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

Hiroaki Matsubara

Abstract—Since the discovery of nonpeptidic ligands, the receptors for angiotensin (Ang) II have been classified into 2 subtypes (Ang II type 1 receptor [AT\(_1\)-R] and Ang II type 2 receptor [AT\(_2\)-R]). AT\(_1\)-R mediates most of the cardiovascular actions of Ang II. AT\(_2\)-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 AT\(_2\)-R can be modulated by pathological states associated with tissue remodeling or inflammation. In failing hearts or neointima formation after vascular injury, AT\(_2\)-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 AT\(_2\)-R. Cardiac myocyte- or vascular smooth muscle–specific mice that overexpress AT\(_2\)-R display an inhibition of Ang II-induced chronotropic or pressor actions, suggesting the role of AT\(_2\)-R on the activity of cardiac pacemaker cells and the maintenance of vascular resistance. AT\(_2\)-R also activates the kinin/nitric oxide/cGMP system in the cardiovascular and renal systems, resulting in AT\(_2\)-R–mediated cardioprotection, vasodilation, and pressure natriuresis. These effects, transmitted by AT\(_2\)-R, are mainly exerted by stimulation of protein tyrosine or serine/threonine phosphatases in a Gi protein–dependent manner. The expression level of AT\(_2\)-R is much higher in human hearts than in rodent hearts, and the AT\(_2\)-R–mediated actions are likely enhanced, especially by clinical application of AT\(_1\)-R antagonists. Thus, in this review, the regulation of AT\(_2\)-R expression, its cellular localization, its pathological role in cardiovascular and kidney diseases, and pharmacotherapeutic effects of AT\(_2\)-R stimulation are discussed. (Circ Res. 1998;83:1182-1191.)

Key Words: angiotensin II receptor | angiotensin II type 2 receptor | angiotensin II AT\(_2\) receptor | angiotensin II type 1 receptor | angiotensin II AT\(_1\) 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 (AT\(_1\)-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 [AT\(_2\)-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 AT\(_2\)-R was isolated by expression cloning from PC12 cells\(^8\) and whole fetus.\(^9\) Knockout mice for the AT\(_2\)-R gene were developed, and studies of these mice suggested that AT\(_2\)-R has a physiological role in blood pressure control and emotional instability and fearfulness.\(^10\),\(^11\) In addition, an AT\(_2\)-R–mediated inhibitory effect on the growth-promoting signals has been found.\(^5\)–\(^6\) Treatment with AT\(_2\)-R antagonists causes a marked elevation of levels of plasma Ang II, which selectively binds to AT\(_2\)-R and exerts as yet undefined effects.\(^12\) Thus, elucidation and understanding of AT\(_2\)-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 AT\(_2\)-R as well as potential issues concerning clinical application of AT\(_1\)-R antagonists.

Structural Features of AT\(_2\)-R

The second isoform of the Ang II receptor, AT\(_2\)-R, has been defined as the receptor that binds specifically to CGP42112 and to a series of
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 (glomerular 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/STATS</td>
</tr>
<tr>
<td><strong>Function</strong></td>
<td>Vasodilatation, aldosterone secretion, hypertension, cellular growth, catecholamine release</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>
</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>

Figure 1. Proposed AT2-R-mediated effects on cardiovascular and renal system. PP2A indicates serine/threonine phosphatase 2A; I[K+]d, delayed rectifier K+ current; I[K+]o, transient outward K+ current; Ca2+/CaMK; calcium calmodulin kinase; MLCK, myosin light chain kinase; and EDHF, endothelium-derived hyperpolarizing factor.

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 with a 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 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.15 The AT2-R gene has been characterized in both the mouse16,17 and human.18–20 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 AT1α-R or AT1β-R, found in the AT1-R family.9,9

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 AT2-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, 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.27,28 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...
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 AT2-R induction during the process of inflammation.29 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 fetal mesenchymal tissues. Although these multiple factors regulate the expression of AT2-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, AT2-R expression is increased markedly. This gene regulation is exerted at the transcriptional level.22,23 Horiuchi et al46,47 demonstrated that interferon regulatory factor-1, the expression of which is increased during confluent state and by serum depletion, binds to the interferon regulatory factory-binding motif of the AT2-R gene promoter region and upregulates its gene transcription.46

**AT2-R-Mediated Effects on Ang II–Induced Mitogen Signaling, Apoptosis, Kinin/Nitric Oxide/eGMP 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 phosphatase (PTP) to inhibit cell proliferation and differentiation.41 NIH/3T3 cells stably expressing AT2-R, Ozawa et al42 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 al43,44 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.45

**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. Jianak et al49 reported the selective activation of AT2-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 myocytes49 or fibroblasts50,51 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.53 Furthermore, Masaki et al44 ascertained that AT2-R significantly inhibits AT1-R–mediated ERK activation in perfused mouse hearts overexpressing AT2-R. The AT2-R–mediated antiproliferative effect on DNA synthesis was also demonstrated in embryonic renalomedullary interstitial cells.54 The mechanism of AT2-R participation in this process might be associated with reduced ERK activation caused by the activation of ERK phosphatase 1 (MKP-1), a dual-specificity phosphatase that acts on both phosphothreonine and phosphosterylone. Horiuchi et al55 reported that AT2-R stimulation dephosphorylates Bcl-2 by activating MKP-1 and induces apoptosis in PC12W cells. Bedec et al57 reported that AT2-R–mediated inhibition of serum- and 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 AT1-R.55 In neuronal cultures isolated from neonatal rat hypothalamus or in nondifferentiated NG108-15 cells (neuroblastoma cells), activation of AT2-R reportedly elicits stimulation of outward58,59 and delayed rectifier K+ currents60 and inhibits T-type Ca2+ 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) AT2-R reportedly induces a marked decrease in phosphorylation of several cellular proteins on tyrosine residues.62 Bottari et al63 and Brechler et al64 reported that AT-R stimulation causes activation of a membrane-associated PTP and inhibition of atrial natriuretic peptide-sensitive particular 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.65 Prolonged serum depletion, which is enhanced by Ang II treatment, elicits programmed cell death of R3T3 cells.43,44 The suppression of growth and inhibition of atrial natriuretic peptide-sensitive particular 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

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

AT2-R–mediated activation of the kinin and nitric oxide (NO) system has been reported in bovine endothelial cells,70 isolated rat carotid arteries,72 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 AT2-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 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 al78 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.79 AT2-R–mediated NO production also is involved voltarily in AT2-R–mediated pressure natriuresis an 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. Cardiac fibroblasts isolated from neonatal and adult rat hearts express abundant amounts of AT2-R (~8-fold more than that in myocytes) but not of AT1-R. Although some attempts to detect the AT2-R in adult rodent myocytes by a binding assay or polymerase chain reaction have proved negative, other researchers have obtained positive results. Although autoradiography results also indicated the presence of AT2-R protein in the adult rat myocardium, 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. Thus, the proportion of cardiac AT2-R expressed in situ in the rat heart varies from that detected in cultured cells, possibly reflecting downregulation of AT2-R expression after the isolation of mammalian cells and the growth-dependent regulation of AT2-R expression in cultured cells.

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. 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, however, the proportion of AT2-R to binding sites differs between these studies. Interestingly, Tsutsumi et al. found that AT1-R and AT2-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 the experimental conditions when Ang II receptor densities are measured in tissue samples. This finding means that great care should be taken to factor in the experimental conditions when Ang II receptor densities are measured in tissue samples. Additionally, Rogg et al. reported a relatively high amount of Ang II receptors (118 fmol/mg protein) in the human atria when they used combined fractions including plasma membranes and internalized receptors; other investigators measured Ang II receptors using only membrane fractions. In fact, Regitz-Zagrosek et al. 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. 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 heart has been examined mainly using emulsion autoradiography. The expression of cardiac AT2-R was localized more highly in the fibroblasts present in the interstitial regions than those in the myocardium (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. Although the AT2-R protein might be partially degraded during sample preparation such as during the freezing of tissues, 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 AT2-R expression in human hearts increases during cardiac remodeling as a result of dilated cardiomyopathy or ischemic heart diseases.

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, cultured human cardiac fibroblasts display mainly AT2-R-mediated effects on collagen synthesis. Anytical Ang II binding sites for Ang 1–7 were detected on human cardiac fibroblasts, whereas Ang 3–8 binding sites were present on rabbit cardiac fibroblasts or myocardial membranes of guinea pigs and human hearts. Two subtypes of the AT2-R, AT2α-R and AT2β-R, were encoded in human tissues, and there was heterogeneity in mammalian AT2-R 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, an
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>↑</td>
<td>↑</td>
<td>Artery constriction/dog</td>
<td>113</td>
</tr>
<tr>
<td>↑</td>
<td>↑</td>
<td>Mechanical stretch/rat</td>
<td>81</td>
</tr>
<tr>
<td>↑</td>
<td>→</td>
<td>Aortic banding/rat</td>
<td>89, 90</td>
</tr>
<tr>
<td>↑</td>
<td>→</td>
<td>mRNA Aortic banding/rat</td>
<td>112</td>
</tr>
<tr>
<td>↑</td>
<td>→</td>
<td>mRNA Aortic banding/rat</td>
<td>111</td>
</tr>
</tbody>
</table>

Failing heart

|                | mRNA/protein | Hereditary myopathy/hamster | 52            |
|                | ↑            | Protein Human atrium        | 101, 102      |
|                | ↑            | Protein Human atrium/LV     | 99            |
|                | ↑            | Protein Human atrium        | 102           |
|                | →            | Protein Human atrium/LV     | 98            |
|                | ↑            | mRNA Human LV               | 115           |
|                | →            | Protein Human atrium/LV     | 97            |

Myocardial infarction

|                | mRNA/protein | Rat                        | 88            |
|                | →            | Protein Rat                | 151           |

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.

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. 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...
coupled with competitive binding studies has been used to characterize the distribution of Ang II receptor subtypes in renal tissue. By use of these techniques, the distribution of AT₁-R and AT₂-R subtypes within the kidneys was shown to be species-dependent. For example, in the rat and rabbit kidneys, Ang II receptors are 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.

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

Ichiki et al. and Hein et al. 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, and their basal blood pressure also increased. However, because AT₂-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. 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. Tsutsu et al. and Hein 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-R152 (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.75,97,98,100,115 The activation of the kinin/NO/cGMP system mediated 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 Ang II.
exerted by ACE inhibitors such as activation of the kinin/NO system. In fact, Schieffer et al.\textsuperscript{146} reported that ACE inhibitors and AT2-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.\textsuperscript{147} These beneficial effects might be partly explained by the potential effect mediated by AT2-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 AT2-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 AT2-R should be confirmed by clinical studies.

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