

Molecular and Cellular Mechanisms of Angiotensin II-Mediated Cardiovascular and Renal Diseases

SHOKEI KIM¹ AND HIROSHI IWAO

Department of Pharmacology, Osaka City University Medical School, Abeno-ku, Osaka, Japan

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¹ Address for correspondence: Shokei Kim, MD, PhD, Department of Pharmacology, Osaka City University Medical School, 1-4-3 Asahimachi, Abeno, Osaka 545-8585, Japan. E-mail: kims@med.osaka-cu.ac.jp

Abstract—A growing body of evidence supports the notion that angiotensin II (Ang II), the central product of the renin-angiotensin system, may play a central role not only in the etiology of hypertension but also in the pathophysiology of cardiovascular and renal diseases in humans. In this review, we focus on the role of Ang II in cardiovascular and renal diseases at the molecular and cellular levels and discuss up-to-date evidence concerning the *in vitro* and *in vivo* actions of Ang II and the pharmacological effects of angiotensin receptor antagonists in comparison with angiotensin-converting enzyme inhibitors. Ang II, via AT₁ receptor, directly causes cellular phenotypic changes and cell growth, regulates the gene expression of various bioactive substances (vasoactive hormones, growth factors, extracellular matrix compo-

nents, cytokines, etc.), and activates multiple intracellular signaling cascades (mitogen-activated protein kinase cascades, tyrosine kinases, various transcription factors, etc.) in cardiac myocytes and fibroblasts, vascular endothelial and smooth muscle cells, and renal mesangial cells. These actions are supposed to participate in the pathophysiology of cardiac hypertrophy and remodeling, heart failure, vascular thickening, atherosclerosis, and glomerulosclerosis. Furthermore, *in vivo* recent evidence suggest that the activation of mitogen-activated protein kinases and activator protein-1 by Ang II may play the key role in cardiovascular and renal diseases. However, there are still unresolved questions and controversies on the mechanism of Ang II-mediated cardiovascular and renal diseases.

I. Introduction

Classically, angiotensin II (Ang II),² the central product of the renin-angiotensin system (Fig. 1), is well known to cause potent increases in systemic and local blood pressure via its vasoconstrictive effect, to influence renal tubules to retain sodium and water, and to stimulate aldosterone release from the adrenal gland (Timmermans et al., 1993). A growing body of evidence, from pharmacological investigations and clinical studies on the effects of angiotensin-converting enzyme (ACE) inhibitors, supports the notion that Ang II may play a central role not only in the etiology of hypertension but also in the pathophysiology of cardiac hypertrophy and remodeling, heart failure, vascular thickening, atherosclerosis, and glomerulosclerosis in humans. An excellent and comprehensive review on pharmacology of angiotensin receptor antagonists was published in 1993 (Timmermans et al., 1993). However, since then, a great number of *in vitro* and *in vivo* findings on the functions of Ang II have emerged, showing that Ang II directly causes cell growth, regulates the gene expression of various bioactive substances [vasoactive hormones, growth

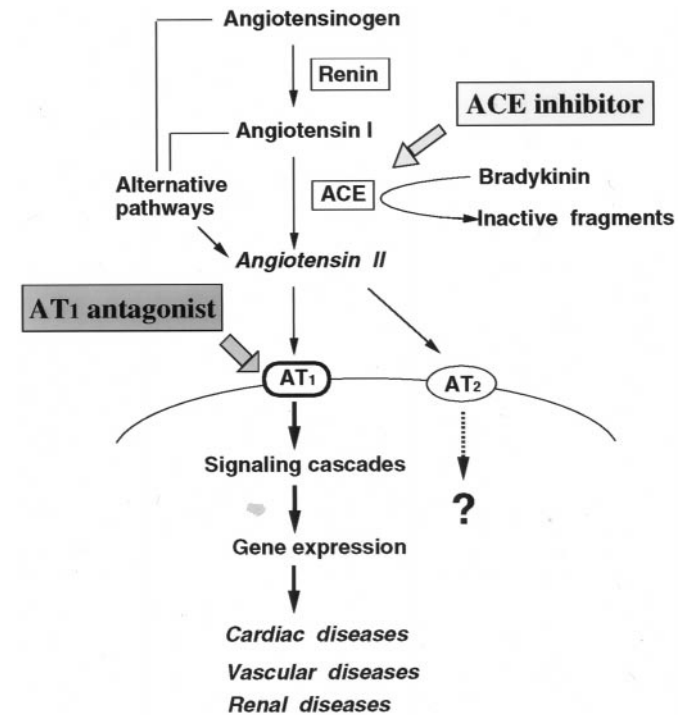


FIG. 1. Cascade of renin-angiotensin system. ACE, angiotensin-converting enzyme; AT₁, AT₁ receptor; AT₂, AT₂ receptor.

factors, extracellular matrix (ECM) components, cytokines, and so on], and activates multiple intracellular signaling cascades [mitogen-activated protein (MAP) kinase cascades, tyrosine kinases, various transcription factors, and so on] in cardiovascular and renal cells. Furthermore, accumulating *in vivo* evidence supports the notion that Ang II may directly cause cardiovascular and renal diseases, independent of its blood pressure-elevating effect. Thus, recent *in vivo* work, coupled with *in vitro* findings, has provided new insights into the molecular and cellular mechanisms of Ang II-mediated cardiovascular and renal diseases. In this review, we focus on the role of Ang II in cardiovascular and renal diseases at the molecular and cellular levels and discuss up-to-date evidence concerning the actions of Ang II and

² Abbreviations: Ang II, angiotensin II; ACE, angiotensin-converting enzyme; AT₁, angiotensin II type 1; ECM, extracellular matrix; MAP, mitogen-activated protein; AT₂, angiotensin II type 2; MHC, myosin heavy chain; ANF, atrial natriuretic factor; PLC, phospholipase C; PKC, protein kinase C; ERK, extracellular signal-regulated kinase; JNK, *c-jun* NH2-terminal kinase; p70S6K, 70-kDa ribosomal S6 kinase; NIDDM, non-insulin-dependent diabetes mellitus; p90RSK, 90-kDa ribosomal S6 kinase; NF- κ B, nuclear factor- κ B; STAT, signal transducer and activator of transcription; TGF- β 1, transforming growth factor- β 1; MEK, mitogen-activated protein kinase kinase; AP-1, activator protein-1; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats; SHRSP, stroke-prone spontaneously hypertensive rats; AC, aorticaval; DM, diabetes mellitus; OLETF, Otsuka Long-Evans Tokushima Fatty; SMC, smooth muscle cell; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; PAI, plasminogen activator inhibitor; PI3-kinase, phosphatidylinositol 3-kinase; KDR/Flk-1, kinase domain-containing receptor/total liver kinase; DOCA, deoxycorticosterone acetate.

the pharmacological effects of angiotensin receptor antagonists. Furthermore, we discuss recent progress, clearly separating the *in vitro* and *in vivo* evidence, because previous data have largely come from *in vitro* studies using cultured cells, and most of these data have not yet been demonstrated to apply to *in vivo* conditions.

II. Classification and Biochemical Characteristics of Angiotensin Receptors

A. AT_1 Receptor

Excellent detailed reviews, dealing with biochemical properties and molecular biology of angiotensin receptors, have been published previously (Brown and Sernia, 1994; Unger et al., 1996). Therefore, in this review, we only briefly discuss the characteristics of angiotensin receptors. The existence of two subtypes of Ang II receptors, including Ang II types 1 and 2 (AT_1 and AT_2) receptors (Fig. 1), was first confirmed by a pharmacological approach using various specific Ang II receptor antagonists (Chiu et al., 1989). The successful cloning of AT_1 receptor in 1991 (Murphy et al., 1991; Sasaki et al., 1991) allowed the development of further research on the structure and function of this receptor. In rats or mice, AT_1 receptor consists of two subtypes, AT_{1a} and AT_{1b} , which have 94% homology with regard to amino acid sequence and have similar pharmacological properties and tissue distribution patterns. AT_1 receptor is a member of the seven transmembrane-spanning, G protein-coupled receptor family; binds to heterotrimeric G proteins; and lacks intrinsic tyrosine kinase activity. Human AT_1 receptor gene is mapped to chromosome 3, and AT_{1a} and AT_{1b} receptor genes in rats are mapped to chromosomes 17 and 2, respectively. AT_1 receptor is ubiquitously and abundantly distributed in adult tissues, including blood vessel, heart, kidney, adrenal gland, liver, brain, and lung. AT_1 receptor mediates all the classic well known effects of Ang II, such as elevation of blood pressure, vasoconstriction, increase in cardiac contractility, aldosterone release from the adrenal gland, facilitation of catecholamine release from nerve endings, renal sodium and water absorption, and so on, as reviewed previously in detail (Timmermans et al., 1993). In addition, recent accumulating *in vitro* and *in vivo* evidence supports the notion that Ang II, mediated by AT_1 receptor, may participate directly in the pathogenesis of various cardiovascular and renal diseases, and this evidence is the focus of this review. Thus, the molecular and cellular actions of Ang II in cardiovascular and renal diseases are almost exclusively mediated by AT_1 receptor. Numerous selective and potent nonpeptide AT_1 receptor antagonists have been developed, such as losartan, candesartan, valsartan, irbesartan, eprosartan, telmisartan, tasosartan, and others, and in recent years, several of these compounds, including losartan, candesartan, valsartan, and others, have been in use

clinically for the treatment of hypertension (Bauer and Reams, 1995; Johnston, 1995; Pitt and Konstam, 1998).

B. AT_2 Receptor

Molecular cloning, structural features, regulation of gene expression, and the possible functions of AT_2 receptor have been reviewed elsewhere (Unger et al., 1996; Matsubara, 1998). Selective AT_2 receptor ligands include PD123177, PD123319, CGP42112, L-162,686, L-162,638, EXP801, and CGP42112A. The cDNA and genomic DNAs of human, rat, and mouse AT_2 receptors have been cloned. AT_2 receptor is ubiquitously expressed in developing fetal tissues, suggesting a possible role of this receptor in fetal development and organ morphogenesis. In contrast, AT_2 receptor expression rapidly decreases after birth, and in the adult, expression of this receptor is limited mainly to the uterus, ovary, certain brain nuclei, heart, and adrenal medulla. The AT_2 receptor gene is localized as a single copy on the X chromosome. Unlike the AT_1 receptor, which has been shown to have subtypes in rats and mice, there is no evidence for subtypes of the AT_2 receptor. Although a comparison of amino acid sequences of AT_{1a} and AT_2 receptors in rats, deduced from nucleotide sequences, shows a low homology between these receptors (32%), AT_2 receptor is also a seven-transmembrane domain receptor. Recent work showed that AT_2 receptor is coupled to the G protein G_i (Hayashida et al., 1996; Zhang and Pratt, 1996). In various cell lines, AT_2 receptor-activated protein tyrosine phosphatase was shown to inhibit cell growth (Matsubara, 1998) or induce programmed cell death (apoptosis) (Yamada et al., 1996). AT_2 receptor inhibited AT_1 receptor-mediated cell growth, demonstrating an antagonistic action. However, there have also been conflicting findings regarding these receptors. In contrast to extensive data on the molecular and cellular functions and pathophysiological significance of AT_1 receptor, the role of AT_2 receptor in cardiovascular and renal diseases remains to be defined. At present, an AT_2 receptor ligand has not been developed for clinical use. However, because up-to-date evidence suggests that AT_2 receptor may contribute to the pharmacological differences between AT_1 receptor antagonists and ACE inhibitors, we discuss the potential functional role of AT_2 receptor in VI. *AT_1 Receptor Antagonists versus Angiotensin-Converting Enzyme Inhibitors.*

III. Molecular and Cellular Actions of Angiotensin II in Heart

A. Molecular Characteristics of Pathological Cardiac Hypertrophy

We first discuss the characteristics of pathological cardiac hypertrophy as background for understanding the role of Ang II in cardiac diseases. The characteristics of pathological cardiac hypertrophy have been reviewed

in detail previously (Schwartz et al., 1993; Parker, 1995). Generally, pathological left ventricular hypertrophy is characterized not only by an increase in myocyte size (quantitative change) but also by myocyte gene reprogramming (qualitative change), as shown by enhanced expression of fetal phenotypes of genes such as β -myosin heavy chain (β -MHC), skeletal α -actin, and atrial natriuretic factor (ANF). In the cardiac ventricle of most mammalian species, including humans, MHC consists of two isoforms: α - and β -MHCs. In the rat, α -MHC is the predominant isoform in adult hearts (Lompre et al., 1984), has high Ca^{2+} and actin-activated ATPase activity, and is associated with increased shortening velocity of the cardiac fibers (Barany, 1967; Alpert and Mulieri, 1982). On the other hand, β -MHC is the predominant isoform in fetal hearts (Lompre et al., 1984), has lower ATPase activity, and is associated with slower shortening velocity (Barany, 1967; Alpert and Mulieri, 1982). Therefore, changes in the ratio of β -MHC (fetal phenotype) to α -MHC (adult phenotype) in the cardiac ventricle significantly alter the contractile properties of the heart. Cardiac sarcomeric actin is also composed of two isoforms: cardiac α -actin and skeletal α -actin. Cardiac α -actin is predominantly expressed in adult rat hearts, whereas skeletal α -actin is normally expressed in fetal and neonatal rat hearts (Minty et al., 1982; Mayer et al., 1984). Because skeletal α -actin has greater contractility than cardiac α -actin (Hewett et al., 1994), the ratio of skeletal α -actin to cardiac α -actin in the ventricle plays a significant role in cardiac function. Thus, an increase in ventricular myocyte expression of fetal isoforms of contractile proteins (β -MHC and skeletal α -actin), which often occurs in the hypertrophic heart (Schwartz et al., 1986; Izumo et al., 1987), modulates cardiac performance. Furthermore, in addition to showing enhanced expression of β -MHC and skeletal α -actin, hypertrophic ventricular myocytes are also characterized by significant up-regulation of ANF (Izumo et al., 1988), which is scarcely expressed in normal adult ventricular myocytes.

Another important property of pathological cardiac hypertrophy is increased accumulation of ECM proteins such as collagen (particularly collagen types I and III) and fibronectin in the interstitium and around blood vessels within the heart. These changes play a central role in ventricular fibrosis or remodeling. The detailed characteristics and significance of these processes have been reviewed elsewhere (Brilla et al., 1992; Pelouch et al., 1993; Weber et al., 1994b; Weber, 1997). Increased interstitial collagen deposition in the heart enhances cardiac stiffness (Abrahams et al., 1987; Doering et al., 1988; Jalil et al., 1989) and results in diastolic dysfunction (Pelouch et al., 1993; Weber et al., 1994b). Fibronectin is localized on the surface of cardiac myocytes, connects cardiac myocytes to perimycytic collagen (Ahumada and Saffitz, 1984), and is thought to affect cardiac systolic and diastolic functions. Thus, increased

ECM accumulation, as well as the above-mentioned ventricular myocyte gene reprogramming, plays a critical role in the impairment of cardiac performance and pathophysiology of cardiac failure. ECM proteins within the heart are predominantly produced by fibroblasts. Notably, unlike cardiac myocytes, cardiac fibroblasts proliferate and increase the production of ECM proteins when the heart is exposed to hypertrophic stimuli such as hemodynamic overload. Thus, cardiac fibroblasts and cardiac myocytes play key roles in the development of pathological cardiac hypertrophy and dysfunction. Accumulating *in vitro* and *in vivo* evidence supports the concept that Ang II is involved in all of these important processes of pathological cardiac hypertrophy, including myocyte hypertrophy, myocyte gene reprogramming, fibroblast proliferation, and ECM protein accumulation, as described later.

B. Cultured Cardiac Myocytes

The molecular mechanism of hypertrophy of cultured cardiac myocytes has been extensively studied, mostly using neonatal rat cardiac myocytes, and was reviewed recently (Baker et al., 1992; Sadoshima and Izumo, 1997; Sugden and Clerk, 1998a). In this article, we briefly discuss the effects of Ang II on cultured cardiac myocyte gene expressions and signaling transduction cascades only to highlight their significance for pathological cardiac hypertrophy. Although cardiac myocytes express both AT_1 and AT_2 receptors (Booz and Baker, 1996), almost all of the biological responses to Ang II reported so far are mediated by AT_1 receptor. The effects of Ang II, which we describe in this section, are apparently all mediated by this receptor. Accumulating evidence has established that Ang II causes hypertrophy of neonatal cardiac myocytes (Baker and Aceto, 1990; Baker et al., 1992; Sadoshima and Izumo, 1993, 1997) and adult myocytes (Wada et al., 1996; Liu et al., 1998; Ritchie et al., 1998). Ang II directly induced the fetal phenotype of gene expressions, such as those of β -MHC, skeletal α -actin, and ANF, in neonatal rat cardiac myocytes, indicating the direct involvement of AT_1 receptor in cardiac gene reprogramming *in vitro* (Sadoshima and Izumo, 1993). Furthermore, Ang II stimulated the expression of immediate-early genes, including *c-fos*, *c-jun*, *jun B*, *Egr-1*, and *c-myc* (Sadoshima and Izumo, 1993). However, it is unclear whether the induction of these immediate-early genes is necessary for myocyte hypertrophy or gene reprogramming by Ang II, and the significance of induction of these immediate-early genes in hypertrophy remains to be determined.

Interestingly, Ang II, via AT_1 receptor, activates a diversity of intracellular signaling cascades in neonatal rat cardiac myocytes, although the role of these signaling cascades in myocyte hypertrophy or gene reprogramming remains to be elucidated. Cardiac myocyte AT_1 receptor couples to a heterotrimeric G protein, G_q . As with other G protein-coupled receptors, Ang II stimu-

lates phosphatidylinositol-specific phospholipase C (PLC)- β isoform through G_q , and the activation of PLC causes increases in inositol triphosphate and diacylglycerol, which in turn lead to an increase in release of Ca^{2+} from intracellular stores and activation of protein kinase C (PKC), respectively. Besides the above-mentioned basic cascades via G_q , it has been reported that Ang II in neonatal rat cardiac myocytes activates tyrosine kinases (Sadoshima et al., 1995), extracellular signal-regulated kinases (ERKs), c-Jun amino-terminal kinases (JNKs; Kudoh et al., 1997), 70-kDa ribosomal S6 kinase (p70S6K; Takano et al., 1996), 90-kDa ribosomal S6 kinase (p90RSK; Sadoshima and Izumo, 1995), p21ras, and phospholipases A_2 and D and increases phosphatidic acid and arachidonic acid, as mentioned in a previous review (Sadoshima and Izumo, 1997). Although AT_1 receptor has no intrinsic tyrosine kinase activity, the addition of Ang II (10^{-7} M) to neonatal rat cardiac myocytes induced rapid phosphorylation of Janus kinase 2, Tyk2, signal transducer and activator of transcription (STAT) $_1$ and STAT $_2$ in the early stage up to 30 min, and phosphorylated STAT $_3$ in the late stage at 120 min (Kodama et al., 1998), although the significance of these observations is unknown. Ang II (10^{-7} M) activated RhoA, which is responsible for Ang II-induced sarcomeric actin organization and ANF expression (Aoki et al., 1998). However, it remains unclear whether Ang II can indeed simultaneously activate all of the above-mentioned signaling cascades in myocytes. Furthermore, it remains to be determined to what extent each signaling cascade is involved in Ang II-induced cardiac myocyte hypertrophy and gene reprogramming seen in pathological cardiac hypertrophy in vivo.

We emphasize that despite abundant evidence for the activation of multiple signaling cascades by Ang II, the molecular mechanism of hypertrophy of cultured neonatal rat myocytes by Ang II is poorly understood. Ang II (10^{-6} M) activated p70S6K in myocytes, and inhibition of this activation by rapamycin (0.5 ng/ml), an immunosuppressant, abolished the Ang II-induced increase in protein synthesis, without blocking ERK activation or *c-fos* mRNA induction, confirming the contribution of p70S6K to Ang II-induced hypertrophy (Takano et al., 1996). However, p70S6 kinase was not involved in Ang II-induced myocyte gene reprogramming, as shown by the lack of effect of rapamycin on Ang II-induced skeletal α -actin, β -MHC, or ANF expression (Sadoshima and Izumo, 1995). Notably, no information is available on the role of ERK activation in Ang II-induced myocyte hypertrophy or gene expression, although ERK activation is thought to be important for myocyte hypertrophy in response to other hypertrophic stimuli, such as phenylephrine (Force et al., 1996; Glennon et al., 1996; Sugden and Clerk, 1998a). Ang II (100 nM) induced the generation of reactive oxygen intermediates in neonatal rat cardiac myocytes, and the antioxidant, butylated hydroxyanisole (10 μ M), significantly inhibited Ang II-

induced myocyte enlargement and increased 3H -leucine incorporation, suggesting an important role of reactive oxygen intermediates in Ang II-induced myocyte hypertrophy (Nakamura et al., 1998). Ang II also stimulated endothelin-1 production in neonatal rat myocytes by activating PKC, which is involved in Ang II-induced myocyte hypertrophy via an autocrine mechanism (Ito et al., 1993).

C. Neonatal versus Adult Cardiac Myocytes

So far, most studies on the effects of Ang II on cardiac myocytes have been carried out using cultured neonatal rat cardiac myocytes. These cells have the ability to divide with serum stimulation, whereas adult rat cardiac myocytes are terminally differentiated and have no ability to divide, indicating a significant difference in phenotype between neonatal and adult cardiac myocytes. In fact, Schunkert et al. (1995) showed that the Ang II-induced increase in protein synthesis of isolated perfused adult rat heart was not accompanied by induction of *c-fos* and *c-jun* expression, in contrast to the situation in neonatal myocytes. Stretching of neonatal rat cardiac myocytes, the most popular in vitro model for investigation of the effect of load on cardiac myocytes, causes myocyte hypertrophy and gene reprogramming (Sadoshima and Izumo, 1997). It has been reported that Ang II secreted from myocytes plays a central role in stretch-induced hypertrophy, functioning as an autocrine in the neonatal rat myocyte culture system (Sadoshima et al., 1993). On the other hand, recent studies using isolated perfused adult rat heart preparation (Thienelt et al., 1997) or isolated perfused adult feline heart (Kent and McDermott, 1996) showed that increases in protein synthesis or *c-fos* and *c-myc* mRNA in the heart that were induced by systolic pressure overload were not prevented by an AT_1 receptor antagonist, which does not support a role for AT_1 receptor in pressure-induced acute growth responses in the adult heart. Thus, the data appear to be inconsistent for neonatal versus adult myocytes regarding the molecular mechanism of Ang II-induced hypertrophic response. The findings for neonatal myocytes should be interpreted with caution.

D. Cultured Cardiac Fibroblasts

The heart is composed of not only cardiac myocytes but also nonmyocyte cells, particularly fibroblasts. Unlike cardiac myocytes, cardiac fibroblasts can proliferate even in the adult heart. Furthermore, cardiac fibroblasts play a major role in the production of ECM proteins such as fibronectin and collagen (Dostal et al., 1996). Therefore, fibroblasts are critical for the development of cardiac fibrosis. Neonatal rat cardiac fibroblasts possess abundant AT_1 receptors. On the other hand, AT_2 receptors were undetectable in neonatal or adult rat cardiac fibroblasts (Villarreal et al., 1993; Crabos et al., 1994), indicating that the responses of cardiac fibroblasts to

Ang II are due to AT₁ receptor. Unlike the situation with neonatal myocytes, Ang II treatment stimulated the proliferation of neonatal rat cardiac fibroblasts, as shown by significant increases in [³H]phenylalanine incorporation, [³H]thymidine uptake, and cell number (Schorb et al., 1993). These mitogenic effects of Ang II were completely blocked by losartan but not by PD123319, demonstrating the primary role of AT₁ receptor in Ang II-induced cardiac fibroblast proliferation. Ang II also exerted mitogenic effects on adult cardiac fibroblasts (Crabos et al., 1994). As in the case of neonatal cardiac myocytes, Ang II increased mRNA levels for *c-fos*, *c-jun*, *jun B*, *Egr-1*, and *c-myc* in cardiac fibroblasts via AT₁ receptor (Sadoshima and Izumo, 1993). Furthermore, Ang II increased collagen type I mRNA and the synthesis and secretion of collagen (Crabos et al., 1994), as well as mRNA expression and protein secretion of transforming growth factor- β 1 (TGF- β 1; Campbell and Katwa, 1997) and fibronectin (Iwami et al., 1996). Ang II also increased cardiac fibroblast osteopontin expression, and stimulation of cardiac fibroblast DNA synthesis by Ang II was completely blocked by antibodies against osteopontin and β 3 integrin, suggesting that Ang II-induced cardiac fibroblast proliferation may require osteopontin engagement of β 3 integrin (Ashizawa et al., 1996).

AT₁ receptor in cardiac fibroblasts couples to G_i, in contrast to AT₁ receptor in cardiac myocytes, which couples to G_q. Ang II signaling cascades differ significantly between cardiac fibroblasts and myocytes (Zou et al., 1998). Ang II (10⁻⁶ M) activated the STAT signaling pathway as shown by gel mobility shift assay (Bhat et al., 1994). Immunoblot analysis showed that Ang II induced tyrosine phosphorylation of ERK, focal adhesion kinase, and Shc in neonatal rat cardiac fibroblasts, which was mediated not by PKC, as shown by the lack of inhibition with phorbol ester-sensitive PKC down-regulation, but rather by a G_i protein as shown by partial inhibition with pertussis toxin pretreatment (100 ng/ml; Schorb et al., 1994). Ang II (10⁻⁶ M) activated ERK, and pretreatment with the specific MAP kinase kinase (MEK) inhibitor PD98059 (5 × 10⁻⁵ M) completely blocked the Ang II-induced increase in thymidine incorporation, suggesting that Ang II-induced DNA synthesis in cardiac fibroblasts is mediated by MEK and, probably, ERK (Zou et al., 1998). However, the signaling cascade underlying the production of ECM proteins in cardiac fibroblasts by Ang II remains unclear.

E. Effects of In Vivo Angiotensin II Infusion on Heart

Figure 2 illustrates the cardiac molecular and cellular effects of Ang II in vivo. Ang II infusion in rats can induce cardiac hypertrophy via AT₁ receptor, independent of its blood pressure-elevating effect (Dostal and Baker, 1992). Furthermore, continuous infusion of Ang II (200 ng/min i.p.) in adult rats, while causing a moderate increase in blood pressure, produced both myocyte

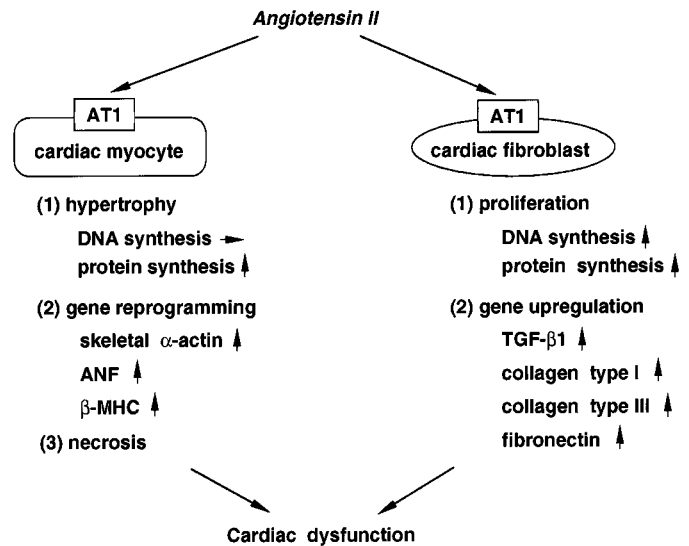


FIG. 2. Proposed mechanism of AT₁ receptor-mediated pathological cardiac hypertrophy. Ang II induces cellular hypertrophy, gene reprogramming or necrosis in cardiac myocytes, and cellular proliferation and up-regulation of fibrosis-associated genes in cardiac fibroblasts, which are all mediated via AT₁ receptor.

necrosis and myocytolysis, as shown by labeling of cardiac myocytes with exogenously administered monoclonal anti-myosin antibody; this subsequently caused cardiac fibroblast proliferation and resulted in significant scar formation, indicating the cardiotoxic effects of Ang II in vivo (Tan et al., 1991). Ang II (200 ng/kg/min s.c.) infusion in rats caused a small and gradual increase in blood pressure; elevated left ventricular mRNAs for skeletal α -actin, β -MHC, ANF, and fibronectin, preceding an increase in left ventricular mass; and elevated TGF- β 1 and types I and III collagen mRNA levels (Kim et al., 1995c); these increases were completely inhibited by candesartan cilexetil (3 mg/kg/day p.o.) but not by hydralazine (10 mg/kg/day). Thus, Ang II in vivo, via AT₁ receptor, directly induces cardiac myocyte hypertrophy and gene reprogramming, and probably fibroblast proliferation and subsequent fibrosis as well, independent of the elevation of blood pressure (Fig. 2), indicating the key role of Ang II in the development of pathological cardiac hypertrophy.

Investigations of the in vivo effects of Ang II on cardiac intracellular signaling cascades are essential to elucidate the molecular mechanism underlying Ang II-induced pathological cardiac hypertrophy. In contrast to the detailed in vitro studies on cultured myocytes and fibroblasts described above, the action of Ang II on cardiac signaling cascades in vivo is poorly understood. Accumulating in vitro evidence on cultured cardiac myocytes or fibroblasts suggests that MAP kinases, including ERK or JNK, may be responsible for myocyte hypertrophy and gene reprogramming or fibroblast proliferation (Force et al., 1996; Sugden and Clerk, 1998b; Wang et al., 1998). Recent work on the effects of Ang II infusion in vivo in conscious rats showed that Ang

II-induced cardiac activation of JNK occurs in a more sensitive manner than that of ERK, and JNK activation by Ang II without ERK activation is followed by activation of activator protein-1 (AP-1; composed of c-Fos and c-Jun proteins) (Yano et al., 1998). Importantly, AP-1 regulates the expression of various genes by binding the AP-1 consensus sequence present in their promoter regions. Interestingly, fetal phenotypes of cardiac genes such as skeletal α -actin and ANF (Karin, 1995; Force et al., 1996), and cardiac fibrosis-associated genes such as TGF- β 1 (Kim et al., 1989) and collagen type I (Katai et al., 1992) have AP-1 responsive sequences in their promoter regions. Indeed, AP-1 activation has been demonstrated to lead to increased promoter activity of skeletal α -actin (Bishopric et al., 1992) and TGF- β 1 (Kim et al., 1990). Therefore, it is intriguing to postulate that JNK activation, in part through activation of AP-1, may be implicated in Ang II-induced cardiac hypertrophic response in vivo (Fig. 3). Thus, important differences in the molecular mechanism of Ang II-induced cardiac hypertrophy exist between the adult heart in vivo and neonatal cardiac myocytes in vitro. However, it remains unclear whether the increased MAP kinase induced by Ang II infusion originates in myocytes, fibroblasts, or both. Further detailed work is needed to demonstrate whether activation of JNK/AP-1 by Ang II infusion is responsible for the above-mentioned cardiac hypertrophy-associated gene expressions.

Ang II, via AT₁ receptor, is known to facilitate the release of norepinephrine from cardiac sympathetic nerve terminals (Zimmerman, 1981; Rump et al., 1994). In situ and in vitro studies in dogs have demonstrated that Ang II enhances cardiac myocyte function via AT₁ receptor present in intrinsic adrenergic neurons (Horackova and Armour, 1997). In Ang II-infused rats, surgical cardiac sympathectomy or treatment with atenolol, a β ₁-adrenergic receptor blocker, significantly

prevented cardiac myocyte necrosis, showing that Ang II-induced cardiac damage is at least in part mediated by catecholamine release from cardiac sympathetic neurons (Henegar et al., 1998). Thus, the activation of cardiac sympathetic neurons by Ang II also contributes to pathological cardiac hypertrophy.

F. Effects of Angiotensin Blockade on Experimental Cardiac Diseases

1. Spontaneously Hypertensive Rats and Other Hypertensive Models. As shown in Table 1, molecular phenotypes of pathological cardiac hypertrophy differ among various types of cardiac diseases. In spontaneously hypertensive rats (SHR), the most popular model of human essential hypertension, left ventricular mRNAs for skeletal α -actin, ANF, and collagen types I and III were higher and α -MHC mRNA levels were lower than those in normotensive control Wistar-Kyoto rats (WKY; Ohta et al., 1996a). Thus, SHR exhibited not only cardiac hypertrophy but also cardiac gene reprogramming. As shown in Table 2, an AT₁ receptor antagonist (SC-52458) or an ACE inhibitor (imidapril) with a mildly hypotensive effect (~30 mm Hg) attenuated the increases in cardiac ANF and collagen types I and III mRNAs and significantly normalized the decreased α -MHC mRNA. On the other hand, a calcium channel blocker or an α ₁-adrenergic blocker had no effect on these mRNA expressions in SHR, despite blood pressure-lowering effects comparable with those of SC-52458 and imidapril. These observations show that cardiac gene reprogramming in SHR can be attributed at least in part to direct AT₁ receptor activation by Ang II. Furthermore, the combination of doxazosin with atenolol suppressed cardiac collagen types I and III expressions, indicating that the enhanced collagen expression was also in part mediated by β -adrenergic receptor (Table 2; Ohta et al., 1996a). Treatment of SHR with M17055, a diuretic, normalized only cardiac collagen type III (Kim et al., 1996a).

TGR(mRen2)27, a transgenic hypertensive rat strain carrying the murine Ren-2 gene, is characterized by a lack of increase in plasma and renal renin concentrations and a high adrenal renin concentration, as in the case of SHR (Mullins et al., 1990). Ren-2 transgene was shown to be expressed in the cardiac tissue of TGR(mRen2)27 (Lee et al., 1995). Interestingly, the pattern of altered cardiac gene expression in TGR(mRen2)27 was similar to that in SHR, as mentioned. As shown by a comparison of the effects of an AT₁ receptor antagonist (candesartan cilexetil) with those of a calcium channel blocker (manidipine), a β -adrenergic blocker (atenolol), and an α -adrenergic blocker (doxazosin), the cardiac AT₁ receptor, but not high blood pressure, was involved in cardiac hypertrophy and gene reprogramming in TGR(mRen2)27, and the cardiac renin-angiotensin system via AT₁ receptor may be involved in cardiac hypertrophy and gene expressions in this transgenic rat (Ohta

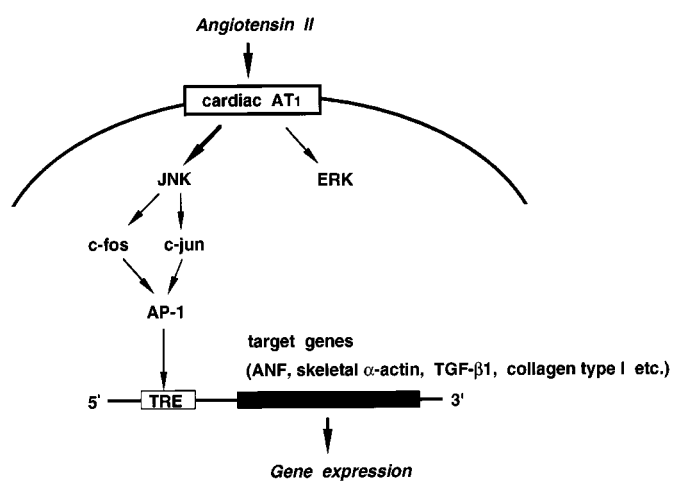


FIG. 3. Proposed in vivo molecular mechanism of cardiac hypertrophy and remodeling-associated gene expressions via AT₁ receptor. Ang II in vivo activates cardiac JNK more potently than ERK, and JNK activation is postulated to induce the activation of AP-1, leading to target gene expressions. TRE, TPA-responsive element.

TABLE 1
Differential molecular phenotypes among various rat cardiac disease models

	SHR	SHRSP	Aortic Banding	Coronary Artery Ligation	Aortocaval Shunt	OLETF
Disease	Moderate hypertension	Severe hypertension	Acute pressure overload	Myocardial infarction	Volume overload	Diabetes
LV mRNA						
β -MHC	→	↑	↑	↑	→	↑
α -MHC	↓	↓	↓	→	→	↓
Skeletal α -actin	↑	↑	↑	↑	→	→
ANF	↑	↑	↑	↑	↑	↑
TGF- β 1	→	↑	↑	↑	→	↑
Collagen I	↑	↑	↑	↑	→	↑
Collagen III	↑	↑	↑	↑	→	↑
Reference	Ohta et al., 1996a	Kim et al., 1996b	Parker et al., 1991; Villarreal et al., 1992	Yoshiyama et al., 1999	Kim et al., 1997b	Yagi et al., 1997

LV, left ventricular; ↑, up-regulation; →, no change; ↓, down-regulation.

TABLE 2
Effects of various antihypertensive drugs on left ventricular gene expressions in SHR

	AT ₁ Receptor Antagonist	ACE Inhibitor	Ca ²⁺ Channel Blocker	α -Blocker	α -Blocker + β -Blocker	Diuretic
Drug (dosage in mg/kg/day)	SC-52458 (30)	Imidapril (5)	Manidipine (3)	Doxazosin (5)	Doxazosin (5) + atenolol (200)	M17055 (5)
Period	7 days	7 days	7 days	7 days	7 days	5 weeks
Decrease in blood pressure (mm Hg)	28	33	36	33	39	22–27
α -MHC	Yes	Yes	No	No	No	No
Skeletal α -actin	No	No	No	No	No	No
ANF	Yes	Yes	No	No	No	No
Collagen I	Yes	Yes	No	No	Yes	No
Collagen III	Yes	Yes	No	No	Yes	Yes

Yes, significant normalization of gene expression; no, failure of normalization of gene expression.

et al., 1996b). Therefore, this appears to be a useful model for study of the role of the cardiac renin-angiotensin system in cardiac disease.

As shown in Table 1, stroke-prone SHR (SHRSP), a substrain of SHR that is regarded as a primary model of human malignant hypertension, showed a different cardiac molecular phenotype from SHR or TGR(mRen2)27, as shown by increased expressions of β -MHC and TGF- β 1 mRNAs (Kim et al., 1995b, 1996b). Losartan (30 mg/kg/day) induced regression of left ventricular hypertrophy in SHRSP and normalized the altered cardiac expressions of α -MHC, skeletal α -actin, ANF, TGF- β 1, and collagen types I and III in SHRSP to a greater extent than amlodipine (5 mg/kg/day) despite comparable hypotensive effects (Kim et al., 1996b). However, the increase in β -MHC mRNA levels in SHRSP was not significantly inhibited by losartan or amlodipine. Thus, up-regulation of β -MHC mRNA in SHRSP seems to be due to neither Ang II nor hypertension, although the mechanism remains unknown. Left ventricular ERK and JNK activities were chronically higher in SHRSP than in WKY (Izumi et al., 1998). Treatment of SHRSP with losartan (30 or 50 mg/kg/day) significantly inhibited the increase in left ventricular JNK activity, along with the regression of left ventricular hypertrophy, and did not lower cardiac ERK activity. Furthermore, hydralazine (50 mg/kg/day) treatment, which completely normalized blood pressure, caused neither significant regression of cardiac hypertrophy nor decreased cardiac JNK or ERK activity. Thus, the increased cardiac JNK

activity in SHRSP is at least in part directly mediated by AT₁ receptor and JNK may contribute to pathological cardiac hypertrophy in SHRSP. Further research on JNK may provide new insights into the in vivo molecular mechanism of Ang II-mediated pathological cardiac hypertrophy.

2. Acute Pressure Overload Model. So far, acute pressure overload, produced by aortic banding or coarctation, has been the most popular model for study of the mechanism of cardiac hypertrophy in vivo. This model is characterized by acute cardiac hypertrophy induced by rapid and severe pressure overload to the heart, in contrast to the above-mentioned slow and mild development of cardiac hypertrophy in hypertensive rats such as SHR or SHRSP. Therefore, it should be noted that this model is rather artificial and may not be a suitable physiological model of human cardiac hypertrophy, which develops slowly and chronically over the long term. Acute pressure overload in rats due to aortic banding rapidly caused left ventricular increases in mRNAs for β -MHC, skeletal α -actin, ANF, TGF- β 1, and collagen types I and III and a reciprocal decrease in α -MHC mRNA (Table 1; Parker and Schneider, 1991; Villarreal and Dillmann, 1992; Parker, 1995). In this model, as in SHRSP, the decrease in left ventricular α -MHC mRNA occurred earlier than the increase in β -MHC mRNA. Treatment with losartan (10 mg/kg/day) suppressed left ventricular hypertrophy and ANF and TGF- β 1 up-regulation after aortic coarctation, suggesting that AT₁ receptor may participate in cardiac hypertrophy and ANF and

TGF- β 1 up-regulation in this model (Everett et al., 1994). However, the effects of losartan on genes other than ANF and TGF- β 1 were not examined in this study. Acute pressure overload, produced by abdominal aortic constriction in rats, caused tyrosine phosphorylation of left ventricular JAK1, JAK2, Tyk2, STAT₁, STAT₂, and STAT₃, whereas treatment with an AT₁ receptor antagonist (30 mg/kg/day E4177) or an ACE inhibitor (10 mg/kg/day cilazapril) suppressed tyrosine phosphorylation of Tyk2 completely and that of JAK2 partially but failed to inhibit JAK1 (Pan et al., 1997). Thus, cardiac AT₁ receptor may directly contribute to the activation of JAK2 and Tyk2 in acute pressure-overloaded rats. Recently, AT_{1a} receptor knockout mice were successfully developed (Sugaya et al., 1995), in which cardiac AT₁ receptor mRNA levels (probably due to AT_{1b} receptor) are less than 10% of those in wild-type mice (Harada et al., 1998a). In contrast to the contribution of AT₁ receptor to pressure overload-induced cardiac hypertrophy in adult rats, acute pressure overload by aortic constriction elicited significant activation of ERK and increased the expression of immediate-early genes and the fetal phenotype of genes such as β -MHC and ANF, myocyte hypertrophy, and fibrosis in the heart of both AT_{1a} knockout and wild-type mice (Harada et al., 1998a,b). However, blockade of pressure overload cardiac hypertrophy in adult animals with an AT₁ receptor antagonist is not at all equivalent to the pressure overload model using AT₁ receptor knockout animals. It is possible that deletion of the AT_{1a} receptor gene from early embryogenesis promotes a compensatory ability to use pathways other than AT₁ receptor for cardiac hypertrophic response, which may explain the sufficient response to hypertrophic stimuli seen in knock-out mice as well as wild-type mice. Furthermore, the possible contribution of AT_{1b} receptor to cardiac hypertrophy in AT_{1a} receptor knockout mice cannot be excluded. Thus, the findings obtained using this model should be interpreted with caution.

3. *Myocardial Infarction.* Myocardial infarction is the most common cause of heart failure. Clinically, ACE inhibitors have proved effective in reducing death and complications, improving symptomatic status, and attenuating the progressive nature of cardiac failure in symptomatic patients with ventricular diastolic and/or systolic dysfunction (The CONSENSUS Trial Study Group, 1987; The SOLVD Investigators, 1991). AT₁ receptor antagonists had beneficial effects similar to those of ACE inhibitors on cardiac dysfunction, hypertrophy, and fibrosis after myocardial infarction in the rat (Schieffer et al., 1994; Weber, 1997). Interestingly, as shown in Table 1, the molecular phenotype in nonischemic left ventricular myocardium of myocardial infarcted rats differed from that of hypertensive or acute pressure-overloaded models, as shown by the lack of change in α -MHC mRNA (Hanatani et al., 1995; Yoshiyama et al., 1999). An AT₁ receptor antagonist (1 and

10 mg/kg/day candesartan cilexetil) and an ACE inhibitor (1 and 10 mg/kg/day imidapril) significantly and similarly suppressed cardiac hypertrophy and the increased mRNA expressions of β -MHC, ANP, and skeletal α -actin in nonischemic left ventricular myocardium at 1 and 4 weeks and collagen types I and III mRNA up-regulation at 4 weeks after coronary arterial ligation (Yoshiyama et al., 1999). Furthermore, these molecular effects of candesartan cilexetil and imidapril were associated with improvements in cardiac systolic and diastolic dysfunction, as assessed with Doppler echocardiography. These findings support the notion that normalization of the molecular phenotype in the nonischemic left ventricle after myocardial infarction may be linked to improvement of cardiac dysfunction. Furthermore, unlike the situation in SHRSP, up-regulation of β -MHC in the left ventricle with myocardial infarction seems to be mediated by AT₁ receptor.

4. *Volume Overload Model.* Previous work on the effects of AT₁ receptor antagonist (40 mg/kg/day losartan) treatment on rat cardiac hypertrophy due to aortocaval shunt showed the contribution of AT₁ receptor to the development of cardiac hypertrophy and dysfunction in the volume overload model (Ruzicka et al., 1994b). As shown in Table 1, the molecular phenotype in cardiac volume overload was characterized only by increases in left ventricular ANF and collagen type III mRNAs, indicating that the volume-overloaded heart has a very unique molecular phenotype (Kim et al., 1997b). The lack of increase in collagen type I and TGF- β 1 expressions may explain why volume overload due to aortocaval shunt does not increase cardiac collagen accumulation (Ruzicka et al., 1994a), in contrast to the increased collagen accumulation seen in other hypertrophic models, such as hypertension, acute pressure overload, or myocardial infarction. Treatment with an AT₁ receptor antagonist (10 mg/kg/day CS-866) or an ACE inhibitor (10 mg/kg/day temocapril) for 7 days decreased left ventricular mass and ANF and collagen type III mRNAs in this model (Kim et al., 1997b). Together with the lack of change in plasma renin activity or aldosterone in this model, these findings suggest that cardiac AT₁ receptor is at least partly involved in not only cardiac hypertrophy but also altered gene expression caused by volume overload.

5. *Diabetes.* Clinical data show that myocardial dysfunction frequently occurs in patients with diabetes mellitus (DM), even in the absence of coronary artery disease, supporting the concept of primary diabetic cardiomyopathy (Regan et al., 1977; Mahgoub and Abd-Elfattah, 1998). Furthermore, abnormalities in cardiac function, including decreased cardiac contractility, are reported to occur in streptozotocin-induced diabetic animals (Feuvray et al., 1979; Litwin et al., 1990). However, the mechanism responsible for cardiomyopathy in DM, particularly NIDDM, is poorly understood. Sechi et al. (1994) found that hyperglycemia leads to increases in

cardiac AT₁ receptor density and mRNA levels without altering plasma renin concentrations, suggesting possible activation of the cardiac renin-angiotensin system in diabetic rats. Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a recently developed model of human NIDDM (Kawano et al., 1992), show a unique cardiac molecular phenotype (Table 1; Yagi et al., 1997). An AT₁ receptor antagonist (10 mg/kg/day E-4177) and an ACE inhibitor (1 and 10 mg/kg/day cilazapril) similarly prevented left ventricular up-regulation of TGF- β 1 mRNA and down-regulation of α -MHC mRNA in OLETF rats, suggesting that AT₁ receptor may be involved in these gene expressions (Kim et al., 1997c).

IV. Molecular and Cellular Actions of Angiotensin II in Blood Vessels

A. Cultured Smooth Muscle Cells

Ang II has been established to stimulate protein synthesis and induce cellular hypertrophy in cultured vascular smooth muscle cells (SMCs) via AT₁ receptor (Geisterfer et al., 1988; Berk et al., 1989). Because the abnormal growth of vascular SMCs plays a major role in the development of various vascular diseases such as hypertension and atherosclerosis (Gibbons and Dzau, 1994), this article focuses on the signal transduction underlying Ang II-induced vascular SMC growth. Cultured vascular SMCs express AT₁ receptor but not AT₂ receptor. A growing body of evidence shows that AT₁ receptor activation in cultured vascular SMCs, coupled to the G protein G_q, causes not only activation of PLC- β leading to increases in diacylglycerol and intracellular calcium but also activation of multiple signal transduction cascades. Many excellent reviews that focus on the mechanism of Ang II-activated signal transduction in vascular SMC have recently been published (Schieffer et al., 1996; Berk and Corson, 1997; Griendling et al., 1997; Bernstein et al., 1998).

Ang II treatment of cultured rat aortic SMCs caused activation of ERK (Duff et al., 1992; Tsuda et al., 1992), p70S6K (Giasson and Meloche, 1995), and p90RSK (Takahashi et al., 1997) and phosphorylation of multiple protein tyrosine residues (Molloy et al., 1993), including focal adhesion kinase (Polte et al., 1994), paxillin (Leduc and Meloche, 1995), PLC- γ (Marrero et al., 1994), JAK2, STAT₁ (Marrero et al., 1995), c-Src (Ishida et al., 1995), p130 CAS (Sayeski et al., 1998), and Pyk2. Furthermore, Ang II has recently been reported to activate JNK (Schmitz et al., 1998) and p38 (Kusuhara et al., 1998). However, little is known about the role of these signal cascades in Ang II-induced hypertrophic response. Treatment of rat aortic SMCs with two tyrosine kinase inhibitors, genistein and herbimycin A, completely abolished the stimulatory effect of Ang II (100 nM) on protein synthesis, without affecting Ang II-stimulated inositol triphosphate production, Ca²⁺ mobilization, ERK activation, or *c-fos* mRNA induction (Leduc et al., 1995).

Thus, the tyrosine kinase pathway contributes to Ang II-induced cellular hypertrophy in rat aortic SMCs, independent of PLC activation, ERK activation, or *c-fos* induction. Rapamycin, an immunosuppressant drug that selectively blocks S6 kinase, significantly inhibited Ang II (100 nM)-induced protein synthesis as well as activation of p70S6K, without inhibiting activation of ERK or induction of *c-fos* mRNA (Giasson and Meloche, 1995). Thus, p70S6K plays a critical role in the hypertrophic response of vascular SMCs to Ang II. Without affecting p70S6 kinase activity, PLC activity, or protein tyrosine phosphorylation, a specific MEK inhibitor, PD98059 (30 μ M), inhibited Ang II (100 nM)-induced protein synthesis in rat aortic SMCs by 70%, and PD98059 (30 μ M) and rapamycin (10 ng/ml) exerted additive inhibitory effects on Ang II-induced protein synthesis, suggesting that activation of MEK and probably activation of ERK are obligatory steps for Ang II-induced hypertrophy in vascular SMCs, with a mechanism distinct from that of p70S6K (Servant et al., 1996). Ang II also promoted the activation of both NADH and NADPH oxidases in cultured rat aortic SMCs, associated with an increase in superoxide generation, whereas treatment with an NADPH oxidase inhibitor (10 μ M diphenylene iodinium) or an NADH oxidase inhibitor (50 μ M quinacrine) led to inhibition of both Ang II (100 nM)-induced protein synthesis and superoxide generation (Griendling et al., 1994). Thus, Ang II-induced superoxide generation seems to function as a second messenger implicated in cellular hypertrophy of vascular SMCs. Furthermore, Ang II induced tyrosine phosphorylation of epidermal growth factor (EGF) receptor and its association with Shc and Grb2 in rat aortic SMCs, whereas treatment with AG1478, a specific EGF receptor kinase inhibitor, inhibited Ang II-induced ERK activation and protein synthesis, supporting the notion that Ang II causes cellular hypertrophy, at least in part mediated by EGF receptor transactivation (Eguchi et al., 1998). Thus, tyrosine kinases, p70S6K, ERK, superoxide, or EGF receptor, via different mechanisms, may be responsible for Ang II-induced cellular hypertrophy in vascular SMCs. However, it remains unresolved whether these signal cascades are indeed all simultaneously activated by Ang II in vivo. Tyrosine phosphorylation of platelet-derived growth factor (PDGF) β -receptor by Ang II in vascular SMCs has been also reported, although the role of transactivation of this receptor is unknown (Linseman et al., 1995).

Long-term treatment with Ang II can induce delayed mitogenic effects on cultured rat aortic SMCs, as shown by the observation that Ang II increased DNA synthesis 5- to 8-fold in vascular SMCs after 48 h, followed by an increase in cell number after 5 days (Weber et al., 1994a). This delayed mitogenic effect of Ang II could be inhibited by suramin, a compound shown to interfere with autocrine cell transformation, suggesting the involvement of suramin-sensitive autocrine growth factors

in Ang II-induced delayed proliferation. Furthermore, Ang II-induced proliferation of vascular SMCs was suggested to be mediated by the autocrine action of PDGF or basic fibroblast growth factor (bFGF) released into the medium (Gibbons et al., 1992). In porcine coronary SMC, Ang II (10^{-6} M) also induced cell proliferation after 96 h and activated phosphatidylinositol 3-kinase (PI3-kinase), a heterodimeric protein composed of 85- and 110-kDa subunits that catalyzes the synthesis of 3-phosphorylated phosphoinositides, whereas LY294002 (10^{-5} to 10^{-10} M), a specific inhibitor of PI3-kinase, inhibited the increases in both RNA and DNA synthesis as well as the increase in cell number generated by Ang II stimulation, supporting the important role of PI3-kinase in Ang II-induced vascular SMC proliferation (Saward and Zahradka, 1997).

Vascular diseases are triggered and developed by a variety of factors, such as growth factors, ECM proteins, cytokines, or chemokines. Accumulating evidence indicates that Ang II in vascular SMCs regulates various gene expressions implicated in vascular diseases. Ang II stimulated the induction of various growth factors, including TGF- β 1 mRNA and protein (Gibbons et al., 1992), PDGF mRNA (Naftilan et al., 1989), bFGF (Gibbons et al., 1992), vascular endothelial growth factor mRNA (VEGF; Williams et al., 1995), and insulin-like growth factor-I mRNA and protein (Delafontaine and Lou, 1993). Ang II-induced TGF- β 1 mRNA expression in vascular SMCs was mediated by activation of ERK and AP-1 (Hamaguchi et al., 1999). Ang II also stimulated induction of various kinds of ECM components, including fibronectin mRNA and protein (Tamura et al., 1998), collagen protein (Kato et al., 1991), laminin, and tenascin mRNA and protein (Sharifi et al., 1992). Ang II increased glucose transporter GLUT- I mRNA and activity in rat aortic SMCs (Low et al., 1992). Ang II stimulated mRNA expression and activity of plasminogen activator inhibitor (PAI)-1 and -2 in rat aortic SMCs, suggesting the involvement of Ang II in thrombosis (Feener et al., 1995). Ang II also stimulated the induction of monocyte chemoattractant protein-1 (MCP-1) mRNA and protein in rat aortic SMCs, which was due to activation of tyrosine kinases and ERK or generation of superoxide, suggesting the participation of Ang II in monocyte infiltration into the vessel wall (Chen et al., 1998). It is intriguing that these factors may play an important role in Ang II-mediated vascular diseases in an autocrine or a paracrine manner.

B. Cultured Endothelial Cells

As in rat aortic SMCs, Ang II promoted PAI-1 and PAI-2 mRNA and protein expressions in rat microvessel endothelial cells isolated from epididymal fat pads via AT₁ receptor (Feener et al., 1995). Thus, AT₁ receptor in endothelial cells may regulate plasminogen activation. In coronary endothelial cells from explanted human heart, Ang II, via AT₁ receptor, induced concentration-

dependent increases in E-selectin mRNA and protein and significantly increased leukocyte adhesion at wall shear stress. This adhesion to endothelial cells was due to E-selectin expression, as demonstrated by the inhibition of leukocyte adhesion with anti-E-selectin antibody (Grafe et al., 1997). Therefore, AT₁ receptor in endothelial cells may participate in leukocyte accumulation in the vessel wall, which is a hallmark of early atherosclerosis and plaque progression. Ang II has also been shown to induce expression of endothelin-1 mRNA and protein in bovine carotid arterial endothelial cells via AT₁ receptor (Imai et al., 1992). In bovine retinal microcapillary endothelial cells, Ang II alone had no effect on cell growth or tube formation, but Ang II in a dose-dependent manner induced significant increases in mRNA and protein for kinase domain-containing receptor/total liver kinase (KDR/Flk-1), which is a VEGF receptor, and significantly enhanced VEGF-induced cell proliferation and tube formation, mediated by AT₁ receptor (Otani et al., 1998). These findings suggest that AT₁ receptor may contribute to the development of diabetic retinopathy by enhancing VEGF-induced angiogenic activity.

C. Effects of In Vivo Angiotensin II Infusion on Vascular Tissues

Several in vivo experiments have shown that Ang II can induce vascular SMC proliferation in vivo (Daemen et al., 1991; Griffin et al., 1991; Simon and Altman, 1992; Su et al., 1998). Interestingly, Ang II infusion (200 ng/min i.p.) in rats subjected to balloon injury 2 weeks earlier showed that the proliferative influence of Ang II is more remarkable in neointimal SMCs than in SMCs of the underlying media or of the normal vessel wall, indicating that the proliferative activity of Ang II in vivo depends on the preexisting proliferative status of vascular SMCs (Daemen et al., 1991). Ang II infusion (200 ng/kg/min s.c. for 10–12 days) in rats increased mesenteric vascular media width, media cross-sectional area, and media/lumen ratio, and these changes were not inhibited by treatment with hydralazine despite normalization of blood pressure, indicating that Ang II caused vascular growth in vivo at least in part in a direct manner (Griffin et al., 1991).

Despite detailed investigations into the molecular mechanism of Ang II-mediated vascular SMC growth in vitro, this process is poorly understood. Ang II infusion, at least in part independent of its blood pressure-elevating effect, increased aortic mRNA and protein expression of fibronectin, which is an ECM protein that induces phenotypic change of vascular SMCs from a contractile to a synthetic phenotype (Kim et al., 1994a). bFGF may play a key role in Ang II-mediated vascular SMC replication in vivo, as shown by the observation that i.v. injection of anti-bFGF antibody significantly inhibited the mitogenic effect of Ang II infusion on rat carotid arteries (Su et al., 1998). Ang II (0.7 mg/kg/day)

infusion in rats doubled superoxide production in rat aorta (mainly media) by activation of NADH/NADPH oxidase, which was completely blocked by treatment with losartan (25 mg/kg/day; Rajagopalan et al., 1996; Laursen et al., 1997). On the other hand, norepinephrine (2.8 mg/kg/day) infusion did not increase vascular superoxide production, despite a hypertensive effect comparable with that of Ang II, suggesting that SMC growth due to Ang II may be specifically mediated by increased superoxide generation. Ang II infusion (0.7 mg/kg/day s.c.) in rats increased heme oxygenase-1 (HO-1) mRNA and protein in the endothelium and adventitia of the aorta, which was prevented by treatment with losartan (25 mg/kg/day; Ishizaka et al., 1997). Because HO-1 is an oxidant-sensitive gene, it is possible that increased oxidative stress is a trigger for HO-1 mRNA up-regulation in Ang II-infused rat aorta and that HO-1 may serve to abrogate this increased stress caused by Ang II. Ang II infusion (0.75 mg/kg/day s.c.) stimulated aortic thrombin receptor mRNA expression in rats, which was blocked by either losartan or heparin-binding chimera of human Cu/Zn superoxide dismutase but not by normalization of blood pressure with hydralazine treatment, suggesting that Ang II increases vascular thrombin receptor by AT₁ receptor-mediated superoxide production and may be implicated in the pathophysiology of atherosclerosis by thrombin cascade activation (Capers et al., 1997). The i.p. injection of Ang II (10⁻⁷ M once daily for 2 days) in mice resulted in increased oxidized low-density lipoprotein (LDL) uptake by peritoneal macrophages and increased macrophage proteoglycan content, suggesting that Ang II may accelerate atherosclerosis by promoting foam cell formation and cholesterol accumulation in the vascular wall (Keidar and Attias, 1997).

D. Effects of Angiotensin Blockade on Experimental Vascular Diseases

1. *Hypertensive Rats.* Table 3 summarizes the in vivo molecular effects of AT₁ receptor antagonists on various rat vascular diseases. Accumulating evidence indicates that TGF-β1 and various ECM proteins are responsible for the development of vascular remodeling. Aortic TGF-β1, fibronectin, and collagen type IV mRNA levels are

higher in SHR than in WKY, and all of these elevated mRNAs in the aorta of SHR were significantly reduced by an ACE inhibitor, alacepril (50 mg/kg/day), or an AT₁ receptor antagonist, SC-52458 (50 mg/kg/day; Ohta et al., 1994). In the mesenteric artery and aorta of SHRSP, the increases in mRNA levels for TGF-β1; fibronectin; collagen types I, III, and IV; and laminin were suppressed by candesartan (1 and 10 mg/kg) or enalapril (10 mg/kg; Kim et al., 1995b). Importantly, these effects were associated with the suppression of medial hypertrophy of the aorta and mesenteric artery of SHRSP, suggesting that AT₁ receptor may be responsible for vascular thickening via stimulation of TGF-β1 and ECM protein expressions.

Aortic ERK and JNK activities are significantly increased with the development of hypertension, and in particular, aortic ERK activity is gradually and chronically enhanced in the development of hypertension and is associated with an increase in aortic weight (Kim et al., 1997a). Unlike the case in vascular tissue, there was no significant increase in ERK or JNK activity in other tissues such as liver, stomach, spleen, or lung of hypertensive rats. A previous report showed no increase in vascular PKC activity in hypertensive rats (Silver et al., 1992). Thus, the increase in vascular MAP kinase activity seems to be a specific event, rather than nonspecific, in hypertensive rats. Furthermore, losartan (30 and 50 mg/kg/day) significantly decreased aortic ERK activity in SHRSP to a greater extent than nifedipine (45 mg/kg/day), although both drugs caused similar hypotensive effects (Hamaguchi et al., 1999). These findings raise the possibility that increased aortic ERK activity in hypertensive rats may be due in part to AT₁ receptor and may be implicated in vascular remodeling that accompanies hypertension.

2. *Balloon Injury.* Arterial injury produced by balloon angioplasty causes proliferation and migration of medial smooth muscle cells, leading to progressive neointimal thickening and narrowing of the vascular lumen (Gibbons and Dzau, 1994). This arterial repair process has been shown to be associated with significant induction of various kinds of genes, including immediate-early genes (Miano et al., 1990), growth factors, and ECM

TABLE 3
In vivo molecular effects of AT₁ receptor antagonists on various rat vascular disease models

Rat Model	AT ₁ Receptor Antagonist	Dosage mg/kg/day	Suppression	Reference
SHR	SC-52458	50	TGF-β1, collagen type IV, and fibronectin mRNAs	Ohta et al., 1994
SHRSP	Candesartan	0.1, 1, and 10	TGF-β1, collagen types I, III, and IV, fibronectin, and laminin mRNAs	Kim et al., 1995b
Balloon injury	Candesartan	10	<i>c-fos</i> , <i>c-jun</i> , <i>Egr-1</i> , ODC, and fibronectin mRNAs	Kim et al., 1995a
Balloon injury	E4177	20	JNK activity, ERK activity, and AP-1 activity	Kim et al., 1998
Balloon injury	Candesartan	10	PDGF-α and -β receptor tyrosine phosphorylation	Abe et al., 1997
OLETF rats	E4177	10	TGF-β1	Kim et al., 1997c

components (Majesky et al., 1990), suggesting the importance of these genes in the formation of neointima. Ang II, via AT₁ receptor, is responsible for neointima formation in rat artery after balloon injury (Powell et al., 1989; Prescott et al., 1991). As shown in Table 3, candesartan cilexetil (10 mg/kg/day) significantly inhibited induction of *c-fos*, *c-jun*, and *Egr-1* mRNAs after balloon injury and significantly inhibited fibronectin mRNA, suggesting that inhibition of fibronectin expression by AT₁ receptor antagonist may be in part responsible for inhibition of vascular smooth muscle cell proliferation and/or migration (Kim et al., 1995a). Furthermore, arterial JNK and ERK activities were rapidly and transiently increased with peaks at 5 min (Kim et al., 1998). Treatment with AT₁ receptor antagonist (20 mg/kg/day E4177) significantly inhibited the activation of JNK and ERK in injured artery, demonstrating that Ang II, via AT₁ receptor, is responsible for balloon injury-induced arterial JNK and ERK activations (Fig. 4). Notably, inhibition of JNK activation by AT₁ receptor antagonist was greater than 80%, whereas that of ERK activation was about 40%, suggesting that JNK activation may be due more to AT₁ receptor than ERK activation. Furthermore, after activation of JNK and ERK, transcription factor AP-1 complex, composed of c-Fos and c-Jun proteins, was activated with a peak at 3 h after injury, and this was blocked 47% by E4177 (Fig. 5). These findings support the notion that AT₁ receptor seems to participate in neointima formation after balloon injury, probably mediated by activation of JNK or ERK followed by AP-1 activation. The inhibition of neointima in balloon-

injured artery by AT₁ receptor antagonist also may be in part due to suppression of PDGF receptor activation, as shown by the findings that PDGF receptor tyrosine phosphorylation was enhanced in injured artery and was suppressed by candesartan cilexetil (10 mg/kg/day) but not by amlodipine (10 mg/kg/day; Abe et al., 1997).

Unlike in the balloon-injured rat carotid artery model, Ang II may not play a role in the development of neointimal thickening in the pig (Huckle et al., 1996) or baboon (Hanson et al., 1991). Furthermore, human trials of the effects of cilazapril (an ACE inhibitor) on restenosis yielded negative results (The MERCATOR Study Group, 1992). There may be species differences in the role of Ang II in neointimal hyperplasia, and it remains unclear whether Ang II contributes to the development of restenosis in humans.

3. *Other Models.* ACE inhibitors have been reported to exert potent antiatherosclerotic actions in Watanabe heritable hyperlipidemic rabbits (Chobanian et al., 1990) and high-cholesterol-fed rabbits (Schuh et al., 1993). In the latter model, treatment with an AT₁ receptor antagonist (20 mg/kg/day E4177) as well as an ACE inhibitor (10 mg/kg/day enalapril) for 5 weeks reduced aortic cholesterol content (Sugano et al., 1996), whereas an other study showed that treatment with an AT₁ receptor antagonist (30 mg/kg/day SC-51316 p.o.) for 3 months did not significantly attenuate aortic atherosclerosis (Schuh et al., 1993). Thus, the role of AT₁ receptor in hyperlipidemic atherosclerosis in rabbits is controversial. However, recent work showed that AT₁ receptor mRNA expression and binding were significantly in-

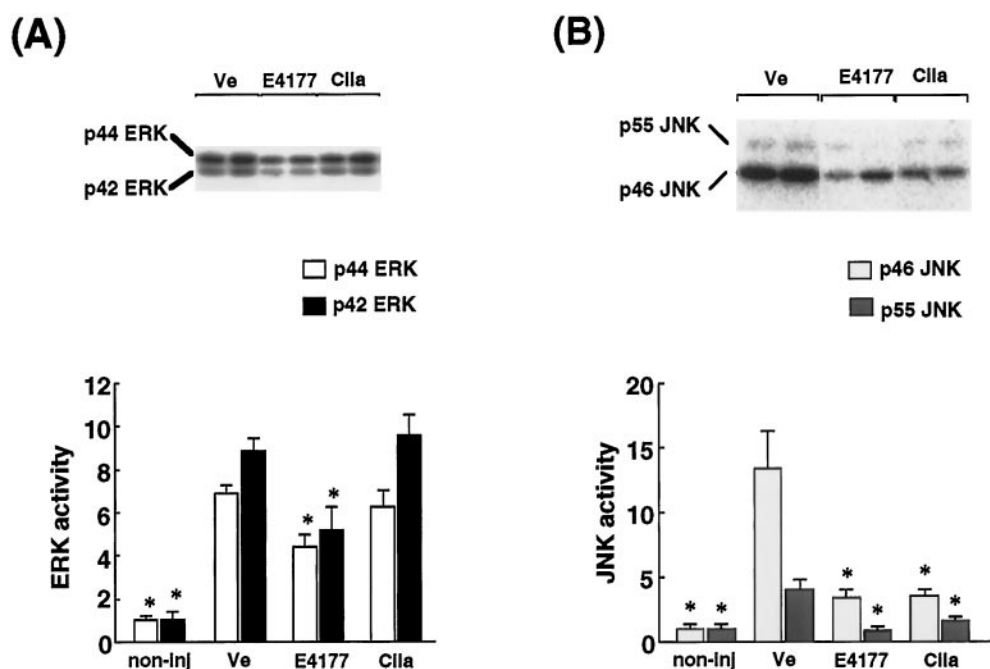


FIG. 4. Inhibition of arterial ERK (A) and JNK (B) in rat balloon-injured artery by E4177 (20 mg/kg/day) and cilazapril (10 mg/kg/day). Top panels indicate representative autoradiograms of ERK or JNK activities. non-inj, noninjured control carotid artery; Ve, balloon-injured artery treated with vehicle; E4177, balloon-injured artery treated with E4177; Cila, balloon-injured artery treated with cilazapril. Values are mean \pm S.E. * P < .01 versus Ve.

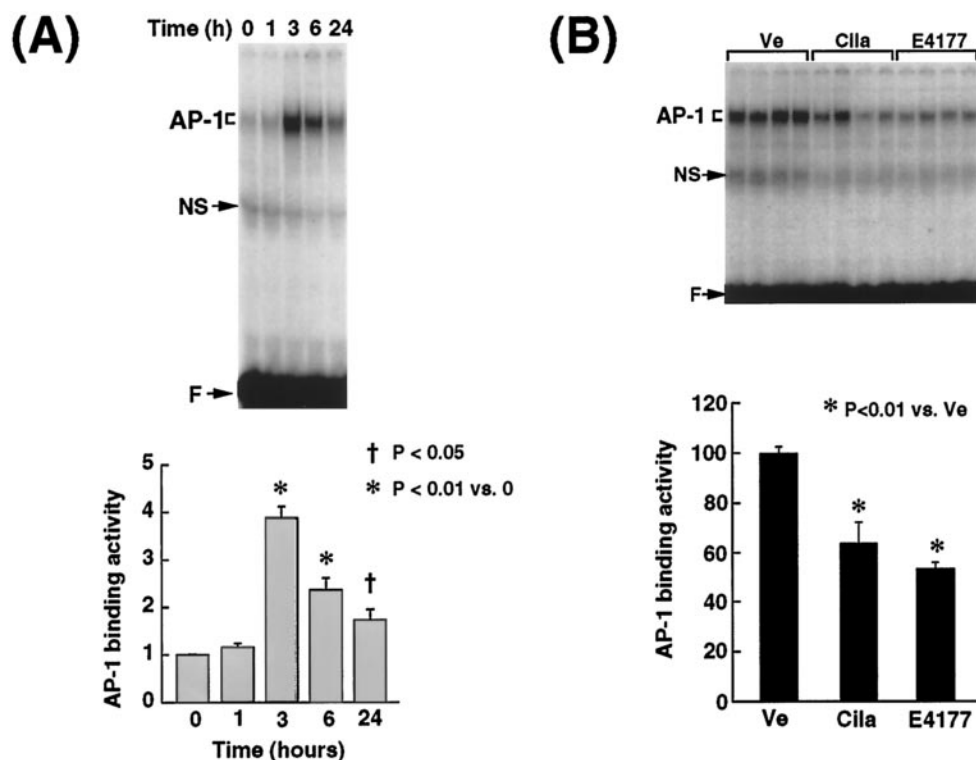


FIG. 5. Time course of activation of AP-1 in rat balloon-injured artery (A) and its inhibition by E4177 (20 mg/kg/day) and cilazapril (10 mg/kg/day) (B). Ve, balloon-injured artery of rats treated with vehicle; Cila, balloon-injured artery of rats treated with cilazapril; E4177, balloon-injured artery of rats treated with E4177; NS, nonspecific binding; F, free probe. Values are mean \pm S.E.

creased mainly in the media and to some extent in the intima of atherosclerotic aorta from high-cholesterol-fed rabbits (Yang et al., 1998). Furthermore, without affecting plasma cholesterol levels, losartan (25 mg/kg/day for 3 months) in apolipoprotein E-deficient mice with severe hypercholesterolemia and extensive atherosclerosis increased the resistance of LDL to CuSO₄-induced oxidative modification, and this was associated with a reduction in atherosclerotic lesion area by 80% (Keidar et al., 1997). Thus, AT₁ receptor antagonists may have beneficial effects on atherosclerosis due to inhibition of LDL lipid peroxidation. DM is also an important risk factor for atherosclerosis. Cilazapril (1 and 10 mg/kg/day) and E4177 (10 mg/kg/day) prevented wall thickening and increased perivascular fibrosis in arterioles and small coronary arteries of OLETF rats, suggesting an important role of AT₁ receptor in diabetic vascular disease (Kim et al., 1997c). However, the molecular mechanism underlying the contribution of AT₁ receptor to diabetic vascular lesions remains to be elucidated.

V. Molecular and Cellular Actions of Angiotensin II in Kidney

A. Cultured Glomerular Cells

Glomerular diseases are mainly caused by glomerular hypertension, DM, or inflammation and are commonly characterized by mesangial cell overgrowth and excessive accumulation of ECM proteins. The development of

such glomerular injuries is largely responsible for glomerular capillary obliteration and decline in the glomerular filtration rate (GFR), ultimately leading to renal failure, as reviewed elsewhere (Wesson, 1998). Thus, glomerular diseases are the most clinically important kidney diseases. Accumulating evidence suggests that mesangial cells, which are a major determinant in regulation of GFR, play a key role in progression from glomerular injury to glomerulosclerosis, due to their overgrowth and synthesis of excess ECM proteins. Therefore, in this review, we focus on the molecular effects of Ang II on glomerular mesangial cells.

Interestingly, Ang II concentrations within the kidney are ~1000-fold higher than those in circulating blood, suggesting the presence of an intrarenal renin-angiotensin system (Seikaly et al., 1990; Ingelfinger and Dzau, 1991). Ang II plays a critical role not only in the regulation of GFR but also in the development of glomerulosclerosis by increasing glomerular capillary pressure caused by preferential constriction of efferent arterioles (Miller et al., 1991). The hemodynamic effects of Ang II in the kidney have been extensively reviewed elsewhere (Ichikawa and Harris, 1991; Stockand and Sansom, 1998). Besides its unique hemodynamic effects in the kidney, Ang II has been shown to have various important direct actions on mesangial cells, and these nonhemodynamic effects of Ang II may play a crucial role in Ang II-mediated glomerular injury. Mesangial cells ex-

clusively express AT₁ receptor but not AT₂ receptor, and mesangial AT₁ receptor is coupled to G protein, causing activation of PLC (Douglas and Hopfer, 1994; Matsubara, 1998). In cultured murine mesangial cells, Ang II (10⁻⁶ M) stimulated cellular hypertrophy, as indicated by increases in cell size and total protein content and synthesis, without an increase in [³H]thymidine incorporation, and these effects were blocked by losartan (Anderson et al., 1993). On the other hand, other investigators showed that Ang II caused not only cellular hypertrophy but also proliferation in mesangial cells (Wolf et al., 1992; Gomez-Garre et al., 1996). However, the molecular mechanism responsible for Ang II-mediated mesangial cell growth is poorly understood. Ang II activated ERK (Huwiler et al., 1995; Anderson et al., 1996) and JNK (Huwiler et al., 1998) in rat mesangial cells, although the role of ERK or JNK activation has not been defined. Ang II (10⁻⁵ to 10⁻⁸ M) dose dependently increased superoxide anion production in rat mesangial cells, in parallel with mesangial cell hypertrophy and proliferation, and the latter effects were completely abolished by blockade of superoxide anion production with diphenyleneiodonium chloride (10⁻⁵ M; Jaimes et al., 1998). Furthermore, stimulation of superoxide anion production by Ang II in mesangial cells was completely blocked by PD98059 (50 μM) or calphostin C (1 μM) but not by tyrosine kinase inhibitors, herbimycin A (1 μM) and genistein (10 μM), suggesting that ERK cascade and PKC participate in mesangial cell growth by enhancing superoxide anion production.

Accumulating evidence supports the notion that TGF-β1 plays a key role in progression of glomerulosclerosis by directly enhancing mesangial cell hypertrophy and ECM production (Border and Ruoslahti, 1992; Border and Noble, 1994). Glomerulosclerosis due to Ang II is postulated to be closely linked to TGF-β1, as previously reviewed (Peters and Noble, 1997; Border and Noble, 1998). The treatment of rat mesangial cells with Ang II (10⁻⁶ M) increased mRNA and protein levels for TGF-β1 and ECM components including biglycan, fibronectin, and collagen type I, and neutralizing antibody to TGF-β1 blocked both Ang II-induced mesangial cell hypertrophy and ECM expression, demonstrating that newly synthesized and released TGF-β1 is involved in Ang II-induced mesangial cell hypertrophy and ECM production in an autocrine manner (Kagami et al., 1994). Ang II-induced fibronectin production in rat mesangial cells was mediated by PKC, as indicated by inhibition with staurosporine (10⁻⁷ M) or H-7 (10⁻⁶ M; Gomez-Garre et al., 1996). Ang II treatment of rat mesangial cells increased PAI-1 mRNA and protein, and anti-TGF-β1 antibody inhibited Ang II-induced PAI-1 production, demonstrating the contribution of TGF-β1 to Ang II-induced PAI-1 synthesis (Kagami et al., 1997). All of these in vitro findings support the notion that TGF-β1 may be a key mediator in Ang II-induced glomerulosclerosis. Exposure of rat mesangial cells to Ang

II increased nuclear factor-κB (NF-κB) DNA-binding activity, and subsequently increased MCP-1 mRNA and protein; pyrrolidine dithiocarbamate (200 μM), an antioxidant capable of inhibiting NF-κB activation, abolished Ang II (10⁻⁷ M)-induced NF-κB activation and MCP-1 gene expression, suggesting that NF-κB activation may be responsible for MCP-1 induction by Ang II (Ruiz-Ortega et al., 1998). Thus, Ang II also seems to be responsible for inflammatory responses of mesangial cells.

Glomerular cells are composed of not only mesangial cells but also endothelial and epithelial cells, although little is known about the role of these cells in glomerular injury. Notably, cultured rat glomerular endothelial cells were found to possess not only AT₁ receptor but also AT₂ receptor, and Ang II treatment stimulated RANTES (chemokine with chemoattractant properties for macrophages/monocytes) mRNA and protein synthesis; these processes were blocked by AT₂ receptor ligand PD123177 or CGP-42112 but not by AT₁ receptor antagonist losartan, suggesting a possible role of endothelial AT₂ receptor in Ang II-induced RANTES expression (Wolf et al., 1997). Thus, glomerular endothelial AT₂ receptor may be implicated in the development of glomerular inflammation. Glomerular epithelial cells, which play an important role as a glomerular filtration barrier, have both AT₁ and AT₂ receptors, and Ang II was shown to increase cAMP accumulation in these cells via AT₁ receptor but not AT₂ receptor (Sharma et al., 1998), although the significance of this observation is unknown.

B. Effects of In Vivo Angiotensin II Infusion on Kidney

Ang II infusion in vivo in rats was shown to cause glomerulosclerosis (Miller et al., 1991), and several important findings on cellular and molecular mechanisms of glomerular injury by Ang II infusion have been previously reported. In various types of glomerular diseases in rats and humans, glomerular mesangial cells undergo phenotypic changes, as shown by expression of significant amounts of α-smooth muscle actin, which is not expressed in normal mesangial cells (Johnson et al., 1992b). Furthermore, glomerular diseases are also characterized by a significant increase in the expression of desmin, an intermediate filament, by glomerular epithelial cells. Thus, phenotypic changes in glomerular mesangial and epithelial cells are believed to play an important role in the pathophysiology of glomerular diseases. Continuous Ang II infusion (200 ng/min s.c.) in rats led to dramatic up-regulations of α-smooth muscle actin in glomerular mesangial cells and desmin in epithelial cells, indicating that Ang II caused the phenotypic changes in these glomerular cells (Johnson et al., 1992a). However, the significance of these phenotypic changes induced by Ang II remains to be clarified. Continuous administration of Ang II (100 ng/min s.c.) in rats for 7 days caused increases in glomerular mRNAs for

TGF- β 1 and collagen type I (Kagami et al., 1994). The i.p. infusion of Ang II (500 ng/h) in rats for 4 days significantly stimulated glomerular mRNA and protein expression of RANTES and increased glomerular macrophage/monocyte influx. Notably, oral treatment with PD123177 (50 mg/l drinking water) did not affect blood pressure but did attenuate glomerular RANTES expression or glomerular macrophage/monocyte influx, showing the involvement of Ang II in glomerular chemotaxis of macrophages/monocytes through local RANTES induction via AT₂ receptor (Wolf et al., 1997). Acute i.v. infusion of Ang II (100 or 1000 ng/kg/min) in conscious rats rapidly and transiently induced activation of glomerular ERK and JNK, followed by an increase in glomerular AP-1 binding activity (Hamaguchi et al., 1998). Thus, as in cardiovascular diseases, the ERK and JNK signaling cascades, via activation of AP-1, may be implicated in the development of Ang II-induced glomerular injury.

C. Effects of Angiotensin Blockade on Experimental Glomerular Diseases

Table 4 shows the potential molecular mechanisms underlying the beneficial effects of AT₁ receptor antagonists in a variety of renal disease models. In deoxycorticosterone acetate (DOCA)-salt hypertensive rats or SHRSP, which prominently exhibit progressive glomerulosclerosis, renal TGF- β 1 and ECM protein mRNA levels are higher than those in normotensive control rats (Kim et al., 1994b,c). Treatment of DOCA-salt hypertensive rats with candesartan cilexetil (1 mg/kg/day) or enalapril (10 mg/kg/day), although not decreasing blood pressure, significantly reduced urinary protein and albumin excretion and induced histological improvement in renal lesions, in association with decreases in renal cortical mRNA levels for TGF- β 1, fibronectin, laminin, and collagen types I, III, and IV (Fig. 6; Kim et al., 1994c). Also in SHRSP, candesartan cilexetil (0.1 mg/kg/day), without significantly decreasing blood pressure, significantly reduced renal TGF- β 1, fibronectin, laminin, and collagen types I, III, and IV mRNA levels (Kim et al., 1994b). These findings provide in vivo evidence implicating Ang II, via AT₁ receptor, in renal injury in these hypertensive models, due to enhanced renal TGF- β 1 and ECM expressions.

Nephropathy is a major cause of complications and death in DM. AT₁ receptor antagonists as well as ACE inhibitors have been shown to decrease proteinuria and

slow the progression of diabetic glomerular injury in streptozotocin-induced diabetic rats (the most popular model of insulin-dependent DM; Remuzzi et al., 1993; Wolf and Ziyadeh, 1997) and OLETF rats, an NIDDM model (Kim et al., 1997c). Thus, it is possible that AT₁ receptor blockade may be effective for treating nephropathy in NIDDM patients as well as in IDDM patients, although the molecular mechanism is unknown.

In rats with subtotal renal ablation (remnant kidney model), candesartan cilexetil (1 mg/kg/day) significantly reduced the expression of glomerular α -smooth muscle actin and desmin, while decreasing urinary albumin excretion and inducing histological improvement of glomerulosclerosis (Hamaguchi et al., 1996). These findings suggest a contribution of the AT₁ receptor to glomerular cell phenotypic changes in the remnant kidney model. In the rat remnant kidney model with a 40% protein diet, treatment with losartan (180 mg/l drinking water) for 8 or 14 days attenuated increases in renal TGF- β 1 mRNA and protein and improved glomerulosclerosis, whereas treatment with a combination of reserpine, hydralazine, and hydrochlorothiazide, despite hypotensive effects comparable with those of losartan, failed to lessen renal TGF- β 1 expression or glomerulosclerosis (Junaid et al., 1997). Thus, Ang II may participate in glomerulosclerosis in the remnant kidney model by enhancing glomerular TGF- β 1 expression.

In Thy 1.1 glomerulonephritic rats, losartan or enalapril treatment significantly, but not completely, lowered glomerular TGF- β 1, fibronectin, and PAI-1 mRNAs and proteins (Peters et al., 1998). In rat nephropathy induced by a daily s.c. injection of 15 mg/kg cyclosporin A (an important immunosuppressive drug), the administration of losartan (10 mg/kg) ameliorated renal lesions, with simultaneous reductions in renal cortical TGF- β 1, PAI-1, collagen types I and IV, and biglycan (Shihab et al., 1997). Thus, Ang II-mediated TGF- β 1 up-regulation may contribute to glomerulosclerosis induced by hypertension, subtotal nephrectomy, glomerulonephritis, or cyclosporin A nephropathy.

VI. AT₁ Receptor Antagonists versus Angiotensin-Converting Enzyme Inhibitors

A. Pharmacological Differences

In humans, ACE inhibitors have been demonstrated to be powerful agents for the treatment of hypertension,

TABLE 4
Molecular effects of AT₁ receptor antagonist on various glomerular disease models

Disease Model	AT ₁ Receptor Antagonist (dosage)	Suppression	Reference
DOCA-salt HT	Candesartan (1 mg/kg/day)	TGF- β 1, FN, Co I, Co III, Co IV, laminin	Kim et al., 1994c
SHRSP	Candesartan (0.1 mg/kg/day)	TGF- β 1, FN, Co I, Co III, Co IV, laminin	Kim et al., 1994b
Remnant kidney	Losartan (180 mg/L DW)	TGF- β 1	Junaid et al., 1997
Remnant kidney	Candesartan (1 mg/kg/day)	Phenotypic change (α -SMA, desmin)	Hamaguchi et al., 1996
Thy 1.1 GN	Losartan (500 mg/L DW)	TGF- β 1, FN, PAI-1	Peters et al., 1998
CsA nephropathy	Losartan (10 mg/kg/day)	TGF- β 1, PAI-1, Co I, Co IV, biglycan	Shihab et al., 1997

HT, hypertensive rats; GN, glomerulonephritis; CsA, cyclosporin A; DW, drinking water; FN, fibronectin; Co, collagen; α -SMA, α -smooth muscle actin.

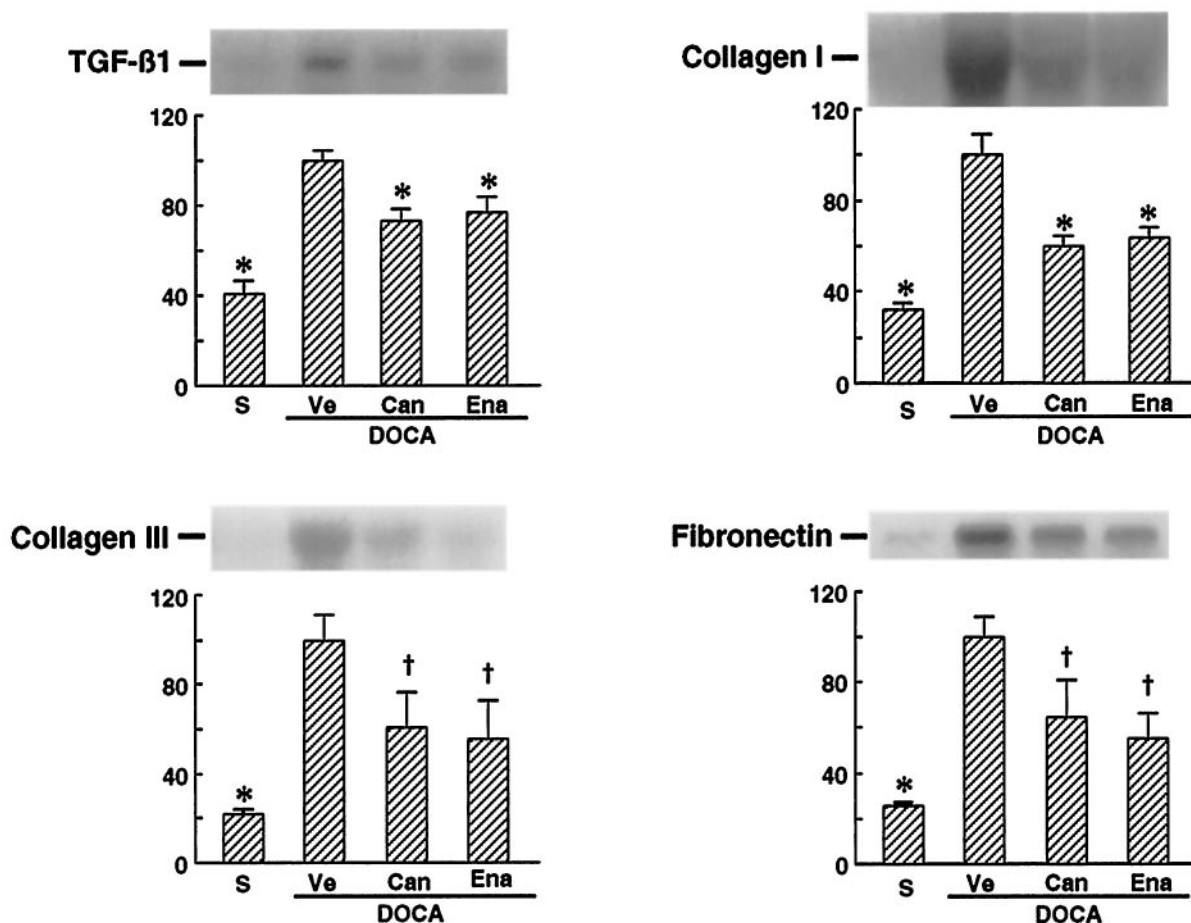


FIG. 6. Inhibitory effects of candesartan cilexetil (1 mg/kg/day) and enalapril (10 mg/kg/day) on renal cortical TGF-β1, collagen types I and III, and fibronectin in DOCA-salt hypertensive rats. S, sham-operated group; Ve, DOCA-salt rats treated with vehicle; Can, DOCA-salt rats treated with candesartan cilexetil; Ena, DOCA-salt rats treated with enalapril. Values are mean ± S.E. *P < .05, †P < .01 versus Ve.

congestive heart failure (The CONSENSUS Trial Study Group, 1987; The SOLVD Investigators, 1991), and renal diseases (Maschio et al., 1996) and the prevention of myocardial infarction in patients with NIDDM (Estacio et al., 1998). Recently, in elderly patients with heart failure, treatment with losartan was associated with a lower mortality rate than that found with captopril (Pitt et al., 1997). Therefore, it is a very important clinical question whether AT₁ receptor antagonists have different pharmacological actions than ACE inhibitors. Table 5 summarizes possible pharmacological differences between AT₁ receptor antagonists and ACE inhibitors. It

has been well established that ACE inhibitors not only attenuate ACE-mediated formation of Ang II in the circulation and peripheral tissues but also allow the accumulation of bradykinin through the inhibition of its degradation, because ACE is identical with kininase II (Linz et al., 1995; Waeber and Brunner, 1996). The recent availability of the specific bradykinin B₂ receptor antagonist icatibant has allowed researchers to determine whether various pharmacological effects of ACE inhibitors are due to accumulated bradykinin by examining the effects of icatibant on pharmacological actions of ACE inhibitors in vitro in cultured cells or in various disease models. As reviewed in detail by Linz et al. (1995), accumulated bradykinin in tissues due to ACE inhibition contributes to blood pressure reduction in renovascular hypertensive rats, prevention of balloon injury-induced neointima formation in rat carotid artery, improvement of cardiac performance, increase in coronary blood flow, prevention of ischemia/reperfusion-induced arrhythmias, and regression of left ventricular hypertrophy in pressure-overloaded rats by aortic coarctation. Recently, it has been reported that bradykinin contributes to ACE inhibitor-induced vasodilation in the

TABLE 5

Comparison of in vivo pharmacological actions between AT₁ receptor antagonist and ACE inhibitor

	AT ₁ Receptor Antagonist	ACE Inhibitor
AT ₁ receptor	Direct inhibition	Indirect inhibition
ACE activity	No effect	Direct inhibition
Plasma renin	↑	↓
Plasma Ang II	↑	↓
AT ₂ receptor	Stimulation	Inhibition
Bradykinin	→	↑
Dry cough	No	Yes

↑, increase; →, no change; ↓, decrease.

human forearm, as shown by inhibition with icatibant (Hornig et al., 1997). Furthermore, in both normotensive and hypertensive humans, bradykinin was shown to be responsible for the short-term hypotensive effect of captopril (25 mg/day), as indicated by attenuation of this effect with coadministration of icatibant (Gainer et al., 1998). Thus, accumulation of tissue bradykinin by ACE inhibitors probably produces significant beneficial effects in certain cardiovascular diseases. However, AT₁ receptor antagonists have no such action. In this regard, ACE inhibitors seem to be superior to AT₁ receptor antagonists.

On the other hand, accumulating evidence indicates the existence of an alternative pathway for generation of Ang II, independent of ACE, in cardiovascular and renal tissues of various species, including humans (Hollenberg et al., 1998). In particular, chymase, which is a potent and specific Ang II-forming serine protease that is not affected by ACE inhibitors, has been identified in human cardiac and vascular tissues in high concentrations, suggesting that ACE inhibitors may not completely block Ang II production in human tissues (Urata et al., 1990, 1993; Shiota et al., 1993; Liao and Husain, 1995). Thus, with regard to inhibition of Ang II action via AT₁ receptor, AT₁ receptor antagonists may be superior to ACE inhibitors.

Chronic blockade of AT₁ receptor results in increased plasma renin activity and Ang II levels due to loss of the negative feedback mechanism of renin release via AT₁ receptor. Increased plasma Ang II is expected to compete with and displace AT₁ receptor antagonists at the receptor site, which may attenuate the inhibition of AT₁ receptor by these agents. Furthermore, at the same time, increased circulating Ang II is expected to activate AT₂ receptor. Interestingly, in failing human hearts, the ratio of AT₂ to AT₁ receptor is increased because of down-regulation of AT₁ receptor (Asano et al., 1997). Therefore, AT₂ receptor activation may play a key role in the pharmacological differences between AT₁ receptor antagonists and ACE inhibitors, as discussed later.

B. Is the AT₂ Receptor Beneficial or Detrimental?

There are conflicting data concerning the role of AT₂ receptor in cardiovascular and renal diseases. Furthermore, it is not easy to determine whether the effects of AT₂ receptor-selective ligands, particularly in vivo effects, represent antagonistic or agonistic actions, because the molecular, cellular, and physiological functions of AT₂ receptor have not been clearly identified. In this article, we discuss conflicting data on the functions of AT₂ receptor. The recent successful development of AT₂ receptor gene-manipulated animals has provided evidence regarding the function of AT₂ receptor in vivo (Hein, 1998). AT₂ receptor gene knockout mice exhibited a higher basal blood pressure than wild-type mice and enhanced pressor sensitivity in response to i.v. infusion of Ang II compared with the wild type, suggesting that

AT₂ receptor has an opposing effect to AT₁ receptor on blood pressure in vivo (Hein et al., 1995; Ichiki et al., 1995). However, the mechanism for the hypotensive effect of AT₂ receptor remains unresolved, because AT₂ receptor expression is negligible in normal vascular tissues. Cardiac-specific overexpression of AT₂ receptor in mice produced no obvious cardiac morphological changes, but increases in blood pressure and heart rate in this transgenic model in response to Ang II infusion were significantly less than those of wild-type mice, suggesting that AT₂ receptor may decrease the sensitivity of pacemaker cells to Ang II (Masaki et al., 1998). Gene transfer of AT₂ receptor in rat carotid artery in vivo significantly decreased neointimal formation induced by balloon injury, showing the antiproliferative effect of AT₂ receptor on vascular smooth muscle cells in vivo (Nakajima et al., 1995). In rats with heart failure induced by myocardial infarction, treatment with AT₁ receptor antagonist (1.5 mg/kg/day L-158809) significantly improved cardiac hypertrophy and remodeling and cardiac dysfunction, and these effects were partially blocked by AT₂ receptor antagonist (10 mg/kg/day PD123319 via osmotic minipump), suggesting that AT₂ receptor may have the cardioprotective effects (Liu et al., 1997). Furthermore, recent in vitro studies showed that in contrast to AT₁ receptor, under certain experimental conditions, AT₂ receptor exerts antiproliferative effects on neonatal rat cardiac myocytes (Booz and Baker, 1996), fibroblasts (Ohkubo et al., 1997), and rat coronary endothelial cells (Stoll et al., 1995). All of these findings support the concept that AT₂ receptor activation may produce beneficial effects in cardiovascular diseases. An up-to-date review on the functions of this receptor, which emphasizes the beneficial role of AT₂ receptor in cardiovascular and renal diseases, was published recently (Matsubara, 1998).

However, it should be noted that conflicting data have also been reported. In global, no-flow ischemia in left atrium-perfused isolated working rat hearts, PD123,319 (0.3 μM) administered before ischemia improved the recovery of left ventricular work and efficiency, whereas losartan (1 μM) blocked the recovery of left ventricular work and depressed efficiency, suggesting that AT₂ receptor is involved in mechanical dysfunction after ischemia/reperfusion in the heart (Ford et al., 1996). The administration of PD123319 (30 mg/kg/day) in rats receiving Ang II infusion (120 ng/kg/min) had no effect on hemodynamic parameters or cardiac hypertrophy but antagonized the effects of Ang II on smooth muscle cell growth and ECM expression, whereas the administration of losartan (10 mg/kg/day) prevented Ang II-induced hemodynamic effects but did not significantly affect Ang II-induced vascular hypertrophy (Levy et al., 1996). Therefore, vascular hypertrophy and remodeling by Ang II in vivo may be mediated by AT₂ receptor rather than AT₁ receptor. Furthermore, Ang II infusion (200 ng/kg/min) in rats decreased the proliferation of left and right

ventricular and right atrial myoendothelial cells, and these effects were not at all blocked by losartan treatment (10 mg/kg/day), indicating an important role of non-AT₁ receptor in the suppression of myoendothelial proliferation, although this study did not examine the effects of AT₂ receptor ligands (McEwan et al., 1998). These in vivo data support the concept that AT₂ receptor activation may have detrimental effects in cardiovascular diseases. The role of AT₂ receptor is still controversial and probably differs among various cardiovascular diseases.

C. Combination Therapy

Theoretically, monotherapy with an AT₁ receptor antagonist or ACE inhibitor may be insufficient to block the renin-angiotensin system in vivo. Indeed, in mildly salt-depleted normotensive healthy volunteers, a combination of single oral doses of captopril (50 mg) and losartan (50 mg) induced additive blood pressure reduction, whereas a combination of single oral doses of 50 mg losartan with 10 mg enalapril decreased blood pressure and stimulated plasma renin activity to a greater extent than enalapril alone at 10 to 20 mg (Azizi et al., 1997). Furthermore, recent animal studies that compared the effects of combined AT₁ receptor antagonist and ACE inhibitor at a wide range of doses with those of each agent alone with regard to blood pressure and cardiac weight in SHR demonstrated that a low-dose combination of losartan (3 mg/kg/day) and enalapril (3 mg/kg/day) induced greater reductions in blood pressure and left ventricular weight/body weight ratio in SHR than monotherapy with higher doses (each 10 mg/kg/day) and had effects comparable with those of monotherapy with 20 mg/kg/day enalapril or 30 mg/kg/day losartan (Menard et al., 1997). In this work, as expected, the combination of enalapril with losartan in SHR prevented the increase in circulating Ang II levels observed with losartan alone, which is expected to prevent the AT₂ receptor activation that is induced by losartan alone. Furthermore, in SHR, hypotensive effects and regression of cardiac hypertrophy due to ACE inhibition are not mediated by bradykinin, as shown by the lack of effect of icatibant on the hypotensive action of ACE inhibitors in SHR (Linz et al., 1995). Therefore, these synergistic effects of combined ACE inhibitor and AT₁ receptor antagonist in SHR seem to be due not to bradykinin or AT₂ receptor but rather to more potent blockade of AT₁ receptor action. These findings clearly demonstrate that combination therapy with an AT₁ receptor antagonist and an ACE inhibitor is more effective for inhibiting the renin-angiotensin system than either agent alone and may be more powerful for cardiovascular protection than either monotherapy. However, detailed comparisons of combination therapy with monotherapy, using a broad ranges of doses and long-term treatment periods in both animal models and humans, are essential to establish

the advantages and mechanisms of combination therapy using both types of drugs.

VII. Conclusions

There remain unresolved questions regarding the pathophysiological mechanisms of Ang II-mediated cardiovascular and renal diseases.

Although both cultured cardiac myocytes and fibroblasts in vitro significantly respond to Ang II via AT₁ receptor, the relative importance of myocytes and fibroblasts in pathological cardiac hypertrophy in vivo remains unclear. Interestingly, recent in vitro findings suggest that cardiac myocyte hypertrophy due to Ang II may be mediated by paracrine action of TGF- β 1, endothelin-1, or an unknown factor released from fibroblasts rather than by direct action (Sil and Sen, 1997; Gray et al., 1998). Thus, further study on cross-talk between cardiac myocytes and fibroblasts is needed to elucidate the mechanism underlying Ang II-induced pathological cardiac hypertrophy. Furthermore, it remains controversial whether cardiac Ang II actually participates in cardiac hypertrophy due to pressure overload.

Ang II, via AT₁ receptor, promotes phenotypic change and growth of cardiac, vascular, and glomerular cells and induces expression of various growth factors, ECM proteins, cytokines, and chemokines. These actions seem to play a central role in the molecular mechanism of cardiovascular hypertrophy and remodeling or glomerulosclerosis. Although AT₁ receptor is coupled to G protein and has no intrinsic tyrosine kinase activity, recent in vitro evidence suggests that AT₁ receptor activation leads to activation of not only PLC but also a variety of intracellular signal transduction cascades and transactivation of EGF or PDGF receptor. However, even in in vitro experiments, the role of these multiple signaling cascades in the above-mentioned molecular effects of Ang II remains to be elucidated, and little is known about whether AT₁ receptor activation significantly and simultaneously activates these multiple signal cascades under in vivo conditions. Future studies, particularly in vivo experiments, must determine which signal cascades activated by Ang II are responsible for cellular phenotypic change, cellular growth, and gene expression.

AT₁ receptor-mediated ERK or JNK activation may play some role in the pathophysiology of cardiovascular and renal diseases, partially mediated by activation of AP-1. Unfortunately, at present, specific and potent pharmacological inhibitors of MAP kinases or AP-1 are not available for use in in vivo experiments. Therefore, in vivo inhibition of MAP kinase activation with dominant interfering mutants of MAP kinases, using gene transfer techniques, is a powerful strategy for elucidating the role of MAP kinases in the pathophysiology of cardiovascular and renal diseases. This approach may provide important clues to in vivo molecular mecha-

nisms of Ang II-mediated cardiovascular and renal diseases.

In vivo, Ang II exerts systemic effects, including vasoconstriction, elevation of systemic blood pressure, glomerular hypertension, activation of sympathetic nervous system, and retention of sodium and body fluids. Undoubtedly, these well known systemic effects of Ang II, with various direct molecular effects, significantly participate in the pathophysiology of cardiovascular and renal diseases in an additive or a synergistic manner. It remains unclear which is more responsible for Ang II-mediated cardiovascular and renal diseases: the molecular and cellular effects or the systemic effects. To answer this question, elucidation of the relative roles of local and circulating renin-angiotensin systems is essential. Furthermore, in vivo experiments using transgenic or knockout animals targeted to angiotensin receptor genes in a tissue-specific manner will begin to elucidate the importance of local actions of Ang II in cardiovascular and renal diseases, distinct from systemic effects. Elucidation of these questions should provide new concepts regarding the pathophysiological mechanisms of cardiovascular and renal diseases.

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