thickening and SMC proliferation relative to WT mice.</td>
<td>AT1 inhibits coronary artery remodeling in nonocclusive inflammatory injury.</td>
</tr>
<tr>
<td>AT1-/- mice28</td>
<td>Aortic banding led to greater arterial thickening and perivascular fibrosis relative to WT mice.</td>
<td>AT1 mediates an inhibitory effect on coronary arterial remodeling.</td>
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<tr>
<td>Studies suggesting that AT1 may induce or oppose remodeling</td>
<td>Losartan and enalapril both reduced BP and collagen concentration to the levels found in WT rats, but PD123319 had no effect. Enalapril and PD123319 reduced media cross-sectional area of aorta relative to untreated SHR, but losartan had no effect.</td>
<td>AT1, but not AT2, plays a role in remodeling of matrix tissue. AT2 may play a role in hypertrophy of aortic SMCs in SHR.</td>
</tr>
<tr>
<td>SHR30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SMC indicates smooth muscle cell; AT2-/-, AT2-deficient mice; LVH, left ventricular hypertrophy; WT, wild-type; and SHR, spontaneously hypertensive rat.

Possible explanation for some of these discrepancies is provided by a study that investigated the effects of Ang II on apoptosis of 2 morphologically different rat aortic smooth muscle cell phenotypes.53 Ang II induced apoptosis of epithelioid cells but not spindle cells, and this apoptosis was mediated by AT1 but not AT2. Thus, the ability of Ang II to mediate apoptosis in vascular smooth muscle cells may depend on the cell phenotype.

In a study of spontaneously hypertensive rats, AT2 was shown (using PD123319) to stimulate apoptosis of smooth muscle cells in vivo in the presence of AT1 blockade with losartan.44 PD123319 given alone did not affect growth or apoptosis.

More recently, in a study in AT2-/- (Agtr2-/-Y) and wild-type mice with surgically induced hindlimb ischemia, we showed that AT2 exerted an antiangiogenic effect that was associated with activation of apoptosis.45 We speculate that AT2 may control vessel growth associated with tissue ischemia via activation of the apoptotic reaction. AT2 may also directly or indirectly modulate other cellular pathways that

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Muscle cells,24 cardiomyocytes,35 glomerular epithelial cells,36 and renal proximal tubular cells.37 In accordance with this finding, blockade of AT1 with irbesartan and of AT2 with PD123319 prevented Ang II–induced apoptosis in cultured cardiomyocytes.35 A study in cultured cell lines that express abundant AT2 but not AT1 showed that AT2 mediates apoptosis.38 AT2 is expressed in the adult rat kidney and was shown to promote apoptosis and cellular proliferation in proximal tubular epithelial cells.39 Recently, it was shown that activation of AT2 could induce vascular cell apoptosis and thus participate in the early phase of vascular remodeling in spontaneously hypertensive rats subjected to chronic AT1 blockade with losartan.40

However, some studies have reported opposing results. Studies in transgenic mice overexpressing AT1 have indicated that Ang II infusion does not induce apoptosis in isolated cardiomyocytes.41 Similarly, AT1 blockade after acute ischemia-reperfusion in isolated rat hearts was associated with increased AT2 protein expression and cardioprotection, but there was no increase in apoptosis of cardiomyocytes.42 A
are involved in regulating angiogenesis. In contrast to this finding that AT₂ inhibits angiogenesis, the ACE inhibitor quinaprilat was found to promote angiogenesis in a rabbit model of hindlimb ischemia, and the low-dose combination of the ACE inhibitor perindopril and the diuretic indapamide increased revascularization in rat ischemic legs. A separate study in mice deficient for the bradykinin B₁ receptor (B₁−/− mice) suggested that this proangiogenic effect of ACE inhibition is mediated by B₂ signaling.

Angiogenesis is a necessary corrective process for overcoming the effects of long-term ischemia. Any disruption of angiogenesis that may arise from stimulation of AT₂ in the context of AT₁ blockade could therefore have serious implications in ischemic tissues such as a diseased myocardium or in lower limbs affected with peripheral arterial disease.

**Implications for Therapy With Inhibitors of the RAS**

As described above, the increased stimulation of AT₂ that occurs in the presence of AT₁ blockade was believed to contribute to the benefits of ARBs, not just through control of BP but also through the antihypertrophic and antifibrotic effects of such stimulation. In addition, Ang II can be produced by alternative pathways and is not completely blocked by ACE inhibitor therapy. For these 2 reasons, it was thought that ARBs might offer greater benefits than ACE inhibitors in patients with cardiovascular disease.

However, it is now clear that the effects of AT₂ are context dependent. There are species-dependent and vessel type–dependent differences in the vascular responses to AT₂ stimulation, and it is therefore difficult to predict the effects in human beings of long-term overstimulation of AT₂ resulting from ARB therapy. This information has to be gathered from the results of clinical trials in real patients.

In this regard, the Evaluation of Losartan In The Elderly (ELITE) II trial demonstrated that the AT₁ receptor blocker losartan was not superior to the ACE inhibitor captopril in reducing morbidity and mortality in patients with heart failure. On the contrary, total mortality, myocardial infarction, and stroke all showed a nonsignificant trend toward lower rates in the captopril group, and the difference for sudden death was close to significant (hazard ratio for losartan versus captopril 1.30, 95% CI 1.00 to 1.69). Because a benefit of losartan had been predicted on the basis of the smaller preceding ELITE trial, the results of ELITE II show that it is unwise to predict clinical outcomes based on any one mechanism of action, especially with drugs that affect complex systems. The Optimal Trial in Myocardial Infarction with the Angiotensin II Antagonist Losartan (OPTIMAAL) also failed to show an advantage for ARBs over ACE inhibitors; in fact, most end points showed a trend in favor of captopril over losartan, with cardiovascular death significantly lower in the captopril group. The trial was designed to show superiority or noninferiority of losartan relative to captopril, but it did not do so. These results appear to indicate that ARBs do not in fact offer benefits in reducing cardiovascular end points relative to ACE inhibitors, although there have also been suggestions that a suboptimal dosage of losartan may have been used in both trials. A higher dose of losartan was used in the Losartan Intervention For End point reduction in hypertension (LIFE) study, in which losartan was superior to atenolol (a non-RAS blocker) in reducing overall cardiovascular morbidity and, in particular, stroke.

Two reasons for supposing that ARBs may have been more successful than ACE inhibitors in treating patients with cardiac disease have been mentioned above. However, a third factor, the effect on bradykinin, is likely to favor ACE inhibitor above ARB therapy. ACE inhibition prevents the breakdown of bradykinin, a peptide that in turn has vasodilator and other favorable effects. Interestingly, a recent study using knockout mice lacking the B₁ receptor for bradykinin suggests that these receptors play an essential role in the host defense response to ischemic injury.

Combined treatment with an ACE inhibitor and an ARB could theoretically offer more benefit than either drug alone by combining the more complete RAS system blockade that is provided with the ARB with the potentiation of bradykinin provided by the ACE inhibitor. However, the Valsartan in Acute Myocardial Infarction Trial (VALIANT), which compared the use of valsartan, captopril, or a combination of the 2 drugs in patients with myocardial infarction associated with heart failure and/or left ventricular dysfunction, evidenced that valsartan is as effective as captopril and a combination of drugs did not improve survival. In this regard, interesting results on the progression of renal disease have recently been reported by Nakao et al. Renal disease was outside the scope of the present review, but it is well known that the RAS has an important role in the progression of nondiabetic renal disease, and both AT₁ and AT₂ are present in the kidney. Nakao et al. compared the effect of 3 years’ treatment with high doses of trandolapril, losartan, or a combination of the 2 drugs on renal end points in 263 patients with nondiabetic renal disease. With combination therapy, there was an improvement in event-free survival and a further decrease in urinary protein excretion relative to therapy with either agent alone. This suggests that the addition of trandolapril to losartan had a beneficial effect, possibly through a reduction in AT₂ activity, an increase in bradykinin activity, or both.

**Conclusions**

The experimental findings reviewed in this article point to a need for a more in-depth understanding of the role of AT₂ in the cardiovascular system. On the one hand, selective blockade of AT₁ and resultant overstimulation of AT₂ by ARBs may be predicted to have a beneficial effect in mediating vasodilation and controlling BP in hypertension. However, accumulating evidence now suggests that long-term AT₂ stimulation might also exert a hypertrophic and antiangiogenic influence on cardiovascular tissues. Thus, the long-term consequences of ARB therapy might be less beneficial than has been previously supposed and could even be harmful in some circumstances. The potential consequences of such effects, if found to be clinically important, might include cardiac hypertrophy, vascular fibrosis, and a decrease in neovascularization in hypoxic tissues such as the myocardium.

In large-scale randomized clinical trials to date, ARBs have failed to live up to the high expectations that they would
prove superior to ACE inhibitors in the context of chronic heart failure. The concept that increased stimulation of AT2 may have harmful as well as beneficial effects provides a possible explanation for this finding.

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**Key Words:** receptors ■ angiotensin ■ cardiovascular diseases ■ hypertrophy

Lévy AT2 Angiotensin Receptors in CVD 13
Can Angiotensin II Type 2 Receptors Have Deleterious Effects in Cardiovascular Disease?: Implications for Therapeutic Blockade of the Renin–Angiotensin System

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