Angiotensin II Receptor Blockade and End-Organ Protection

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The renin-angiotensin system (RAS) is a widely studied hormonal system that comprises substrate-enzyme interactions, the end result of which is production of the active peptide angiotensin II (Ang II). Because Ang II affects blood pressure control, sodium and water homeostasis, and cardiovascular function and structure, a great deal of research effort has been directed toward blocking the RAS. Angiotensin II may also be involved in end-organ damage in hypertension, heart failure, and vascular disease. At least two subtypes of angiotensin II receptors have been identified: AT₁ and AT₂. The AT₁ mediates all of the known actions of Ang II on blood pressure control. Additionally, research has indicated that the AT₁ receptor modulates cardiac contractility and glomerular filtration, and increases renal tubular sodium reabsorption, and cardiac and vascular hypertrophy. Less is known regarding the function of the AT₂ receptor. Evidence suggests that the AT₂ receptor inhibits cell proliferation and reverses AT₁-induced hypertrophy. Indeed, these receptors are thought to exert opposing effects.

Angiotensin II AT₁ receptor antagonists (AT₁RA) inhibit the RAS at the receptor level by specifically blocking the AT₁ receptor subtype. These drugs induce a dose-dependent blockade of Ang II effects, resulting in reduced blood pressure, urinary protein, and glomerular sclerosis. It is postulated that AT₁RA may provide end-organ protection by blocking Ang II effects via the AT₁ receptor, yet leaving the AT₂ receptor unopposed. Consequently, these agents may reduce the morbidity and mortality that result from myocardial infarction (MI) and other conditions resulting from structural alterations in the heart, kidney, and vasculature. Am J Hypertens 1999; 12:150S–156S © 1999 American Journal of Hypertension, Ltd.

KEY WORDS: Renin-angiotensin system, end-organ protection, angiotensin receptors, AT₁ receptor antagonists, ACE inhibitors, hypertension, congestive heart failure, myocardial infarction, left ventricular hypertrophy, vasculature.
include cardiac left ventricular hypertrophy (LVH) and structural alterations of the vasculature, heart, and kidney (eg, neointima formation, postinfarct remodeling, and nephrosclerosis). In this regard, Ang II has been recognized as a growth-promoting factor that contributes to structural alterations in various organs.

**DEVELOPMENT OF ANG II RECEPTOR BINDING SUBSTANCES**

In an endeavor to block the RAS specifically at the level of its receptors, Ang II-receptor antagonists were developed. As the first of its class, saralasin (Sar1 Ile8-Ang II) was introduced in 1971. However, saralasin and other antagonists, such as sarlesin or sarmesin, were all based on a peptide structure that precludes oral application. Besides their lack of bioavailability, these peptide-based Ang II-receptor antagonists also featured the disadvantage of having a short duration of action and high intrinsic activity by themselves. Furthermore, because they were unable to discriminate between the different Ang II receptor subtypes, they unselectively blocked all available Ang II receptors. In 1982, imidazole-5-acetic acid derivatives were introduced, and it was soon recognized that these compounds were capable of antagonizing Ang II-induced vasoconstriction in isolated vessels. This was the basis for the further development of highly specific and selective angiotensin AT1 receptor binding substances such as losartan, valsartan, irbesartan, candesartan, eprosartan, telmisartan, and others, as well as AT2 receptor ligands/antagonists (eg, PD123177, PD123319, and CGP42112). The novelty of these substances is twofold. First, they express the AT1 receptor subtype. Subsequently, the role of the AT1 receptor has been well documented after the cloning of the receptor and localization of the gene. The binding of Ang II to the AT1 receptor is responsible for most of the peripheral and central effects of Ang II on blood pressure, osmoregulation, and cell growth, and thus for the contribution of the RAS to cardiovascular and renal pathology. Distribution of the AT1 receptor in adults is ubiquitous, including the vasculature, kidney, adrenal gland, heart, liver, and brain. This receptor is involved in vasoconstriction, cardiac contractility, aldosterone and arginine vasopressin release, renal salt retention, vascular hypertrophy, and cardiac hypertrophy. In rodents (but not in humans), the AT1 receptor subtype exists in two isoforms: AT1a and AT1b. Although the AT1a and AT1b subtypes are expressed more or less equally in the spleen, liver, and kidneys, the AT1a receptor seems to be predominant in vascular smooth muscle, heart, lung, ovary, and hypothalamus. Angiotensin II type 1 receptors are found mainly in vascular smooth muscle, suggesting that this subtype plays a role in vasoconstriction. On the other hand, as the AT1b receptor subtype seems to prevail in the anterior pituitary, adrenal gland, uterus, and several periventricular brain areas, this receptor may be involved in hormonal secretion and central osmotic control.

The AT1 receptor belongs to the superfamily of a 7-transmembrane domain and G-protein–coupled receptors and has an estimated molecular weight of 65 kilodaltons. It interacts with various G-proteins and is coupled to one of the two heteromeric G-proteins: Gq or G11. The binding of Ang II to specific sites on the extracellular and membrane-spanning portions of the AT1-receptor results in the release of the α subunit of the G-protein and the subsequent activation of phospholipase C (PLC) via Gi or inhibition of adenylate cyclase via Gi, respectively. The PLC activation results in the generation of 1, 4, 5-trisphosphate and diacylglycerol, leading to a subsequent activation of protein kinase C and an increase in intracellular calcium levels via L-type calcium channels.

**ANGIOTENSIN II RECEPTOR SUBTYPES**

The availability of these highly specific and selective AT1 receptor antagonists was the basis for the identification of Ang II receptor subtypes. Although in the past experimental and clinical efforts were guided by the belief that the effects of Ang II were mediated by a single receptor, it is now known that this is not the case. Two main angiotensin receptor subtypes have been characterized—AT1 and AT2—which display a heterogeneous distribution in peripheral tissues and in the brain. Although they belong to the same receptor family, their signal transduction and function differ.

**The AT1 Receptor** Evidence that the AT1 is responsible for virtually all the known classic actions of Ang II came early, with the observation that vascular smooth muscle cells predominantly, if not exclusively, express the AT1 receptor subtype. Subsequently, the role of the AT1 receptor has been well documented after the cloning of the receptor and localization of the gene. The binding of Ang II to the AT1 receptor is responsible for most of the peripheral and central effects of Ang II on blood pressure, osmoregulation, and cell growth, and thus for the contribution of the RAS to cardiovascular and renal pathology. Distribution of the AT1 receptor in adults is ubiquitous, including the vasculature, kidney, adrenal gland, heart, liver, and brain. This receptor is involved in vasoconstriction, cardiac contractility, aldosterone and arginine vasopressin release, renal salt retention, vascular hypertrophy, and cardiac hypertrophy. In rodents (but not in humans), the AT1 receptor subtype exists in two isoforms: AT1a and AT1b. Although the AT1a and AT1b subtypes are expressed more or less equally in the spleen, liver, and kidneys, the AT1a receptor seems to be predominant in vascular smooth muscle, heart, lung, ovary, and hypothalamus. Angiotensin II type 1 receptors are found mainly in vascular smooth muscle, suggesting that this subtype plays a role in vasoconstriction. On the other hand, as the AT1b receptor subtype seems to prevail in the anterior pituitary, adrenal gland, uterus, and several periventricular brain areas, this receptor may be involved in hormonal secretion and central osmotic control.

**The AT2 Receptor** Far less is known about the signaling pathways and physiologic actions of the AT2 receptor subtype. Ubiquitous in fetal tissue, the AT2 receptor in adults is present in high concentrations only in the adrenal medulla, uterus, ovary, vascular endothelium, and distinct brain areas. The distribution of the AT2 receptor in humans. Increased expression or dominance of the AT2 receptor has also been documented in pathophysiologic conditions such as cardiac failure, postinfarct repair, and skin and nervous system lesions. Figure 2 shows the comparative expression of the AT1 and AT2 genes in the left ventricle of rat hearts after acute
myocardial infarction. Consequently, the AT2 receptor is believed to be involved in the inhibition of cell proliferation, differentiation and development, regeneration, and apoptosis.\textsuperscript{1,20,21} Although the AT2 receptor has been cloned recently, its molecular structure and signal transduction pathway are far from being completely understood. The rat AT2 receptor cDNA encodes for a 363-amino acid protein that has a 7-transmembrane topology and only a 32\% to 34\% homology in its amino acid sequence to the AT1 receptor. It is still controversial whether or not the AT2 receptor is coupled to G-proteins, and how it signals.

Recent evidence from studies in rat kidney and aorta suggests that Ang II can also interact with the bradykinin/nitric oxide system by an AT2 receptor-dependent mechanism.\textsuperscript{25–27} These diverse data have been extended by findings in AT2 receptor knockout mice.\textsuperscript{28,29} These mice show an increased sensitivity to the pressor actions of Ang II, and an altered exploratory behavior and drinking response to water deprivation.\textsuperscript{28,29} Basal blood pressure in AT2 knockout mice was increased in one study,\textsuperscript{28} but remained unaltered in another study.\textsuperscript{29}

**INHIBITION OF THE RENIN-ANGIOTENSIN SYSTEM**

The effectiveness of angiotensin-converting enzyme (ACE) inhibitors in treating hypertension, congestive heart failure (CHF), myocardial infarction (MI), and diabetic nephropathy demonstrates the benefits of inhibition of the RAS.\textsuperscript{1} Recently, angiotensin II AT\textsubscript{1} receptor antagonists (AT1RA) have been introduced as orally active antihypertensive agents. In animal studies, they have been found to improve cardiac, vascular, and renal structure and function to a similar degree as ACE inhibitors.\textsuperscript{26,30} Although ACE inhibitors and AT1RA have different mechanisms of action, they both diminish the effects of Ang II by differential blockade of the RAS. Angiotensin-converting enzyme inhibitors work by inhibiting the ACE-induced enzymatic conversion of the inactive decapeptide Ang I to the active octapeptide Ang II. However, because Ang II can also be generated by non-ACE enzymes, such as chymase,\textsuperscript{31} the functional RAS blockade with ACE inhibitors may not be complete. Further, ACE is not a specific enzyme with multiple potential substrates, including bradykinin and tachykinins such as substance P. Angiotensin II AT\textsubscript{1} receptor antagonists block the AT\textsubscript{1} receptor, do not interfere directly with bradykinin metabolism, and leave the AT2 receptor unopposed. Moreover, blockade of the Ang II-induced– and AT\textsubscript{1} receptor-mediated feedback inhibition of renin release from the kidney results in increased plasma renin activity and, consequently, increased plasma Ang II levels.\textsuperscript{27,32} Therefore, AT1RA may have an advantage over ACE inhibitors in that

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**FIGURE 1.** Distribution of the AT\textsubscript{2} receptor, which is ubiquitous in fetal tissue, and is present in high concentrations in adults only in the adrenal medulla, uterus, ovary, vascular endothelium, and distinct brain areas.

**FIGURE 2.** AT\textsubscript{1} and AT\textsubscript{2} gene expression in the left ventricle of rat hearts after acute myocardial infarction. Solid bars, infarcted animals; open bars, sham-operated animals.\textsuperscript{17}
they selectively inhibit the AT$_1$, allowing for Ang II to bind to the AT$_2$ receptor. This potentially enhances the beneficial opposing effects of these receptors on endothelial proliferation, vasoconstriction, and tissue repair.$^2$

Recent findings regarding the generation of Ang II in the heart points to the importance of the potential ability of AT$_1$RA to exploit the beneficial opposing effects of the Ang II receptors. In untreated pigs, tissue Ang II levels in various parts of the heart were found to be five times higher than the level in plasma, whereas the tissue Ang I level was $75\%$ of the level in plasma.$^{33}$ After the administration of the ACE inhibitor captopril, Ang II plasma levels fell, but levels in tissue were unaffected. Levels of Ang I and renin rose in both plasma and tissue, whereas tissue levels of angiotensinogen fell and plasma levels remained unchanged. These findings indicate that cardiac Ang II can be produced at the tissue level by conversion of in situ-synthesized rather than blood-derived Ang I. These data also suggest that the heart can maintain Ang II production despite decreased circulating Ang II. When viewed in the context of previous stated findings of increased expression or dominance of the AT$_2$ receptor in cardiac failure and postinfarct repair, these findings may indicate a role for AT$_2$RA in cardiac conditions.

**HYPERTENSION**

Blocking the effects of plasma Ang II by inhibiting AT$_1$ receptors clearly appears to be an effective method of blood pressure control. The AT$_1$RA class has been found to result in control of blood pressure to at least the same degree as that attained with members of other antihypertensive classes while resulting in significantly greater tolerability.$^{34}$

**Left Ventricular Hypertrophy** Left ventricular hypertrophy is a strong independent risk factor for the development of ischemic heart disease and CHF. Angiotensin II is speculated to have a major role in the development of cardiovascular hypertrophy, which occurs in most forms of hypertension. Both ACE inhibitors and AT$_1$RA can antagonize this hypertrophic effect.$^{35,36}$ Furthermore, there is evidence from animal experiments and human trials that AT$_1$RA can reverse hypertrophy to at least the same degree as $\beta$-blockers. In one trial, the AT$_1$RA irbesartan reduced hypertrophy sooner and to a numerically greater extent than did atenolol, despite similar reductions in blood pressure.$^{37}$ Several studies have shown that ACE inhibition increases the density and length of capillaries in the hypertrophied myocardium of spontaneously hypertensive rats.$^{37-40}$ The imbalance in the growth of the myocardium and coronary capillaries caused by Ang II may at least be one of the mechanisms leading to the circumstances seen in the myocardial hypertrophy of hypertension; that is, that the abnormally scarce capillary net cannot provide a sufficient blood flow for the hypertrophied myocardium, leading to ischemia, cardiac dysrhythmia, and heart failure. In a study to determine how antihypertensive drugs reduce ventricular hypertrophy, thereby improving cardiac function, stroke-prone spontaneously hypertensive rats were treated in utero to the age of 20 weeks with either the ACE inhibitor ramipril or the AT$_1$RA losartan.$^{26,40,41}$ Both losartan and high-dose ramipril reduced systolic blood pressure to almost normotensive levels and reduced LVH. Low- and high-dose ramipril significantly increased cardiac capillary length density and improved cardiac and vascular function and metabolism when compared with vehicle-treated animals. Losartan did not significantly alter cardiac capillary length density, possibly due to AT$_2$ receptor-mediated inhibition of capillary endothelial growth. However, the AT$_1$RA produced near-identical cardiodynamic improvement and protection as the ACE inhibitor.$^{26,41}$ It is possible to speculate that AT$_1$RA may play a role in this regard, because the AT$_2$ receptor remains unopposed and may be stimulated in the face of AT$_1$ receptor antagonism and increased Ang II levels.

**ATHEROSCLEROSIS**

Recent interest has focused on the role of Ang II, acting at the AT$_1$ receptor in the vasculature, specifically in the processes of atherosclerosis. Angiotensin II has been shown to increase superoxide production in the vascular wall of rats.$^{42}$ Cotreatment with a chimeric heparin-binding form of the recombinant human Cu/Zn superoxide dismutase and losartan improved the circumstances and reduced superoxide formation, suggesting that superoxide is one of the factors implicated in the atherogenic process. Experiments in Watanabe hyperlipidemic rabbits demonstrated that treatment with the AT$_1$RA irbesartan resulted in inhibition of aortic atherosclerosis,$^{43}$ a decrease in aortic intimal surface involvement, and a significantly reduced cholesterol content. Based on these encouraging results, it will be important to determine whether the AT$_1$ receptor blockade improves the pathogenesis of atherosclerosis in humans.

**WOUND HEALING AND TISSUE REPAIR**

Angiotensin II receptor subtypes can counteract each other with respect to their growth-modulating actions. For example, whereas cell proliferation is induced by AT$_1$ receptor activation in several cell types, it can be inhibited by AT$_2$ stimulation.$^{7}$ In wound healing and tissue repair, including MI, skin lesions, and central and peripheral nerve injury, a dramatic increase in AT$_2$ receptor expression occurs. The strong develop-
mental regulation and postnatal loss of AT$_2$ receptors also indicate the role of AT$_2$ receptors in the development of various tissues. These findings led to the postulation that Ang II acts via AT$_2$ receptors as a modulator of programs affecting cellular life. The antagonistic actions of AT$_1$ and AT$_2$ in terms of growth mediation and the signal transduction cascades that lead to either apoptosis or nerve-fiber regeneration are not well understood. However, involvement of AT$_2$ receptors is widely accepted in both neuroregeneration and programmed cell death. Direct in vivo evidence for an AT$_2$-receptor-mediated neurotrophic action of Ang II has been provided in a recent study demonstrating axonal regeneration of retinal ganglion cells after optic nerve crush.

**STROKE**

Stroke is considered the primary hazard of hypertension. To address the role of AT$_1$RA in stroke, we first conducted a study comparing the effects of oral or intravenous (iv) irbesartan and losartan administered peripherally or centrally on vasopressin release, drinking response, and c-Fos expression in the brains of normotensive Wistar rats. The results showed that both irbesartan and losartan injected iv 30 min before intracerebroventricularly (icv) administered Ang II reduced the Ang II-induced release of vasopressin. Irbesartan, but not losartan, administered iv, attenuated the icv Ang II-induced drinking response. When the AT$_1$ antagonists were administered orally 60 min before icv Ang II, irbesartan was more effective than losartan in inhibiting the icv Ang II-induced release of vasopressin into the circulation and Ang II-induced drinking. These findings indicate that irbesartan crosses the blood-brain barrier more readily than losartan up to 1 h after it is administered peripherally.

Additionally, 24 h after a 90-min occlusion of the middle cerebral artery, brain neurocytochemistry and examination of the immediate early genes or inducible transcription factors were assessed in rats. They were continuously treated icv with irbesartan or losartan 5 days before the induction of cerebral ischemia. Treatment with irbesartan and losartan icv improved neurologic status and reduced expression of c-Fos in the brains of rats exposed to ischemia. These data are encouraging and point to the need for further studies of the effects of AT$_1$RA in neurologic disorders such as stroke.

**MYOCARDIAL INFARCTION**

After acute myocardial infarction (MI) and heart failure, local generation of Ang II may become dysfunctional and contribute to fibrosis and remodeling. The Evaluation of Losartan In The Elderly (ELITE) study compared treatment with an ACE inhibitor (captopril) and an AT$_1$RA (losartan) in elderly subjects who had moderately severe CHF. The ACE inhibitor and AT$_1$RA did not differ with respect to the primary endpoint of the study, which was renal dysfunction. However, results suggested that there was a 30% decrease in all-cause mortality resulting from a decrease in sudden death in patients treated with the AT$_1$RA compared with those treated with the ACE inhibitor. This result needs to be corroborated in further studies but could be attributable to the role of Ang II in the heart.

The effectiveness of AT$_1$RA in improving survival of CHF has not been clearly established. Richer-Giudicelli et al. in a study of Wistar rats with MI, found that the AT$_1$RA irbesartan increased survival in a dose-dependent manner in postischemic rats with CHF. This effect resulted mainly from improvements in systemic and coronary hemodynamics, including left ventricular end diastolic pressure, total peripheral resistance, and coronary blood flow. Several human trials confirmed the benefits of the AT$_1$RA on parameters such as exercise treadmill time, left ventricular ejection fraction, and cardiothoracic ratio.

**CONCLUSIONS**

Though much is known about the effects of the RAS, recent evidence has shed new light on this important system and has pointed to the role of at least two Ang II receptors that seem to mediate opposing effects. The AT$_1$ receptor is responsible for all the known deleterious actions of Ang II on blood pressure, while also potentially contributing to increased cardiac contractility, glomerular filtration, tubular sodium reabsorption, and cardiovascular hypertrophy. Conversely, it is now believed that the AT$_2$ receptor may mediate opposing effects to the AT$_1$ receptor, such as inhibition of cell proliferation, differentiation, tissue repair, and programmed cell death.

The recent development of AT$_1$RA has broadened the therapeutic choice in hypertension, and may provide additional end-organ protection. This assumption can best be appreciated through the pharmacology of the AT$_1$RA. These compounds result in more complete blockade of the AT$_1$-receptor-mediated effects of Ang II than ACE inhibitors, while leaving the AT$_2$ receptor unopposed.

Experiments performed in various animal models and cell lines have indicated that unopposed AT$_2$ receptors may result in improved tissue regeneration, although clinical trials have yet to be carried out. These preliminary findings seem to concur with the observation that the AT$_2$ receptor results in antiproliferation and may shift cells into differentiation. In certain circumstances such as tissue repair or regeneration, it has been shown to be regenerative *in vitro* and *in vivo*, pushing cells into a regeneration program.
Therefore, this receptor is crucial after tissue injury such as that seen in MI.

Although current consensus maintains that lowering blood pressure is beneficial regardless of the antihypertensive agent used, more controversial is whether pharmacologic blockade of the RAS confers additional benefits beyond lowering blood pressure. It is unknown whether blocking the RAS with ACE inhibitors or AT1RA confers superior end-organ protection. Clinical trials are needed to determine the extent of the benefits offered by AT1RA, the potential of which appears impressive.

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