Pathophysiological Role of Angiotensin II Type 2 Receptor in Cardiovascular and Renal Diseases

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Abstract—Since the discovery of nonpeptidic ligands, the receptors for angiotensin (Ang) II have been classified into 2 subtypes (Ang II type 1 receptor [AT₁-R] and Ang II type 2 receptor [AT₂-R]). AT₁-R mediates most of the cardiovascular actions of Ang II. AT₂-R is expressed at very high levels in the developing fetus. Its expression is very low in the cardiovascular system of the adult. The expression of AT₂-R can be modulated by pathological states associated with tissue remodeling or inflammation. In failing hearts or neointima formation after vascular injury, AT₂-R is reexpressed in cells proliferating in interstitial regions or neointima and exerts an inhibitory effect on Ang II-induced mitogen signals or synthesis of extracellular matrix proteins, resulting in attenuation of the tissue remodeling. An extreme form of cell growth inhibition ends in programmed cell death, and this process, which is initiated by the withdrawal of growth factors, is also enhanced by AT₂-R. Cardiac myocyte- or vascular smooth muscle–specific mice that overexpress AT₂-R display an inhibition of Ang II-induced chronotropic or pressor actions, suggesting the role of AT₂-R on the activity of cardiac pacemaker cells and the maintenance of vascular resistance. AT₂-R also activates the kinin/nitric oxide/cGMP system in the cardiovascular and renal systems, resulting in AT₂-R–mediated cardioprotection, vasodilation, and pressure natriuresis. These effects, transmitted by AT₂-R, are mainly exerted by stimulation of protein tyrosine or serine/threonine phosphatases in a Gi protein–dependent manner. The expression level of AT₂-R is much higher in human hearts than in rodent hearts, and the AT₂-R–mediated actions are likely enhanced, especially by clinical application of AT₁-R antagonists. Thus, in this review, the regulation of AT₂-R expression, its cellular localization, its pathological role in cardiovascular and kidney diseases, and pharmacotherapeutic effects of AT₂-R stimulation are discussed. (Circ Res. 1998;83:1182-1191.)

Key Words: angiotensin II receptor ■ angiotensin II type 2 receptor ■ angiotensin II AT₂ receptor ■ angiotensin II type 1 receptor ■ angiotensin II AT₁ receptor

The biological effects caused by circulating angiotensin (Ang) II are diverse, widespread, and play a critical role in the regulation of the cardiovascular and renal systems (Figure 1). The components of the renin-angiotensin system are present in peripheral tissues such as vasculature, kidneys, adrenal glands, and hearts, all of which locally produce Ang II.1 Ang II produced in the peripheral tissues binds to specific receptors through the autocrine or paracrine system and exerts growth-promoting effects on the tissue remodeling process.2 The well-known Ang II actions such as the regulation of blood pressure and water-electrolyte balance have been attributed mainly to the activation of various signal-transduction pathways modulated by Ang II type 1 receptor (AT₁-R). However, the discovery of highly selective, peptidic, and nonpeptidic ligands such as CGP42112A and PD123319 has led to the identification of a second subtype (Ang II type 2 receptor [AT₂-R]).3–7 This receptor is expressed at very high levels in the developing fetus. By contrast, in the adult, its expression is restricted to the adrenals, uterus, ovary, heart, and specialized nuclei in the brain. Initially, the cDNA for AT₂-R was isolated by expression cloning from PC12 cells8 and whole fetus.9 Knockout mice for the AT₂-R gene were developed, and studies of these mice suggested that AT₂-R has a physiological role in blood pressure control and emotional instability and fearfulness.10,11 In addition, an AT₂-R–mediated inhibitory effect on the growth-promoting signals has been found.5,6 Treatment with AT₂-R antagonists causes a marked elevation of levels of plasma Ang II, which selectively binds to AT₂-R and exerts as yet undefined effects.12 Thus, elucidation and understanding of AT₂-R–mediated pathological actions have important pharmacotherapeutic implications. This review examines the results of studies into the structure-function, transcriptional control and gene expression, signal-transduction mechanism, cellular distribution, and pathophysiological roles of AT₂-R as well as potential issues concerning clinical application of AT₁-R antagonists.

Structural Features of AT₂-R
The second isoform of the Ang II receptor, AT₂-R, has been defined as the receptor that binds specifically to CGP42112 and to a series of...
Developmental roles for AT2-R. This receptor had been thought not to be a seven-transmembrane receptor, given that its ligand binding affinity was not reduced by stable GTP analogues, and it did not contain no intron in its coding region. This excludes the possibility of multiple forms of AT2-R encoded by several homologous genes or delivered by alternative splicing. Genomic Southern blot analyses also have indicated that there are no other subtypes to the AT2-R gene family, such as ATn,-R or ATp,-R, found in the AT1-R family.

**Regulation of AT2-R Gene Transcription and Expression**

The gene expression of AT2-R is regulated by multiple factors (Table 1). Increase in intracellular Ca2+ levels by Ca2+ ionophore and activation of protein kinase C (PKC) by phorbol ester markedly downregulated the AT2-R mRNA level in PC12 cells.21 Norepinephrine and Ang II (via AT1-R), which elevate Ca2+ levels and activate PKC, downregulate the AT2-R expression in cardiac myocytes.22 Growth factors (epidermal growth factor, nerve growth factor, and platelet-derived growth factor) also downregulate AT2-R expression in PC12 cells.22,23 R3T3 cells,24,25 and vascular smooth muscle cells (VSMCs).26 Stimulation of growth factor receptors results in the formation of an activator protein AP-1 complex including Fos and Jun transcription factors, which in turn binds to the AP-1 site of the promoter region of many genes. This effect is mimicked by PKC activation with phorbol ester. Considering that both growth factors and phorbol ester suppress the AT1-R mRNA expression, their effects may converge on the AP-1 site. In agreement with this hypothesis, the AP-1 site was shown to be present in the promoter region of rat AT2-R.23 and AT2-R gene transcription assessed by nuclear runoff assays is inhibited by growth factors and phorbol ester.22,23 The promoter region of the AT2-R gene possesses a glucocorticoid response element, CAA/T/enhancer-binding protein, nuclear factor-interleukin 6, AP-1, and a cAMP response element, suggesting a transcriptional regulation by glucocorticoids, cytokines, phorbol esters, and cAMP.23,27 Interestingly, the inhibition of AT2-R expression by glucocorticoid or cAMP analogues is regulated at the gene transcription level.22,23 Thus, multiple factors downregulate AT2-R expression. Ichiki et al24,28 and Kambayashi et al29 reported

**Table 1. Pharmacology, Regulation, and Physiological Function of AT1-R and AT2-R**

<table>
<thead>
<tr>
<th></th>
<th>AT1-R</th>
<th>AT2-R</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Distribution</strong></td>
<td>Artery, liver, kidney, adrenal gland, heart, brain (glial cell)</td>
<td>Fetus, brain (neuron), myometrium, kidney, lung, heart</td>
</tr>
<tr>
<td><strong>Ligand</strong></td>
<td>Losartan, TCV116, valsartan</td>
<td>PD123319, CGP42112A</td>
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<td><strong>Amino acid</strong></td>
<td>359</td>
<td>363</td>
</tr>
<tr>
<td><strong>Chromosome</strong></td>
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<td>X chromosome</td>
</tr>
<tr>
<td><strong>Exon</strong></td>
<td>5 (human), 3 (rat)</td>
<td>3 (human, rat)</td>
</tr>
<tr>
<td><strong>Signaling</strong></td>
<td>Gq/Gi coupling, PLC activation (Ca2+/IP3), Ras/ERK, JAK2/STATS</td>
<td>Gi coupling, tyrosine phosphatase, serine/threonine phosphatase, kinase/NO/cGMP</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Vasoconstriction, aldosterone secretion, hypertension, cellular growth, catecholamine release</td>
<td>Voltage-gated L, K⁺ channel activation, T-type Ca2⁺ channel inhibition, growth inhibition, apoptosis induction, vasodilation</td>
</tr>
<tr>
<td><strong>Null mice</strong></td>
<td>Anomalous development of renal calyx, decrease in blood pressure, hypertension of renin-producing cell</td>
<td>Increase in basal blood pressure, hyperresponse to Ang II pressor action, exploratory behavior, anomalous development of ureter</td>
</tr>
<tr>
<td><strong>Cardiac overexpression</strong></td>
<td>Bradycardia and arrhythmia, atrial hyperplasia</td>
<td>Negative chronotropic action, inhibition of AT1-R-mediated ERK activity</td>
</tr>
<tr>
<td><strong>Arterial overexpression</strong></td>
<td>Unestablished</td>
<td>Hyporesponse to Ang II pressor action, decrease in arterial vascular resistance</td>
</tr>
<tr>
<td><strong>Regulation</strong></td>
<td>Upregulation by glucocorticoid, cAMP, cytokines, cardiac hypertrophy, myocardial infarction</td>
<td>Downregulation by glucocorticoids, growth factors, phorbol ester, Ca2⁺ ionophore, Gq-coupled receptor stimulation</td>
</tr>
</tbody>
</table>

Figure 1. Proposed AT2-R-mediated effects on cardiovascular and renal system. PP2A indicates serin/threonine phosphatase 2A; IKr, delayed rectifier K⁺ current; Is, transient outward K⁺ current; Ca2⁺/CaMK; calcium calmodulin kinase; MLCK, myosin light chain kinase; and EDHF, endothelium-derived hyperpolarizing factor. PD compounds. Its abundance in mesenchymal tissues of the developing fetus, such as the uterus, the adrenal medulla, pheochromocytoma, and specific brain regions suggested neuronal and developmental roles for AT2-R. This receptor had been thought not to be a seven-transmembrane receptor, given that its ligand binding affinity was not reduced by stable GTP analogues, and it did not show agonist-induced internalization. The cDNA isolated from PC12 cells or rat fetus by expression cloning encoded a protein with a 363 amino-acid residue, which corresponded to a theoretical molecular weight of 41 303, and it showed a seven-transmembrane domain receptor that included the highly conserved sequence Asp141-Arg142-Tyr143 in the N-terminal region of the second extracellular loop and the conservation of residues known to be crucial for binding in other G protein-coupled receptors. However, it shared only a 32% amino-acid sequence identity with AT1a-R. Mutational analysis of AT1a-R indicated that the Tyr108 in extracellular loop 1, the Arg1082 in extracellular loop, and the Asp297 in extracellular loop 3 play an important role in Ang II binding to AT1-R, whereas mutations of Arg182 and Asp297, but not Tyr108, drastically impaired Ang II binding to AT2-R. The AT2-R gene has been characterized in both the mouse16,17 and human. It exists as a single copy localized on the X chromosome in both species and contains no intron in its coding region. This excludes the possibility of multiple forms of AT2-R encoded by several homologous genes or delivered by alternative splicing. Genomic Southern blot analyses also have indicated that there are no other subtypes to the AT2-R gene family, such as ATn-R or ATp-R, found in the AT1-R family.
the upregulation of the AT$_2$-R gene by interleukin (IL)-1$\beta$, insulin, and insulin-like growth factors in R3T3 cells and VSMCs. IL-1$\beta$ is one of the important cytokines mediating inflammation, and it may be involved in AT$_2$-R induction during the process of inflammation. Fetal mesenchymal fibroblasts, which highly express AT$_2$-R, express a substantial amount of insulin-like growth factor-I and its receptor$^{11}$; insulin may be important for the abundant expression of AT$_2$-R in fetal mesenchymal tissues. Although these multiple factors regulate the expression of AT$_2$-R, the molecular mechanisms responsible for hormonal control and cell-specific expression of the AT$_2$-R gene are less defined than those for control and expression of the AT$_1$-R gene.$^{5,25-28}$ Expression of the AT$_2$-R gene is dependent on growth state. When PC12 cells,$^{22}$ R3T3 cells,$^{36,37}$ or mesangial cells$^{38}$ reach a confluent quiescent state, AT$_2$-R expression is increased markedly. This gene regulation is exerted at the transcriptional level.$^{22,23}$ Horiuchi et al$^{46-47}$ demonstrated that interferon regulatory factor-1, the expression of which is increased during confluent state and by serum depletion, binds to the interferon regulatory factor-binding motif of the AT$_2$-R gene promoter region and upregulates its gene transcription.

**AT$_2$-R–Mediated Effects on Ang II–Induced Mitogen Signaling, Apoptosis, Kinin/Nitric Oxide/cGMP System**

Involvement of Phosphotyrosine Phosphatase

In contrast with AT$_1$-R, the AT$_2$-R–mediated signaling mechanism is not well established. Before the structure of AT$_2$-R had been determined, numerous studies attempted to elucidate signal transduction pathways associated with this subtype. Cell lines such as PC12W cells, neuroblastoma (NG108-15, NIE-115), or fibroblasts (R3T3) expressing only AT$_2$-R were used as models for signaling studies. In the PC12W and NIE-115 cells, AT$_2$-R activates phosphotyrosine phosphatase (PTP) to inhibit cell proliferation$^{39,40}$ and differentiation.$^{41}$ NIH3T3 cells stably expressing AT$_2$-R, Ozawa et al$^{42}$ also found the AT$_2$-R–mediated inhibitory effect on serum-stimulated cell growth in a pertussis toxin-sensitive manner. Using a membrane-associated fraction in the postnuclear fraction isolated from R3T3 cells, Tsuzuki et al$^{43-44}$ reported that AT$_2$-R stimulation caused activation of PTP when both nitrophenylphosphate and the peptide substrate Raytide containing a phosphotyrosine residue were used as targets. They also described the suppression of growth stimulation of R3T3 fibroblasts by basic fibroblast growth factor. This suppression correlated dose-dependently with AT$_2$-R–mediated signals, suggesting the role of PTP in AT$_2$-R–mediated inhibition of cellular growth.$^{45}$

**Inhibition of AT$_1$-R–Mediated Extracellular Signal-Regulated Kinase Activation**

In cells expressing AT$_1$-R, Ang II activates extracellular signal-regulated kinase (ERK), which leads to a mitogenic or hypertrophic response through activation of tyrosine kinase system.$^{45,46}$ With use of coronary endothelial cells expressing both AT$_1$-R and AT$_2$-R$^{47}$ or an overexpression model of AT$_1$-R in VSMC,$^{48}$ AT$_2$-R was shown to have an inhibitory effect on AT$_1$-R–mediated growth-promoting action assessed by DNA synthesis, and in the latter experiment, AT$_2$-R decreased the AT$_1$-R–mediated ERK activity. Janiak et al$^{49}$ reported selective activation of AT$_2$-R as an effective approach for the suppression of neointima formation after balloon catheterization. AT$_2$-R stimulation inhibits AT$_1$-R–mediated DNA and cell growth in myocytes$^{50}$ or fibroblasts.$^{51,52}$ Isolated from neonatal rat hearts and in cardiac fibroblasts from myopathic hamsters.$^{32}$ The inhibition of DNA synthesis by AT$_2$-R was also reported in zona glomerulosa cells from rat adrenal glands.$^{33}$ Furthermore, Masaki et al$^{54}$ ascertained that AT$_2$-R significantly inhibits AT$_1$-R–mediated ERK activation in perfused mouse hearts overexpressing AT$_2$-R. The AT$_2$-R–mediated antiproliferative effect on DNA synthesis was also demonstrated in embryonic renalomedullary interstitial cells.$^{55}$ The mechanism of AT$_2$-R participation in this process might be associated with reduced ERK activity caused by the activation of ERK phosphatase 1 (MKP-1), a dual-specificity phosphatase that acts on both phosphosorynine and phosphothreonine. Horiuichi et al$^{66}$ reported that AT$_2$-R stimulation dephosphorylates Bcl-2 by activating MKP-1 and induces apoptosis in PC12W cells. Bedec et al$^{57}$ reported that AT$_2$-R–mediated inhibition of serum- or growth-factor-induced ERK activity in NIE-115 cells is associated with vanadate-sensitive PTP, in which catalytic activity of Src homology 2 domain phosphatase-1 (SHP-1), a soluble PTP, is an early transducer of the AT$_2$-R signaling pathway. These investigators also found that expression of MKP-1 is not modified by AT$_1$-R. In neuronal cultures isolated from neonatal rat hypothalamus or in nondifferentiated NG108-15 cells (neuroblastoma cells), activation of AT$_2$-R reportedly elicits stimulation of outward$^{58,59}$ and delayed rectifier $K^+$ currents$^{60}$ and inhibits T-type Ca$^{2+}$ current.$^{61}$ These effects in neuronal cultures appear to be due to activation of serine/threonine phosphatase mediated in a Gi protein–dependent manner. In contrast, in neuroblastoma (NIE-115) AT$_2$-R reportedly induces a marked decrease in phosphorylation of several cellular proteins on tyrosine residues.$^{62}$ Bottari et al$^{63}$ and Brechler et al$^{64}$ reported that AT$_2$-R stimulation causes activation of a membrane-associated PTP and inhibition of atrial natriuretic peptide–sensitive particular guanyl cyclase via a G protein–independent pathway. These studies show that AT$_2$-R inactivates ERK and that ERK activity can be used as a sensitive index of AT$_2$-R action, rather than used to directly determine PTP activity, which is difficult because of the high background contribution of several PTPs.$^5$

**AT$_2$-R–Mediated Induction of Apoptosis**

An extreme way of cell growth inhibition might direct cells into programmed cell death. AT$_2$-R has been associated with apoptotic changes in rat ovarian granulosa cells in culture.$^{65}$ Prolonged serum depletion, which is enhanced by Ang II treatment, elicits programmed cell death of R3T3 cells.$^{36,37}$ PC12W cells also undergo apoptosis upon depletion of nerve growth factor, which also is enhanced by Ang II, and this effect was attenuated by inhibition of MKP-1 function and by the PTP inhibitor orthovanadate.$^{66}$ Horiuchi et al$^{46}$ reported that ERK plays a critical role in inhibiting apoptosis in PC12W cells by phosphorylating Bcl-2 and that AT$_2$-R inhibits ERK activation, resulting in the inactivation of Bcl-2 and the induction of apoptosis. Hayashida et al$^{67}$ showed that a synthetic peptide containing a 22-residue sequence from the third cytosolic loop of rat AT$_2$-R suppresses the ERK activity when transferred into VSMCs, and that this inhibition is reversed by pertussis toxin or orthovanadate, suggesting that the third cytosolic loop of AT$_2$-R plays a role in the activation of a Gi-mediated PTP that inhibits ERK. Zhang and Pratt$^{68}$ also reported the direct binding of AT$_2$-R to immunoprecipitated Gi$_i$ and Gi$_o$ proteins. In contrast, Cigola et al$^{69}$ reported that the stimulation of AT$_2$-R but not AT$_1$-R induces apoptosis by Ca$^{2+}$–dependent endonuclease in neonatal rat myocytes. No apoptotic changes were observed in cardiac myocytes from transgenic mice expressing AT$_2$-R specifically in the heart.$^5$

**Involvement of Bradykinin/Nitric Oxide/cGMP Systems**

AT$_2$-R–mediated activation of the kinin and nitric oxide (NO) system has been reported in bovine endothelial cells,$^{70}$ isolated rat carotid arteries,$^{71}$ canine microvessels from coronary arteries,$^{72}$ rat aortic strips,$^{73}$ rat kidney,$^{74}$ and rat heart.$^{75}$ In a rat model of heart failure due to myocardial infarction,$^{76}$ the reduced cardiac function and cardiac fibrosis were improved by an AT$_2$-R antagonist as well as by a Bradykinin receptor antagonist. In stroke-prone spontaneously hypertensive rats,$^{77}$ aortic cGMP production stimulated by Ang II infusion was inhibited by an AT$_2$-R antagonist as well as by a Bradykinin receptor antagonist or NO synthase inhibitor, suggesting the involvement of bradykinin and NO in the AT$_2$-R signaling. Siragy et al$^{78}$ reported using a microdialysis technique that AT$_2$-R stimulates renal production of cGMP in response to Na depletion, and that this effect is mediated by NO production.$^{77}$ AT$_2$-R–mediated NO production also is involved vitally in AT$_2$-R–mediated pressure natriuresis and diuresis.$^{78,79}$
Expression and Cellular Localization of AT2-R in the Heart

Inhibition of Ang II production by angiotensin-converting enzyme (ACE) inhibitors is a major new approach for treatment of hypertension, cardiovascular remodeling, heart failure, and chronic renal diseases. Both AT1-R and AT2-R have been observed in cardiac myocytes isolated from neonatal rat hearts,90,108,111 Cardiac fibroblasts isolated from neonatal and adult rat hearts express abundant amounts of AT1-R (108-fold more than that in myocytes)102 but not of AT2-R.81–84 Although some attempts to detect the AT2-R in adult rodent myocytes by a binding assay or polymerase chain reaction have proved negative,84–86 other researchers have obtained positive results.24,87–90 Although autoradiography results also indicated the presence of AT2-R protein in the adult rat myocardium,91 an in situ hybridization study did not detect AT2-R mRNA in cardiac muscle of adult rat but did show it in the annulus of all valves during the perinatal period.92 Thus, the proportion of cardiac AT2-R expressed in situ in the rat heart varies from that detected in cultured cells,93 possibly reflecting downregulation of AT2-R expression after the isolation of mammalian cells84,94 and the growth-dependent regulation of AT2-R expression in cultured cells.21,66

The expression pattern of AT2-R in the human heart is quite different from that in the rat heart, and human adult hearts express substantial amounts of AT2-R. Tsutsumi et al.95,99,102 found that AT2-R receptors in human left ventricular tissues were in the range of 3.8 to 17.3 fmol/mg protein, and that the proportion of AT2-R to the total Ang II receptors using only membrane fractions. In fact, Regitz-Zagrosek et al.97 found that the Ang II receptor densities determined with combined fractions were increased markedly compared with those found by using only membranes. Urata et al.103 reported that the basal portion of human left ventricular tissues contained higher densities of Ang II receptors than those in other portions. Ang II receptor densities are affected by the methodological differences, such as dissimilarities in the purity of membrane fractions, the portion of ventricles examined, or membrane preparation.

Cellular localization of AT2-R in the human heart has been examined mainly using emulsion autoradiography.95,99,102 The expression of cardiac AT2-R was localized more highly in the fibroblasts present in the interstitial regions than those in the myocardium95,99,102 (Figure 2). In contrast, AT1-R is localized most abundantly in nerves distributed in the myocardium, and its expression level in the myocardium itself or interstitial regions is very low,95,99,102 although the AT1-R protein might be partially degraded during sample preparation such as during the freezing of tissues.93 These findings imply that the expression of AT1-R in the heart increases in parallel with the progression of interstitial fibrosis, which corresponds to the reports that AT1-R expression in human hearts increases during cardiac remodeling as a result of dilated cardiomyopathy or ischemic heart diseases.95,99,102 AT1-R expression in human hearts also reportedly increases in parallel with intracardiac filling pressures.104 The results of ligand binding and autoradiographic studies on human heart tissues appear to differ from those obtained in studies of isolated cardiac cells. Although AT1-R binding sites are localized in interstitial regions in human hearts in situ,95,99,102 cultured human cardiac fibroblasts display mainly AT1-R-mediated effects on collagen synthesis.104 Atypical Ang II binding sites for Ang 1–7 were detected on human cardiac fibroblasts,105,106 whereas Ang 3–8 binding sites were present on rabbit cardiac fibroblasts107 or myocardial membranes of guinea pig and rabbit hearts.108 Two subtypes of the AT1-R, AT1a-R and AT1b-R, were encoded in human tissues,109 and there was heterogeneity in mammalian AT1-R,110 but these reports are isolated ones, which are not followed up by confirmation.

Regulation of Cardiac AT2-R Expression During Cardiac Remodeling

Both AT1-R and AT2-R are upregulated to different extents in pathological conditions, such as cardiac hypertrophy,111–113 an
TABLE 2. Expression of AT₁-R and AT₂-R in Cardiovascular Diseases

<table>
<thead>
<tr>
<th>Clinical State</th>
<th>AT₁-R</th>
<th>AT₂-R</th>
<th>Experimental Model</th>
<th>Reference No.</th>
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<td>Cardiac hypertrophy</td>
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<td>mRNA/protein</td>
<td>SHR/2KIC RHR/rat</td>
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<tr>
<td></td>
<td>↑ ↑</td>
<td>mRNA/protein</td>
<td>Artery constriction/dog</td>
<td>113</td>
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<td></td>
<td>↑ ↑</td>
<td>mRNA/protein</td>
<td>Mechanical stretch/rat</td>
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<td>↑ →</td>
<td>mRNA</td>
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<tr>
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<tr>
<td></td>
<td>↓ →</td>
<td>mRNA</td>
<td>Human atrium/LV</td>
<td>115</td>
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<tr>
<td>Myocardial infarction</td>
<td>↑ ↑</td>
<td>mRNA/protein</td>
<td>Rat</td>
<td>88</td>
</tr>
<tr>
<td></td>
<td>→ →</td>
<td>mRNA/protein</td>
<td>Protein</td>
<td>151</td>
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</table>

Studies that simultaneously determined the expression level of AT₁-R and AT₂-R are provided. SHR indicates spontaneously hypertensive rats; RHR, renovascular hypertensive rats, and LV, left ventricle.

Pathophysiological Role of AT₂-R in Remodeling of the Heart

The dominant expression of AT₂-R in cardiac fibroblasts has interesting pharmacological implications for the predicted actions of AT₂-R antagonists. Because circulating Ang II levels are increased by administration of AT₁-R antagonists and because Ang II preferentially binds to cardiac AT₂-R, AT₂-R–mediated actions are expected to be activated in failing hearts, especially in cardiac fibroblasts. Using fibroblasts from myopathic hamster hearts, Ohkubo et al. determined that AT₂-R has an inhibitory effect on AT₁-R–mediated synthesis of DNA and extracellular collagenous protein, such as fibronectin and collagen type I, and that AT₂-R stimulation inhibits the progression of interstitial fibrosis in myopathic lesions. Several other studies also demonstrated an antigrowth role of AT₂-R in the cardiovascular system, shown by its inhibition of the proliferation of rat coronary endothelial cells and transfected VSMCs, its modulation of arterial hypertrophy, and fibrosis in Ang II–induced hypertensive rats and its prevention of Ang II–induced growth of cultured neonatal rat myocytes. AT₂-R–mediated apoptosis might be one mechanism by which AT₂-R induces an antigrowth effect. Indeed, apoptotic myocytes have been found to occur relatively frequently in the border zone of infarcted human hearts, and researchers have speculated that AT₂-R may affect changes in myocardial structure by mediating apoptosis. Liu et al. reported that the cardioprotective action of AT₂-R antagonists is mainly exerted by the selective stimulation of AT₂-R, which is mediated partly by the kinin/NO system. Ang II–induced cardiac fibrosis is increased by chronic inhibition of NO synthase, and generation of coronary kinin mediates NO release after Ang II receptor stimulation. ATR2-R–mediated activation of the kinin/NO system also is involved in pressure natriuresis and diuresis of the kidneys.

The AT₂-R in cardiac myocytes may have an additional action distinct from the effect in cardiac fibroblasts. In mice developed for cardiomyocyte-specific overexpression of AT₂-R, with use of an α-myosin heavy chain promoter, Ang II–induced positive chronotropic action is inhibited (Figure 3). In myocytes from neonatal rat hearts expressing both AT₁-R and AT₂-R, Booz et al. found that AT₂-R stimulation inhibits Ang II–induced myocyte hypertrophy by decreasing the protein-to-DNA ratio and increasing protein degradation. Kijima et al. reported that AT₂-R in hypertrophic myocytes at least partially exerts an inhibitory effect on AT₁-R–mediated positive chronotropic or hypertrophic actions by showing upregulation of AT₁-R in stretch-induced myocyte hypertrophy.

Cellular Localization and Function of AT₂-R in the Kidneys

Ang II has multiple effects on renal function, including modulation of renal blood flow, glomerular filtration rate, tubular epithelial transport, renin release, and cellular growth. Autoradiography
that AT 2 -R mRNA is not present in rat or mouse kidneys. However, essentially of the AT1 -R subtype, 126–130 whereas both AT 1 -R and AT 2 -R mRNA signals were present in the medullary thick ascending limb and medullary collecting ducts. Within the rat glomerulus, AT1 -R mRNA was localized predominantly in the glomeruli, the renal tubules, and the renal vasculature.134,135 These observations in the rat kidney were further confirmed at mRNA levels using polymerase chain reaction and in situ hybridization; strong AT 1 -R mRNA signals were highly localized in interlobular arteries, 142 suggesting a role in the local regulation of renal blood flow. AT 2 -R, on the other hand, is the major subtype in large cortical blood vessels. In contrast, Sechi et al130 reported that AT 2 -R is present primarily in both glomeruli and cortical blood vessels and that AT 2 -R protein is not expressed in the human kidney. Analysis of the human renal cortex using in situ hybridization showed strong AT 2 -R mRNA signals localized in interlobular arteries and tubulointerstitial fibrous regions and weaker signals detected in glomeruli and proximal tubules.142 AT 2 -R mRNA signals were highly localized in interlobular arteries,142 suggesting a role of AT 2 -R in renal blood flow, which agrees with the diuretic effect of AT 2 -R antagonists.143 The findings from a study of AT 2 -R null mice indicate AT 2 -R involvement in the formation of the embryonic ureter by the promotion of the mesenchymal cell apoptosis.144 Lo et al109 isolated tubular function from hemodynamic action by maintaining a constant renal blood flow and found that the AT 2 -R antagonist PD123319 markedly and rapidly increased diuresis and natriuresis from the rat kidney. Inagami et al19 also described AT 2 -R knockout mice as not having a diuretic response to PD123319. Siragy et al145 reported that AT 2 -R stimulates renal production of cGMP in response to Na depletion and that this effect is mediated by NO production.77 Madrid et al146 also observed that the activation of the NO/cGMP system by AT 2 -R impaired pressure diuresis. Arima et al147 reported, with use of microperfused afferent arteriole, that selective activation of AT 2 -R causes endothelium-dependent vasodilation via a cytochrome P-450 pathway, possibly by epoxycosa-
trienoids, which suggests that glomerular blood flow is partly regulated by the AT 2 -R in an endothelium-dependent manner.

Pathophysiological Function of the AT 2 -R Identified by Gene-Manipulated Animals

Ichiki et al30 and Hein et al148 used targeted deletion to eliminate the gene encoding the AT 2 -R in mice. The resultant AT 2 -R null mice exhibited elevated pressor sensitivity in response to intravenous infusion of Ang II,9,10 and their basal blood pressure also increased.9 However, because AT 2 -R expression in the adult vasculature is very low, the underlying mechanism remains unclear. Cardiac targeted overexpression of AT 2 -R in mice was recently generated using an α-myosin heavy chain promoter.44 No obvious morphological changes were observed in the heart, electrocardiograms were normal, and no arrhythmia or conduction block was seen. Infusion of Ang II increased blood pressure and heart rate dose-dependently in wild-type mice, whereas in AT 2 -R transgenic mice, these hemodynamic responses were inhibited markedly without affecting cardiac contractility (Figure 3). This effect was observed at a physiological Ang II concentration range that did not stimulate catecholamine release, suggesting that AT 2 -R decreases the sensitivity of pacemaker cells to Ang II. Tsutsui et al have used a VSMC-specific α-actin promoter to generate transgenic mice that overexpress the AT 2 -R in the vascular system (unpublished data, 1998). Although the basal blood pressure of this transgenic mouse does not differ from that in wild-type mice, the pressor effect by chronic Ang II infusion was abolished, and Ang II–induced contraction of the abdominal artery was significantly increased by an AT 2 -R inhibitor. The increased sensitivity of AT 2 -R knockout mice to the pressor effect of Ang II might be explained partly by lack of AT 2 -R–mediated negative chronotropic action as well as by the reduction of vascular resistance.

Summary and Clinical Applications for AT 2 -R Antagonists

In the near future, several AT 2 -R antagonists will be used for treatment of cardiovascular and renal diseases. However, clinicians should understand the differences in pharmacological effects between AT 2 -R antagonists and ACE inhibitors. Treatment with AT 2 -R antagonists causes an increase in plasma Ang II level, which selectively stimulates AT 2 -R.152 (Figure 4). As mentioned above, AT 2 -R stimulation inhibits cardiac fibroblast growth and extracellular matrix formation and exerts a negative chronotropic effect, indicating that AT 2 -R stimulation has a novel cardioprotective effect. Moreover, in human hearts, the distribution ratio of the AT 2 -R is high, and its expression is increased further during heart failure by downregulation of the AT 2 -R.95,97,98,103,115 The activation of the kinin/NO/cGMP system mediated through the AT 2 -R is involved partially or dominantly in the cardiovascular and renal effects of the AT 2 -R, raising the possibility that AT 2 -R stimulation has a similar effect to that...
exerted by ACE inhibitors such as activation of the kinin/NO system. In fact, Schieffer et al reported that ACE inhibitors and AT1-R antagonists are equally effective in preventing ventricular remodeling after myocardial infarction. Very recently, an evaluation of losartan in the elderly compared the effectiveness of the AT1-R antagonist losartan and the ACE inhibitor captopril in elderly heart failure patients, and the results showed that losartan was more beneficial than captopril as evidenced by a lower rate of sudden death and hospitalization. These beneficial effects might be partly explained by the potential effect mediated by AT1-R. Because blockade of the renin-angiotensin system is essential for the management of patients with heart failure or renal diseases and because AT1-R antagonists probably will be used widely for treatment of patients with cardiovascular and renal diseases in the near future, this novel tissue-protective effect of AT1-R should be confirmed by clinical studies.

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References


Angiotensin II Type 2 Receptor


105. Neuss M, Regitz-Zagrosek V, Hildebrandt A, Fleck E. Human cardiac fibroblasts express an angiotensin receptor with unusual binding characteristics which is coupled to cellular proliferation. *Biochem Biophys Res Commun*. 1994;204:1334–1339.


Keiser JA, Bjork FA, Hodges JC, Taylor DQ Jr. Renal hemodynamic and excretory response to PD123319 and losartan, nonpeptide AT\textsubscript{1} and AT\textsubscript{2} subtype-selective antagonists. J Pharmacol Exp Ther. 1992;263:1154–1160.

Miyazaki Y, Nishimura H, Harris RC, McKanna JM, Inagami T, Ichikawa I. Angiotensin regulates embryonic development of the ureter via type 1 (AT\textsubscript{1}) and type 2 (AT\textsubscript{2}) receptors. J Am Soc Nephrol. 1997;8:405A. Abstract.


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