Recent Progress in Angiotensin II Type 2 Receptor Research in the Cardiovascular System

Masatsugu Horiuchi, Masahiro Akishita, Victor J. Dzau

Abstract—Angiotensin II (Ang II) plays an important role in regulating cardiovascular hemodynamics and structure. Multiple lines of evidence have suggested the existence of Ang II receptor subtypes, and at least 2 distinct receptor subtypes have been defined on the basis of their differential pharmacological and biochemical properties and designated as type 1 (AT₁) and type 2 (AT₂) receptors. To date, most of the known effects of Ang II in adult tissues are attributable to the AT₁ receptor. Recent cloning of the AT₂ receptor contributes to reveal its physiological functions, but many functions of the AT₂ receptor are still an enigma. AT₁ and AT₂ receptors belong to the 7-transmembrane, G protein–coupled receptor family. However, accumulating evidence demonstrates that the function and signaling mechanisms of these receptor subtypes are quite different, and these receptors may exert opposite effects in terms of cell growth and blood pressure regulation. We will review the role of the AT₂ receptor in the cardiovascular system and the molecular and cellular mechanisms of AT₂ receptor action. *(Hypertension. 1999;33:613-621.)*

Key Words: angiotensin II • apoptosis • blood vessels • cell growth • heart • receptors • signaling

Angiotensin II (Ang II) has significant influence on the heart and blood vessels through its effects on systemic hemodynamics and blood volume. Ang II also exerts long-term structural effects through its direct hypertrophic and proliferative growth actions.¹² Multiple lines of evidence have suggested the existence of Ang II receptor subtypes, but it was only recently that at least 2 distinct receptor subtypes were defined on the basis of their differential pharmacological and biochemical properties and designated as type 1 (AT₁) and type 2 (AT₂) receptors.³⁴ Subsequent cloning of these 2 receptors⁵–⁸ fostered renewed interest in the biochemical, pharmacology, and physiology of Ang II receptors.

To date, extensive pharmacological evidence indicates that most of the known effects of Ang II in adult cardiovascular tissues are attributable to the AT₁ receptor, but less is known about the AT₂ receptor. As shown in the Table, accumulating evidence revealed that this receptor acts as an antagonistic receptor against AT₁ receptor, ie, AT₂ receptor exerts antiproliferative, antihypertrophic, and proapoptotic effects.

**Vascular Effect**

**Vascular Development**

It has been shown that rat and mouse vascular AT₂ receptor mRNA is expressed at very low levels in the aorta during early embryonic development (up to embryonic day 15) but at high levels during the later stages of development (embryonic days 16 to 21) and in the neonate,⁹¹⁰ whereas the AT₁ receptor in aorta is expressed at relatively constant levels from the first point tested (embryonic day 10) throughout development and the neonatal period and into the adult stage. After birth, AT₂ receptor levels decline rapidly. Shanmugam et al¹¹ confirmed this observation using in situ hybridization. The high level of AT₂ receptor mRNA expression in the outer medial-adventitial region persisted after birth in the neonate, but AT₂ receptor mRNA expression was absent in the tunica media and was decreased in the tunica adventitia 10 days after birth and had almost completely disappeared at 22 days after birth.

To further elucidate the physiological significance of the effects mediated by the AT₂ receptor in fetal vasculature, we examined the effects of angiotensin receptor blockade on the rates of DNA synthesis in the developing aorta when AT₂ receptor is expressed. The physiological role of the AT₂ receptor in the developing fetal aorta was examined by PD123319 infusion in utero (embryonic days 15 to 21).⁹ At embryonic day 15, when the AT₂ receptor is not expressed and aortic DNA synthesis rates are at or near maximum, before the developmentally regulated decrease in DNA synthesis, PD123319 has no effect on DNA synthesis. However, when the growth rates in the fetal aorta are declining and the AT₂ receptor is expressed (embryonic days 16 to 21), PD123319 attenuates the reduction in aortic DNA synthesis. These results suggest strongly that the AT₂ receptor mediates an antigrowth effect on the aorta in vivo. The opposing effects of the AT₁ and AT₂ receptor subtypes suggest an antagonistic interaction between these receptors in their effect on vascular structure (Figure 1). On the basis of these data, one might conclude that the AT₂ receptor modulates the...
growth of the blood vessel, perhaps by controlling the growth-stimulatory effects of developmentally regulated growth factors or by other mechanisms such as apoptosis.

To examine further the role of the AT2 receptor, we have generated the AT2 receptor knockout mouse using homologous recombination. The structural consequences of vascular AT2 receptor expression in vascular development in the knockout mouse still await detailed analysis. Recent studies using neuronal cells suggest that AT2 receptor activation may enhance differentiation in PC12W cells (a rat pheochromocytoma cell line) and in NG108-15 cells. Accordingly, we examined, in the AT2 receptor knockout mouse, vascular differentiation that drives the alteration in expression of numerous proteins, notably the constituents of the contractile apparatus. One of the hallmarks of development and differentiation of the vessel wall is the regulation of the synthesis of smooth muscle–specific proteins. We have demonstrated that the expression of h-caldesmon and calponin is delayed in the aorta of the AT2 receptor knockout mouse, which suggests that the AT2 receptor enhances the differentiation of vascular smooth muscle cells (VSMCs). To date, a careful morphological examination of the relationship between the expression of AT2 receptor in the vessel wall and apoptosis, differentiation, and fibrosis, for example, has not been completed. Furthermore, a detailed morphometric analysis of vascular structure, including thickness of the vessel layers and internal and external diameters of large, medium, and small arteries and arterioles, is required. In summary, current data suggest that the AT2 receptor plays a role in VSMC growth inhibition and differentiation during late gestation, thereby influencing the structure and function of the blood vessels (Figure 1).

### Vascular Remodeling

#### Growth

In adults with certain pathological conditions such as vascular balloon “injury” or inflammation induced by cuff placement, the AT2 receptor is reexpressed (Figure 1). Our group examined further the function of the AT2 receptor using a gain-of-function approach. Adult rat aortic VSMCs expressing very low levels of endogenous AT2 receptors were transfected with the AT2 receptor expression vector, and its effect on cell growth was examined. Ang II significantly increased the cell number in the control vector–transfected VSMCs. This increase was abolished with the AT1 receptor antagonist DuP753, thereby demonstrating that AT1 receptor activation enhances VSMC growth. On the other hand, in the cells coexpressing the AT2 receptor, Ang II treatment had little or no effect on cell number. Treatment of these VSMCs with the AT2 receptor antagonist PD123319 unmasked the growth effect of Ang II exerted through the AT1 receptor. Consistent with our results, Stoll et al also observed an antiproliferative influence of the AT2 receptor on cultured coronary endothelial cells. Goto et al reported that the cultured mesangial cells prepared from stroke-prone spontaneously hypertensive rats (SHR) showed lower expression of AT2 receptor and higher proliferation activity than those of normotensive Wistar-Kyoto rats, suggesting that AT2 receptor

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### Physiological Roles of AT2 Receptor in Cardiovascular System

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LVED indicates left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; and EF, ejection fraction.

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**Figure 1.** Effect of AT2 receptor in vasculogenesis and vascular remodeling. AT2 receptor is abundantly and widely expressed in the fetal vasculature and contributes to physiological vascular development. Disruption of AT2 receptor gene results in an increase in basal blood pressure as well as increased vasoconstriction in response to a vasoactive substance such as Ang II. Upregulation of the AT2 receptor in diseased vessels is induced by injury and inflammation. AT2 receptor disruption results in increased neointimal formation. KO indicates knockout.
may exert antiproliferative effect in mesangial cells. Moreover, antiproliferative effects of \( \text{AT}_2 \) receptor were also shown in mouse fibroblast R3T3 cells and in PC12W cells (rat pheochromocytoma cell line).\(^9\) In contrast, Otsuka et al\(^{21} \) observed very recently that mRNA expression for both \( \text{AT}_1 \) and \( \text{AT}_2 \) receptors was enhanced in the aorta of SHR and demonstrated that treatment with PD123319 reduced the media cross-sectional area of the aorta, whereas losartan reduced the arterial systolic blood pressure and the collagen concentration. They suggest that \( \text{AT}_1 \) receptor, but not \( \text{AT}_2 \) receptor, plays a crucial role in the remodeling of matrix tissue, while \( \text{AT}_2 \) receptor plays a role in the development of hypertrophy of smooth muscle in aorta in SHR.

Our group examined the effects of the expression of the transfected \( \text{AT}_2 \) receptor expression vector on adult VSMCs in vivo using the rat carotid injury model.\(^9\) The \( \text{AT}_2 \) receptor vector or control vector was transfected into the balloon-injured rat carotid artery by the hemagogglutinating virus of Japan–liposome method at the time of surgery. The neointimal area (expressed as a ratio of medial area) of the vessels transfected with and expressing the \( \text{AT}_2 \) receptor transgene was significantly smaller (70% decrease) than that of the untransfected or the control vector–transfected vessels. This inhibitory effect on the development of the neointimal lesion could be blocked with the \( \text{AT}_2 \) receptor antagonist PD123319.

To define the role of the endogenous \( \text{AT}_2 \) receptor in vascular disease, we applied the mouse model of vascular disease induced by polyethylene cuff placement.\(^{15} \) Our experiments using this model of cuff-wrapped mouse femoral artery revealed that the upregulation of the \( \text{AT}_2 \) receptor was preceded by an increase in inflammatory cytokines and that both \( \text{AT}_2 \) receptor knockout mice and wild-type mice developed neointima in the femoral artery, but the lesion was twice as large in the knockout mice as in the wild-type mice. On the other hand, Levy et al\(^{22} \) reported that chronic blockade of \( \text{AT}_1 \) receptor by losartan in rats receiving Ang II resulted in normal arterial pressure, but it induced significant aortic hypertrophy and fibrosis, and that chronic blockade of \( \text{AT}_2 \) receptor by PD123319 in Ang II–induced hypertensive rats had no effect on arterial pressure but antagonized the effect of Ang II on arterial hypertrophy and fibrosis, suggesting that in vivo vasotrophic effects of Ang II are at least partially mediated by \( \text{AT}_2 \) subtype receptors. These apparent differences in functions of \( \text{AT}_1 \) receptor are partly due to the difference in the species and/or experimental models, and these issues must be addressed in the near future.

**Apoptosis**

Because of evidence that apoptosis is critical for cardiovascular remodeling, we examined the effect of Ang II on apoptosis in VSMCs. After serum growth factor depletion, cultured VSMCs showed morphological changes typical of apoptosis and internucleosomal DNA fragmentation, and Ang II inhibited the onset of apoptosis through the \( \text{AT}_1 \) receptor.\(^{23} \) In contrast, as we demonstrated using \( \text{AT}_2 \) receptor–transfected VSMCs, selective \( \text{AT}_2 \) receptor stimulation enhanced apoptosis after serum starvation.\(^{16} \) In addition, we have demonstrated that \( \text{AT}_2 \) receptor exerts a proapoptotic effect in neonatal cardiomyocytes, PC12W cells, and R3T3 mouse fibroblasts.\(^{24–27} \) Recently, Dimmeler et al\(^{28} \) reported that Ang II induces apoptosis of human umbilical venous endothelial cells by activation of the caspase cascade and that simultaneous blockade of both \( \text{AT}_1 \) and \( \text{AT}_2 \) receptors prevents Ang II–induced apoptosis, whereas selective agonistic stimulation of the \( \text{AT}_2 \) receptor alone induces apoptosis. Moreover, Li et al\(^{29} \) demonstrated that Ang II induces apoptosis in the skin fibroblasts of the mouse embryo but not in those prepared from \( \text{AT}_2 \) receptor knockout mice.

**Blood Pressure**

Ichiki et al\(^{30} \) recently reported that mice lacking the gene encoding the \( \text{AT}_2 \) receptor have higher blood pressure than the wild-type control, while Munzenmaier and Greene\(^{31} \) reported that \( \text{AT}_2 \) receptor blockade augments the pressor effect of Ang II in the rat. Consistent with these results, we observed that the \( \text{AT}_2 \) receptor knockout mouse exhibits an enhanced acute blood pressure response to low-dose Ang II infusion.\(^{12} \) These findings suggest that the \( \text{AT}_2 \) receptor mediates vasodilation. Neither the target vasculature nor the underlying mechanism, however, is well understood. Since the vascular \( \text{AT}_2 \) receptor was minimally expressed in the vasculature when the blood pressure and Ang II infusion studies were performed (3- to 5-month-old mice), the data suggest that transient and developmentally regulated \( \text{AT}_2 \) receptor expression exerts a long-term effect on blood pressure, possibly through its influence on vascular structure (Figure 1).

Moreover, Arima et al\(^{32} \) directly examined the \( \text{AT}_2 \) receptor–mediated effect of Ang II on renal arterioles. They isolated and microperfused the rabbit glomerular afferent arteriole, which is a vascular segment crucial to the control of glomerular hemodynamics, and examined whether the \( \text{AT}_2 \) receptor is involved in the vasodilation and, if so, by what mechanism. They showed that the \( \text{AT}_2 \) receptor mediates vasodilation and that dilation was abolished by either disrupting the endothelium or inhibiting the cytochrome P-450 pathway, which suggests that afferent arteriole activation of the \( \text{AT}_2 \) receptor causes endothelium-dependent vasodilation via a cytochrome P-450 pathway, possibly by epoxyeicosa-trienoic acids. In contrast, it has been reported that stimulation of renal \( \text{AT}_2 \) receptors in anesthetized rats has no effect on total renal blood flow but blunts the pressure natriuresis.\(^{33} \) Siragy and Carey\(^{34,35} \) have demonstrated that activation of the renin-angiotensin system during sodium depletion increases renal nitric oxide (NO) production through stimulation by Ang II at the \( \text{AT}_2 \) receptor and renal production of cGMP and that \( \text{AT}_2 \) receptor blockade potentiates \( \text{AT}_1 \) receptor–induced prostaglandin E\(_2\) production. NO release by \( \text{AT}_2 \) receptor stimulation was also reported in dog coronary microvessels and large coronary arteries.\(^{36} \) Consistent with these results, Gohlke and colleagues\(^{37} \) demonstrated that \( \text{AT}_2 \) receptor–mediated cGMP production in hypertensive rat aorta is mediated by bradykinin and NO.

In addition, \( \text{AT}_2 \) receptors may be involved in salt conservation. Madrid et al\(^{38} \) examined the effect of an Ang II \( \text{AT}_1 \) or \( \text{AT}_2 \) receptor antagonist on the impairment of the pressure diuresis and natriuresis response produced by NO synthesis blockade by \( \text{N}^\omega\)-nitro-L-arginine methyl ester (L-NAME).
They observed that, in rats given L-NAME, valsartan elevated baseline excretory values at all renal perfusion pressure, but it had no effect on the sensitivity of the pressure diuresis and natriuresis response. However, the administration of PD-123319 to L-NAME–pretreated rats shifted the slopes of the pressure diuresis and natriuresis responses toward control values, indicating that the impairment produced by NO synthesis blockade on pressure diuresis is dependent on the activation of AT$_2$ angiotensin receptors. Ozono et al$^{40}$ demonstrated that Ang II mediates jejunal sodium and water absorption by an action at the AT$_2$ receptor involving cGMP production, while Ang II inhibits absorption through the AT$_1$ receptor.$^{9}$ They also showed that dietary sodium depletion increased the AT$_2$ receptor expression in mature adult rat kidneys.

Myocardial Effect

**Cellular Effects**

The function of the AT$_2$ receptor in the myocardium has not been well defined. In recent studies, AT$_2$ receptor stimulation in cultured rat neonatal cardiomyocytes and fibroblasts inhibited AT$_1$ receptor–dependent growth.$^{41,42}$ Consistent with this observation, in our preliminary experiments the AT$_2$ receptor blocked apoptosis and the AT$_2$ receptor enhanced apoptosis in cultured neonatal rat cardiomyocytes.$^{35}$ These suggesting that Ang II exerts simultaneous opposing effects on cell growth through 2 different receptor subtypes. In contrast, Kajstura and colleagues$^{43}$ reported that Ang II induced apoptosis in cultured rat neonatal ventricular myocytes through a protein kinase C–mediated mechanism, an effect that was blocked by losartan. Moreover, having found that p53 increased angiotensinogen and AT$_1$ receptor expression in cultured adult rat ventricular myocytes, they postulated that p53 induced myocyte apoptosis through activation of the myocyte angiotensin system and that stretch-mediated release of Ang II in adult myocytes is coupled with apoptosis and the activation of p53, which may be responsible for the prolonged upregulation of the local renin-angiotensin system and the increased susceptibility of myocytes to undergo apoptosis.$^{44,45}$ The basis for the discrepancy in our observation on AT$_1$ versus AT$_2$ receptor effect on myocyte apoptosis is unclear. It may be partially dependent on the cell characteristics and the conditions of the cell culture experiment. Further investigation to clarify this issue and its physiological relevance is clearly warranted.

**AT$_2$ Receptor in Cardiomyocytes**

If AT$_1$ and AT$_2$ receptors exert antagonistic action on myocardial biology, especially growth, then the relative expression of these receptors and their ratios under different cardiac pathological conditions may be important in determining myocardial function and structure. Cardiac expression of AT$_1$ and AT$_2$ receptor subtypes is species dependent, and changes in their relative proportion may influence myocardial hypertrophy and fibrosis. The density of the myocardial AT$_2$ receptor was shown to be increased in experimental myocardial infarction 1 day after infarction in the infarcted portion, and AT$_2$ receptor expression was further upregulated 7 days after infarction in both the infarcted and the noninfarcted portions.$^{46}$ In the hypertrophied rat heart, the ratio of AT$_2$ to AT$_1$ receptor densities is increased.$^{47}$ In failing Bio14.6 cardiomyopathic hamster hearts, AT$_2$ receptor expression has been reported to increase in cardiac fibroblasts in fibrous regions, in turn exerting an anti-AT$_1$ receptor effect on the progression of interstitial fibrosis during cardiac remodeling by inhibiting both fibrillar collagen metabolism and growth of cardiac fibroblasts.$^{48}$ In contrast, AT$_1$ receptor expression increased in the hypertrophy stage and then decreased to the control level during heart failure. Moreover, changes in AT$_2$ receptor expression in human cardiac diseases have been reported.$^{49}$ In failing human heart, the relative ratio of AT$_2$ receptor expression to AT$_1$ receptor has been reported to be higher than in the normal heart.$^{50}$ Wharton et al$^{51}$ carefully examined the expression and localization of the AT$_2$ receptor in human diseased hearts and reported that endocardial, interstitial, perivascular, and infarcted regions in the ventricles of patients with end-stage ischemic heart disease or dilated cardiomyopathy exhibited a significantly greater density of high-affinity AT$_2$ binding sites than adjacent noninfarcted myocardium. Regions displaying the relative increase in AT$_2$ binding sites corresponded to areas of fibroblast proliferation and collagen deposition. The border zone between infarcted and noninfarcted myocardium characteristically contained numerous microvessels exhibiting perivascular AT$_2$ receptors. In contrast, AT$_1$ binding sites were localized to nerves, occurred at relatively low density in coronary vessels, and represented only 23% to 29% of myocardial Ang II binding sites. Taken together, these results suggest that the AT$_2$ receptor plays some role in cardiovascular remodeling in humans.

**Cardiac Function**

Given these associations, if we postulate that the AT$_2$ receptor contributes to the pathogenesis of cardiac diseases and the subsequent remodeling process, then treatment with the selective AT$_2$ receptor antagonist may have interesting cardiac remodeling effects that have not heretofore been appreciated. Using a model of heart failure induced by myocardial infarction in rats, Liu and colleagues$^{52}$ demonstrated that a significant increase in left ventricular end-diastolic and endsystolic volume and a decrease in ejection fraction, interstitial collagen deposition, and cardiomyocyte size were all improved by AT$_2$ receptor antagonist and that these effects were blocked by the AT$_2$ receptor antagonist. They speculate that in heart failure, blockade of AT$_1$ receptors increases both renin and angiotensin; this angiotensin stimulates the AT$_2$ receptor, which in turn is part of the therapeutic effect of the AT$_1$ receptor antagonist.

Using an α-myosin heavy chain promoter, Masaki and colleagues$^{53}$ developed a mouse model that shows cardiac-specific overexpression of the AT$_2$ receptor gene, which resulted in decreased sensitivity to AT$_1$-receptor mediated pressor and chronotropic actions. They saw no obvious morphological change in the myocardium and no significant difference in cardiac development or ratio of heart to body weight between wild-type and transgenic mice.
Apoptosis is controlled in part by a family of cytoplasmic proteins, the Bcl-2 protein family. Phosphorylation/dephosphorylation of Bcl-2 family proteins such as Bcl-2 and Bad is reported as a mechanism of posttranscriptional regulation of their function.62–65 Consistent with these reports, we demonstrated in PC12W cells that nerve growth factor (NGF) activated Bcl-2 by ERK-dependent phosphorylation and that AT2 receptor inhibits NGF-mediated Bcl-2 phosphorylation by inhibiting ERK activity, thereby resulting in the induction of apoptosis.27 We also observed that serum depletion in PC12W cells increased the Bax mRNA expression, while NGF decreased Bax expression and AT2 receptor stimulation increased it.66 Moreover, we found that AT2 receptor stimulation increased de novo ceramide production through PTPase activation.67

Pertussis toxin treatment attenuated AT2 receptor–mediated ERK inactivation and resulted in the inhibition of the growth-inhibitory and proapoptotic effects of this receptor.24,56 Using coimmunoprecipitation studies with antibodies specific for various G protein α subunits, Zhang and Pratt68 found that only antibodies specific for Gαi were able to coimmunoprecipitate AT2 receptor binding sites in the membrane prepared from rat fetus. Moreover, we demonstrated that transfection of the synthetic intracellular third loop peptide of the AT2 receptor into rat adult aortic VSMCs resulted in ERK inactivation and growth inhibition and that the 125I-labeled third loop peptide of AT2 receptor was immunoprecipitated with anti-Gαi antibody.56 Taken together, these results suggest that the AT2 receptor is a G protein–coupled receptor and that the intracellular third loop domain of the AT2 receptor is closely linked with the cellular signaling pathways in which Gαi is involved, and this interaction results in the ERK inactivation. Consistent with these observation, Kang and colleagues69 reported that the AT2 receptor stimulates potassium current in neurons cultured from rat hypothalamus and brain stem.58 We also observed a decrease in ERK activity in the heart of AT2 receptor transgenic mice,51 which suggests that the ERK inactivation by the AT2 receptor has a physiological role in vivo.

Specific phosphatases that couple with AT2 receptor have not been identified. An immediate early gene product known as 3CH134 was identified recently as a phosphatase specific for MAP kinase and named MAP kinase phosphatase-1 (MKP-1).69 A reduction of MKP-1 has been reported in rat VSMC after vascular injury60 and in the rat aorta in acute hypertension elicited by stress or vasoactive substances,61 thus suggesting the important role of this enzyme in vascular remodeling and hypertension. Our finding in PC12W cells that pretreatment with antisense oligonucleotide of MKP-1 inhibited the proapoptotic effect of the AT2 receptor24,27 suggests that MKP-1 is an AT2 receptor–activated phosphatase. In NIE-115 neuroblastoma cells and in Chinese hamster ovary cells expressing recombinant human AT2 receptor, Ang II rapidly stimulates the catalytic activity of SH-PTP1, a soluble PTPase that has been implicated in termination of signaling by cytokine and growth factor receptors; SH-PTP1 activation resulted in ERK inactivation.57 It is intriguing to find other target substrates in addition to ERK, which are regulated by AT2 receptor–activated phosphatases.

**Transcriptional Regulation of AT2 Receptor Expression**

To understand the molecular mechanism of the developmental and growth regulation of AT2 receptor expression,
Angiotensinogen

Renin

Ang I

Inactive Peptides

Bradykinin

AT1 Receptor

Blocker

AT2 Receptor

Cytokines

Growth Factors

AT2 Receptor

Phosphatases

Ceramide

NO

AT2 Receptor

ICF-1

AT2 Receptor Gene

Phosphatases

Ceramide

NO

Figure 3. Transcriptional control of AT2 receptor, signaling mechanism of AT2 receptor-mediated growth inhibition, and possible role of AT1 receptor blockade. When AT1 receptor is blocked, plasma renin and angiotensins may increase, and therefore increased angiotensins may act preferably on AT2 receptor. AT2 receptor stimulation activates phosphatase(s) such as MKP-1, which results in the inhibition of ERK and Bcl-2 dephosphorylation. Moreover, AT2 receptor stimulation increases ceramide production. AT2 receptor-mediated NO production may also act as a proapoptotic factor. In apoptotic cells, IRF-1 is upregulated and increases AT2 receptor, IL-1β-converting enzyme (ICE), and inducible nitric oxide synthase (NOS) expression, leading to apoptosis. AT1 receptor expression is also modulated by multiple growth factors, including Ang II in both positive and negative directions.

we cloned the mouse AT2 receptor gene, analyzed its structure, and examined its promoter activity.\textsuperscript{70} We used R3T3 cells, a mouse fibroblast cell line, in our model since these cells express only AT2 subtype binding sites and the expression of AT2 receptor sites in these cells is modulated by the growth state of the cells, ie, AT2 receptor expression is low in the growing state and becomes high in the confluent state.\textsuperscript{71,72} Promoter/luciferase reporter deletion analysis of the AT2 receptor in R3T3 cells showed a putative negative regulatory region located between positions −453 and −225 that plays an important role in the transcriptional control of AT2 receptor gene expression along with the cell growth. The expression of AT2 receptor in R3T3 cells is transcriptionally regulated by the competitive binding of interferon regulatory factor (IRF)-1 and IRF-2, ie, IRF-1 increases growth-dependent AT2 receptor expression in mouse fibroblast R3T3 cells, whereas IRF-2 inhibits it.\textsuperscript{70} Moreover, upregulation of IRF-1 in apoptotic R3T3 cells results in the increased expression of AT2 receptor, thereby exerting proapoptotic effects.\textsuperscript{26} Thus, it is intriguing to note that the same transcriptional factor, IRF-1, activates the expression of inducible nitric oxide synthase\textsuperscript{73} and interleukin (IL)-1β-converting enzyme\textsuperscript{74,75} both of which are involved with apoptosis (Figure 3).

Ichiki and colleagues\textsuperscript{76} also examined the effects of several growth factors on the expression of AT2 receptor mRNA in R3T3 cells and observed that serum (10%), fibroblast growth factor, phorbol ester, and lysophosphatidic acid reduced AT2 receptor expression, whereas IL-1β and insulin enhanced it, thus suggesting that AT2 receptor expression is modulated by multiple growth factors in both positive and negative directions. They also proposed the presence of potential cis DNA elements that respond to IL-1β (CCAAT enhancer binding protein site), insulin [insulin response sequence of phospho(enol)pyruvate carboxykinase gene], and phorbol ester (AP-1 site) in the promoter region of the mouse AT2 receptor gene. Moreover, it has been reported that Ang II enhances the number of AT2 receptor in R3T3 cells.\textsuperscript{72,77}

In contrast, the mechanism of AT2 receptor expression in VSMCs and cardiomyocytes is poorly understood. Expression of the AT2 receptor in the fetal aorta is substantial, while that in the adult aorta and cultured VSMCs is very low or even absent. Kambayashi and colleagues\textsuperscript{78} reported that prolonged serum depletion (6 to 8 days) with a supplement of insulin induced expression of AT2 receptor mRNA in cultured VSMCs from Wistar-Kyoto rats, but receptor expression could not be induced in VSMCs prepared from SHR.\textsuperscript{79} They also reported that insulin-like growth factor upregulates AT2 receptor expression in cultured VSMCs. Moreover, vasoactive substances with the protein kinase C–calcium pathway, such as norepinephrine and Ang II, have been reported to downregulate the AT2 mRNA level in cultured rat neonatal myocytes.\textsuperscript{80}

Summary

Angiotensin-converting enzyme (ACE) inhibitor has been widely used for the treatment of hypertension.\textsuperscript{81–83} It has been well established that ACE inhibitors improve cardiac function and remodeling and prolong survival in patients with heart failure. AT1 receptor antagonists constitute an exciting and important new class of antihypertensive drug and are already used in antihypertension treatment. The effect of AT1 receptor antagonist may not be entirely due to blockade of the AT1 receptor.\textsuperscript{84} When AT1 receptor is blocked, plasma renin and angiotensins increase,\textsuperscript{85} and therefore increased angiotensins may act preferably on AT2 receptor (Figure 3). If the AT2 receptor contributes to the pathogenesis and consequent remodeling of cardiovascular diseases in humans, AT1 receptor antagonist may have some specific effects in the treatment of cardiovascular diseases. Indeed, cardioprotective effects and improved cardiac functions by AT1 receptor antagonist have been reported in heart failure in experimental animal models.\textsuperscript{86–89} Liu and colleagues,\textsuperscript{52} using a model of heart failure induced by myocardial infarction in rats, demonstrated that AT1 receptor antagonist improved cardiac functions and decreased interstitial collagen deposition and cardiomyocyte size and that these effects were blocked by

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the AT₁ antagonist, suggesting that the part of the effect of AT₁ receptor antagonist was due to the stimulation of AT₂ receptor. However, there are no reports demonstrating the clear differences between the long-term effects of AT₁ receptor antagonists and ACE inhibitors in the treatment of hypertension and cardiovascular diseases. One of the reasons is due to the fact that the cardioprotective effect of ACE inhibitor may be due to the renin-angiotensin system and/or inhibition of kinin destruction. Moreover, detailed localization and time course of AT₂ receptor expression in cardiovascular diseases as well as the factors regulating AT₂ receptor in vivo including receptor ligands must be elucidated. In summary, it is now conceivable that AT₂ receptor plays some roles in the pathogenesis and the remodeling of cardiovascular diseases, and further understanding of AT₂ receptor may contribute to new therapeutic strategies for cardiovascular diseases and hypertension.

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