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Chapter 186

Angiotensin II Peptides

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ABSTRACT

Much evidence supports the notion that angiotensin II (Ang II), the central product of the renin–angiotensin system (RAS), may play a central role not only in the etiology of hypertension but also in the pathophysiology of cardiovascular diseases in humans. Ang II, via the Ang II type 1 receptor, directly causes cellular phenotypic changes and cell growth, regulates the gene expression of various bioactive substances, and activates multiple intracellular signaling cascades in cardiac myocytes and fibroblasts, as well as vascular endothelial and smooth muscle cells. Recently, new factors have been discovered, such as angiotensin-converting enzyme 2, angiotensin-(1-7), and its receptor Mas. This section summarizes the current knowledge about the broad RAS in the pathophysiology of cardiac hypertrophy and remodeling, heart failure, vascular thickening, and atherosclerosis.

DISCOVERY

Research on the renin–angiotensin system (RAS) began with the discovery of renin from the kidney by Tiegerstedt and Bergman in 1898. In 1940, a peptide that had vasoconstrictive effects in the RAS was discovered, and it was named *hypertensin* by Braun-Menendez in Argentina, and *angionin* by Page and Helmer in the United States. These two terms persisted for about 20 years, until it was agreed to rename the pressor substance *angiotensin*. In the 1950s, two forms of angiotensin were recognized, the first a decapeptide (angiotensin I: Ang I) and the second an octapeptide (angiotensin II: Ang II). Skeggs et al. in the United States reported that Ang II was formed by the enzymatic cleavage of Ang I by another enzyme, termed *angiotensin-converting enzyme* (ACE). Schwyzer and Bumpus succeeded in the synthesis of Ang II in 1957. Gross suggested, in 1958, that the RAS was involved in the regulation of aldosterone secretion, and then Davis, Genet, Laragh et al. proved his hypothesis.

In the early 1970s, polypeptides either inhibiting the formation of Ang II or blocking Ang II receptors were discovered, but these were not orally active. Cushman and Ondetti succeeded in the development of captopril, the orally active ACE inhibitor in 1977. After that, many experimental and clinical studies with ACE inhibitors uncovered additional roles for the RAS in the pathophysiology of hypertension, heart failure, and vascular diseases. Furthermore, losartan (Dup 753), an orally active, highly selective and potent nonpeptide Ang II receptor blocker (ARB), was developed in 1988, and the cloning of Ang II receptors, type 1 (AT_1R) and type 2 (AT_2R) was accomplished in the early 1990s.

Angiotensin-(1-7) [Ang-(1-7)] was discovered in 1988 by Santos et al., and angiotensin-converting enzyme 2 (ACE2) was identified in 2000, which catalyzes the conversion of Ang I [Ang-(1-10)] to Ang-(1-9) by the removal of a single carboxy-terminal amino acid. ACE2 is an essential regulator of heart function and a functional receptor for the SARS coronavirus.

STRUCTURE OF THE PEPTIDES AND COMPONENT OF RAS

The RAS plays an important role in the regulation of arterial blood pressure. Renin is an enzyme that acts on angiotensinogen to catalyze the formation of Ang I. Ang I is then cleaved by ACE to yield Ang II. A representation of the biochemical pathways of RAS is shown in Fig. 1.

The major element of the rate of Ang II production is the amount of renin released by the kidney. Renin is synthesized, stored, and secreted into the renal arterial circulation by the granular juxtaglomerular cells. The secretion of renin is controlled predominantly by three pathways: The first mechanism controlling renin release is the intrarenal macula densa pathway and the second is the intrarenal baroreceptor pathway. The third mechanism is the β-adrenergic receptor pathway, which is mediated by the release of norepinephrine from postganglionic sympathetic nerve terminals. An increase in renin secretion enhances the formation of Ang II, and Ang II stimulates the AT_1R on juxtaglomerular cells to inhibit renin release.

The substrate for renin is angiotensinogen, an abundant α_2-globulin that circulates in the plasma. The primary

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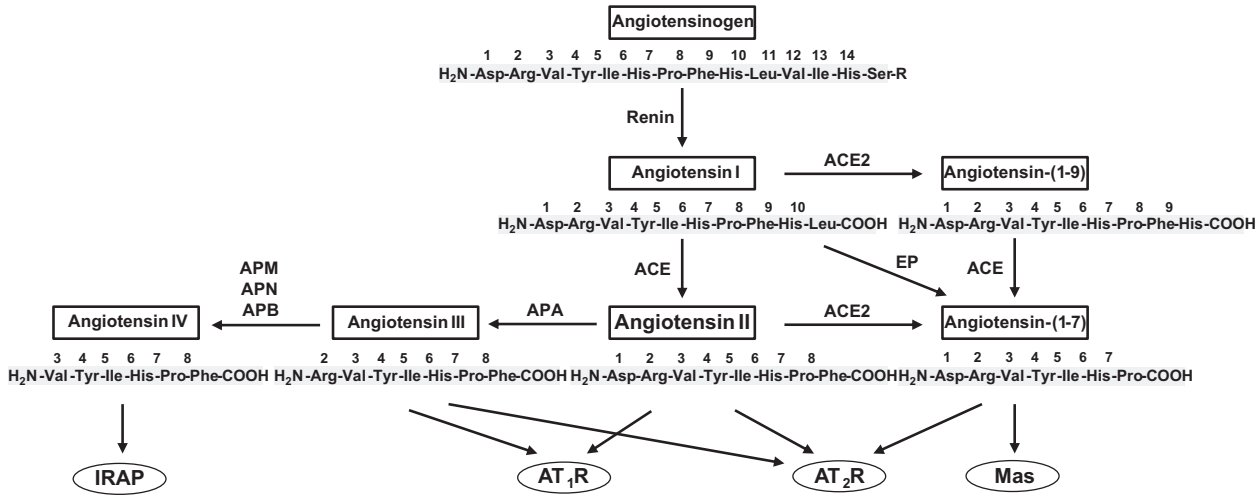

FIGURE 1 Formation of RAS peptides. ACE, angiotensin-converting enzyme; EP, endopeptidase; APA, B, M, N; aminopeptidase A, B, M, N; IRAP, insulin-regulated aminopeptidases.

structure of angiotensinogen has been deduced by molecular cloning. Angiotensinogen is synthesized primarily in the liver, although mRNA that encodes the protein is abundant in fat, certain regions of the central nervous system, and the kidney. The rate of Ang II synthesis can be influenced by changes in angiotensinogen levels.

ACE was discovered in plasma as the factor responsible for conversion of Ang I to Ang II. The ACE gene contains, in intron 16, an insertion/deletion polymorphism that explains 47% of the phenotypic variance in serum ACE levels. Individuals homozygous for the deletion allele have higher levels of serum ACE.

Ang I is rapidly converted to Ang II, when given intravenously. Ang III [Ang-(2-8)] can be formed by the action of aminopeptidase on Ang II. Ang III Ang II cause qualitatively similar effects. Ang III is approximately as potent as Ang II in stimulating the secretion of aldosterone. However, Ang III is only 25% as potent as Ang II in elevating blood pressure. Ang IV [Ang-(3-8)] is generated by the sequential cleavage of two amino acid residues from the amino terminus of Ang II by aminopeptidases localized to the endothelial surface.

There are bypass pathways in the RAS to produce Ang II besides the main enzymes such as renin and ACE. Arakawa et al. showed that trypsin and kallikrein can produce Ang II. One of the most famous enzymes in such bypass pathways is a heart chymase that is secreted by mast cells.

DISTRIBUTION OF RAS, PROCESSING, AND ENDOGENOUS FORM IN THE CARDIOVASCULAR SYSTEM

Circulating renin of renal origin acts on circulating angiotensinogen of hepatic origin to produce Ang I in the plasma, and circulating Ang I is converted by plasma ACE and pulmonary endothelial ACE to Ang II. Then, Ang II is delivered to its target organs via blood flow. This traditional pathway is called the circulating RAS. However, heart, blood vessels, and several other tissues contain and/or synthesize components of the RAS, called the tissue (local) RAS.

Many tissues, including heart, blood vessels, brain, kidney, and adrenal gland, express mRNAs for renin, angiotensinogen, and/or ACE. Furthermore, several other Ang I cleavage products have been found in the cultured cell types from these tissues, for example, Ang-(1-9), Ang-(1-7), Ang III, and Ang IV (Fig. 1). Ang I and Ang II have been localized in atria and ventricles. Therefore, it seems that the tissue RAS exists independently of the renal/hepatic-based system.

Several serine proteases, such as tonin and cathepsin G, have been shown to hydrolyze Ang II precursors. An aspartyl protease with cathepsin D-like properties was shown to convert angiotensinogen to Ang I. Conversion of Ang I to Ang II in human and dog cardiac ventricles may occur by heart chymase. Unlike ACE, human heart chymase shows high specificity for Ang I and does not degrade bradykinin or vasoactive intestinal peptide. It is likely that the relative contribution of ACE and chymase to cardiac Ang II formation varies with the cardiac chamber in the human heart, because ACE levels are the highest in the atria and chymase levels are the highest in ventricles. It is recently believed that chymase plays a crucial role in various tissues because chymase can not only produce Ang II but also convert precursors of transforming growth factor (TGF)-β and matrix metalloproteinase-9 to their active forms, whose increases damage various organs.[30]

RECEPTORS AND THEIR DISTRIBUTION IN THE CARDIOVASCULAR SYSTEM

The effects of angiotensins are exerted through specific cell surface receptors. There are two subtypes of Ang II receptors, types 1 and 2 (AT_1R and AT_2R). The successful

cloning of the AT_1R in 1991 and the AT_2R in 1993 allowed the development of further research on the structure and function of this receptor.

The AT_1R consists of two subtypes, $AT_{1a}R$ and $AT_{1b}R$, which have 94% homology with regard to amino acid sequence and have similar pharmacological properties and tissue distribution patterns. The AT_1R is a member of the seven-transmembrane-spanning, G protein-coupled receptor (GPCR) family; it binds to heterotrimeric G proteins and lacks intrinsic tyrosine kinase activity. The human AT_1R gene is mapped to chromosome 3, and the $AT_{1a}R$ and $AT_{1b}R$ genes in rats are mapped to chromosomes 17 and 2, respectively. The AT_1R is ubiquitously and abundantly distributed in adult tissues, including blood vessel, heart, kidney, adrenal gland, liver, brain, and the lung. The AT_1R 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, and renal sodium and water absorption. Therefore, the AT_1R has a role in atherosclerosis, congestive cardiac failure, and several acute and chronic inflammatory diseases, conditions in which inflammation is known to play a significant role (Fig. 2). Numerous selective and potent nonpeptide AT_1R antagonists, such as losartan, candesartan, valsartan, irbesartan, eprosartan, telmisartan, tasosartan, have been developed.

In contrast, the AT_2R has high affinity for PD123177, PD123319, CGP42112, L-162,686, L-162,638, and CGP42112A. The cDNA and genomic DNAs of human, rat, and mouse AT_2Rs have been cloned. The AT_2R is mainly expressed in developing fetal tissues, and it is believed to have an essential role in physiological vascular development. The AT_2R expression rapidly decreases after birth,[19] and, in the adult, expression of this receptor is limited mainly to the uterus, ovary, certain brain nuclei, heart, and adrenal medulla. Unlike the AT_1R, which has been shown to have subtypes in rats and mice, there is no evidence for subtypes of the AT_2R. Although a comparison of amino acid sequences of $AT_{1a}R$ and AT_2R in rats, deduced from nucleotide sequences, shows a low homology between these receptors (32%), The AT_2R is also a seven-transmembrane domain receptor. In various cell lines, the AT_2R-activated protein tyrosine phosphatase was shown to inhibit cell growth or induce programmed cell death (apoptosis). The AT_2R inhibited AT_1R-mediated cell growth, demonstrating an antagonistic action. It is believed that the expression balance of AT_1 and the AT_2 is important for cardiovascular diseases.[8] There have also been conflicting findings regarding these receptors. For example, it is controversial whether cardiac hypertrophy is promoted by the AT_2R. However, the AT_2R plays a cardioprotective role on left ventricular function after myocardial infarction. In contrast to extensive data on the molecular and cellular functions and pathophysiological significance of the AT_1R, the role of the AT_2R in cardiovascular diseases remains to be defined. At present, the implications of overstimulation of AT_2R by long-term use of ARB are being investigated in clinical trials.[15]

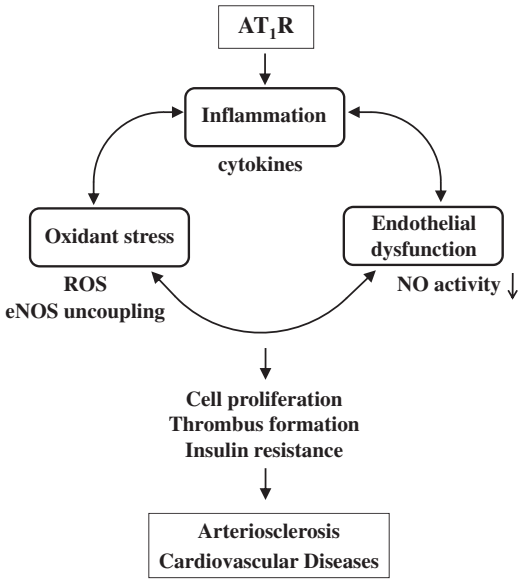

FIGURE 2 Proposed mechanism of the Ang II-mediated pathological cardiac hypertrophy. ROS, reactive oxygen species; eNOS, endothelial nitric oxide synthase.

It has been suggested that Ang-(1-7) mediates its effects by interacting with the GPCR Mas, a prototypic seven-transmembrane domain receptor, which is predominantly expressed not only in the brain and testis but is also found in the kidney, heart, and blood vessels.

The Ang AT_4 receptor was originally defined as the specific, high-affinity binding site for Ang IV. Therefore, it was suspected to be classically a GPCR. And then, Albiston et al. have identified it as the transmembrane enzyme, insulin-regulated aminopeptidase (IRAP),[3] an abundant protein that is found in specialized vesicles containing the insulin-sensitive glucose transporter GLUT4.

BIOLOGICAL ACTIONS AND PATHOPHYSIOLOGICAL IMPLICATION IN THE CARDIOVASCULAR SYSTEM

Molecular Characteristics of Pathological Cardiac Hypertrophy

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 peptide (ANP). In the cardiac ventricle of most mammalian species, MHC consists of two isoforms, α- and β-MHCs.

In the rat, α-MHC is the predominant isoform in adult hearts, whereas β-MHC is the predominant isoform in fetal hearts. Therefore, changes in the ratio of β-MHC to α-MHC 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. The ratio of skeletal α-actin to cardiac α-actin in the ventricle plays a significant role in cardiac function, because skeletal α-actin has greater contractility than cardiac α-actin. Furthermore, in addition to showing enhanced expression of β-MHC and skeletal α-actin, hypertrophic ventricular myocytes are also characterized by significant upregulation of ANP, which is scarcely expressed in normal adult ventricular myocytes.

Another important property of pathological cardiac hypertrophy is increased accumulation of extracellular matrix (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.[21,34] Increased interstitial collagen deposition in the heart enhances cardiac stiffness and results in diastolic dysfunction. Fibronectin is localized on the surface of cardiac myocytes, connects cardiac myocytes to perimyocytic collagen and is believed to affect cardiac systolic and diastolic functions. Thus, increased ECM accumulation, and the abovementioned ventricular myocyte gene reprogramming play critical roles in the impairment of cardiac performance and pathophysiology of cardiac failure. ECM proteins within the heart are predominantly produced by fibroblasts. 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 these important processes of pathological cardiac hypertrophy, including myocyte hypertrophy, myocyte gene reprogramming, fibroblast proliferation, and ECM protein accumulation.

Effect of AT_1R on Cardiac Remodeling (Fig. 2)

Activated AT_1R can activate multiple signaling pathways. The acute vasoconstrictor function of Ang II is primarily mediated through AT_1R by classical G protein-dependent signaling mechanisms. Investigations of the effects of Ang II on cardiac intracellular signaling cascades are essential to elucidate the molecular mechanism underlying Ang II-induced pathological cardiac hypertrophy. Accumulating *in vitro* evidence on cultured cardiac myocytes or fibroblasts suggests that mitogen-activated protein (MAP) kinases, including extracellular signal-regulated kinase (ERK), c-jun amino terminal kinase (JNK) and p38MAP kinase (p38MAPK), may be responsible for myocyte hypertrophy and gene reprogramming or fibroblast proliferation. Ang II-induced cardiac activation of JNK occurs in a more sensitive manner than that of ERK.

The ERK cascade activates platelet-derived growth factor (PDGF), epidermal growth factor receptor (EGFR), insulin receptor pathways, and nonreceptor tyrosine kinases belonging to the c-Src family, prolinerich tyrosine kinase 2, focal adhesion kinase, and janus kinases (JAKs).[18] Besides the G protein-dependent effects of Ang II activated AT_1R, the activated AT_1R can also stimulate G protein-independent signal transduction mechanisms by directly associating with signaling molecules, such as β-arrestins, JAK, Cdc42, and Src.[10]

JNK activation by Ang II without ERK activation is followed by activation of activator protein-1 (AP-1). 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 ANP, and cardiac fibrosis-associated genes such as TGF-β1 and collagen type I, 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 and TGF-β1. Therefore, it is intriguing to postulate that JNK activation, in part through the activation of AP-1, may be implicated in Ang II-induced cardiac hypertrophic response *in vivo*. An *in vivo* study showed that apoptosis signal-regulating kinase 1 (ASK1), one of the MAP kinase kinase kinases, plays an important role in Ang II-induced cardiac hypertrophy and remodeling, including cardiomyocyte hypertrophy, cardiac hypertrophy-related mRNA upregulation, cardiomyocyte apoptosis, interstitial fibrosis, coronary arterial remodeling, and collagen gene upregulation.[11]

The RAS, TGF-β, and β-adrenergic system network mediates this myocardial remodeling. TGF-β1 mRNA and protein expression is augmented in cardiomyocytes and cardiac fibroblasts, which in these situations transdifferentiate to myofibroblast phenotype, following progressive diastolic dysfunction. Ang II caused a significant increase in TGF-β1 mRNA. Chronic administration of Ang II-induced TGF-β1 protein expression in myocardium.[14] Ang II-induced hypertrophic growth of cardiomyocytes is mediated by TGF-β1, which is downstream to Ang II in network.[26] Mice expressing constitutively hyperactive TGF-β1 had a chronic hypertrophy, fibrosis, and cardiac dysfunction that was resistant to the ARB telmisartan but blocked by TGF-β antagonist.[24] The Ang II-mediated cardiac remodeling mechanism is thus dependent on TGF-β1. From all the studies performed so far, it is clear that Ang II/TGF-β1 induced autocrine–paracrine cellular responses in cardiac fibroblasts, in the myocardial

interstitium, and cardiomyocytes cause cardiac hypertrophy. Ang II-induced TGF-β1 signaling also induces translocation of Smad proteins into the nucleus to drive transcription of fibrotic marker proteins such as collagen, fibronectin, and connective tissue growth factor (CTGF).[17] CTGF is a profibrotic factor that stimulates TGF-β1 responses mediating fibrosis and apoptosis.[22] The inflammatory cytokines such as interleukin-8, interleukin-6, vascular cell adhesion molecule-1, and monocyte chemoattractant protein-1, are believed to have important roles in the coupling acute ischemic episodes and chronic myocardial remodeling with chronic angiotensin receptor stimulation.[9]

Ang II facilitates the release of norepinephrine from cardiac sympathetic nerve terminals. In Ang II-infused rats, surgical cardiac sympathectomy or treatment with 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. Thus, the activation of cardiac sympathetic neurons by Ang II also contributes to pathological cardiac hypertrophy.

Ang II, via the AT_1R, is also known to be a potent mediator of oxidative stress and reactive oxygen species (ROS)-mediated signaling. A large body of evidence indicates that ROS plays a major part in the initiation and progression of cardiovascular dysfunctions associated with diseases such as hyperlipidemia, diabetes mellitus, hypertension, ischemic heart disease, and chronic heart failure.[32] In addition, Ang II signaling activates membrane NAD(P)H oxidases in VSMCs to produce ROS, which mediate the pleiotrophic effects of Ang II.[28] Ang II signaling mediated activation of NAD(P)H oxidases involves the upstream mediators Src/EGFR/PI3K/Rac-1 and PLD/PKC/p47phox phosphorylation.[28,33] Transcription factors such as nuclear factor-κB, AP-1, and Nrf2 that are implicated in the pathogenesis of atherosclerosis are activated by ROS.[20] ROS may cause vessel inflammation by inducing the release of cytokines and causing an increase in the expression of the leukocyte adhesion molecules in the cell membranes. This may increase recruitment of monocytes to the area of endothelial damage. Thus, activated AT_1R-induced ROS production can lead to changes in structural and functional properties of the vasculature and is the central aspect of vascular pathobiology in hypertension, diabetes, and cardiovascular diseases.[7]

Effect of AT_1R on Vascular Remodeling

Numerous *in vivo* experiments have shown that Ang II can induce VSMC proliferation *in vivo*.[13] Ang II infusion increased mesenteric vascular media width, media cross-sectional area, and media/lumen ratio. Despite detailed investigations into the molecular mechanism of Ang II-mediated VSMC 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 VSMCs from a contractile to a synthetic phenotype. Basic fibroblast growth factor (bFGF) may play a key role in Ang II-mediated VSMC replication *in vivo*, as shown by the observation that the injection of anti-bFGF antibody significantly inhibited the mitogenic effect of Ang II infusion on rat carotid arteries. Ang II infusion in rats doubled superoxide production in rat aorta by activation of NAD(P)H oxidase. On the other hand, norepinephrine infusion did not increase vascular superoxide production, despite a hypertensive effect comparable to that of Ang II, suggesting that VSMC growth due to Ang II may be specifically mediated by increased superoxide generation. Ang II infusion in rats increased heme oxygenase-1 (HO-1) mRNA and protein in the endothelium.[16] Because HO-1 is an oxidant-sensitive gene, it is possible that increased oxidative stress is a trigger for HO-1 mRNA upregulation in the Ang II-infused rat aorta and that HO-1 may serve to abrogate this increased stress caused by Ang II. Ang II infusion stimulated aortic thrombin receptor mRNA expression in rats, which was blocked by either ARB or the 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 AT_1R-mediated superoxide production and may be implicated in the pathophysiology of atherosclerosis by thrombin cascade activation. The injection of Ang II resulted in increased oxidized low-density lipoprotein 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.

Endothelial dysfunction in the context of hypertension and cardiovascular diseases is partly dependent on the production of ROS. AT_1R-induced ROS not only burns nitric oxide (NO) but also reduces NO formation in the endothelium by oxidizing tetrahydrobiopterin, a crucial cofactor for endothelial NO synthase (eNOS).[27] Superoxide anion, a highly reactive ROS, is known to cause breakdown of endothelial-derived relaxing factor and dysfunction of endothelium. Regenerated endothelium has an impaired ability to release endothelial-derived relaxing factors, in particular NO. Activation of angiotensin receptors triggers an efflux of cytosolic calcium in endothelial cells (ECs). Calcium efflux activates PLA-2 to release arachidonic acid, which is metabolized by cyclooxygenase (COX) to generate various eicosanoids including endoperoxides and various prostaglandins that activate thromboxane A2/prostanoid receptors located on the VSMCs causing contractions. Endothelial COX activity also produces ROS. In addition, uric acid, despite its known antioxidant effects, may cause

human vascular EC dysfunction by local activation of RAS, inducing oxidative stress. ROS may, in turn, activate local RAS of whole vasculature, including EC, smooth muscle cells, fibroblast-enhancing Ang II production and concomitant stimulation of AT_1R to create a positive feedback loop between local RAS and ROS. Thus, in hypertensive cardiovascular diseases, endothelial dysfunction is characterized by blunted endothelium-dependent vasodilations or enhanced endothelium-dependent contractions.[7,31]

Effects of in AT_2R on Cardiovascular System

Generally, AT_2R is believed to promote apoptosis and inhibit proliferation and hypertrophy. Unlike AT_1R, AT_2R contributes to maintenance of blood pressure by controlling the vascular tone through vasodilatation. Available evidence indicates links between AT_2R and bradykinin B_2 receptor (B_2R) in NO production in vasodilation.[5,12] AT_2R stimulation by Ang II leads to an increase in cGMP levels through a mechanism involving B_2R, causing NO release. AT_2R activation stimulates bradykinin formation by activating kininogenases. Chronic infusion of Ang II into AT_2R overexpressing mice completely abolished the AT_1R-mediated pressor effect, suggesting vasodilatation by AT_2R. This was blocked by one of the inhibitors of B_2R and NOS. In addition, aortic explants from these transgenic mice overexpressing AT_2R showed greatly increased cGMP/NO production and diminished AT_1R-induced vascular constriction. Removal of endothelium or treatment with the inhibitors of B_2R and NOS abolished these AT_2R-mediated effects, indicating that endothelium is the primary site of effect manifestation by AT_2R. The AT_2R in aortic SMCs also stimulates the production of bradykinin, which stimulates the NO/cGMP system to promote vasodilation. In the AT_2R-deficient mice, bradykinin-induced dilation is seriously impaired. Bradykinin-induced dilation is inhibited by AT_2R antagonist but not by Losartan, an ARB. Thus, AT_2R and B_2R may form functional heterodimers, as recently shown, and this might lead to increased NO and cGMP production.[1] The physical association between the dimerized AT_2R and B_2R initiates changes in intracellular phosphoprotein signaling activities and enhanced production of NO and cGMP.[7,15]

Growth and apoptosis are also two opposite components of tissue remodeling. Cardiac remodeling happens in certain physiological or pathological circumstances, such as exercise, aging, hypertension, and myocardial infarction. It is known that although AT_2R expression decreases sharply after birth, it can increase again in some pathophysiological conditions. Stimulation of AT_2R expression may inhibit neointima formation, cell proliferation, inflammation in vascular injury, myocardial infarction, and ischemic diseases, suggesting its protective role. Conversely, in AT_2R-deficient mice, recovery from acute myocardial infarction was reduced compared to the wild-type mice.[2] Although neointima formation and VSMC proliferation after vascular injury were exaggerated in AT_2R-deficient mice, they were suppressed in $AT_{1a}R$-deficient mice. AT_2R possibly exerts antiproliferative effects and proapoptotic effects in VSMCs by controlling $AT_{1a}R$.[29] The number of apoptotic cells in the injured artery was increased significantly in $AT_{1a}R$-deficient mice but decreased in AT_2R-deficient mice, indicating that AT_2R mediates apoptosis. Selective AT_2R stimulation also inhibited restenosis after balloon angioplasty. Thus, experimental studies have shown a beneficial role for the reexpression of the AT_2R after vascular injury leading to inhibition of cell growth and promotion of apoptosis. Gene transfer studies *in vivo* have also supported that AT_2R expression attenuated neointima accumulation in injured carotid arteries by antagonizing growth-promoting effects of AT_1R. Vascular injury studies using mice with disrupted AT_2R expression showed enhanced neointimal formation compared with wild-type mice, suggesting a protective role for AT_2R.

AT_2R expression is upregulated in several disease conditions. In the left ventricle-specific AT_2R overexpression mice, elevated left ventricular endodiastolic pressure, increased systolic and diastolic dimensions, severely depressed left ventricular fractional shortening, and wall thinning were observed, suggesting that ventricular myocyte-specific expression of AT_2R promotes the development of dilated cardiomyopathy and heart failure *in vivo*. *In vivo* ectopic AT_2R overexpression in postnatal left ventricular myocardium is thus detrimental, explaining the sharp decline of AT_2R after birth. If prolonged ARB treatment turns out to be completely beneficial through clinical trials, then it is either a dosage effect or it depends on the time and site of AT_2R expression. The implications of overstimulation of AT_2R by long-term use of ARB are being investigated in clinical trials.

Effects of in Mas on Cardiovascular System

Ang-(1-7) mediates its effects by interacting with the GRCR, Mas,[25] a prototypic seven-transmembrane domain receptor, which is not only predominantly expressed in the brain and testis but is also found in the kidney, heart, and blood vessels.[4] Moreover, several studies have shown that the interaction of Ang-(1-7) with Mas induces the protective cardiovascular actions such as NO release, Akt phosphorylation and vasodilation.

Ang-(1-7) also functions as an antithrombotic, antiproliferative, and antioxidative agent. Although the mechanisms of action in the vascular system has not yet been well established, several studies have focused on control of the NO and O_2^- balance, which regulates cardiovascular function. A reduction in superoxide dismutase and catalase activity in Mas-deficient mice demonstrates impaired antioxidant properties in these animals.[35] Moreover, ROS

levels are increased in Mas-deficient animals. The Mas-deficient mice exhibit impaired *in vivo* endothelial function and increased blood pressure. The transgenic rats overexpressing human ACE2 in VSMCs, showed improved endothelial function and decreased vasoconstriction response to Ang II. Furthermore, the infusion of AVE 0991, a Mas agonist, increases the hypotensive effect of bradykinin in rats; one plausible mechanism for this effect involves an increase in NO levels.[6]

ACE2 protects endothelial cells from Ang II-mediated macrophage infiltration and oxidative stress in an Ang-(1-7)-dependent manner in atherosclerosis. The long-term Ang-(1-7) treatment induces atheroprotective effects in ApoE-deficient mice, an animal model for atherosclerosis. The improvement in endothelial function resulted from an increase in NO bioavailability, as the heptapeptide increased NOS expression and decreased O_2^- production. The genetic deletion of ACE2 significantly increases plaque formation in ApoE-deficient mice, which is decreased by targeted vascular ACE2 overexpression. Consequently, these actions of the ACE2-Ang-(1-7)-Mas system may be exploited in the treatment of atherosclerosis and other vascular diseases.

Thus, the ACE2-Ang-(1-7)-Mas system has opposite functions of the classical RAS in tissues responsible for blood pressure regulation and cardiovascular homeostasis. It decreases the production of ROS and increases eNOS, improving the antioxidant capacity of cardiovascular tissues. The ACE2-Ang-(1-7)-Mas system may become an important therapeutic target for cardiovascular protection.[23]

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