II.1 Ang II produced in the peripheral tissues binds to specific adrenal glands, and hearts, all of which locally produce Ang II are present in peripheral tissues such as vasculature, kidneys, (Figure 1). The components of the renin-angiotensin system in the regulation of the cardiovascular and renal systems (AT1-R). However, the discovery of highly selective, peptide, and nonpeptidic ligands such as PD123319 has led to the identification of a second subtype (Ang II type 2 receptor [AT2-R].) This receptor 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 AT2-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.)

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, peptide, 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.²,⁵,⁶ 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 AT2-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

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Figure 1. Proposed AT2-R–mediated effects on cardiovascular and renal system. PD compounds.4 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.3–7,13,14 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.3,5 The cDNA isolated from PC12 cells8 or rat fetus 9 by expression cloning encoded a protein of molecular weight of 41 303, and it showed a seven-transmembrane structure with a 363 amino-acid residue, which corresponded to a theoretical molecular weight of 43 500. This gene family, such as AT1a-R or AT1b-R, found in the AT1-R family.8,9

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### 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 The promoter region of the AT2-R gene possesses a glucocorticoid response element, CAAT/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>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>
</tr>
<tr>
<td><strong>Ligand</strong></td>
<td>Losartan, TCV116, valsartan</td>
</tr>
<tr>
<td><strong>Amino acid</strong></td>
<td>359</td>
</tr>
<tr>
<td><strong>Chromosome</strong></td>
<td>No. 3 (human), No. 17 (rat)</td>
</tr>
<tr>
<td><strong>Exon</strong></td>
<td>5 (human), 3 (rat)</td>
</tr>
<tr>
<td><strong>Signaling</strong></td>
<td>Gq/Gi coupling, PLC activation (Ca2+/IP3), Ras/ERK, JAK2/STAT5</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Vasoconstriction, aldosterone secretion, hypertrophy, cellular growth, catecholamine release</td>
</tr>
<tr>
<td><strong>Null mice</strong></td>
<td>Anomalous development of renal calyx, decrease in blood pressure, hypertrophy of renin-producing cell</td>
</tr>
<tr>
<td><strong>Cardiac overexpression</strong></td>
<td>Bradycardia and arrhythmia, atrial hyperplasia</td>
</tr>
<tr>
<td><strong>Arterial overexpression</strong></td>
<td>Unestablished</td>
</tr>
<tr>
<td><strong>Regulation</strong></td>
<td>Upregulation by glucocorticoid, cAMP, cytokines, cardiac hypertrophy, myocardial infarction</td>
</tr>
</tbody>
</table>

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the upregulation of the AT₂-R gene by interleukin (IL)-1β, insulin, and insulin-like growth factors in R3T3 cells and VSMCs. IL-1β is one of the important cytokines mediating inflammation, and it may be involved in AT₁-R induction during the process of inflammation.³⁰ Fetal mesenchymal fibroblasts, which highly express AT₂-R, express a substantial amount of insulin-like growth factor-I and its receptor; insulin may be important for the abundant expression of AT₂-R in feto mesenchymal tissues. Although these multiple factors regulate the expression of AT₁-R, the molecular mechanisms responsible for hormonal control and cell-specific expression of the AT₂-R gene are less defined than those for control and expression of the AT₁-R gene.³²⁻³⁵ Expression of the AT₂-R gene is dependent on growth state. When PC12 cells,²² R3T3 cells,³⁶⁻³⁷ or mesangial cells³⁸ reach a confluent quiescent state, AT₁-R expression is increased markedly. This gene regulation is exerted at the transcriptional level.²²,²³ Horiuchi et al.⁴⁻⁵⁰ demonstrated that interferon regulatory factor-1, the expression of which is increased during confluent state and by serum depletion, binds to the interferon regulatory factory-binding motif of the AT₁-R gene promoter region and upregulates its gene transcription.

**AT₂-R–Mediated Effects on Ang II–Induced Mitogen Signaling, Apoptosis, Kinin/Nitric Oxide/cGMP System**

**Involvement of Phosphotyrosine Phosphatase**

In contrast with AT₁-R, the AT₂-R–mediated signaling mechanism is not well established. Before the structure of AT₂-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 AT₂-R were used as models for signaling studies. In the PC12W and NIE-115 cells, AT₂-R activates phosphotyrosine phosphorylation (PTP) to inhibit cell proliferation³⁹⁻⁴¹ and differentiation.⁴¹ N1H7T3 cells stably expressing AT₂-R, Ozawa et al.⁴² also found the AT₂-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 AT₂-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 AT₂-R–mediated signals, suggesting the role of PTP in AT₂-R–mediated inhibition of cellular growth.⁴³

**Inhibition of AT₁-R–Mediated Extracellular Signal-Regulated Kinase Activation**

In cells expressing AT₁-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 AT₁-R and AT₂-R, or an overexpression model of AT₂-R in VSMC,⁴⁸ AT₂-R was shown to have an inhibitory effect on AT₁-R–mediated growth-promoting action assessed by DNA synthesis, and in the latter experiment, AT₂-R decreased the AT₁-R–mediated ERK activity. Janiak et al.⁴⁹ reported the selective activation of AT₁-R as an effective approach for the suppression of neointima formation after balloon catheterization. AT₂-R stimulation inhibits AT₁-R–mediated DNA and cell growth in myocytes⁵⁰ or fibroblasts⁵¹⁻⁵² isolated from neonatal rat hearts and in cardiac fibroblasts from myopathic hamsters.⁵² The inhibition of DNA synthesis by AT₂-R was also reported in zona glomerulosa cells from rat adrenal glands.³¹,³² Furthermore, Masaki et al.⁴² ascertainment that AT₂-R significantly inhibits AT₁-R–mediated ERK activation in perfused mouse hearts overexpressing AT₁-R. The AT₁-R–mediated antiproliferative effect on DNA synthesis was also demonstrated in embryonic renodemequatorial interstitial cells.⁵⁵ The mechanism of AT₁-R–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 phosphotyrosine and phosphothreonine. Horiiuchi et al.⁶⁶ reported that AT₂-R stimulation dephosphorylates Bcl-2 by activating MKP-1 and induces apoptosis in PC12W cells. Bedec et al.⁶⁷ reported that AT₂-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 AT₂-R signaling pathway. These investigators also found that expression of MKP-1 is not modified by AT₂-R.⁶⁷ In neuronal cultures isolated from neonatal rat hypothalamus or in nondifferentiated NG108-15 cells (neuroblastoma cells), activation of AT₂-R reportedly elicits stimulation of outward²⁸,⁶⁹ and delayed rectifier K⁺ currents⁷⁰ and inhibits T-type Ca²⁺ current.⁷¹ 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) AT₂-R reportedly induces a marked decrease in phosphorylation of several cellular proteins on tyrosine residues.⁷² Bottari et al.⁷³ and Brechler et al.⁷⁴ reported that AT₂-R stimulation causes activation of a membrane-associated PTP and inhibition of atrial natriuretic peptide-sensitive particulate guanylate cyclase via a G protein–independent pathway. These studies show that AT₂-R inactivates ERK and that ERK activity can be used as a sensitive index of AT₂-R action, rather than used to directly determine PTP activity, which is difficult because of the high background contribution of several PTPs.⁵

**AT₂-R–Mediated Induction of Apoptosis**

An extreme way of cell growth inhibition might direct cells into programmed cell death. AT₂-R has been associated with apoptotic changes in rat ovarian granulosa cells in culture.⁶⁹ Prolonged serum depletion, which is enhanced by Ang II treatment, elicits programmed cell death of R3T3 cells.⁶⁶ 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.⁶⁰ Horiuchi et al.⁶⁶ reported that ERK plays a critical role in inhibiting apoptosis in PC12W cells by phosphorylating Bcl-2 and that AT₂-R inhibits ERK activation, resulting in the inactivation of Bcl-2 and the induction of apoptosis. Hayashida et al.⁷⁰ showed that a synthetic peptide containing a 22-residue sequence from the third cystolic loop of rat AT₂-R suppresses the ERK activity when transferred into VSMCs, and that this inhibition is reversed by pertussis toxin or orthovanadate, suggesting that the third cystolic loop of AT₂-R plays a role in the activation of a Gi-mediated PTP that inhibits ERK. Zhang and Pratt⁷⁰ also reported the direct binding of AT₂-R to immunoprecipitated Gα₂ and Gα₃ proteins. In contrast, Cigola et al.⁷⁶ reported that the stimulation of AT₂-R but not AT₁-R induces apoptosis by Ca²⁺–dependent endonuclease in neonatal rat myocytes. No apoptotic changes were observed in cardiac myocytes from transgenic mice expressing AT₂-R specifically in the heart.⁵⁴

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

AT₂-R–mediated activation of the kinin and nitric oxide (NO) system has been reported in bovine endothelial cells,⁷⁶ isolated rat carotid arteries,⁷⁷ canine microvessels from coronary arteries,²² rat aortic strips,³¹,³² rat kidney,²⁴ and rat heart.⁷⁸ In a rat model of heart failure due to myocardial infarction,³⁴ the reduced cardiac function and cardiac fibrosis were improved by an AT₂-R antagonist as well as by a bradykinin receptor antagonist. In stroke-prone spontaneously hypertensive rats,⁷⁹ aortic cGMP production stimulated by Ang II infusion was inhibited by an AT₂-R antagonist as well as by a bradykinin receptor antagonist or NO synthase inhibitor, suggesting the involvement of bradykinin and NO in the AT₂-R signaling. Siragy et al.⁸⁰ reported using a microdialysis technique that AT₂-R stimulates renal production of cGMP in response to Na depletion, and that this effect is mediated by NO production.⁸⁰ AT₂-R–mediated NO production also involves activation of AT₂-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 demonstrated in cardiac myocytes isolated from neonatal rat hearts.50,80,81 Cardiac fibroblasts isolated from neonatal and adult rat hearts express abundant amounts of AT1-R (~8-fold more than that in myocytes)80 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 found that the amount of Ang II receptors in human left ventricular tissues was in the range of 3.8 to 17.3 fmol/mg protein, and that the proportion of AT2-R to the total Ang II binding sites was about 41%. The amount of Ang II receptors is similar to the amounts reported by others.96–100 However, the proportion of AT2-R to binding sites differs between these studies. Interestingly, Tsutsumi et al.95 found that AT2-R and AT1-R decreased by as much as by 30±2.7% and 8.2±1.4%, respectively, during the freezing of tissue samples. This finding means that great care should be taken to factor in pathological conditions when Ang II receptor densities are measured in tissue samples. In addition, Rögge et al.101 reported a relatively high amount of Ang II receptors (118 fmol/mg protein) in the human atria where they used combined fractions including plasma membranes and internalized receptors; other investigators102–105 measured Ang II receptors using only membrane fractions. In fact, Rejz´c-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.98 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 AT2-R protein might be partially degraded during sample preparation such as during the freezing of tissues.93 These findings imply that the expression of AT2-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 AT2-R expression in human hearts also reportedly increases in parallel with intracardiac filling pressures.103 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 AT2-R binding sites are localized in interstitial regions in human hearts in situ,95,99,102 cultured human cardiac fibroblasts display mainly AT2-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,87,111–113 an

Figure 2. Emulsion autoradiography of Ang II receptors in human failing hearts. Adjacent sections obtained from a patient with dilated cardiomyopathy were stained with hematoxylin-cosin or incubated with [125I]-(Sar1,Ile8)-Ang II (0.25 nmol/L), dipped in emulsion, developed, and then stained with Kernechtrot. On sections stained with hematoxylin-cosin (No. 1), the fibrous regions are indicated by arrows. Adjacent sections were incubated with [125I]-(Sar1,Ile8)-Ang II in the absence of competitors (No. 2, total Ang II binding) and in the presence of PD123319 (0.1 µmol/L) (No. 3), losartan (0.1 µmol/L) (No. 4), or Ang II (3 µmol/L) (No. 5, nonspecific binding). Binding sites were localized in regions with interstitial fibrosis, whereas fewer were seen in the surrounding myocardium. The Ang II binding sites in fibrous regions were strongly inhibited by PD123319 (No. 3) but not by losartan (No. 4). (Figure was previously published in Tsutsumi et al [Circ Res. 1998;83:1035–1046]).
Angiotsin II Type 2 Receptor

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TABLE 2. Expression of AT1-R and AT2-R in Cardiovascular Diseases

<table>
<thead>
<tr>
<th>AT1-R</th>
<th>AT2-R</th>
<th>Experimental Model</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>mRNA/protein</td>
<td>mRNA/protein</td>
<td>SHR/2K-1C RHR/rat</td>
<td>87</td>
</tr>
<tr>
<td>mRNA/protein</td>
<td>mRNA/protein</td>
<td>Artery constriction/dog</td>
<td>113</td>
</tr>
<tr>
<td>mRNA/protein</td>
<td>mRNA/protein</td>
<td>Mechanical stretch/rat</td>
<td>81</td>
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<tr>
<td>mRNA/protein</td>
<td>mRNA</td>
<td>Aortic banding/rat</td>
<td>89, 90</td>
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<tr>
<td>mRNA/protein</td>
<td>mRNA</td>
<td>Aortic banding/rat</td>
<td>112</td>
</tr>
<tr>
<td>mRNA/protein</td>
<td>mRNA</td>
<td>Aortic banding/rat</td>
<td>111</td>
</tr>
</tbody>
</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 AT1-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 fiberonectin 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 AT1-R induces an antigrowth effect, and generation of coronary kinin mediates NO release after Ang II receptor stimulation.

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 AT1-R stimulation inhibits Ang II–induced myocyte hypertrophy by decreasing the protein-to-DNA ratio and increasing protein degradation. Kijima 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 fibroblasts has an inhibitory effect on AT1-R–mediated synthesis of DNA and extracellular collagenous protein, and 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 AT1-R induces an antigrowth effect, 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.

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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.
AT2-R mRNA is not present in rat or mouse kidneys. However, AT2-R mRNA is essentially of the AT1-R subtype, whereas both AT1-R and AT2-R are present within the kidney of the opossum and primates.

The distribution of renal Ang II receptors has been studied most extensively in the rat, where the AT1-R is localized in mesangial areas, predominantly at the vascular pole and between AT1-R antagonists and ACE inhibitors. Treatment with AT1-R antagonists causes an increase in plasma Ang II level, which selectively stimulates AT2-R12 (Figure 4). As mentioned above, AT2-R stimulation inhibits cardiac fibroblast growth and extracellular matrix formation and exerts a negative chronotropic effect, indicating that AT2-R stimulation has a novel cardioprotective effect.

Pathophysiological Function of the AT2-R Identified by Gene-Manipulated Animals

Ichiki et al9 and Hein et al10 used targeted deletion to eliminate the gene encoding the AT2-R in mice. The resultant AT2-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 AT1-R expression in the adult vasculature is very low, the underlying mechanism remains unclear. Cardiac targeted overexpression of AT2-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 AT2-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 AT2-R decreases the sensitivity of pacemaker cells to Ang II. Tsutsuemi et al have used a VSMC-specific α-actin promoter to generate transgenic mice that overexpress the AT2-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 AT2-R inhibitor. The increased sensitivity of AT2-R knockout mice to the pressor effect of Ang II might be explained partly by lack of AT2-R-mediated negative chronotropic action as well as by the reduction of vascular resistance.

Summary and Clinical Applications for AT2-R Antagonists

In the near future, several AT2-R antagonists will be used for treatment of cardiovascular and renal diseases. However, clinicians should understand the differences in pharmacological effects between AT2-R antagonists and ACE inhibitors. Treatment with AT2-R antagonists causes an increase in plasma Ang II level, which selectively stimulates AT2-R12 (Figure 4). As mentioned above, AT2-R stimulation inhibits cardiac fibroblast growth and extracellular matrix formation and exerts a negative chronotropic effect, indicating that AT2-R stimulation has a novel cardioprotective effect. Moreover, in human hearts, the distribution ratio of the AT2-R is high, and its expression is increased further during heart failure by downregulation of the AT2-R.35,97,98,103,115 The activation of the kinin/NO/cGMP system meditated through the AT2-R is involved partially or dominantly in the cardiovascular and renal effects of the AT2-R, raising the possibility that AT2-R stimulation has a similar effect to that of the AT1-R.

Human kidney. Analysis of the human renal cortex using in situ hybridization showed strong AT2-R mRNA signals localized in interlobular arteries and tubulointerstitial fibrous regions and weaker signals detected in glomeruli and proximal tubules.142 AT2-R mRNA signals were highly localized in interlobular arteries,142 suggesting a role of AT2-R in renal blood flow, which agrees with the diuretic effect of AT2-R antagonists.143 The findings from a study of AT2-R null mice indicate AT2-R involvement in the formation of the embryonic ureter by the promotion of the mesenchymal cell apoptosis.144 Lo et al145 isolated tubular function from hemodynamic action by maintaining a constant renal blood flow and found that the AT2-R antagonist PD123319 markedly and rapidly increased diuresis and natriuresis from the rat kidney. Inagami et al also described AT2-R knockout mice as not having a diuretic response to PD123319.

Siragy et al44 reported that AT2-R stimulates renal production of cGMP in response to Na depletion and that this effect is mediated by NO production.77 Madrid et al45 also observed that the activation of the NO/cGMP system by AT2-R impaired pressure diuresis. Arima et al46 reported, with use of microperfused afferent arteriole, that selective activation of AT2-R causes endothelium-dependent vasodilation via a cytochrome P-450 pathway, possibly by epoxyeicosatrienoic acids, which suggests that glomerular blood flow is partly regulated by the AT2-R in an endothelium-dependent manner.

Figure 3. Attenuated response of cardiac-specific overexpression of AT2-R to Ang II–induced pressor (upper panel) and chronotropic (lower panel) actions. Mice were anesthetized with pentobarbital, and blood pressure and heart rate were directly measured with catheters placed in the carotid artery. After 10 minutes of captopril administration (30 mg/kg body weight), Ang II diluted in saline was infused directly into the catheter at different doses in a volume of 10 μL. To examine the effect of PD123319, PD123319 (10 mg/kg) was infused into mice (n=8) pretreated with captopril, and after 20 minutes, Ang II was infused. The results are expressed as means±SE. P<0.01, P<0.05 vs the levels in wild-type mice.
exerted by ACE inhibitors such as activation of the kinin/NO system. In fact, Schieffer et al.\textsuperscript{146} reported that ACE inhibitors and AT\textsubscript{1}-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 AT\textsubscript{1}-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.\textsuperscript{147} These beneficial effects might be partly explained by the potential effect mediated by AT\textsubscript{2}-R. Because blockade of the renin-angiotensin system is essential for the management of patients with heart failure or renal diseases\textsuperscript{148–151} and because AT\textsubscript{1}-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 AT\textsubscript{2}-R should be confirmed by clinical studies.

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Angiotensin II Type 2 Receptor


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