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The Renin Angiotensin System in Cardiovascular Disease

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Preface

The renin angiotensin system (RAS) is a key regulatory mechanism in the body and is known to play a critical role in cardiovascular physiology and pathophysiology. This system is accountable for maintaining arterial blood pressure and electrolyte homeostasis, as well as regulating organ perfusion. Under pathological conditions, RAS elicits its response by triggering oxidative stress, inflammation, as well as functional and structural remodeling of both the blood vessels and myocardium. By inducing cardiovascular damage, it participates in the pathogenesis of cardiovascular abnormalities such as hypertension, ischemic heart disease, and heart failure. It is pointed out that cardiovascular diseases are the number one cause of death worldwide, with the morbidity and mortality rate of more than 36% of all deaths. An in-depth study of the RAS, under both experimental and clinical settings, has revealed the therapeutic efficacy of the blockade of this system by various pharmacological agents in reducing cardiovascular and related events. Treatment of the patients having cardiovascular abnormalities with angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor (AT_1R) blockers, alone or in combination, has significantly improved the global mortality and morbidity. It is also well known that the RAS is present in both the peripheral system and different tissues and is involved in the formation of angiotensin II (Ang II) due to the participation of various components, including ACE. The circulating Ang II affects different organs in the body upon binding to its receptor Ang II type 1, located at the plasma membrane. It then activates signal transduction mechanisms in the cell leading to a variety of biological effects such as vasoconstriction, sodium retention, hypertension, aldosterone and vasopressin production, platelet activation, endothelial dysfunction, cell growth, connective tissue formation, as well as inflammatory, fibrotic, and oxidative stress activities. On the other hand, the activation of the angiotensin II type 2 receptor mitigates the growth-promoting and other effects of the angiotensin II type 1 receptor stimulation. Thus, RAS can be seen to exert multifactorial (both harmful and beneficial) effects in the body.

Since several studies underlying physiological, pathological as well as pharmacological aspects of RAS components have remained a focus of cardiovascular health and disease over the past decades, the present book intends to summarize the current status regarding the role of RAS in cardiovascular diseases. Due to a direct link to neurohormonal dysregulation and cellular dysfunction, excessive activation of RAS and its interaction with different hormones are considered essential modulatory mechanisms, which are involved in inducing cardiovascular abnormalities. This book describes the role of RAS in the pathophysiology of hypertension, atherosclerosis, and ischemic heart disease with particular focus on vascular remodeling, cardiac dysfunction, arrhythmias, and heart failure under various stressful conditions such as pressure and volume overload as well as myocardial infarction. In this context, the molecular and biochemical regulation, genetic and mechanistic approaches, as well as signaling transduction pathways in the pathogenesis of these diseases are discussed. Despite the progress and achievements, in the area of neurohormonal treatments, the growing global burden imposed by these diseases is emphasized. A framework of current and futuristic treatment options targeting RAS for the prevention or reversal of cardiovascular and infectious diseases, as well as the translational success for controlling the expression of RAS, is addressed. In view of the fact that prolonged activation of RAS is known to induce inflammation, metabolic alterations, oxidative stress, and Ca^{2+}-handling abnormalities, some information on these mechanisms of defects with or without ACE inhibitors and AT_1R antagonists treatments of diseased subjects is included.

This book is assembled to contain 25 chapters by international experts in the field of RAS in cardiovascular health and disease. Overall, this monograph provides recent developments in our understanding of alterations in RAS due to different risk factors for cardiovascular disease, as well as highlighting the potential pharmacological approaches for targeting RAS to improve cardiac function in clinical situations. The 25 chapters of this book are arranged in three different parts, namely: Part I–Modulatory Aspects of Renin Angiotensin System (9 chapters), Part II–Pathophysiological Aspects of the Renin Angiotensin System (9 chapters), and Part III–Pharmacotherapeutic Aspects of Renin Angiotensin System (7 chapters). All these three parts of the book are interrelated because each chapter will hopefully motivate investigators to develop innovative approaches to prevent cardiovascular diseases due to the involvement of RAS. This book will also be particularly useful for medical students, fellows, residents, graduate students, and health professionals. It is our contention that the state-of-the-art information about the involvement of RAS in the pathogenesis of cardiovascular disease, as well as the molecular and cellular basis of its therapy provided in this book, could be helpful to biomedical and clinical researchers. Particularly, the development of newer and more effective treatments that can help in decreasing the disease burden while enhancing the quality and life expectancy would prove to be challenging.

First of all, we are indebted to all contributors for their time and efforts in preparing articles for this book. We are grateful to the St. Boniface Hospital Albrechtsen Research Centre for providing infrastructural support for this project. We wish to thank Dr. Gonzalo Cordova most sincerely for his leadership, understanding, and efforts in promoting this project on the Role of RAS in Cardiovascular Disease. We also express our gratitude to Mr. Rajan Muthu and his team for their excellent assistance in the development as well as production of this book.

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Part I
Modulatory Aspects of Renin Angiotensin System

Chapter 1
Renin–Angiotensin–Aldosterone System and LOX-1 Interaction in Hypertension with a Focus on Modulation of the Immune System

Weijia Cheng, Fang Shao, Jawahar L. Mehta, and Xianwei Wang

Abstract The renin–angiotensin–aldosterone system (RAAS) plays a key role in the pathological process of many cardiovascular diseases, including hypertension. Angiotensin II (Ang II) and its two receptors, Ang II type 1 receptor (AT1R) and Ang II type 2 receptor (AT2R), exert many physiological and pathophysiological effects. Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) is responsile for the uptake and degradation of oxidized low density lipoprotein (ox-LDL). The activation of LOX-1 is involved in various cardiovascular diseases including hypertension. The interplay of LOX-1 and AT1R or AT2R has been recently shown to play important part in hypertension and the related cardiovascular diseases such myocardial infarction and stroke. There is increasing evidence that the immune system is a critical mediator in these physiopathological processes. This chapter reviews the role of the immune system in the crosstalk between RAAS and LOX-1 in the genesis of hypertension and the related pathological consequences.

Keywords Renin–angiotensin–aldosterone system · Hypertension · Angiotensin II · Ang II type 1 receptor (AT1R) · Ang II type 2 receptor (AT2R) · Lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1)

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Introduction

Hypertension is one of the most important risk factors for the development of cardiovascular diseases including myocardial infarction (MI), ischemic stroke, chronic heart failure, and atherosclerosis. The RAAS is also a major regulator of blood pressure and salt-water homeostasis [1–5]. Ang II and its receptors are critical for mediating vasoconstrictor responses, excitation of peripheral sympathetic nerve and release of arginine vasopressin. Ang II induces its downstream signaling by activating two of its major G protein coupled receptors, AT1R and AT2R [6–8]. The subsequent G protein-mediated signaling induces most of the effects of RAAS. These two receptors have distinct functions in both physiological and pathophysiological regulation of blood pressure. Ang II-AT1R complex induces several molecular and cellular events while the activation of AT2R has the opposite effects.

AT1R is evolutionarily expressed in various species including humans, rats, mice, dogs and pigs. Its relatively high expression has been found in heart, kidney, blood vessels, liver, lung, brain, gastric mucosa, adrenal gland, ovine, placenta and adipose tissues. Among the cell types, AT1R is expressed in cardiomyocytes, endothelial cells, vascular smooth muscle cells (VSMCs), monocytes, fibroblasts, neurons, embryonic stem cells, intestinal epithelial cells, T lymphocytes and podocytes.

AT1R expression has been determined to be affected in many pathological conditions, including coronary atherosclerotic plaque, myocardial infarct and peri-infarct regions, failing heart, hypertensive heart, several tissues in type 2 diabetics as well as in various tumor tissues [9].

The human AT1R gene is located in chromosome 3q21-3q25 and its encoded protein is a 359 amino acids protein with a 41 KD molecular mass. The protein belongs to G protein-coupled receptor (GPCR) protein superfamily with extracellular N-terminus, seven-transmembrane domains and intracellular C-terminus. The serine/threonine and tyrosine-rich residues located in the intracellular C terminus are the phosphorylation sites of protein kinase C (PKC). Similar to other GPCRs, AT1R is also a protein that initiates downstream signaling pathway by interacting with different adaptor G protein, Gq/11, G12/13, and Gi/o. The activated Gq/11 mainly induces the activation PLC-β and hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP2) on the plasma membrane. The generation of the second messenger inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) are subsequently induced, leading to an increase of cytosolic calcium concentration. The increased intracellular calcium subsequently causes the activation of PKC dependent signaling pathway [10, 11]. In cardiomyocytes, calcium-loaded calmodulin (CaM) induces the activation of Calcineurin (phosphatase-2B), which then enhances the expression of hypertrophic markers such as atrial natriuretic peptide (ANP), β-myosin heavy chain (β-MHC) and α-skeletal muscle (α-SKA) [12, 13]. G12/13 mainly triggers the activation of PLC and Rho kinases. Rho kinases are involved in various cellular functions including cell morphology, polarity, cytoskeletal remodeling, cell–cell adhesion, cell proliferation and cell migration. Rac, a member of the Rho family, is a component of the nicotinamide adenine dinucleotide phosphate

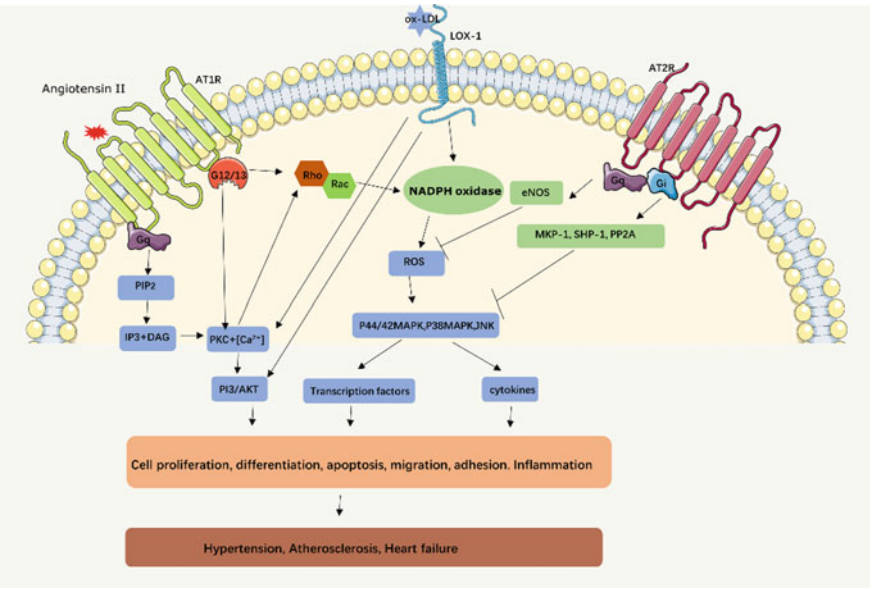

Fig. 1.1 Activation of AT1R results in the induction of PKC-PI3K and ROS-MAPKs with subsequently production of cytokines and transcription factors. Activation of AT2R has the opposite effect by promoting the activation of eNOS, MKP-1, SHP-1 and PP2A thus opposing the effects of AT1R-mediated signaling. Ox-LDL and LOX-1 interaction leads to the activation of PKC-PI3K and ROS-MAPKs. All the above signaling pathways facilitate cellular functions including cell proliferation, differentiation, apoptosis, migration, adhesion and inflammation which are critical in the pathogenesis of different cardiovascular diseases such as hypertension, atherosclerosis and heart failure

(NADPH) oxidase complex which is involved in the production of reactive oxygen species (ROS). ROS, in turn, enhance NAPDH oxidase activity through RhoA and Rac [12, 14] (Fig. 1.1).

Similar to the calcium signal, ROS act as potent secondary messengers in G12/13- and Gq/11-induced signaling pathways. Several studies have shown that AT1R-mediated ROS production is essential for its downstream signaling, mitogen-activated protein kinases (MAPKs) such as p38 MAPK, P42/44 MAPK and JNK [15, 16] (Fig. 1.1). MAPKs are important signals in ATR-mediated cell functions such as cell proliferation, differentiation, apoptosis and inflammation [17]. In addition to Ang II-AT1R-G12/13-Rho/Rac-ROS-JNK/p38MAPK pathway, Ang II-AT1R–Gq–Ca^{2+}/PKC-PI3K-Akt pathway is another primary signaling pathway in Ang II-AT1R-mediated cell activities. The activation of PKC induced by Gq/11 activates PI3 Kinase (PI3K) and its downstream effector Akt. This PKC-PI3K-Akt pathway is often involved in many pathological conditions including hypertension, heart failure, ischemic stroke, diabetes and inflammation [18–21] (Fig. 1.1). Gi/o proteins inhibit adenylyl cyclase and suppress the activation of PKA in some specific tissues.

Besides its primary effect in regulation of systemic blood pressure and salt-water balance, increasing evidence suggests that Ang II-AT1R complex participates in various immune responses. AT1R activation facilitates the release of various inflammatory cytokines such as Interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), tumor necrosis factor-α (TNF-α), and initiates inflammatory response. Expression of matrix-metalloprotease 2 (MMP2) in vascular cells plays a role in the attraction and recruitment of monocytes and T cells.

TNF-α increases MMP2 expression in vascular cells and plays a pivotal role in the recruitment of infiltrating inflammatory cells such as T cells and monocytes. The differentiation process of monocyte-derived macrophages is regulated by Ang II-induced cytokines [22, 23]. Ang II-induced inflammatory responses and vascular remodeling, both contribute to endothelial dysfunction and further promotes the formation of arteriosclerotic plaques and abdominal aortic aneurisms [24–27].

AT2R is an alternative component of Ang II-mediated downstream physiological functions. The receptor also belongs to seven transmembrane GPCRs. AT2R is generally expressed in low levels in a variety of organs such as heart, brain, kidney, pancreas and skin [28–34]. Similar to AT1R, AT2R expression is also enhanced in various pathological conditions including myocardial infarction, type 2 diabetes, chronic heart failure and inflammation. AT2R mediates its signal transduction by interacting with Gq/11 and Gi/o. However, the downstream signal cascades are different from those induced by AT1R. So far, three AT2R-mediated signalling pathways have been identified. The first molecular event is the activation of Src homology-2 domain-containing Tyr phosphatase-1 (SHP-1), protein phosphatase 2A (PP2A), and mitogen-activated protein (MAP) kinase phosphatase (MKP)-I with a subsequent inactivation of MAPKs including ERK1/2, JNK and p38 (Fig. 1.1). The inactivation of MAPKs is important in cell growth, proliferation and apoptosis [24]. The second signal transduction cascade is cyclic guanine 3′,5′-monophosphate (cGMP)/nitric oxide (NO) pathway [24–26]. Numerous studies have suggested that NO and its downstream effector cGMP are critical for several physiological process such as vasodilation, cell growth, apoptosis, and inflammation. AT2R activation promotes NO generation by facilitating the expression of endothelial nitric oxide synthase (eNOS). The accumulation of NO initiates the activation of cGMP, and induction of vasodilation, cell migration and proliferation. The last signaling pathway is stimulation of phospholipase A2 (PLA2) and release of arachidonic acid (AA). The enhanced PLA2 activity and AA release activate lipid signaling pathways [24, 35].

The role of AT2R in the development of blood pressure is controversial. Generally, AT2R regulates blood pressure by negatively controlling AT1R's effects. A variety of hypertension models, including renal hypertension, genetic modification hypertension and L-NAME-induced hypertension, have been used to identify the role of AT2R in the regulation of blood pressure [36–39]. AT2R-mediated blood pressure-lowering effect has been demonstrated in a hypertension model, renal wrap hypertensive rats [40]. Additional observations have been made in the normotensive Sprague–Dawley rats [41]. A long-term study suggested that AT2R ligand CGP42112A alone had only a mild blood pressure-lowering and vasodilatory effect in the conscious rats which could be enhanced by combination with Ang II receptor blockers [41, 42]. Two

pharmacologic studies showed that AT2R blocker PD123319 amplified the blood pressure increase in response to Ang II in both brain and uterine arteries [43, 44]. More definitive evidence for the role of AT2R activation in blood pressure was shown in the AT2R-deficient mice. The AT2R-deficient mice had the elevated basal blood pressure, sustained hypertension and sodium excretion. These mice also displayed an enhanced vasopressor response upon Ang II administration [45]. In addition, long-term administration of Ang II to the AT2R overexpressing mice totally eliminated the AT1R-mediated pressor effect [46]. AT2R opposes the pressor actions of Ang II by regulating the generation of cGMP and NO in various tissues including mesenteric, renal, coronary, cerebral and uterine vascular beds.

Dyslipidemia is observed in a large proportion of hypertensive patients that leads to high levels of oxidized low-density lipoprotein (ox-LDL) [47, 48]. LOX-1, is a major single type II transmembrane receptor for binding and uptake of ox-LDL in various tissues and cell types including VSMCs, endothelial cells, monocytes, macrophages, cardiomyocytes and platelets [49–51]. The expression of LOX-1 is extremely low under physiological conditions but is upregulated in a series of pathological states, for example, hyperlipidemia, diabetic nephropathy, hypertension, atherosclerosis, myocardial infarction and chronic renal failure [52–59].

LOX-1 belongs to class E scavenger receptor with a C-type lectin like domain and totally contains four functional domains: a short N-terminal cytoplasmic domain, a connecting neck region, a single transmembrane domain and the extracellular C-terminal ligand binding domain which is the functional domain for recognizing its stimuli.

The elevated level of soluble form of LOX-1 (sLOX-1) in blood has been proposed as a biomarker for several cardiovascular diseases including acute coronary syndrome. sLOX-1 is derived from shortening of the full-length LOX-1 through proteolytic cleavage [60, 61]. Increased sLOX-1 levels have been shown in hypertension, and sLOX-1 levels have been thought of as a diagnostic predictor for early endothelial damage in hypertension [62]. sLOX-1 level also acts as a biomarker for plaque instability in patients with acute coronary syndrome and sLOX-1 concentration is higher in the coronary circulation compared to the systemic circulation [63, 64]. sLOX-1 levels are also much higher in early disease onset of systemic lupus erythematosus patients, indicating that sLOX-1 may be a diagnostic marker in SLE patients with high cardiovascular risk. The elevated sLOX-1 levels correlate with the upregulated levels of IL-8, high-density lipoprotein, ox-LDL and high-sensitivity C-reactive protein as well as reduced IFN-γ. Membrane LOX-1 expression is significantly increased in CD14+ and CD16+ monocytes in patients with systemic lupus erythematosus. Ox-LDL enhances the susceptibility of monocytes to DNA-IC stimulation. Monocytes challenged with both ox-LDL and DNA-immune complex (DNA-IC) result in TNF-α, IL-1β and IL-6 release. Polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) are important immune regulators facilitating progression of tumor or different autoimmune diseases [60–62]. LOX-1 expression is increased in low density granulocytes (LDGs) from patients with systemic lupus erythematosus. These LDGs have similar phenotype as MDSCs.

Ox-LDL-LOX-1 signaling facilitates the generation of neutrophil extracellular traps (NETs) in LDGs, promoting endothelial injury and inflammatory responses [65].

After binding of ox-LDL to LOX-1, the complex internalizes to the cytoplasmic compartments and elicits its intracellular signaling, PKC activation, which further induces the subsequent signals including MAPKs (p38, p42/44, ERK, JNK), nuclear factor-kappaB (NF-κB), p21-activated kinase (PAK), nuclear translocation of the transcription factor (Nrf2) and activating protein-1 (AP-1). The activation of PI3K/Akt, and protein tyrosine kinase (PTK), AT1R, sirtuin-1 (SIRT1) and NADPH oxidase are also observed [66–77]. The NF-κB-mediated ROS generation is prompted by the induction of NADPH oxidase 2 (Nox2) and Nox4 which are the main regulators in vessel walls [78]. Ox-LDL increases the activity of epithelial sodium channel (ENaC) in endothelial cells through LOX-1-NADPH oxidase-ROS production. The activation of ENaC can be inhibited by the increased NO. Since LOX-1-PI3K/Akt-mediated decrease of eNOS-NO production was also been determined, blocking the activity of ENaC and increasing NO production may protect the ox-LDL-LOX-1-caused endothelial dysfunction [79]. It has also been shown that rapamycin downregulates phosphorylation of its mechanistic target mammalian target of rapamycin (mTOR) and downstream NF-κB activation, resulting in an attenuated LOX-1 expression and, ox-LDL uptake in endothelial cells. This finding suggests an important role of mTOR in ox-LDL-mediated LOX-1 expression [80]. In addition, the activation of PKC-CD40/CD40L and NADPH oxidase-ROS signaling pathways leads to an upregulated inflammatory response and LOX-1 expression, facilitating a vicious effect in LOX-1 activation [71]. A study confirmed the positive function of ROS formation in the process of atherosclerosis [60]. NF-κB activation is also pivotal for the expression of adhesion molecules and chemokines [75, 76]. Also, induction of PKC, p-p38MAPK, p42/44 MAPK, p-JNK, NF-κB, AP-1and Nrf2 upregulates LOX-1 expression in human VSMCs and macrophages and amplifies LOX-1-mediated cellular responses [70, 72], suggesting a positive feedback loop between above-mentioned signaling activators and LOX-1 expression.

There is evidence that the inhibition of LOX-1 suppresses ox-LDL- or TNF-α-mediated NOD-like receptor pyrin domain containing (NLRP3) inflammasome activation in endothelial cells [81]. In addition, LOX-1 mediates NLRP3 activation through the upregulated ROS generation in SMCs and monocytes treated with xanthine oxidase [33]. Clinical data suggests that electronegative LDL cholesterol (L5-LDL) is significantly increased in patients with ST-segment elevation myocardial infarction. In these studies, L5-LDL induced the activation of caspase-1, NF-κB and IL-1β, which was impaired by the downregulation of NLRP3 or LOX-1 in human macrophages. Furthermore, blocking LOX-1 with a specific antibody suppressed IL-1β generation, indicating the critical role of LOX-1 in the activation of NLRP3 inflammasomes [82].

LOX-1 has also been demonstrated to be involved in numerous physiological and pathological events, including inflammation, cell proliferation, differentiation, apoptosis, autophagy, the enhanced production of inducible nitric oxide synthase (iNOS), MCP-1, MMP1, ROS, adhesion molecules and proinflammatory cytokines, as well as the downregulated secretion of endothelial nitric oxide synthase (eNOS) and NO.

LOX-1 activation is also critical in the pathogenesis of the CVD-related diseases such as atherosclerosis, ischemic stroke, acute coronary syndrome, chronic renal failure, myocardial infarction, hypertension, obesity, hyperlipidemia, diabetic nephropathy, and arthritis [59, 66, 70, 83–89]. In mice with sustained hypertension, loss of LOX-1 dramatically decreased the cardiac fibroblast number and expression of fibronectin and procoallagen-1/collagen in the hearts, indicating the essential role of LOX-1 in cytoskeletal organization and growth of cardiac fibroblasts [90]. Recent studies showed that GATA Binding Protein 4 (GATA4) was essential in LOX-1-mediated proliferation of cardiac fibroblasts. Knockdown of LOX-1 inhibits PI3K/Akt activation, GATA4 expression and cardiac fibroblast proliferation and this effect can be restored by overexpressed LOX-1, indicating the important role in the LOX-1-PI3K/Akt-GATA4-induced proliferation of cardiac fibroblasts which contributes to cardiac remodeling [91].

Innate and Adaptive Immune Responses in Hypertension

Numerous studies have indicated that the immune system is involved in the development and maintenance of hypertension. Also, different immune cells including adaptive immune cells such as CD8+ T cells, CD4+ T cells, and B cells as well innate immune cells such as macrophages, monocytes, dendritic cells (DCs) also play a critical role in the pathogenesis of hypertension and the hypertension-induced diseases (Fig. 1.2). The immune system is composed of two subtypes, the innate and adaptive immunity. The innate immune system recognizes pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) by the pathogen recognition receptors (PRRs) (Fig. 1.2). Toll-like receptors (TLRs), the most important PRR expressed on various innate immune cells including neutrophils, monocytes, macrophages, and DCs, are activated by DAMPs from the damaged tissues under the stress condition in the context of hypertension. TLRs were identified in human. TLR2, 4 and 5 are expressed on the cell surface while TLR3, 7 and 9 are located in the intracellular compartments, such as endosome, endolysosome and endoplasmic reticulum (ER). The TLRs utilize different adaptor proteins, myeloid differentiation primary response gene 88 (MyD88), TIR-domain–containing adaptor/MyD88 adaptor–like (TIRAP/Mal), TIR-domain–containing adaptor molecule 1/TIR-domain–containing adaptor-inducing interferon β (TICAM1/TRIF), and TRIF-related adaptor molecule (TRAM) to induce two distinct downstream signalings. Upon binding to its stimulator, TLR2,5,7 and 9 induce MyD88 dependent signaling and TLR3 activates TRIF dependent signaling. In addition, TLR4 is the only TLR that can induce both MyD88 and TRIF signaling pathways. Several groups have shown an enhanced TLR4 expression in different tissues and cells including heart and VSMCs from hypertensive animal models [92–95]. Inhibition of TLR4 by its specific antibody downregulates expression of TLR4 and its downstream MyD88-mediated NF-κB and proinflammatory cytokine signaling pathway, resulting in the decrease of blood pressure in hypertensive animal

models [96]. TLR9 can recognize circulating mitochondrial DNA (mtDNA) that is associated with vascular dysfunction in spontaneously hypertensive rats [97]. In the hypertensive environment, the activation of TLRs by diseases-derived molecules significantly trigger the production of pro-inflammatory cytokines, chemokines, and the generation of ROS, leading to a low-grade inflammation [98] (Fig. 1.2). These pathological inflammatory responses including oxidative stress, vascular injury and remodeling as well as multiple organ damages reveal an association between TLRs and the hypertension-related diseases. Circulating monocytes and tissue resident macrophages also play a key role in the pathogenesis of hypertension. The levels of TNFα and IL-1β are elevated in monocytes from hypertensive patients. The release of TNFα, IL-1β and ROS by macrophages increases the blood pressure by suppressing vasculature endothelial and smooth muscle function with consequent vasoconstriction. The hypertension induced by impaired renal sodium excretion also attributes to higher levels of TNFα, IL-1β and ROS-mediated endothelial cell dysfunction [99].

Furthermore, antigen-presenting cells (APC) can uptake these DAMPs and prime T cells by major histocompatibility complex II (MHC II) dependent antigen presentation pathway, initiating the activation of T and B lymphocytes [100, 101]. The accumulation of memory T cells have been observed in hypertensive patients [102]; this phenomenon has also been identified in animal models [103]. In spontaneously

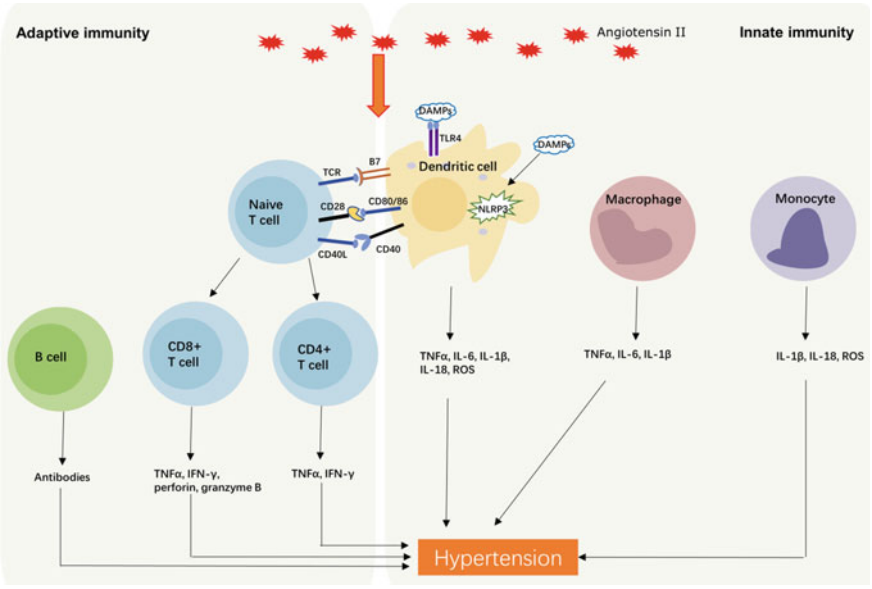

Fig. 1.2 The key role of innate and adaptive immunity in hypertension. Adaptive immune cells including CD8+ T cells, CD4+ T cells, and B cells as well innate immune cells including macrophages, monocytes, dendritic cells (DCs), release cytokines, ROS and antibodies promote the progress of hypertension and hypertension-induced diseases. The NLRP3 inflammasome activation in monocytes and DCs is critical in hypertension

hypertensive rats, reduced numbers of T cells, especially non-helper T cells population, were identified in the thymus [104]. Restoring thymic function inhibited the development of hypertension [105–107]. In RAG1−/− mice that has deficiency in both T and B cell development, Ang II-induced hypertension is impaired and the vascular injury is also suppressed. In another T and B cell deficiency model, SCID (severe combined immunodeficiency) mouse strain, dramatically decreased heart and kidney injury was determined following Ang II challenge [105]. In hypertensive patients, percentage of pro-inflammatory and cytotoxic CD8+ cells were increased together with upregulated production of perforin, granzyme B, interferon-γ (IFN-γ) as well as TNF-α (Fig. 1.2).

The Interplay of LOX-1 and RAS System in Hypertension

There is increasing evidence that the crosstalk between LOX-1 and AT1R plays a key role in pathogenesis of hypertension and hypertension-related diseases. The positive effect of Ang II in the expression of LOX-1 and consequently uptake of ox-LDL has been determined in several in vitro studies [108–112]. This dose-dependent Ang II-mediated response has been determined to be totally inhibited by the AT1R blocker losartan, but not by the AT2R blocker PD123319 [68, 112]. In in vivo studies, losartan showed its capability of inhibiting the elevated expression of LOX-1 in endothelium and neointima of autologous vein grafts and preventing the development of atherosclerosis in vein grafts [54, 57]. Another clinical investigation showed a similar effect of Ang II in LOX-1 expression under the stress of obstructive sleep apnea [113]. High concentration of LDL-cholesterol in hypercholesterolemic state regulates AT1R expression. Synergistic effect between LDL-cholesterol and Ang II in enhanced AT1R levels was observed in the cultured SMCs derived from rat aortas [113]. This effect was shown to be modulated by ox-LDL rather than native-LDL in the cultured SMCs [114]. Thus, ox-LDL mainly acts as a secondary messenger in the increased AT1R expression of hyperlipidemia. Subsequent in vivo experiments showed that AT1R expression was predominantly increased in smooth muscle layers of aortic intima derived from animal models with hypercholesterolemia. Several-fold upregulation of AT1R was shown in the aortic intima of hypercholesterolemic rabbits [115]. Further, the AT1R expression in a myocardial infarction model was shown to be potentiated by high serum cholesterol and reversed to baseline level by atorvastatin [116].

The transmembrane and cytoplasmic domains of LOX-1 are required for the proximity between LOX-1 on cell-surface membranes. Interaction of ox-LDL to LOX-1 induces AT1-dependent signaling pathway by interactions between the intracellular domain of LOX-1 and AT1R. This signaling event in human vascular endothelial cells is attenuated by either inhibiting the expression of AT1R or use of AT1R blocker, leading to relaxation of vascular rings from mouse thoracic aorta. Ox-LDL significantly increases cytosolic G protein which can be inhibited by AT1R blockade [117].

Azilsartan, a potent AT1R antagonist, was shown to inhibit ox-LDL-induced upregulation of LOX-1, MCP-1, and CXCL-1 as well as the reduced production eNOS and NO. In addition, azilsartan blocked the reduced the expression of occludin. This AT1R inhibitor also reversed the enhanced effect of ox-LDL in endothelial monolayer permeability. Further, azilsartan inhibited ox-LDL-mediated activation of Krüppel-like factor 2 (KLF2) which is also required for the activation of Occluding [118]. This study showed the role of AT1R blockade in repair of damaged tight junction.

Loss of LOX-1 significantly decreases Ang II-induced hypertension in the aged mice. LOX-1 deficiency leads to impaired fibrosis and attenuated fibronectin and collagen-3 expression, as well as ROS production rather than the expression of collagen-1 and collagen-4 in the hearts of aged mice. This effect was further determined in the aged mice with sustained hypertension by long-term Ang II infusion [119]. Pyrogallol-phloroglucinol-6,6-bieckol, a derivative of Ecklonia cava (E. cava), dramatically inhibits the excessive expression of adhesion molecule expression, and VSMCs proliferation and migration, and suppresses the increase of blood pressure, lipoprotein and cholesterol. This compound has also been shown to exert an inhibitory effect on high-fat diet or ox-LDL induced activation of LOX-1-PKC-α signaling pathway (Fig. 1.3). This compound reversed the increased expression of mesenchymal cell markers (α-SMA and vimentin) and reduction of endothelial cell markers (PECAM-1 and vWF) in the aorta or endothelial cells. Moreover, this compound reduced intima-media thickness as well as high fat diet-induced hypertension [120].

Stroke-prone spontaneously hypertensive rat is an animal model of severe hypertension and spontaneous stroke. Liang et al. showed that LOX-1 depletion dramatically slowed the development of hypertension-associated cerebral ischemic injury and brain damage, suggesting a potential role of LOX-1 in blood-brain barrier disruption after cerebral ischemia [121]. Grell et al. showed that the expression of LOX-1 to be significantly upregulated in the middle cerebral arteries from spontaneously hypertensive rats compared to wild-type controls, indicating a link between LOX-1 and hypertension-mediated vascular changes and organ injury [122]. LOX-1 deficiency leads to attenuation of angiogenesis in mice infused with Ang II. Lastly, LOX-1 depletion decreases the expression of pro-inflammatory MCP-1 and IL-1β in the hypertensive mice hearts. These observations suggest that LOX-1 is important in Ang II-mediated cardiac angiogenesis in hypertension and its association with inflammation [123].

Several studies have indicated that ROS might be the common downstream mediators in the crosstalk between LOX-1 and AT1R, and ROS generation is NADPH oxidase dependent [124–126]. Activation of AT1R regulates the generation of oxidative stress and this process can be inhibited by AT1R antagonist losartan or the NADPH oxidase (NOX) inhibitor DPI [124, 127]. The increase of LOX-1 expression leads to ROS generation and AT1R expression (and activity) via Akt/eNOS and Ca^{2+} signaling pathways [126]. Ox-LDL and Ang II combination induces capillary tube formation in a ROS dependent manner which is attenuated by NADPH oxidase inhibitor apocynin [38, 190]. The suppression of the NADPH oxidase activity also

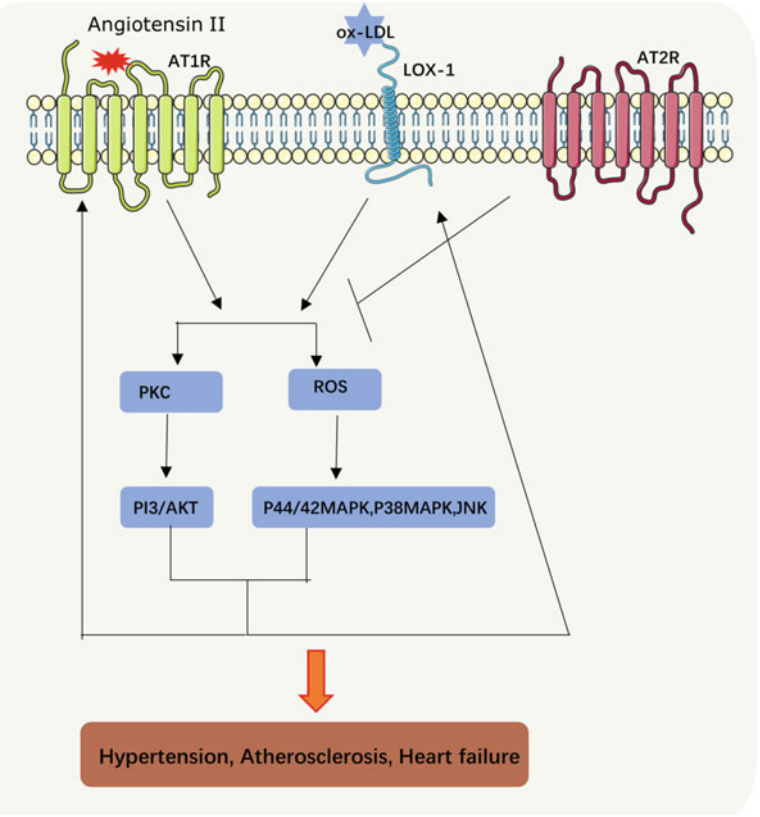

Fig. 1.3 The positive feedback loop of interplay between LOX-1 and AT1R is PKC-PI3K and ROS-MAPKs-dependent. The AT2R in this crosstalk between LOX-1 and AT1R inhibits ROS-MAPKs signaling pathways

impedes the expression of AT1R and LOX-1 [128, 129]. Other intracellular molecular factors including angiotensin-converting enzyme 2 (ACE2) and MAPKs, are also involved in the crosstalk between AT1R and LOX-1 (Fig. 1.3). The increased ACE2 expression and activity in HUVECs and in abdominal aorta lead to the downregulated expression of LOX-1 and AT1R simultaneously. The ACE2 via ROS generation plays a regulatory role in the expression of LOX-1 and AT1R [130, 131]. The ROS generation subsequently induces the activation of ROS-PKC-MAPKs signaling pathway which is critical in the crosstalk of LOX-1 and AT1R [16, 66, 124, 132] (Fig. 1.3). The synergistic function of LOX-1 and AT1R has been demonstrated in the pathogenesis and development of different cardiovascular diseases. For example, several investigators have shown that these two receptors coordinate in the formation of foam cells and progression of atherosclerotic lesions. The increased expression and activity of LOX-1 and AT1R serve as key biomarkers of early atherogenesis. In high cholesterol diet fed animal models, simultaneous activation of AT1R and LOX-1

promotes atherogenesis by activation of ROS generation and vascular inflammation [115, 133, 134].

It has been shown that right ventricular systolic pressure is increased in LOX-1 transgenic mice with the hypoxic-pulmonary hypertension. In a rat model of hypoxic-pulmonary hypertension, expression of LOX-1 was noted to be increased which was responsible for pulmonary vascular remodeling. Knocking down or blocking LOX-1 dramatically decreased pulmonary arterial SMCs dedifferentiation via inhibiting ERK1/2 dependent signaling pathway, suggesting a key role of LOX-1 in the maintenance of pulmonary arterial SMCs phenotype [135, 136]. Furthermore, a recent study showed that LOX-1-NOX-ROS pathway plays a critical role in hypoxic-pulmonary hypertension induced right ventricular hypertrophy and cardiac fibrosis in rats [137].

The interplay between LOX-1 and AT2R has not been determined in detail. Recent studies showed that Ang II-LOX1 interplay might be critical in a variety of diseases including hyperlipidemia, atherosclerosis, hypertension, myocardial infarction, ischemic stroke and inflammation. However, Watanabe et al. observed no interregulatory effect between LOX-1 and AT2R as the increased LOX-1 expression did not influence AT2R expression [138]. These studies suggest that AT1R inhibition, but not AT2R alteration, can block the increased expression of LOX-1 in the cultured endothelial cells [112]. These data may be attributed to the low expression of AT2R in physiologic states and a relatively modest increase in pathologic states.

On the other hand, LDLR deficiency results in significantly enhanced LOX-1 expression. Hu et al. in our laboratory showed that this effect could be partly reversed by AT2R overexpression utilizing recombinant adeno-associated virus type-2 (AAV) with AT2R cDNA (AAV/AT2R). The AT2R overexpression in the LDLR deficient mice also led to decreased atherogenesis in the aorta [109]. The authors of this study suggested that the increased AT2R expression might play a role in stress states, and play an inhibitory role in the pathogenesis of cardiovascular disease states.

Overall, AT2R may act as an anti-atherosclerotic modulator, while antagonizing LOX-1-mediated pro-atherogenic effects [139]. Thus, the imbalance between LOX-1 and AT2R may determine the severity of atherosclerosis. Of note, different subtypes of collagens have been characterized as key components of atherosclerotic plaques and collagen aggregation has been established as a hallmark of development of atherosclerotic plaques [140–143]. Dandapat et al. showed that AT2R overexpression may reduce the expression of collagens as well as MMPs in atherosclerotic plaques suggesting a new mechanism of decreased atherogenesis in the aorta [144]. This is important since LOX-1 facilitates collagen aggregation [77, 145]. Loss of LOX-1 suppresses collagen aggregation and increases the expression and activity of MMP2 and MMP9 in atherosclerotic plaques. LOX-1 deficiency also inhibits components of NADPH oxidase expression and ROS production in the LDLR deficient mice. There is also evidence that AT2R activation results in an attenuated ROS generation via enhanced NO levels [146, 147].

The progression of atherosclerosis has been linked to a continuing inflammatory response [148]. Ox-LDL-LOX-1-mediated downstream immune responses have been shown to be essential in the pathogenesis of atherosclerotic lesions [196]. AT2R activation suppresses inflammatory responses during atherogenesis [83, 149].

Thus, the interplay of these two receptors in vascular inflammation might be an important element in the formation of atherosclerotic plaques. LOX-1 and AT2R interaction might be involved in other pathological process including the increased hypertension and fibrosis [150–152]. Though significant progress has been obtained in the crosstalk of LOX-1 and AT2R in collagen formation and development of atherosclerotic lesions, further work needs to be done in this realm.

In summary, LOX-1 has been termed as a potent pro-inflammatory mediator in Ang II-mediated hypertension via AT1R and AT2R. Further investigations in immune and inflammatory responses mediated by RAAS system and LOX-1 interaction might provide new insight and potential therapeutic targets in hypertension and its related diseases.

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Chapter 2
Renin Angiotensin System and Thyroid Hormone Crosstalk: From Experimental Approaches to Clinical Perspectives

Laura Sabatino, Dario Genovesi, and Cristina Vassalle

Abstract The renin angiotensin system (RAS) plays a central role in the maintenance of regular cardiovascular functions and is involved in several cardiovascular disease. In addition to the circulating RAS, the existence of independently acting local RAS has been demonstrated by biochemical and functional data. At cardiovascular level, classical RAS components seem to be involved in fine-tuned events driving to the settlement of pathological processes, such as cardiac hypertrophy, coronary artery disease and atherosclerosis. In the last decades, in addition to the classical arm of local RAS, new important factors have been described, belonging to the so-called "protective arm" of RAS, with beneficial effects in the settings of cardiovascular diseases. A close relationship has been described between RAS and TH functions, in particular, in the events associated to cardiac hypertrophy induction by TH metabolic derangements. In the present review, main aspects and principal mediators of RAS-TH crosstalk at cardiovascular level have been evaluated.

Keywords Renin angiotensin system · Renin · ACE · Ang II · ACE 2 · Ang (1–7) · AT1R · Mas · Thyroid hormones

Introduction

The Renin Angiotensin System (RAS) is a fundamental controller of cardiovascular functions and is involved in a multitude of cardiovascular diseases, which make RAS an important field of investigation in cardiovascular research. RAS is well known for its role in physiological regulation of blood pressure, extracellular volume and cardiovascular control of neuro-endocrine functions. Chronic hypertension causes mechanical stress, with endothelium, heart, and kidneys as the main organs damaged under

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this condition. In addition, hypertension-induced oxidative stress, chronic inflammation, reparative mechanisms activation lead to end-organ damage. In this context, RAS has been also identified as a critical factor in the pathogenesis of atherosclerosis and it has been associated with inflammatory biomarkers (e.g. COX-2), oxidative stress (e.g., activation of the NADH/NADPH oxidase pathway), increased production of different cytokines (e.g. IL-6, TNF-a) and the recruitment of inflammatory cells to the injured site [1]. In particular, Angiotensin II (Ang II), one of RAS main components, participates in all stages of plaque generation and development, contributing to NO reduction, release of metalloproteinases and other components of the extracellular matrix, and inducing a pro-coagulant milieu (e.g., downregulating the production of tissue plasminogen activator-tPA, acting through bradykinin degradation) [2]. Moreover, oxidized LDL-induced macrophage activation has the potential to locally enhance the angiotensin-converting-enzyme (ACE), which activates RAS, inducing progression of the plaque to a phenotype more vulnerable to rupture and thrombosis [3]. Shortly after myocardial infarction, there is an elevation in Ang II levels, followed by an accumulation, differentiation and stimulation of hematopoietic stem cells to supply the infarcted area of the immune cells.

Cardiomyocyte death promotes the release of damage-associated molecular patterns (DAMPs), cytokines, chemokines, and adhesion molecules through the activation of innate immune pathways, facilitating the recruitment and infiltration of leukocytes (especially monocytes) into the infarcted zone. This fact, although essential for cardiac repair, may also contribute to adverse remodeling and to the onset of heart failure. Monocytes release pro-inflammatory cytokines through the binding of Ang II to type 1 angiotensin-II receptor (AT1R), which induces the phosphorylation of nuclear factor-kappa B (NF-kB) and a pro-inflammatory status mediated by tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL1β) [2]. Moreover, also a RAS-modulated fibroblast activation is elicited in this phase as a part of a repair process [4]. However, once become chronic, this response induces a ventricular remodeling, characterized by progressive hypertrophy of myocytes and interstitial fibrosis, leading to apoptosis, gradual loss of myocytes, and intensified inflammatory events, ultimately resulting in heart failure [5]. The sodium and water retention, together with the vasoconstriction induced by activation of RAS and the sympathetic nervous system (SNS), increases ventricular preload, afterload and wall stress, with the release of natriuretic peptides (NPs), which oppose to RAS and SNS actions that occur in heart failure [6]. In summary, RAS can represent a possible promising target for hypertension, atherosclerosis, and cardiovascular diseases, for which current RAS blocker drugs or new agents may provide effectiveness and further benefits [2].

Even though RAS was initially considered exclusively a circulating system, the finding of RAS components in a broad variety of tissues revealed the existence of a "local RAS" with a paracrine/autocrine function and involved in cell survival, differentiation and inflammation [7]. In particular, local RAS activation in the heart is considered an important modulator of cardiac phenotype and its functional characteristics may be complementary or independent of those served by endocrine RAS.

In the years, several large clinical trials have been conducted to evaluate the effects of inhibition of RAS cascade on post-infarction left ventricular dysfunction and the possible amelioration of the prognosis of failing heart [8, 9]. For all these considerations, during the time, we assisted to an increasing interest towards pharmacological approaches targeting RAS, in order to have a better control on RAS-associated diseases.

Main Components of "Classical" Arm of RAS

Classically, RAS main components are angiotensinogen (AGT), renin, angiotensin converting enzyme (ACE), Ang II and its two receptors (AT1R and AT2R) (Fig. 2.1).

Angiotensinogen

AGT is the unique precursor of all angiotensin peptides and is itself a blood pressure regulator, as observed in many animal models [10, 11]. It is a non-inhibitory serpin [12] and is a first rate-limiting step in the RAS function since the cleavage of N-terminus by the renin releases the decapeptide Ang I, which, in turn, is processed into other bioactive peptides, among which Ang II is the most investigated [13, 14]. AGT is present in the plasma at relatively high concentrations (0.8 M) however, its principal activity is at cellular level, exerting a direct role in the blood pressure control [14, 15]. After its interaction with renin, a complex AGT conformational change occurs, which is itself a driving force for the modulation of AGT release [16]. At molecular level, the highly conserved disulfide (S–S) bridge between Cys 18-Cys 138, which links the N-terminus of AGT molecules is known to have an important functional role [17]. The oxidation of S–S bridge alters the balance between reduced-to-oxidized ratio, resulting in a fourfold increase in the Ang I release, which is sufficient to cause a hypertensive response [18].

Renin

Renin is an aspartyl protease released in the plasma mainly by juxtaglomerular cells. On release in the circulation, renin cleaves AGT at the N-terminus, forming the decapeptide Ang I [19]. Therefore, renin activity controls the levels of Ang I, obtained by circulating AGT storage (which is about 1000 times more concentrated than Ang I and II). The fact that the renin is the rate-limiting enzyme of the RAS makes this factor particularly interesting for pharmacological research of specific inhibitors of the renin-angiotensin cascade in cardiovascular diseases. Despite the promising premises, renin inhibitors so far developed did not show higher efficacy with respect to

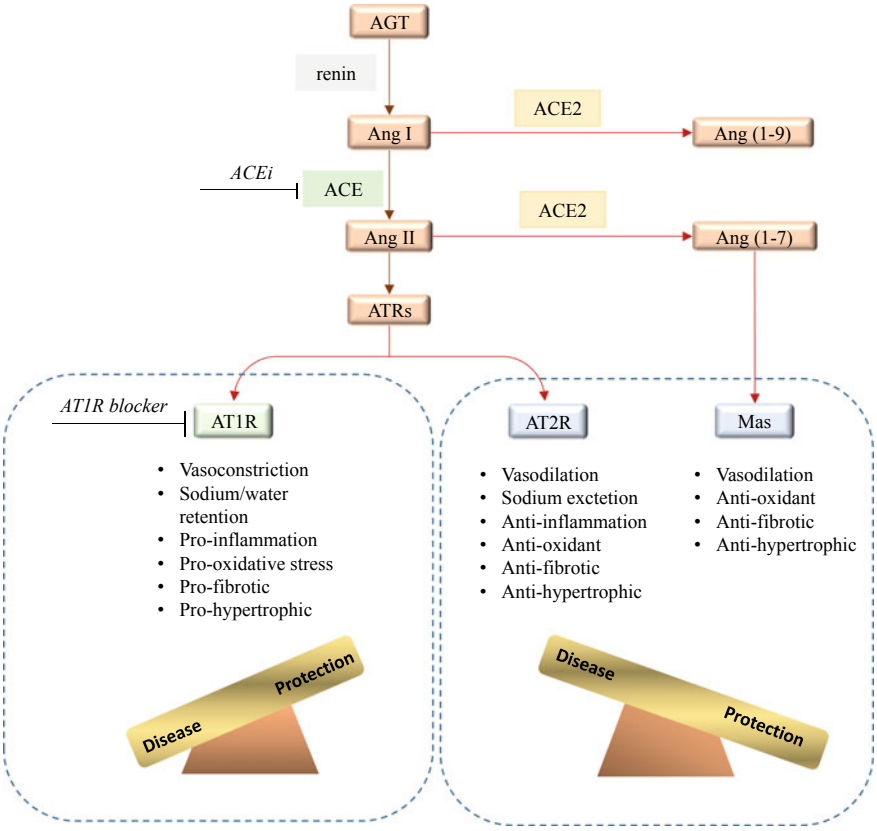

Fig. 2.1 Enzymatic cascade of renin angiotensin system, main factors and biological effects. Renin catalyzes the conversion of Angiotensinogen (AGT) into Angiotensin (Ang). Ang is cleaved by angiotensin-converting enzyme (ACE) to angiotensin II (Ang II), which can be metabolized in angiotensin (1–7) (Ang (1–7)) by angiotensin-converting enzyme 2 (ACE2). Ang can be cleaved by ACE 2 to obtain angiotensin (1–9) (Ang (1–9)). Ang II type I and II receptors are AT1R and AT2R and Ang (1–7) receptor is Mas receptor

better-established ACE inhibitors (ACEi) and angiotensin receptor blockers (ARBs), extensively used in anti-hypertensive treatment. Therefore, renin inhibitors often need a combination therapy in order to obtain a pharmacological acceptable effect [20].

Angiotensin-Converting-Enzyme

ACE converts Ang I into Ang II, a strong vasoconstrictor octapeptide. Differently from renin, ACE activity is not sensitive to variations of Ang II concentration and

its specific inhibitors (ACEi) are among the most used compounds for treatment of hypertension and cardiovascular dysfunctions, reducing the rates of death, myocardial infarction, stroke, revascularization, cardiac arrest, heart failure [21]. ACE has been described in a large spectrum of cell types, tissues and organs where the enzyme permits the local generation of Ang II [22]. In particular, ACE cardiac expression is increased in experimental heart failure and ACE inhibition attenuates cardiac dilation after coronary ligation, thus improving rats' survival and limiting progression of heart failure [23]. Furthermore, in pressure-overloaded rats, administration of low doses of ACEi (which did not interfere with blood pressure) prevented the onset of cardiac hypertrophy [24].

Angiotensin II

Ang II is the main effector of RAS and was initially characterized as a powerful vasorepressor, it is involved in electrolyte homeostasis, vascular muscle contraction, hypertension and coronary heart disease [19]. Successively, it was also considered an important myocardial inotropic modulator, becoming one of the most investigated regulatory factors in cardiovascular pathophysiology [25]. In the failing heart, the excessive exposure of myocardium to Ang II leads to an inotropic unbalancing, progressing to pathological remodeling and apoptosis. In this context, the identification of the molecular events of Ang II-mediated inotropic and apoptotic signaling may constitute a keystone for the development of new therapeutic strategies targeting heart failure [26].

As confirmed by in vivo and in vitro studies, Ang II acts through endocrine, paracrine and intracrine actions and promotes cardiomyocyte growth, fibroblast proliferation, growth factors' production [27, 28].

Administration of Ang II to primary cultured myocytes and fibroblasts induces hypertrophy of myocytes and hyperplasia of fibroblasts and the activation of the the so-called immediate-early genes (IE), such as c-*fos*, c-*jun* and *Egr-1* within a few minutes [28]. In particular, in neonatal rat cardiomyocytes, the AngII-induced c-*fos* gene expression requires activation of multiple phospholipid-derived second messenger system, more specifically, phospholipase C (PLC) and D (PLD) and protein kinase C (PKC) and subsequent release of Ca^{2+} from cellular storage [29]. Furthermore, Ang II induces the "fetal program" with the induction of skeletal-actin and atrial natriuretic factor (ANF) and stimulates AGT expression, probably for a feedback regulation of cardiac growth, and TGF-β1 gene, which is known to be the main inducer of fetal gene expression in cardiomyocytes and of synthesis in extracellular matrix [30].

Ang II Receptors: AT1R and AT2R

The effects of Ang II on cardiac tissue are associated with the activation of specific Ang II receptors, AT1R and AT2R [27]. Whereas cardiac AT1R gene expression is relatively unchanged in the passage from fetal to newborn life, it has observed that AT2R gene expression is particularly high during fetal growth and decreases immediately after birth [27]. AT1R is the more abundant of the two receptors to which are attributed the main physiological and pathological conditions associated with Ang II action. Differently, AT2R is more diffusely expressed in adult tissues and its relevance often moves to the background because of AT1R predominance, therefore, AT2R effects are not evident in experimental contexts, unless AT1R is blocked and AT2R effects unmasked [31]. Ang II receptors are 7-transmembrane domain receptors in the plasma membrane and their activation is coupled to a cascade of intracellular proteins, starting with a G protein [32]. Upon interaction with its ligand, AT1R-Ang II complex is rapidly translocated inside the cell by endosomal vesicles and Ang II, once performed its specific intracellular functions, is degraded [33]. Differently, AT2R is not internalized in the cell and counteracts AT1R-mediated effects on myocardium [33], therefore, the action of both AT1R and AT2R is of central interest, particularly to cardiac pathology. For example, in infarcted rats, it was observed that the beneficial effects of AT1R antagonist on heart function and on hypertrophy reduction are strongly repressed by simultaneous administration of AT2R antagonist [34]. Similarly, AT2R blockade induces early signals of AT1R-mediated myocardial growth in hypertrophied heart in rats, supporting the counteracting role of AT2R versus AT1R [35].

Main Components of "Protective" Arm of RAS

In the last decades, new components of RAS have been described as part of the so-called "protective" arm of RAS and include as main components the enzyme ACE2 (homologous of ACE) and the two products Ang (1–7) (from Ang II) and Ang (1–9) (from AGT) (Fig. 2.1) [36, 37]. Furthermore, the G-protein-coupled receptor Mas was identified as the Ang (1–7) functional receptor [38]. In cardiomyocytes, the administration of Ang (1–7) induces a consistent release of NO, providing direct evidence that endothelial NO synthase (eNOS) and phosphatidylinositol-3-kinase (PI3K)/Akt are downstream mediators for Ang (1–7) signaling [39]. Furthermore, in $Mas^{-/-}$ cardiomyocytes, it was observed a complete absence of Ang (1–7)-mediated NO production, suggesting an important link between Mas receptor and eNOS enzymatic complex in these cell type [40]. Another relevant evidence reported in $Mas^{-/-}$ cardiomyocytes was the dysfunction associated with Ca^{2+} signaling, indicating the important role of Mas in long-term Ca^{2+} handling in cardiac cells [41].

Thyroid Hormone and Renin Angiotensin Systems Crosstalk

Thyroid hormones (THs), tetraiodothyronine (T4) and triiodothyronine (T3) are produced in the thyroid gland and released in the blood stream where, transported almost entirely by specific binding proteins, reach the target organs. T3 is considered the biologically active form of THs and derives from outer ring monodeiodination of T4 molecule, both in thyroid gland and peripheral tissues by specific enzymes called deiodinases [42]. So far, three deiodinases have been described and called D1, D2 and D3, which differ in their catalytic activity and tissue distribution, allowing a fine regulation of TH homeostasis in target cells and promoting the activation (D1 and D2) or inactivation (D3) of THs [43–46].

THs exert multiple effects on the heart and vascular system, where they increase cardiac contractility and arterial relaxation and reduce vascular resistance. TH activity in the cell may act by genomic or non-genomic activities. T3 can act through specific nuclear TH receptors (TRs) that recognize and bind TH response elements (TREs) in the promoter of target genes, directly regulating their expression [47, 48]. Differently, non-genomic effects are more rapid and require the involvement of several intracellular signaling pathways in cardiomyocytes and vascular cells [49].

In addition to genomic and non-genomic actions, several studies have investigated the less debated issue about the role of RAS as important mediator of TH effects on heart and cardiovascular system [50]. Furthermore, the crosstalk between the two systems is considered bidirectional since several in vivo and in vitro studies showed the importance of THs in regulation of expression and function of RAS elements both at circulating and local levels [51].

The interaction between RAS and TH system is particularly evident in thyroidal pathological contexts [52]. Patients with hyperthyroidism show an increased risk of cardiac disease and the activation of RAS is, at least in part, involved in the causal progression of these conditions, in particular of cardiac hypertrophy [53]. In experimental models of hyperthyroidism, the treatment with ACEi or ARBs prevents the cardiac pathological hypertrophy induced by THs [54] and, analogously, in cultured cardiomyocytes, AT1R silencing inhibits hypertrophic effects mediated by THs. Diniz et al. demonstrated a rapid T3-mediated increase of PI3K in cultured cardiomyocytes, and a possible downstream activation of the pathway Akt/GSK-3b/mTOR in the progression of hypertrophic signaling [55]. These experimental approaches permitted to highlight the existence of a dual modality of TH-induced cardiac hypertrophy by mediation of classical RAS: (1) by stimulating the increase of local Ang II concentration and (2) by promoting AT1R expression [55].

Further insights on the molecular mechanisms of T3-induced hypertrophy derived from recent studies on the involvement of some small RNAs (miRNAs) in the regulation of *AT1R* gene expression at cardiomyocyte level, after T3 treatment [56]. MiRNAs are implicated in many aspects of cellular physiological and pathological processes, they are short-non coding RNA molecules acting as post-transcriptional regulators of gene expression by affecting the degradation and translation of target mRNAs [57]. In particular, miR-208a and miR-208b, encoded within *α-MHC* and

β-MHC genes and important in controlling muscle myosin content, are believed to be under AT1R regulation in hyperthyroidism [58, 59]. More in detail, like *β-MHC*, miR-208b is down-regulated in hyperthyroid state whereas, miR-208a, like *α-MHC*, is up-regulated in high TH conditions. In vitro and in vivo experiments showed that AT1R mediates TH-induced cardiac miRNA208a/*α-MHC* up-regulation, whereas it is not clear if AT1R plays a role in down-regulation of miRNA208b/*β-MHC* [59].

In addition, beneficial effects of protective RAS component activation have been investigated and the Ang (1–7)/ACE2/Mas pathway involvement evaluated. Ang (1–7) cardioprotective role has been observed in several animal models and some studies have been performed in order to define a possible association with TH signaling [60].

The discovery of the new components of RAS permitted to develop a novel definition of RAS-mediated mechanisms regulating cardiac homeostasis in cardiovascular diseases [60]. In hyperthyroid state, local Ang (1–7) is increased in hypertrophic heart and prevents deleterious effects of Ang II activation, such as cardiac remodeling, hypertrophy and fibrosis [61, 62]. Cardioprotective effects of Ang (1–7) are generally mediated by Mas receptor, whose deletion provokes detrimental consequences on heart function and structure [63]. Moreover, in vitro and in vivo studies showed that FOXO3, a downstream target of Akt, is important in the counteraction of T3-induced cardiac hypertrophy and that FOXO3 is activated by Ang (1–7) via Mas receptor [64]. FOXO3 is a transcription factor that negatively regulates cardiac growth [65] and upregulates antioxidant genes (superoxide dismutase SOD1 and catalase) and redox-sensitive nuclear factor-kB, NF-kB [66].

The role of AT2R in cardiac functional profile is less clear than AT1R. However, in an ex vivo ischemia/reperfusion (I/R) rat model, it was observed that AT2R may be involved in the response to T3 precondition, improving post-ischemical recovery. AT2R cardioprotector activity requires NO production and Akt pathway mediation [67]. Recently, we evaluated the expression profile of main components of protective and detrimental RAS arms in an I/R rat model, after the infusion of low dose of T3 and observed that, at least in part, throughout gene expression regulation, T3 could be involved in the local cardiac ameliorative response to I/R procedure [68]. These data suggest that strategies targeting AT2R agonists can ameliorate cardiac function and suggest potential therapeutical strategies in heart disease.

THs and RAS interaction has been observed also at systemic vasculature level where the TH increase produces the reduction of systemic vascular resistance (SVR). In contrast, high SVR is observed in TH deficiency or hypothyroidism and is efficiently reversed with TH replacement. Vascular resistance is mainly due to the contractile conditions of smooth muscle cells in the vascular wall (VSMC) and vasoactive factors can act directly modifying the vascular tone or by indirect action, stimulating the release of vasoactive molecules by endothelial cells, such as NO by TRα1-mediated PI3K/Akt/eNOS pathway [69]. More recently, some studies evidences that NO production may occur in VSMCs, through the activation of PI3K/Akt signaling pathway and the involvement of iNOS, a Ca^{2+}-independent enzyme and that requires also the activation of NF-kB [70]. Fukuyama

et al. demonstrated that THs down-regulate AT1R both at transcriptional and post-transcriptional levels in VSMC, thus attenuating Ang II activity and promoting TH anti-atherosclerotic and vasorelaxing effects [71].

Thyroid Hormone and Renin Angiotensin Systems Interaction in Some Organ Diseases

In the present review, the principal interaction of RAS with THs at cardiovascular level have been explored and summarized. However, it is important to take into consideration the possibility that the two systems may functionally interact also in body contexts other than the cardiovascular system.

As an example, thyroid dysfunctions induce important changes in different brain compartments, interfering with their general secretory activities [72] and hypo/hyperthyroidism may directly or indirectly affect the different components of RAS system and, consequently, RAS paracrine and endocrine regulatory activities.

In the liver, THs stimulate renin production, contributing to the increase in plasma renin activity (PRA) [73]. Furthermore, both in hyper and hypothyroid rats, a significant decrease of plasma AGT concentration (PAC) was found, whereas liver AGT content (LAC) showed a significant increase in hyperthyroidism and a marked decrease in hypothyroidism. As PAC is regulated by AGT production by liver and by plasma degradation by renin, the decrease in PAC observed in hyperthyroidism could be due to an increase in PRA, which would overcome the increased synthesis of liver AGT observed in these animals [74].

Several studies indicate that RAS components are expressed in bone and that their action stimulates osteoclast formation and inhibits osteoblast activity, thus inducing increased bone turnover and decreased bone density [75, 76]. Vitamin D, related to the regulation of bone turnover and phospho-calcium homeostasis, results as a negative endocrine regulator of RAS and some evidence suggests an association between vitamin D deficiency and autoimmune thyroid disease [77, 78]. Interestingly, vitamin D has been shown to be associated with an increase of D2 levels and peripheral T4 to T3 conversion in tissue homogenates (from liver, kidney, muscle, femur bone, heart and brain) of diabetic rats [79].

Clinical Features in Thyroid Hormone and Renin Angiotensin Systems Crosstalk

THs have various effects on the heart and vascular system and most of the clinical manifestations of a thyroid dysfunction are due to the cardiovascular hemodynamic alteration induced by THs, whose effects are opposite depending on whether a condition of hyperthyroidism or hypothyroidism occurs [80].

Parameters such as heart rate, cardiac output and systemic vascular resistance are closely related to the thyroid activity. In fact, if on one hand THs have a direct metabolic effect on the increase in oxygen consumption, on the other hand they carry out their actions through interaction with RAS [81]. In particular, blood pressure changes are observed in both hypothyroid and hyperthyroid patients: hypothyroidism is usually associated with reduced cardiac output and reduced blood pressure with low plasma renin activity [80], while hyperthyroidism is frequently associated with hyperdynamic hemodynamic alterations and high blood pressure values are accompanied by RAS hyperactivity [82]. The increase in cardiac output during hyperthyroidism is not the only factor responsible for the increase in blood pressure, in fact, in hyperthyroid mice, ACE blockade does not reduce cardiac output, but normalizes blood pressure values. Furthermore, the prolonged administration of ACEi prevents the onset of hypertension induced by excess of T3 [83].

A severe complication of not-treated hyperthyroidism is thyrotoxic cardiomyopathy. In this condition the heart, essentially working in hyperdynamic state, is unable to exploit its physiological functional reserve since it works at its maximum capacity. Therefore, patients with thyrotoxic cardiomyopathy usually presents tachycardia, dyspnea, exercise intolerance, systolic hypertension and peripheral edema [84]. The pathogenesis of thyrotoxic cardiomyopathy is mainly related to the direct action of THs at cardiac level, causing an increase in heart rate, myocardial contractility and output, as well as at peripheral level, with reduction of vascular resistance, higher basal tissue metabolism and consequent increased needs in energy and oxygen. Moreover, thyrotoxic cardiomyopathy is also related to the TH-induced RAS activation.

The RAS-mediated vasoactive action of THs is strongly associated to the progression of atherosclerosis and in this regard, a condition of euthyroidism seems to be protective because of the presence of a better lipid metabolism and endothelial function, a lower arterial stiffness/inflammation and a down-regulation of the AT1 receptors [85–91].

Furthermore, THs play a key role in kidney development, growth, and function. In hypothyroid patients, a reduction in renal perfusion and glomerular filtration rate (GFR, index of renal filtration capacity and function) is often observed, and it is determined both by the reduction of cardiac output and RAS activity [92]. Functional changes in the kidney due to abnormal TH levels are not only related to extrarenal but also to intrarenal RAS; in particular, in hyperthyroid subjects, the increased intrarenal activity of RAS is partially responsible for the increase in blood pressure associated to the increase in tubular reabsorption of sodium [93].

Conclusions and Perspectives

For many years, biomedical investigations on RAS and THs have been kept separated and independent and specialized researches have long been performed on the two systems. In the last decades, evidence of their functional interconnection in

several pathological contexts has encouraged the search and development of new experimental models towards more effective therapeutical approaches.

Major evidence has been obtained in the cardiovascular settings, where the modulation of RAS and THs may be strategical in the management of different cardiovascular diseases. Analogously, also the THs-RAS crosstalk observed in other functional contexts (e.g., liver, bone, CSN, kidney) may offer new perspective of study to better understand the maladaptive mechanisms driving to pathological conditions and, consequently, new insights in the development of a potential clinical use of drugs targeting RAS elements.

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Chapter 3
Crosstalk Between Abnormal Electrical Activity and Angiotensin II Cell Signaling in the Hyperglycemic Mammalian Heart

Belma Turan

Abstract It has been well-accepted that an up-regulation of not only the systemic but also local cardiac renin angiotensin system (RAS) leads to end-stage damage in the heart under pathological conditions. So, a well-controlled RAS has an important impact on the prevention of morbidity and mortality of patients with cardiovascular disorders. Local cardiac RAS is composed of renin, **angiotensin**-converting enzyme (ACE), **angiotensin I** (Ang I), and it products Ang(1–9), **angiotensin II** (Ang II), and its product Ang(1–7), while the main effector peptide of the RAS is Ang II. Ang II plays prominent roles in cardiovascular pathology through its fundamental roles in the modulation of cellular signaling mechanisms, such as participation in immunity, lipid peroxidation, and insulin resistance. An overall insight to the literature data, activation of cardiac RAS may be pivotal in the pathogenesis of cardiac dysfunction in diabetes, metabolic syndrome, obesity, and/or other types of pathological conditions. Herein, the aim of this review article is particularly focused to discuss the evidence on the role of crosstalk between Ang II cell signaling and abnormal electrical activity and in the hyperglycemic mammalian heart. In the content of the article, it will be discussed characteristics and mechanisms of Ang II-related cardiac remodeling such as effects of Ang II receptor activation on electrical and mechanical activities of the heart under pathological stimuli, in both cellular and organelle level alterations. It will be also documented the literature data related to the roles of Ang II receptors on structural and electrophysiological remodeling in the hyperglycemic and insulin-resistant heart.

Keywords Diabetes · Insulin resistance · Metabolic syndrome · Aging · Heart function · Renin angiotensin system

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Introduction

The mammalian renin angiotensin system (RAS) is a hormone system and acts at multiple targets in organs including the heart, basically to regulate blood pressure and fluid balance in the body. In other phrase, RAS functions in the body via regulating the extracellular fluid volume and therefore is responsible for the homeostatic mechanisms of several organ systems, including the cardiovascular system. A well-controlled RAS has an important impact on the prevention of high percentage morbidity and mortality of patients with cardiovascular system disorders [1, 2]. Until the beginning of 2000, it was still not known whether or not every organ system has its own RAS, and therefore, the existence of a local RAS in the heart was still a controversial issue [3]. Recent studies demonstrated RAS as not only a cardiovascular circulating hormonal system but also a local tissue system. The heart RAS can function either dependently or independently with this circulating system [4–9].

The RAS, besides its systemic importance, has various critical roles in cardiovascular function under both physiological and pathophysiological conditions affecting the directly cardiovascular system and/or several signaling pathways [5, 10, 11]. Therefore, the RAS in the heart behaves as a key contributor to the development and progression of cardiovascular disease, and thereby its inhibition has important benefits in the treatment of cardiovascular disease [12, 13]. Although most of the studies are supporting this statement, however, despite optimal medication with this system inhibitors, the up-regulated systemic and local RAS in the heart may not be well-controlled due to its irreversible effects, and, so, its inhibitors may not always provide expected cardioprotection [14, 15].

The discovery history of the RAS began in 1898 with the studies by Tigerstedt and Bergman [16], and later, this system has wide importance in mammalians, basically in both cardiovascular and renal systems. The classical RAS in the heart is composed of renin, angiotensin-converting enzyme (ACE), angiotensin I (Ang I), and its product, Ang(1–9), angiotensin II (Ang II), and its product Ang(1–7) (Fig. 3.1). In our classical knowledge, the main effector peptide of the RAS is the Ang II, whose synthesis starts with the cleavage of angiotensinogen into Ang I by the renin and then its conversion into Ang II by the ACE [4, 17, 18]. Angiotensin II is localized in different areas of the heart such as the atria, conduction system, valves, coronary vessels, ventricles, fibroblasts, and myocytes [19, 20]. Cardiac angiotensinogen is synthesized in the cardiac muscle, whereas they are lower concentration in the ventricles as compared to atria in the heart. Ang II, besides its role in other systems, plays prominent roles in cardiovascular pathology through its fundamental roles in the modulation of cellular signaling mechanisms, such as participation in cell-to-cell communication, immunity, lipid peroxidation, and insulin resistance.

Taking into consideration the various roles of RAS in several organ functions, one can point out its crucial role in the whole-body homeostasis as well as can emphasize the central role of its activation in many common pathologic conditions including hypertension, heart failure, and renal disease. Various pathological stimuli can activate the myocardial RAS, further leading to elevation of the local Ang II

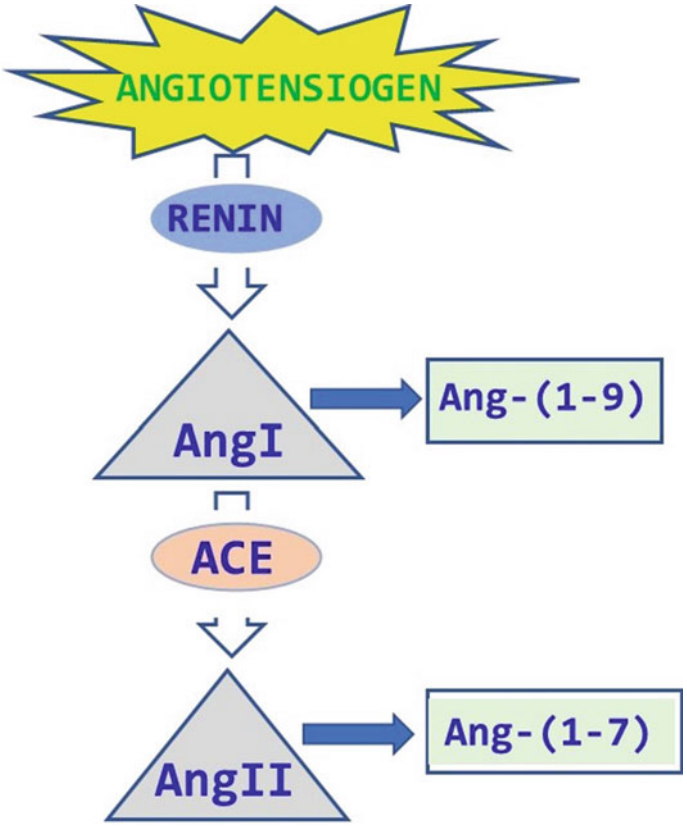

Fig. 3.1 A schematic presentation of the renin angiotensin system (RAS). Angiotensinogen is produced and secreted by the liver and enzymatically cleaved by the protease, renin to form angiotensin I (Ang I). Ang I is cleaved by angiotensin-converting-enzyme (ACE) to form angiotensin II (Ang II). Other bioactive angiotensin peptides are Ang-(1–9) and Ang-(1–7)

level, while its chronic activation can more importantly further lead to induction of pathological cardiac function. In this concept, cellular oxidative metabolic stress and endothelial dysfunction are pivotal in the generalized pathogenesis of cardiovascular disease and are closely linked to circulating and tissue levels of Ang II. Furthermore, the association of the RAS with the endocrine system is particularly illustrated by the prominent role of Ang II in diabetes and metabolic syndrome [21–30].

An overall insight to the literature data, activation of cardiac RAS may be pivotal in the pathogenesis of cardiac dysfunction in diabetes, metabolic syndrome, obesity, and/or other types of pathological conditions. Herein, the aim of this review article is particularly focused to discuss the evidence on the role of crosstalk between Ang II cell signaling and abnormal electrical activity and in the hyperglycemic mammalian heart.

Characteristics and Mechanisms of Ang II-Related Cardiac Remodeling

Physiological, pharmacological, and clinical studies have demonstrated that activation of the RAS is a key mediator of the progression of heart failure. It has been defined with several studies that a classical view of RAS consists of a series of enzymatic reactions, further leading to the generation of Ang II (Fig. 3.1). Studies in later decades demonstrated the discovery of many new components in this system. These new components include ACE2 (a homolog of ACE), which is responsible for the conversion of Ang II to Ang(1–7) [1, 4, 17, 18]. Furthermore, it is accepted that ACE2 is widely expressed in cardiomyocytes, cardiofibroblasts, and endothelial cells. The Ang(1–7) opposes the molecular and cellular effects of Ang II via enhanced susceptibility to heart failure with loss of ACE2 as well as prevention and reversing of the heart failure phenotype with increasing ACE2 level.

Biologically active peptide, ACE, acts on both Ang II type 1 and type 2 receptors (AT_1 receptors and AT_2 receptors; Fig. 3.2) inducing opposite effects on pathological conditions. In the mammalian heart, activation of AT_1 receptors via activation of Ang II promotes mainly depression in contractile activity, vasoconstriction, inflammation, salt and water reabsorption, and oxidative stress [31]. The activation of Ang II/AT_1 receptors and the detrimental effects of this pathway underlies the development of heart failure in various pathological conditions, such as pressure overload, diabetes (both type 1 and type 2), obesity, and post-remodeling (Fig. 3.2). Increases in reactive oxygen species (ROS), hypertrophy, mitogen-activated protein kinase (MAPK), matrix metalloproteinase (MMP), lipotoxicity, and fibrosis are the common altered parameters in the heart under these pathological conditions via detrimental effects of activation of Ang II/AT_1 receptors. Furthermore, cardiac metabolic abnormality (mainly insulin resistance) is existing in diabetes (type 2) and obesity (also in metabolic syndrome, MetS) [32], besides the others via activation of Ang II/AT_1 receptors. These alterations as activation of Ang II/AT_1 receptors, which are members of several intracellular signaling pathways, play important roles in cardiomyocyte dysfunction, as well. Insulin contributes to hemodynamic regulation in the cardiovascular system, by multiple mechanisms, through contribution to either protective or injury in these tissues [33].

Two important hormones, insulin and Ang II (at most via AT_1 receptors) have an impact on the control of metabolic and hemodynamic homeostasis, as systemic and cellular levels [34], as well as their signal transduction pathways, share many downstream effectors and cross-talk at multiple levels [35]. In other words, metabolic disturbances in cells and/or other cardiovascular risk factors stimulate RAS which further leads to increased production of ROS. ROS activate redox-sensitive several signaling pathways including phosphorylation of insulin receptor substrate-1 (IRS-1) at serine residues, then inhibit insulin-stimulated phosphorylation of IRS-1 at tyrosine residues [36, 37]. Through this pathway, as consequence, insulin signaling is inhibited via the phosphatidylinositol 3-kinase (PI3K) pathway, resulting in insulin resistance, hyperinsulinemia, type II diabetes, and other metabolic diseases such as obesity or

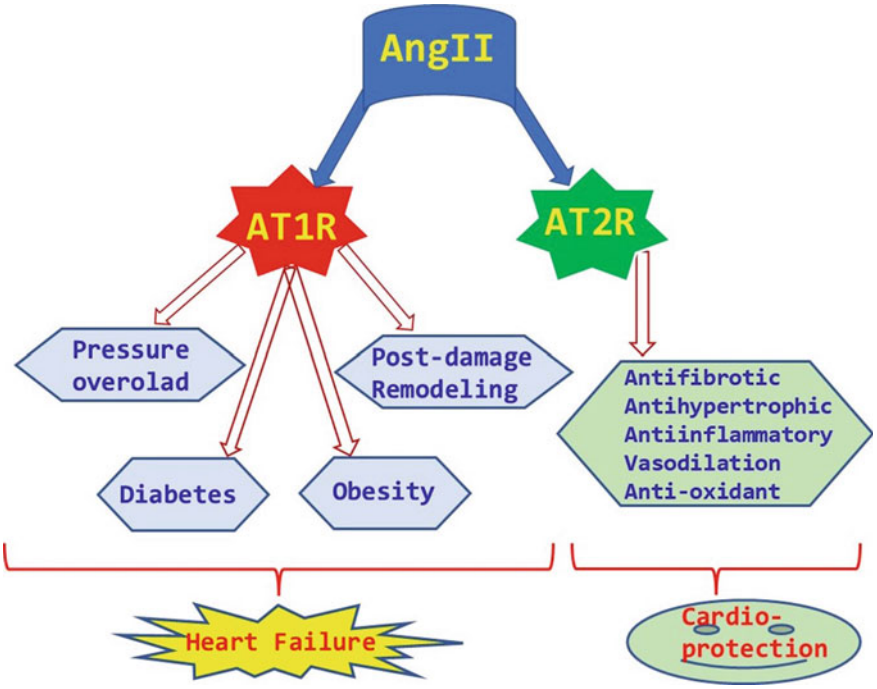

Fig. 3.2 Pathway associated with Angiotensin II receptors and heart function. Ang II binds to both type I (AT_1) and type II (AT_2) receptor subtypes in tissues. The AT_1 receptors, mediating most of the cardiovascular effects of Ang II, included in the development of oxidative stress, vasoconstriction, aldosterone secretion, sympathetic stimulation, cardiac cell hypertrophy, and cell proliferation. On the other hand, there is concomitant stimulation of AT_2 receptors by blocking of AT_1 receptors with angiotensin receptor blockers, which can in turns prevent the pathophysiological effects such as myocardial and vascular improvements

MetS. On the other hand, Ang II/ROS activates the mitogen-activated protein kinase (MAPK) pathway to induce vascular remodeling, which promotes cardiovascular disease, as well [38]. The angiotensin AT_2 receptor has been increasingly recognized as an integrative part of the protective arm of the RAS. Although it shares with the AT_1 receptors the same endogenous ligand, Ang II, it promotes different, and often opposing effects to those of the AT_1 receptors. The current knowledge on the role of the AT_2 receptors in health and disease for the cardiovascular system, the underlying protective molecular mechanisms as well as the therapeutical potential of AT_2 receptors agonism in acute and chronic heart diseases [39]. In this context, it has been shown that deletion of the AT_2 receptors can reduce adipose cell size and protect from diet-induced obesity and insulin resistance [40], while it is upregulated and contribute to Ang II-induced vasodilation in resistance arteries of hypertensive diabetic patients treated with AT_1 receptors blockers [41].

Overall, Ang II is the main biological effector of the RAS, through its major role in cardiovascular homeostasis. Most of the physiological and pathophysiological effects

of Ang II are mediated by the AT_1 receptors, which are widely expressed by most cell types, including heart cells. Although expression of the AT_2 receptors is more limited and occurs predominantly in fetal tissues [42], the later studies, mostly in cellular and adult animal models, showed the distribution of AT_2 receptors in certain areas of various tissues including the cardiovascular system [43, 44]. Interestingly, although the increased AT_2 receptors expression has been observed under pathological conditions, such as myocardial infarction [45] and congestive heart failure [46], its pharmacological potential for the treatment of cardiovascular diseases has only started with the development of selective AT_2 receptors agonists [5].

Ang II Receptor Activation Effects on Electrical and Mechanical Activities of the Heart Under Pathological Stimuli

The RAS, particularly local RAS in the heart, has a critical role in the not only physiological condition but also the pathophysiological condition in mammalians. As mentioned in previous sections, local activated RAS, primarily via effector hormone Ang II, mediates high blood pressure, endothelial dysfunction, metabolic disturbances, and cardiac dysfunction, while Ang II receptor activation is related to the different actions of Ang II in the heart [47]. As shown in Fig. 3.2, Ang II, via AT_1 receptors, can induce G protein- and non-G protein-related signaling pathways, and the activation of AT_1 receptors in the heart can underlie different pathological conditions including diabetes. Furthermore, studies have shown that cardiac AT_1 receptor gene expression level is not changed by age starting from fetal development, whereas the AT_2 receptor gene expression level decreases rapidly after birth [48].

The Ang II synthesis in the heart under pathological conditions [49, 50]. The dynamics of cardiac RAS during any pathological stimuli (i.e. pressure-overload, hypertrophy, myocardial infarction, metabolic disturbances, etc.) can vary and the contributing factors are numerous to these dynamics. For instance, rice in plasma renin level and the end-stage heart failure can appear to be parallel in individuals [51]. Furthermore, it has been shown that activation of Ang II exerts direct actions on cardiac tissue inducing cardiomyocyte hypertrophy and both electrical and mechanical dysfunctions [52]. The contribution of RAS level in heart tissue to ventricular remodeling in myocardial pathology was documented by early several experimental and clinical studies [53]. The cardiac remodeling following exposure to pathological stimuli eventually is maladaptive and result in heart failure further leading to end-stage heart disease. By using the different approaches to this event such as either inhibition of ACE, usage of Ang II antagonists, or both, the role of activated cardiac RAS in the development of heart diseases have been documented even in early studies [54, 55]. Supporting data to the above statements were given by various studies, in which it has been demonstrated the increased mRNA level of angiotensinogen in the

heart tissue of rats after experimental myocardial infarction [56], marked increase in the ACE activity in scar tissue [57], and enhanced production of Ang II in the infarcted heart tissue and fibroblasts [58].

Randomized controlled trials have renewed focuses on the role of Ang II for circulatory shock [59–61]. In these studies, it has been considered that Ang II could increase mean arterial pressure in patients with circulatory shock [59], thereafter, considerable literature surrounds the clinical and experimental use of Ang II [62, 63].

There are marked alterations in the parameters of the surface electrocardiogram (ECG) in patients with cardiac hypertrophy and/or heart failure. Among other alterations, it is commonly observed a prolonged QT interval duration and dispersion as well as increased incidence of arrhythmias in these patients [64]. Although Ang II is an important modulator of fluid balance in the body, cardiac Ang II exerts direct actions on the heart via inducing cardiomyocyte hypertrophy and mechanical dysfunction [65]. Studies performed with experimental animal models and the clinical setting demonstrated important relation between increased level of local Ang II level in the heart and development of cardiac hypertrophy and heart failure [66, 67]. Moreover, some experimental data have demonstrated the efficacy of RAS inhibitors in the treatment of arrhythmias [68]. In addition, clinical evidence has demonstrated the efficacy of ACE inhibition in the reduction of long QT intervals in hypertensive patients with left ventricular hypertrophy [69]. However, there is yet some uncertainty about the underlying mechanisms of a direct action of Ang II activation and electrical remodeling in the heart.

There are also some important experimental animal model studies supporting the above statements related to cardiac RAS and the role of its local activation. The authors examined the role of cardiac Ang II overproduction on cardiac electrophysiological activity by using animal models [70]. In their model animals, the increased Ang II production in cardiac tissues but not any change in circulating Ang II, cardiac hypertrophy, and heart failure were developed via the mediation of AT_1 receptors while the blockade of these receptors could prevent the development of ventricular hypertrophy [71]. In another study, authors demonstrated recovery in blood pressure and reduction in prolonged QT-interval following chronic treatment of an ACE inhibitor [72, 73]. As mentioned above statements, some findings have shown that activation of the AT_1 receptors is involved in both electrical and mechanical as well as structural remodeling of the heart, but these outcomes may be regulated by different pathways downstream of AT_1 receptor activation.

Most of the studies on the key role of the AT_2 receptor have been extensively studied in the development of the embryo while their roles in the adult mammalians remain unanswered yet [74]. Among some early studies in this subject include the investigations performed under in vitro conditions in cultured cells and isolated organs [75–80]. Although experimental studies associated with AT_2 roles focused on their roles in vascular function and blood flow at most, through the production of vasoactive substances, NO, the AT_2 receptors do not seem to play a major role in baseline conditions [81]. However, it has been demonstrated that other vasoactive substances can also mediate AT_2 receptor-dependent dilation in different organs or small arteries [82]. Moreover, it has been also mentioned that the stimulation

of the AT2 receptor can also have a role in shear stress-induced dilation through an endothelial production of NO in isolated arteries even under physiological conditions [83]. Thus, if one can interpret the early findings in the perspective of a chronic AT_1 receptor blockade in patients, it can be suggested the beneficial role of AT_2 receptors overstimulation via their vasodilator effect.

Also, activation and/or overstimulation of Ang II, in addition to their roles in cardiac electrical and mechanical functions, is known as an Ang II-mediated effect on cell growth and apoptosis and to have pro-oxidative and proinflammatory effects [46, 84, 85]. Indeed, Ang II has been shown in both human and animal models to be involved in the development of cardiomyocyte hypertrophy and cardiac fibrosis and the modulation of cardiac fibroblast growth and collagen synthesis [10, 86]. For instance, in in vitro studies, authors have reported that mechanical factors stimulated a local synthesis of Ang II, which in turn induces an increase in the synthesis of extracellular matrix proteins, such as fibronectin and collagen, via the AT_1R in various organ cultured cells [87, 88]. In vivo studies also provided information about the involvement of Ang II in the increase of cardiovascular fibrosis in the induction of hypertension and with the stiffness of the cardiac muscle in the development of diastolic dysfunction [89–91].

Besides, the AT_2 receptors have some important contributions to the control of not only heart function but also cardiovascular structure. In that content, Mifune and coworkers examined the effects of stimulation of AT_2 receptors on collagen synthesis in vascular smooth muscle cells [92]. In that study, they determined a dose- and time-dependent increase in collagen synthesis and a 50% decrease in protein tyrosine phosphatase activity, which were completely inhibited by an AT_2R antagonist while there was no effect with an AT_1 receptors antagonist. These studies together with others in similar experimental conditions suggested that AT_2 receptors stimulation could increase collagen synthesis in vascular smooth muscle cells via a G_i-mediated mechanism through heterogeneous effects of AT_2 receptors stimulation in different tissues [92]. There are also studies related to the effects of AT_2 receptors on extracellular matrix synthesis as well as elevation in collagen and fibronectin synthesis in cardiac tissues in cardiomyopathic animals [46]. Moreover, it has been also shown that the AT_2 receptor density increases by 153% during heart failure and by insulin, whereas AT_1R density increases in the hypertrophy stage and then return to control level during heart failure [46, 82, 93]. Interestingly, it has been shown a marked reduction in ventricular tachycardia risk by altering connexin43 via inhibition of RAS under either an ACE inhibitor or an AT_1 receptor blocker [94]. Taking into consideration the clinical relevance of ventricular tachycardia in the development of sudden cardiac arrest as well as under the light of early and recent documents, overall, it can be pointed out the contribution of local RAS activation through multicentric signaling of Ang II in the cardiovascular system, particularly under pathological conditions [95].

Ang II-Induced Cellular and Organelle Level Alterations in Cardiomyocytes

As mentioned in the previous sections, the Ang II, at both systemic and organ levels, is known to mediate multicentral endogenous effects in cells such as cell growth, apoptosis, oxidations, and inflammations as well as important impacts on organelle function and ultrastructure [82]. Indeed, Ang II is involved in the remodeling of the cardiovascular system under pathological stimuli. For instance, early studies have shown its role in cardiac myocyte loss in ischemia–reperfusion injury and myocardial infarction [20, 96]. Treatment of wild-type experimental animals with an ACE inhibitor or AT_1 receptor blocker induced a marked shortening of AV nodal conduction, whereas no significant change in sinus rate, surface PR and QT intervals, atrial and ventricular refractory periods, or ventricular tachycardia inducibility [94]. Indeed, cardiac electrical remodeling is one of the most serious complications, creating a cardiac microenvironment that evokes fatal ventricular arrhythmias [97].

Various experimental studies are focused on the roles of Ang II activation and its accessories in isolated cardiomyocytes to show the underline mechanism of its contribution to heart dysfunction under pathological conditions. At the cardiomyocyte level, there are serious electrical modifications, generally, including prolongation of the action potential duration (APD) caused by differential expression of ion channels [98–100]. Among them, it is generally observed downregulation of K^+-channels responsible for the APD. However, studies to demonstrate a direct association between Ang II and cardiomyocyte electrical remodeling seem to need more molecular examinations. A supporting data of this statement has been given by researchers to evaluate the direct effects of high levels of intracardiac Ang II on cardiac electrophysiology by using a transgenic mouse model with cardiac-specific and overproduced Ang II [101]. They demonstrated that correlations between the reduced inwardly rectifying potassium current, prolonged APD at 90% repolarization in cardiomyocytes, and prolonged QT interval in surface ECGs in those transgenic mice. In another study, this team also demonstrated an Ang II-mediated cardiac hypertrophy in transgenic mice by analysis of isolated cardiomyocyte isotonic shortening. Their data strongly supported marked phenotypic changes in cardiomyocytes during the adaptive response to chronic cardiac-specific endogenous Ang II stimulation via determination of impaired contractility in the cardiomyocytes isolated from these transgenic mice (developed dilated cardiomyopathy). They demonstrated significantly impaired contractility in these isolated cardiomyocytes, parallel to a downregulation of the sarcoplasmic reticulum (SR) Ca^{2+}-pump (SERCA) and depressed intracellular Ca^{2+} releases under electrical stimulation. Overall, these and other studies clearly show that a chronic Ang II activation in cardiac myocardium, without fluid overload, can produce cardiomyocyte dysfunction in terms of electrical and mechanical activities which are further leading to heart failure. Indeed, these above effects have been described previously in the setting of various pathological conditions, including hypertensive cardiomyopathy, in which the administration of ACE

inhibitors could normalize the abnormal intracellular Ca^{2+}-handling and increase SERCA2a expression [102].

Similarly, it has been demonstrated that a transmural gradient in the amplitude of transient outward K^+-channel current (I_{to}) was induced by an AT_1 receptor activation in canine ventricular cardiomyocytes via a co-internalization of α subunit of this channel protein with the AT_1 receptors [103, 104]. Consequently, these experimental studies demonstrated a cross-correlation between the magnitude of I_{to} and Na/K-pump activity through internalization of the activation of AT_1 receptors in the cardiomyocytes, without any contribution that G protein signaling. Additional studies have shown the role of activated local RAS in cardiomyocytes to increase contractility of the heart [105].

The experimental data demonstrated the multi-signaling pathways of Ang II through AT_1 receptors such as its role in MAP kinases, receptor tyrosine kinases (i.e. insulin receptor), and nonreceptor tyrosine kinases [47]. In addition, AT_1 receptors-mediated NAD(P)H oxidase activation leads to the generation of reactive oxygen species, ROS, widely implicated in vascular inflammation and fibrosis [106]. Most importantly, some studies also mentioned the high-level generation of ROS via AT_1 receptor-mediated NAD(P)H oxidase activation [107–110]. Moreover, in later studies, authors have shown the role of Ang II-induced high-level production of Mitochondrial-ROS to cardiovascular disease [111]. Indeed, although Mitochondrial-ROS plays an important role in normal physiological cell signaling, similar to high glucose, activated Ang II, can cause the overproduction of Mitochondrial-ROS, which leads to the stimulation of NAD(P)H oxidases. Another study is also supporting this action of Ang II via activation of AT_1 receptors results in the initiation of a variety of events, such as the stimulation of phospholipase C, with subsequent activation of protein kinase C (PKC) and release of Ca^{2+} from intracellular stores, including SR and Mitochondria, while tyrosine kinase and MAPK are phosphorylated [112–114]. Other studies also mentioned that Ang II, through the activation of AT_1 receptors and PKC, can lead to apoptosis in cultured neonatal rat ventricular myocytes via activation of p53 [115]. Interestingly, it has been also demonstrated the AT_2 receptor stimulation associated apoptosis, at the cellular level as well [116]. More importantly, some findings implied an interesting contribution of AT_2 receptor blockade to increase the early signals of AT_1 receptor-mediated cardiac growth responses in the heart [117]. Consequently, these studies pointed out a possible counteractive action of AT_2 receptors on the effects of AT_1 receptors, particularly under pathological stimuli.

The COOH-terminal of AT_1 receptors can be phosphorylated with PKC, which leads to their internalization transiently under their Ang II-dependent activation, further leading to GTP hydrolysis-mediated and β-arrestin-dependent mechanisms in cells [118, 119]. Activation of Ang II, at most via AT_1 receptor stimulation, cannot only activate PKC but also inhibit PKA phosphoinositide 3 kinase (PI3K) [120]. These alterations are generally responsible for the development of hypertrophic cardiomyocytes. In this signaling pathway, when the G-protein activation is ended, the β-arrestin associated receptors are internalized to play role in a new signaling

pathway [121]. Supporting, literature documents show new signaling pathways associated with the role of local RAS activation in the heart published in numerous original and updated review articles, describing particularly structural components and functional selectivity of AT_1 receptors [5, 122, 123]. Besides these above subjects, there are the second messengers that are involved in the synthesis of Ang II in the heart under pathological conditions, which play important role in the control of cell communication and inward Ca^{2+} current in cardiomyocytes, as well.

In addition above effects with Ang II activation, its activation is closely associated with induction of fibrosis, an increase in pacemaker activity, and a decrease in conduction velocity in the heart through changes in cellular Ca^{2+} cycling, inhibitions of both inward depolarizing Na^+-current and repolarizing K^+-currents [124]. Supporting these experimental findings, ACE inhibitors and AT_1 receptor blockers could provide cardioprotection preventing the occurrence of antiarrhythmic potentials in humans [125]. Studies from mice with high cardiac Ang II levels showed sudden cardiac arrest, atrial and ventricular, and a marked long QT syndrome with normal blood pressure [126, 127].

Dikalov and Nazarewicz (2013) widely documented and discussed Ang II-induced mitochondrial ROS production and its relevance for cardiovascular disease [111]. The molecular mechanisms of Ang II pathophysiological activity involve the stimulation of NADPH oxidases, which produce superoxide and hydrogen peroxide. Ang II also increases the production of mitochondrial ROS, while the inhibition of Ang II improves mitochondrial function; however, the specific molecular mechanisms of the stimulation of mitochondrial ROS are not clear. However, it has been well documented that AT_1 receptors activate at least two different cell signaling axes, where one, represented by ERK1/2 and its downstream targets, is redox independent, and the other involves the activation of redox-dependent pathways [128]. The activation of the redox-dependent pathways involves the stimulation of NADPH oxidases, leading to ROS production and oxidative stress in cells. This redox-dependent activation further causes hypertrophic cell growth, cell senescence, and cardiovascular remodeling. In line with these findings, the pharmacological inhibition of AT_1 receptors could preserve the energy state of, indicating the significance of AT_1 receptor signaling in the pathology of ischemia-induced heart damage and links AT_1 receptor signaling and mitochondria functions under ischemic mitochondria conditions [129]. However, the Ang II-induced high ROS production is not completely understood and still requires extensive studies.

Roles of Ang II Receptors on Structural and Electrophysiological Remodeling in Hyperglycemic Heart

The actions of Ang II have been implicated in many cardiovascular conditions, including diabetic cardiomyopathy. Supporting this statement, the authors examined whether hyperglycemia activates the cardiac intracellular RAS in vivo and whether angiotensin-receptor-blocker, ACE, or renin inhibitors block synthesis and effects of intracellular Ang II. They demonstrated an intracellular Ang II production in diabetic rats, being correlated with cardiomyocyte apoptosis, oxidative stress, and cardiac fibrosis [130]. These data and others can demonstrate the important involvement of Ang II activation in the development and pathological changes in diabetes and metabolic syndrome (MetS) [26, 131]. More importantly, some studies also mentioned the involvement of mitochondria as the critical contributor to these changes under Ang II activation in diabetes. For instance, it has been recently shown that the AT_1 receptor blockade protected mitochondria via inhibiting high oxidant production, as well as recovered significantly prolonged action potential and depressed potassium currents, and altered Ca^{2+}-homeostasis in type-I diabetic rat cardiomyocytes [131–133]. Furthermore, even early studies have shown the marked recoveries by application of AT_1 receptor blockers, ACE inhibitors, or both in the depressed potassium currents and prolonged action potentials in streptozotocin (STZ)-induced type-I diabetic ventricle cells, at most, due to inhibition of activated PKC [100, 134]. Overall, these experimental data showed that treating STZ-induced type-I diabetic rats with an AT_1 receptor blocker could restore the altered kinetics of cardiac intracellular Ca^{2+}-transients and contractile activity of cardiac tissue. These results have important implications in searching for a better treatment for understanding the physiopathology of diabetic cardiomyopathy with Ang II receptor blockers. Supporting clinical outcomes from LIFE, CHARM, and VALUE trial groups also strengthened the role of blockage of RAS in the prevention of diabetes-associated organ dysfunction [135–137]. In addition, an application of AT_1 receptor blocker could prevent intra-fibrosis, apoptosis, and oxidative stress in tissues from Zucker diabetic rats [138]. Similarly, we determined important recovery in alterations of STZ-induced type-I diabetic rat heart tissue with the treatment of an AT_1 receptor blocker candesartan such as decreases in the number of undulations and lipid droplets, recovery in the heterogeneity of cytoplasm, and decrease in the connective tissue (Fig. 3.3). Furthermore, in in vitro experimental study with hyperglycemic ventricular cultured cardiomyocytes, the candesartan pretreatment induced significant prevention of high ROS production and mitochondrial membrane depolarization. Interestingly, it has been studied whether an ACE2 activator, diminazene aceturate, could improve the STZ-induced electrical changes in ventricular repolarization in hyperglycemic rats through a beneficial effect on long QT and QTc intervals with important cardiovascular benefits [139, 140]. In another study, it has been tested whether Ang II receptor blockade improves cardiovascular function during

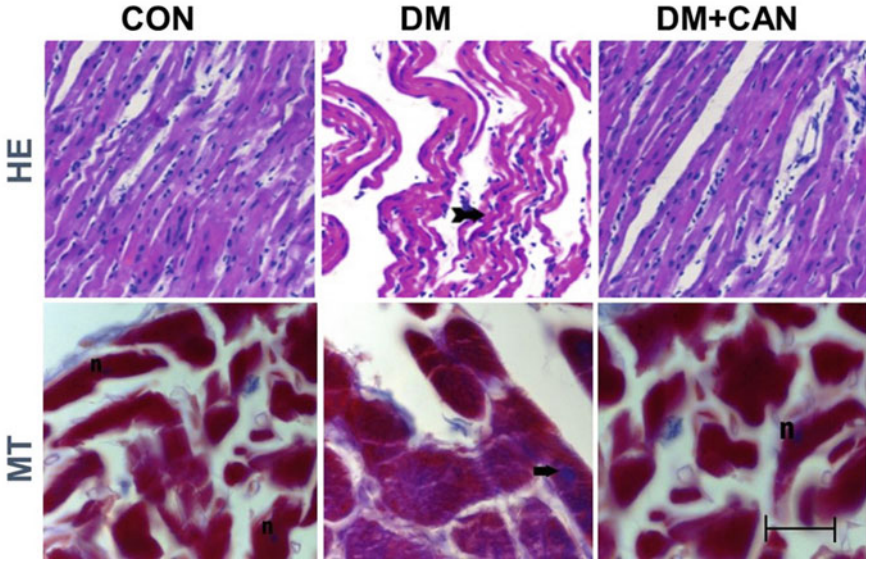

Fig. 3.3 Structural benefits by AT_1 receptors of Ang II blockage in diabetic heart. A treatment of streptozotocin-induced diabetic rats with 5 mg/kg/day candesartan-cilexetil for 4 weeks induced marked recoveries in the damage left venticular part of the heart tissue. Light microscopy examinations represent either hematoxylin and eosin-stained (HE, upper part) or Masson trichrome (MT, lower part)-stained heart sections. Myocardial arrangements in longitudinal sections of control (CON, left), diabetic (DM, middle), and candesartan-cilexetil treated DM rat heart (DM + CAN, right). The abnormal myofibre arrangements and ondulations observed in DM group. There are also increases in thickness of tunica media, foamy cells, and irregular elastic lamellae in DM group. These abnormal observations were markedly disappeared in the candesartan-cilexetil treated DM group. A normal histological structural appearence in normal rat heart (CON group). Shorten symbols: n nucleus, arrow Z-line, arrowhead ondulation. Magnification: Bar is 50 μM

hypoglycemia and they could not observe any benefit with a candesartan treatment on the long QT interval in the ECG [141].

Cellular oxidative metabolic stress and endothelial dysfunction are pivotal in the generalized pathogenesis of cardiovascular disease and are closely linked to circulating and tissue levels of Ang II which has an important impact on remodeling in many ways [142]. As a result of the experimental studies, it can be concluded that Ang II directly acts on cardiomyocytes and through several signaling events, including ROS-generation associated signaling in the cytoplasm and mitochondria of cardiomyocytes [111]. Most of Ang II actions in the heart are mediated by AT_1 receptors-initiated signaling with ROS. Indeed, it has been shown that under pathological conditions such as hyperglycemia and/or hyperinsulinemia, the pharmacological inhibition of AT_1 receptor preserves the energy state of mitochondria, indicating its significance signaling in the pathology of heart damage, and links AT_1 receptor signaling and mitochondria functions under diabetes, as well [111, 129, 143]. For instance, the role of the mitochondrial K_{ATP} channel and its relations with

high ROS production in hyperglycemic cells has been implicated under Ang II activation [144]. Overall, both experimental data and clinical outcomes supported a process in diabetic cardiomyopathy that activated RAS, at most, activation of AT_1 receptors, in the heart and, has impact through associations between endothelium function and insulin signaling transduction pathways as well as the putative role of ACE2-Ang-(1–7)Mas axis in diabetes pathogenesis [28, 29]. In another study, taking into consideration the role of ACE2 and Ang-(1–7) (Ang 1–7)/Mas receptor axis in the modulation of the development of diabetic cardiomyopathy, it has been studied the effects of Ang 1–7 on diabetic cardiomyopathy in diabetic mice. Their results could identify a novel beneficial effect of Ang 1–7 on diabetic cardiomyopathy on the involvement of a reduction in cardiac hypertrophy and lipotoxicity, adipose inflammation, and upregulation of adipose triglyceride lipase, providing a promising therapy for diabetic cardiomyopathy in type-II diabetes [145]. Ang II activation has also an impact on the vascular smooth muscle cells. In this field, authors designed a diabetic-like condition in cardiomyocytes with high glucose, and therefore stimulated Ang II activation via ERK1/2 activation in rat vascular smooth muscle cells which is flowed with an ACE decrease in the same cells [146]. Overall, if one can consider the recent data related to the association between high plasma renin activity and obesity-related diabetes and arterial hypertension, leading to persistent hypertension [147], the role of cardiac RAS in the development of cardiovascular disease in MetS, obesity, and/or diabetes can get more attraction than the before.

Effects of Ang II Receptor Antagonists on Cardiac Remodeling in Insulin-Resistant Elders

Both experimental animal model studies and clinical outcomes have consistently shown that the use of RAS blockers, apart from their antihypertensive effects, could not completely prevent but slows down the progressive age-related organ damage, particularly, insufficient heart function [148–151]. Those effects seem to be maintained, at most, via drugs that inhibit Ang II synthesis/biological activity in target tissues. As mentioned in the previous sections, the mammalian heart has a local Ang II production and plays important role in the induction of RAS-associated cardiac pathophysiology development [67, 152]. Although a direct role of Ang II on the induction of cardiomyocyte hypertrophy and the efficacy of RAS blockade in the treatment of cardiac remodeling has been demonstrated [153], the direct effects of its activation and/or blockade targeting heart function to cause mechanical dysfunction and heart failure independently from hemodynamical alterations remained to be clarified. In this content, Domenighetti, et al. [70] investigated the phenotypic changes in cardiomyocytes from AngII–mediated cardiac hypertrophy induced transgenic mice without elevated blood and performed in vivo analysis of age-dependent cardiac function such as systolic and diastolic dysfunction. Their analysis in isolated

cardiomyocytes also has shown important impaired contractility through downregulation of the SERCA2 and diminution of the amplitude of transient Ca^{2+} releases under electrical stimulation. Their data, overall, demonstrated the role of direct and chronic cardiac Ang II stimulation in the induction of cardiac dysfunction leading to heart failure in aged mammalians.

Interestingly, since the functions of the AT_2 receptors are opposite to those of the AT_1 receptors, AT_2 receptor activation could show a protective role in aging conditions. Supporting this statement, deletion of the AT_2 receptors induced several types of cardiac injury and functional changes [154], whereas their activation could provide marked cardioprotective action [155]. It has been also pointed out a possible pathway for the beneficial role of AT_2 receptors in the aging heart, colocalized with AT_1 receptors on the inner membrane of mitochondria, in which, mitochondrial AT_2 receptor density may decrease in cardiac cells paralleled by an increased expression of mitochondrial AT_1 receptors with aging in the heart [156]. Although various signaling pathways contribute to these effects, experimental data emphasize the role of cellular aging as the consequence of the accumulation of damaged macromolecules, through a modification by excessive ROS via mitochondria in aged tissues [157, 158]. Excessive production of ROS compromises mitochondrial integrity and function, leading to decreases in ATP generation, as well. In this pathway, there is a role of activation of intracellular NADPH oxidase with Ang II via the AT_1 receptors. Although Ang II can regulate oxidative stress under physiological conditions, abnormal activated Ang II-dependent ROS generation can arise associated with age-related activation of local RAS in the heart [159]. Likely, experimental findings have shown induction of cardiac hypertrophy and fibrosis in aged animals while they can be recovered by an application of an Ang II blocker [160]. Additional studies are further supporting the beneficial effects of either ACE inhibitors, Ang II receptor blockers, or both in reducing heart damage during the aging period, at most, their effects through inhibition of high ROS production and preserving of mitochondrial function [161]. The later studies did further support the early results. For instance, it has been documented that decreased age-related cardiac mitochondrial dysfunction in patients could be reversed by treatment of Ang II receptor blockers, at most, through the regulation of mitochondrial redox hemostasis [162]. Furthermore, it has been also demonstrated the cardioprotective effects of RAS blockers against atrial fibrillation in aged patients [163]. Recently, the role of the renin–angiotensin–aldosterone system (RAAS) in the heart is also greatly documented by Mascolo et al. [9].

In conclusion of this section, it has been summarized that mitochondrial dysfunction and oxidative stress are closely associated with activation of local RAS and can underlie many abnormalities in the aging heart. Taking into consideration the existence of high risk for the development of insulin resistance as well as cardiac insufficiencies in aged mammalians at high percentage although, with mild blood glucose level, one has to reconsider the role of activated local RAS in the heart for prevention of cardiac dysfunction during the aging period.

Conclusions and Future Perspectives

Over the half-century, a wide range of publications indicates that Ang II represents a key molecule in physiological and pathological mechanisms of the heart, besides other organs in the human body. At cellular and mitochondrial levels, Ang II regulates energy metabolism and redox state of cells and affects the onset and the progression of cell damage under not only hyperglycemia and hyperinsulinemia but also under the progression of age in mammalians. It is well documented and got consensus that chronic activation of RAS in organs can promote end-stage organ injury and dysfunction, at most, through being associated with increased mitochondrial oxidative stress besides others, So, the use of inhibitors of either ACE, Ang receptor or both, will be able to reduce those associated cardiovascular function under any type of pathological stimuli. A schematic representation of sites of actions of Ang II/ACE inhibitors associated with going beyond the classical paradigms to provide benefits for cardioprotection under pathological stimuli (Fig. 3.4).

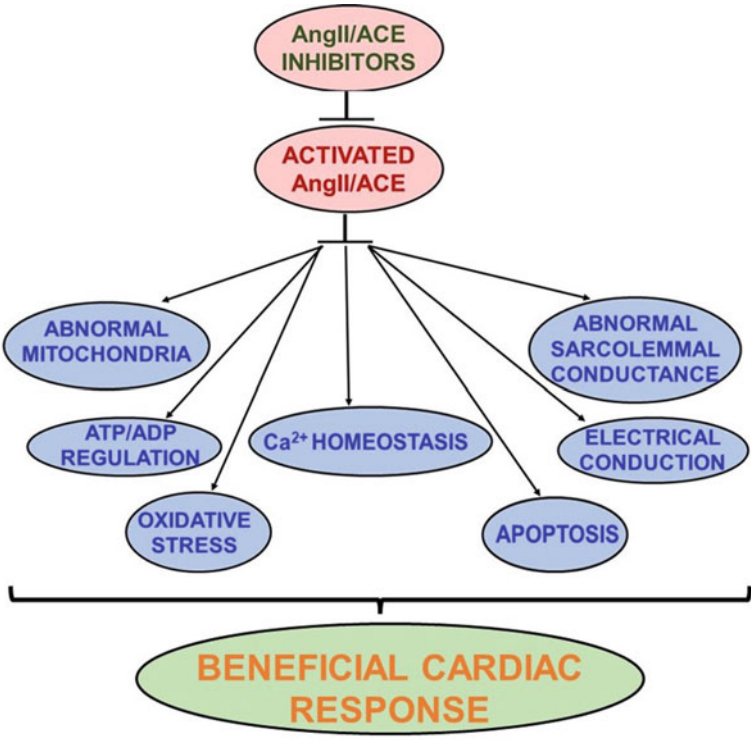

Fig. 3.4 Ang II/ACE inhibition and beneficial cardiac response. A schematic representation of sites of actions of Ang II/ACE inhibitors associated with going beyond the classical paradigms to provide benefits for cardioprotection under pathological stimuli

Therefore, it can be concluded that a well-controlled local RAS activity, via its vital importance because of its links to both mitochondrial function and dysfunction, will be a future focusing field to get important cardioprotection against hyperglycemia, hyperinsulinemia, and even physiological aging. Although many prior studies have advanced our understanding of each of these aging-relevant biological systems, the progress in delineating the molecular mechanisms involved has been rather slow. Given the availability of selective, and relatively safe blockers of RAS, studies focusing on the interface between mitochondria, RAS, and types of pathological stimuli may prove to be very important in the clinical translation of these investigations.

Conflict of Interest The author declares that there is no competing interests.

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Chapter 4
Cardiovascular Physiopathology of Angiotensin II and Its Plasma and Nuclear Envelop Membranes' Receptors

Danielle Jacques and Ghassan Bkaily

Abstract In 1934, the discovery of angiotensin II opened an Eldorado in the field of the cardiovascular system. Until today, this Eldorado is still evolving due to the implication of this octapeptide, not only in normal physiology but also in its effect on the remodeling of the cardiovascular system. The intense scientific research led to the discovery of components that regulates the conversion of Angiotensin I to angiotensin II including angiotensin II converting enzyme and chymase-dependent production of angiotensin II. One important advancement is discovering an inhibitor of the angiotensin II converting enzyme, which is the most clinically used antihypertensive drug. Identifying the receptors of angiotensin II, AT_1, and AT_2, also led to determining the signaling pathways of these two receptors and their contribution to the regulation of the cardiovascular system in health and disease. This made it possible to develop two specific AT_1 and AT_2 receptor antagonists. Its is not until recently that the AT_1 receptor antagonist, losartan, is used as an antihypertensive drug. The role of the AT_2 receptor in the angiotensin II effect is still a matter of debate. These two receptors were also found to be localized at the nuclear envelope membranes, and the normal crosstalk between the plasma and the nuclear envelope membranes angiotensin II receptors seems to be an important factor in the angiotensin II effect. Remodeling this crosstalk may contribute to the angiotensin II effect in cardiovascular diseases. (Dedicated to Prof. Domenico Regoli, a pioneer in the pharmacology of the renin angiotensin system).

Keywords Angiotensin II · AT_1 receptor · AT_2 receptor · Angiotensin II signaling · Nuclear AT_1 · And AT_2 receptors · Calcium · Cardiomyocytes · Vascular endothelium · Vascular smooth muscle · Endocardial endothelial cells

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Introduction

It is in 1934 that in observing a decrease in blood flow through the kidneys causes hypertension in dogs Goldblatt and colleagues [1, 2] hypothesized that this hypertension could be due to renal ischemia that is not controlled by a nervous system but rather by a humoral mechanism [2]. Three years later, Houssay and Fasciolo showed the presence of a vasopressor factor in the blood of renal veins from an ischemic kidney [3–5]. In 1939 and 1940, two groups independently described the presence in the plasma of a vasoconstrictor factor, which was called hypertension [5], and angiotonin [3, 6–9]. It is only in 1958 that there was an agreement between scientists in the field [10] to give this factor a new name: Angiotensin [1]. Since then, this hypertensive octapeptide has gained a lot of interest more particularly at the level of the cardiovascular and renal systems [11]. Under physiological and pathophysiological conditions, Angiotensin II (Ang II) exerts different biological actions in several organs [12]. Indeed, Ang II acts on the kidneys, the cardiovascular system, the adrenal glands, and the central nervous system [12]. In the cardiovascular system, Ang II modulates local and systemic blood pressure via modulating vascular smooth muscle contraction [13, 14]. In addition, several in vivo and in vitro studies have reported the involvement of Ang II in the regulation of the growth of vascular smooth muscle cells (VSMCs) [15–18]. Ang II was also found to induce ventricular cardiomyocytes hypertrophy [13, 14]. This octapeptide was also reported to stimulate the release of aldosterone from the adrenal gland and the retention of sodium and water in the renal tubules [19, 20].

In 1956, the discovery of the presence of an Ang II converting enzyme (ACE) open the way to a new era in the field of Ang II [21]. The enzyme was reported to be present in endothelial cells and converts circulating Angiotensin I (Ang I) to Ang II. Ang II can also be generated from Ang I by a number of other peptidases capable of cleaving the $Phe^{8}His^{9}$ bond of the decapeptide [22]. A novel homolog of ACE named ACE2 has been identified as a receptor for SARS-CoV [23] and COVID-19 [24, 25]. In addition, it has been demonstrated that Ang II modulates the function of cardiomyocytes, vascular endothelial (VECs), and (VSMCs) by increasing intracellular calcium (Ca^{2+}) via activation of both Ang II receptors, AT_1 and AT_2 [26–28].

Ang II Biosynthesis

The synthesis of Ang II occurs mainly in the circulation by sequential proteolytic cleavages of its precursors [29, 30] and involves two main enzymes: renin, an aspartyl proteinase synthesized as prorenin, a proenzyme that continues its maturation into renin in the juxtaglomerular cells of the kidney, before being released into the plasma [31], and ACE, a metalloproteinase also called kininase II [22].

If the classical pathway of Ang II synthesis via the action of renin and ACE remains the major concern of scientists [32], some studies suggest alternative pathways leading to its formation [22]. Indeed, Ang II can be formed directly via the Cathepsin-Tonin pathway [22]. Moreover, a serine proteinase, chymase, discovered in the 1980s, is capable of converting Ang I to Ang II [22, 33]. Chymase has been identified in human heart homogenates [34] and in human, monkey, and dog blood vessels [35, 36]. It should be noted that the importance of chymase in the synthesis of Ang II in these different tissues is highly controversial [22].

Ang II Receptors

The existence of Ang II receptors was first suspected in the 1970s with the discovery that Ang II is able to bind to isolated membranes of the adrenal gland [37, 38]. In 1989, the presence of several types of Ang II receptors became evident [39]. Four types of Ang II receptors have been identified so far: AT_1, AT_2, AT_3, and AT_4 receptors [40]. The AT_1 receptor relays most of the known effects of Ang II such as vasoconstriction, aldosterone release and proliferation of VECs and VSMCs and other cell types [20, 28, 41]. The AT_2 receptor, widely distributed in fetal tissues [42], including human [28, 43], is involved in differentiation, migration, inhibition of proliferation [20, 44, 45], induction of apoptosis [43, 46], endocardial endothelial cells secretion [47] and VSMCs function [41, 48, 49]. In human left and right ventricular endocardial endothelial cells (EECs), Ang II-induced apoptosis in a dose-dependent manner where blockade of the AT_1 receptor with losartan partially but significantly prevented Ang II-induced apoptosis in right ventricular EECs but potentiated the effect in left ventricular EECs (Fig. 4.1a, b). This shows that the contribution of AT_1 receptors to Ang II-induced apoptosis is cell-type dependent [43]. Contrary to AT_1 receptor blockade, inhibition of AT_2 receptors by PD123389 completely blocked Ang II-induced apoptosis (Fig. 4.1a, b). These results show the differences between AT_1 and AT_2 receptors' implication in Ang II-induced apoptosis and demonstrate the importance of AT_2 receptors in endothelial life and death [43]. In addition, stimulation of the AT_2 receptor seems to oppose the actions of Ang II relayed by the AT_1 receptor [50]. The AT_2 receptor is localized at the nuclear membranes level of human VSMCs [48, 49], and this localization seems to be species and cell-type dependent [43, 51]. As for apoptosis, stimulation with Ang II-induced a time-dependent increase of AT_1 receptors density in right ventricular EECs; however, in left ventricular EECs, Ang II-induced a time-dependent decrease in AT_1 receptor density (Fig. 4.2a, b). These results show that Ang II modulates the density of AT_1 receptors and this depends on the cell type [51]. Contrary to AT_1 receptors activation by Ang II, activation of AT_2 receptors induced an increase in this type of receptor in both right and left ventricular EECs (Fig. 4.2c and d). This suggests that the Ang II modulation in the density of its AT_2 receptors does not depend on the type of endothelium [51].

The AT_3 receptor corresponds to an Ang II binding site expressed on a neuroblastoma cell line and is not blocked by specific AT_1 and AT_2 receptor antagonists

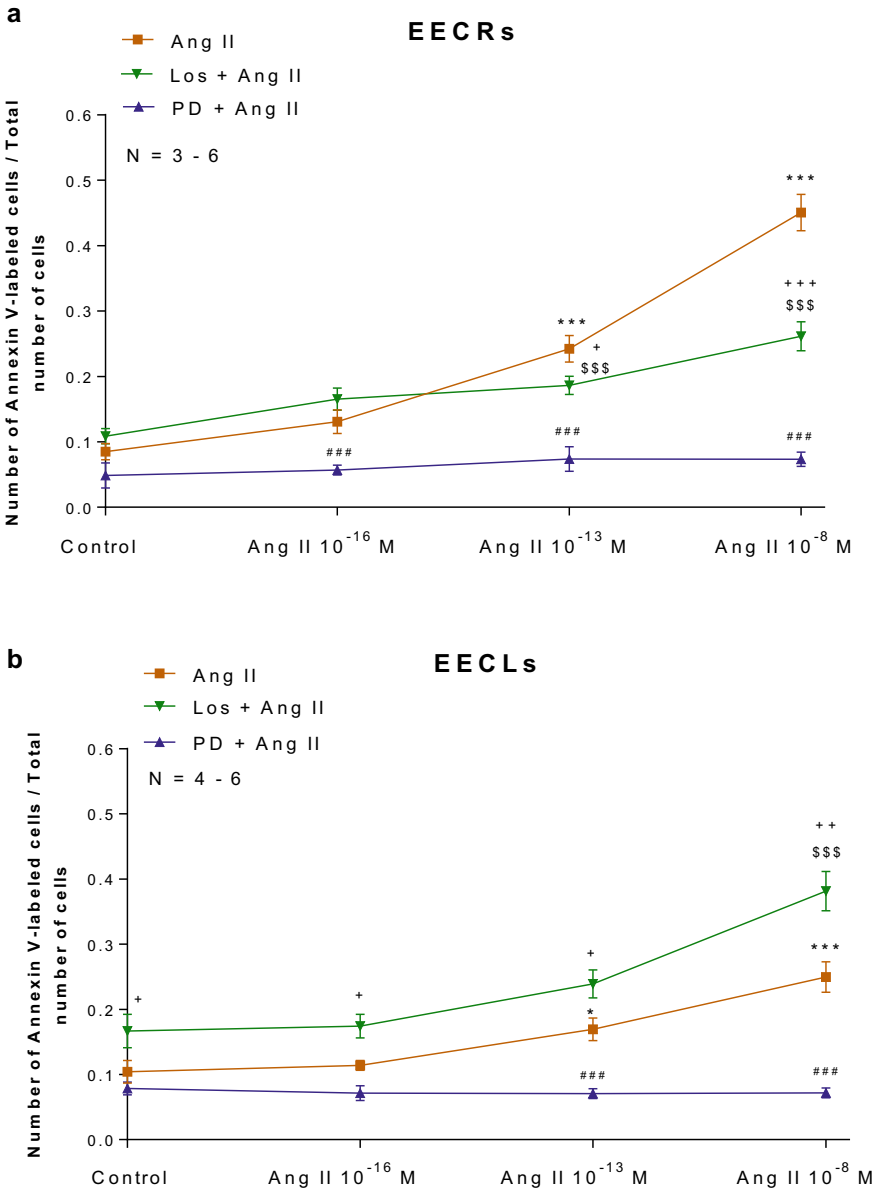

Fig. 4.1 Blockade of AT_2 receptors with AT_2 receptor antagonist PD123319 (10^{-7} M; blue curve) prevents Ang II from inducing apoptosis (labeled with annexin V; orange curve) in both right (EECRs) (**a**) and left (EECLs) (**b**) ventricular endocardial endothelial cells (EECs). However, blockade of AT_1 receptor with losartan (10^{-7} M; green curve) potentiated the Ang II-induced apoptosis effect in EECLs (**b**) and depressed its effect in EECRs (**a**). Values are presented as mean ± SEM. *$p < 0.05$, ***$p < 0.001$ versus control without Ang II; $$$$p < 0.001$ losartan + Ang II versus losartan without Ang II; + $p < 0.05$, + + $p < 0.01$, + + + $p < 0.001$ losartan + Ang II 10^{-8} M vs. Ang II 10^{-8} M. ###$p < 0.001$ PD123319 + Ang II vs. Ang II. N is the number of different experiments (Reproduced with permission from Jacques et al. 2019)

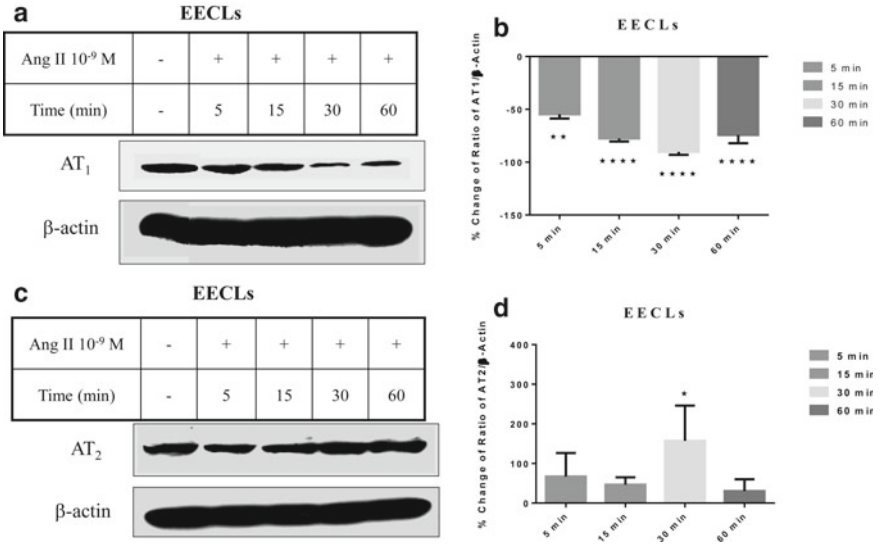

Fig. 4.2 In human left endocardial endothelial cells (hEECLs), Ang II-induced a time-dependent decrease in the density of the AT_1 receptor and an increase in the density of the AT_2 receptor. The results are presented as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{****}p < 0.0001$ versus control (Reproduced and modified with permission from Jacques et al. 2017)

[52]. However, since its initial description, few studies have focused on this receptor, whose presence is widely controversial [40].

The AT_4 receptor has been observed in the human brain [53] and kidney [54], but this receptor does not seem to be present in the cardiovascular system except in bovine endothelial cells [55]. The AT_3 and AT_4 receptors have not yet been cloned [56–58], and the AT_4 receptor seems to be more specific for angiotensin IV than Ang II [40] and is localized in the brain and the kidney [59]. On the other hand, recent studies have demonstrated the presence of a common receptor for Ang II and endothelin-1 (ET-1) (dual ET-1/Ang II receptor). This receptor seems to have two distinct binding sites, one for Ang II and the other for ET-1 [60].

The AT_1 Receptor

The AT_1 receptor, responsible for almost all the effects of Ang II [26, 27, 56–58, 61, 62], has been characterized pharmacologically and cloned in different species [63]. It has been identified in several adult tissues such as blood vessels, heart, kidney, adrenal gland, liver, brain, and lung [14, 26–28, 51, 64]. Several selective non-peptide antagonists of the AT_1 receptor have been developed, such as losartan and candesartan, which are used clinically for the treatment of hypertension [64–70]. In humans, the gene encoding the AT_1 receptor is located on band q22 of chromosome 3 [71, 72].

Unlike humans, rodents have two subtypes of the AT_1 receptor (AT_{1A} and AT_{1B}), encoded by two different genes, located on chromosomes 17q12 and 2q24 [73]. The properties of the AT_{1B} subtype are similar to those of the human AT_1 receptor [56]. The AT_1 receptor is a member of the large family of so-called "serpentine" receptors with seven transmembrane domains coupled to the G protein [40]. It consists of 359 amino acids and has three glycosylation sites, eight phosphorylation sites, and six cysteines. In addition, two disulfide bridges maintain the three-dimensional structure of the receptor [56].

The AT_1 receptor has been identified in human aortic VECs (Fig. 4.3) and VSMCs [28, 48, 49], human EECs (Fig. 4.2) [74], rat aortic and coronary VECs [75, 76], bovine aortic VECs [77], and human umbilical vein VECs [78]. Moreover, the AT_1 receptor relays the majority of the biological actions of Ang II in ECs. Indeed, Ang II induces, via the AT_1 receptor, the proliferation of bovine aortic VECs [77], the increase of PLC and PLA2 in rat aortic VECs, and the modulation of intracellular Ca^{2+} in human VECs [28], VSMCs [41, 48, 49], EECs [51, 74], and umbilical artery VECs [79].

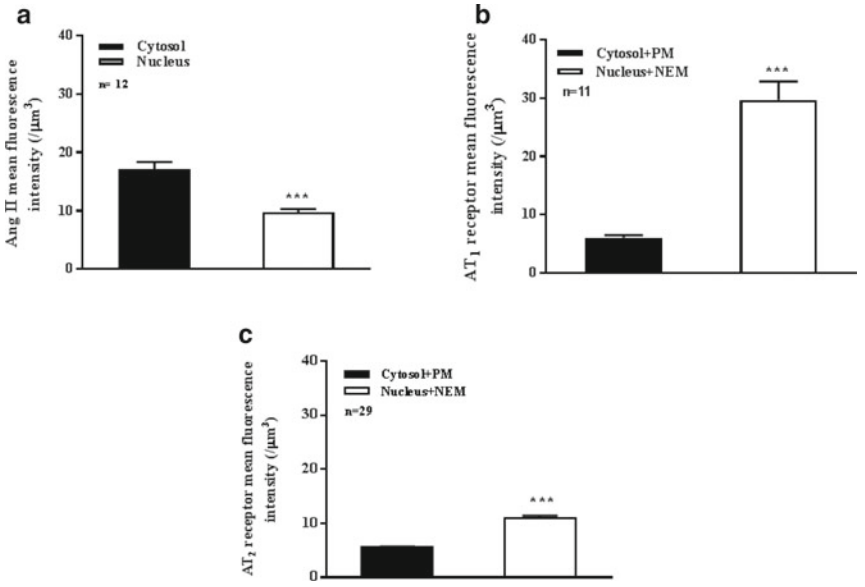

Fig. 4.3 In vascular endothelial cells, the presence of Ang II in the cytosol and the nucleus (**a**) as well as its receptors AT_1 (**b**) and AT_2 (**c**). The relative density of Ang II in the nucleoplasm is lower than in the cytoplasm (**a**). However, the relative density of its receptors AT_1 (**b**) and AT_2 (**c**) is higher in nuclear envelop membranes compared to the plasma membrane. The results are presented as mean ± SEM and n is the number of cells from at least three different experiments. ***$P < 0.001$ (Reproduced with permission from Kamal and al. 2017)

Signaling Pathways Induced by the AT_1 Receptor

The AT_1 receptor exists in two isomeric forms: active and inactive, the active form being stimulated by Ang II binding [80–83]. A kind of equilibrium exists between the active and inactive forms, and any change in this equilibrium allows the coupling of the receptor to different signaling pathways [81].

Following Ang II binding, the AT_1 receptor activates five classical signaling through G-protein coupled receptors (GPCRs) pathway: 1-Activation of Gq protein [82, 83] leading to activation of phospholipase C (PLC), which in turn induce the hydrolysis of phosphoinositides to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [82, 83]. Increased intracellular levels of IP_3 induced release of intracellular Ca^{2+} from the endoplasmic/sarcoplasmic reticulum (ER/SR) [82–84], whereas DAG in the presence of intracellular Ca^{2+} activates protein kinase C (PKC). Increased intracellular Ca^{2+} activates several protein kinases such as Ca^{2+}-calmodulin kinase (CaMK) and transcription factors that modulate the synthesis and/or release of pro- and anticoagulants, growth factors, and vasoactive substances [82, 83, 85, 86]. The activation of CaMK stimulated myosin light chain kinase (MLCK) in the vascular smooth muscle, which induces contraction leading to vasoconstriction and hypertension; 2-Activation of the mitogen-activated protein kinase (MAPK) pathway, which is involved in cell proliferation and differentiation via activation of c-fos, c-myc and c-jun [82, 87]. By activating a phosphorylation cascade involving signal activators and transducers of transcription-1 (STAT1) [82, 83, 88], Janus kinase 2 (JAK2) is able to translate a signal from the membrane surface to the nucleus, where it stimulates the transcription of different genes [82, 89]; 3-Activation of the Akt pathway, which inhibits glycogen synthase kinase 3 and stimulates p70S6 kinase (p70S6K) [82, 90]; and 4-Activation of the phospho-p90RSK (p90RSK) signaling pathway [82, 91].

Furthermore, AT_1 receptor activation stimulates membrane nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and the generation of reactive oxygen species (ROS) [83]. This latter inhibits endothelial nitric oxide synthase (eNOS), leading to endothelial dysfunction [89] and hypertension [83]. The AT_1 receptor can also dimerize with the bradykinin B_2 receptor in VSMCs, leading to an increase in the efficiency of Ang II in IP_3 production [92].

Internalization of the AT_1 Receptor

Several binding studies have demonstrated, in vivo, that Ang II binding to its receptor induces its internalization and sequestration (Fig. 4.4) [41, 93–96]. Specific internalization motifs in the 3rd intracellular loop and in the C-terminal tail of the AT_1 receptor have been identified [97–99]. Internalization occurs via clathrin vesicles through the interaction of the AT_1 receptor with members of the arrestin family [41, 100]. Internalization of the AT_1 receptor may also occur via the caveolin pathway [101] or via uncoated clathrin vesicles [102].

Indeed, Ang II-induced PLC activation is closely linked to AT_1 receptor internalization [103]. On the other hand, the internalization of the AT_1 receptor controls

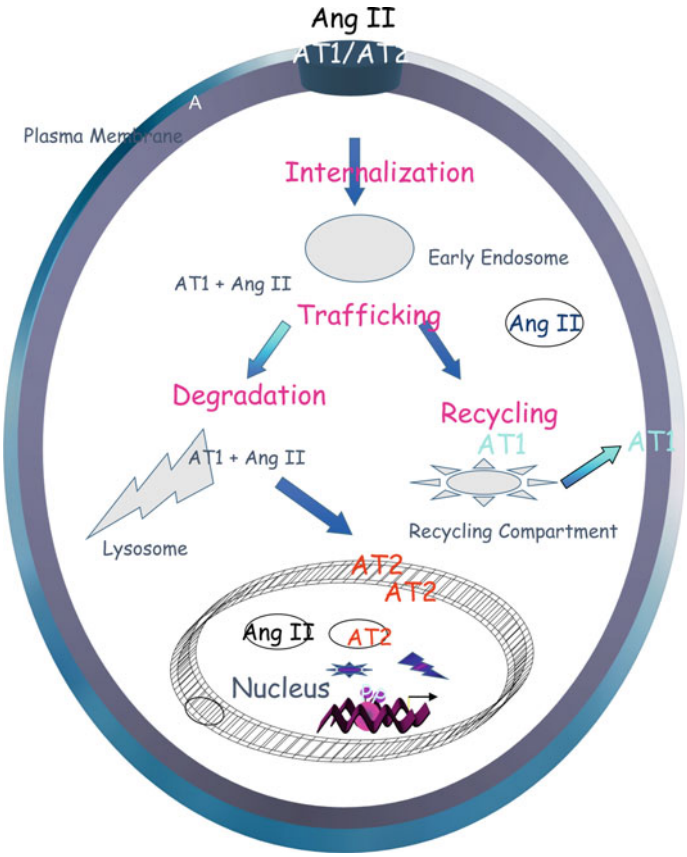

Fig. 4.4 Schematic of internalization of Ang II receptors AT_1 and AT_2 in human vascular smooth muscle cells (hVSMCs) showing their transcellular trafficking. In these types of cells, only AT_2 receptors are present at the nuclear envelope membranes level. AT_1 receptors of the internalized receptors with its ligand are degraded, leaving free Ang II, which binds to AT_2 receptors at the nuclear envelope membranes. AT_1 receptors are also recycled to the plasma membrane (Reproduced and modified with permission from Bkaily et al. 2003)

the number of receptors at the surface membrane and the elimination of Ang II via its degradation in lysosomes (Fig. 4.4) [104]. Studies have also shown that internalized AT_1 receptors are recycled to the surface membrane following ligand removal (Fig. 4.4) [104]. Finally, it has been suggested that internalization may be a way to accumulate Ang II within cells, which in turn activates cytosolic and nuclear AT_1 receptors (Figs. 4.4 and 4.5) [41, 105]. In human VSMCs, Ang II rapidly induces the internalization of the AT_1 receptor, which accumulates at the outer membrane of the nucleus and promotes activation of the nuclear envelop membranes receptors (Fig. 4.5) [41], more particularly AT_2 receptor, which is the main Ang II receptor present at the nuclear level of human aortic VSMCs (Fig. 4.4) [48, 49]. In both human VECs and EECs (Fig. 4.3) as well as VSMCs, Ang II and its receptors, AT_1, and

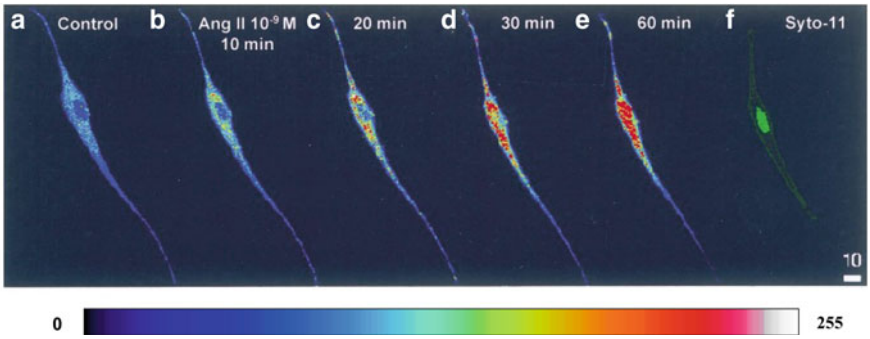

Fig. 4.5 Using hAT_1R-GFP expression and real 3D confocal microscopy showing time-dependent internalization and nuclear translocation of AT_1 receptors upon stimulation with Ang II (10^{-9} M). Panel F shows nuclear labeling with Syto 11 (green color has no measurable meaning). The pseudocolor scale represents the hAT_1R–GFP fluorescence intensity from 0 to 255. In panel F, the green color has no measurable meaning. The white scale bar is in μm (Reproduced with permission from Bkaily et al. 2003)

AT_2, are present at the nuclear level [28, 49, 51]. The level of Ang II was lower in the nucleus compared to the cytosol in human VECs (Fig. 4.3). However, its level in human EECs, is similar in both the cytosol and the nucleoplasm [51]. These results demonstrate that Ang II is present near its receptors, whatever is its localization, including the nuclear envelope membranes. For AT_1 and AT_2 receptors in VECs and EECs, their density is higher in the nuclear envelop membranes when compared to the plasma membrane [28, 51]. However, in human aortic VSMCs, the AT_2 receptors are limited to the nuclear envelope membranes (Fig. 4.4) [48].

AT_2 Receptor

Since its discovery in 1989, the AT_2 receptor has proved to be a real enigma [50, 106]. Its physiological functions are not been clearly defined till now [58]. The AT_2 receptor has been characterized and cloned in different species, including humans, rats, and mice [107, 108]. The AT_2 receptor was reported initially to be abundantly and exclusively present in fetal tissues [106, 109, 110]. In addition, the expression of the AT_2 receptor was reported to decrease considerably after birth, suggesting an important role for this receptor during development [40, 50, 106, 110]. However, in the cardiovascular system, this type of Ang II receptor was reported to be abundantly present in adult human VECs and VSMCs [28, 48, 49]. However, its membrane localization and density depend on the cell type [48, 49]. It was also reported to be present in adult cell types but at a very low density [106, 109, 111–116]. It is also reported that its density may increase in some cardiovascular diseases such as hypertension, heart failure, cardiac fibrosis, stroke, renal diseases, type 1 and 2

diabetes, and atherosclerosis [106, 110]. Its effect was also reported to oppose that of AT_1 receptor activation [92, 106].

The gene coding for the AT_2 receptor is located on the X chromosome in humans and mice [117]. The AT_2 receptor has a high affinity for its antagonist PD 123,319 [118]. The AT_2 receptor does not have specific internalization motifs, which would explain why this receptor is not internalized following its stimulation with Ang II [104].

The AT_1 and AT_2 receptors share only 32–34% amino acid homology [106]. Unlike the AT_1 receptor, the signaling pathways relayed by the AT_2 receptor vary according to cell type [119].

Nuclear Targeting of Ang II Receptors

During the 1980s, several studies demonstrated the presence of Ang II binding sites in the nuclei of rat hepatocytes [120, 121] and rat spleen cells [120]. Some studies have succeeded in proving the presence of intracellular targets of action for Ang II. Indeed, studies have reported that Ang II increases RNA synthesis and is able to bind to chromatin and alter its solubility in isolated nuclei [122, 123]. During the 1990s, several studies targeting the characterization of these intracellular Ang II binding sites followed one another. Studies in rat hepatocytes have shown that the binding profile of Ang II to these receptors at isolated nuclei is different from that of Ang II binding to these receptors at the plasma membrane [105, 124]. However, these receptors resemble those at the surface membrane in their coupling to Gs proteins [105]. Moreover, these nuclear Ang II receptors are functional [41, 125]. Indeed, their stimulation by Ang II induces an increase in Angiotensin-0 and renin mRNA levels in isolated rat hepatocyte nuclei [41]. On the other hand, Haller and his collaborators have demonstrated that, in VSMCs, Ang II injected into the cytoplasm induces, via its binding to specific intracellular receptors, the increase of intracellular Ca^{2+} [126]. Our group has also identified functional nuclear receptors for Ang II in ventricular cardiomyocytes [49]. Their activation induced a transient increase in nucleoplasmic Ca^{2+} (Fig. 4.6).

In 1998, LU and colleagues established that the AT_1 receptor has a nuclear localization sequence (NLS) in its cytoplasmic tail and that Ang II induces a dose-dependent time-dependent nuclear targeting of this receptor in brain neurons [127]. These same studies also demonstrated that, unlike the AT_1 receptor, the AT_2 receptor is not translocated to the nucleus following its stimulation by Ang II [127]. Moreover, the AT_2 receptor does not possess an SLN [128, 129].

Finally, our group and other laboratories have demonstrated the nuclear translocation of the AT_1 receptor coupled on the C-terminal side to a fluorescent protein, green fluorescent protein (GFP), in Chinese hamster ovary cells [130], and in VSMCs isolated from adult human aortas (Fig. 4.5) [41]. Stimulation of Ang II receptors induced a dose-dependent increase in cytosolic and nuclear Ca^{2+} in both human VSMCs (Fig. 4.7a) and VECs (Fig. 4.7b). The EC_{50} of Ang II-induced increase

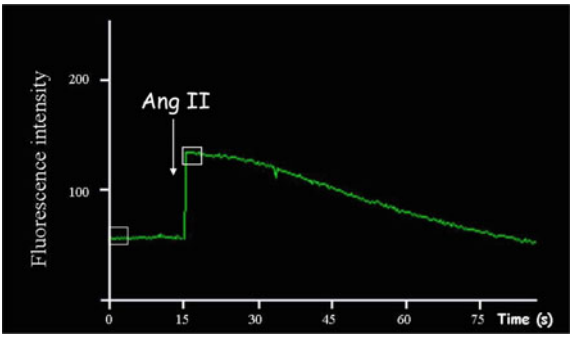

Fig. 4.6 In isolated nuclei, Ang II 10^{-11} mol/L rapidly induced an increase in nucleoplasmic calcium. (Reproduced with permission from Bkaily et al. 2009)

in cytosolic Ca^{2+} is relatively lower at the plasma membrane (2×10^{-11} M) when compared to its effect at the nuclear level (8×10^{-11} M) (Fig. 4.7a). However, in VECs, the EC_{50} of Ang II-induced increase of intracellular Ca^{2+} was similar at the plasma membrane level compared to the nucleus (Fig. 4.7b). The effect of Ang II on cytosolic and nuclear Ca^{2+} reached a plateau at 10^{-10} M (Fig. 4.7b); however, in human VSMCs, the plateau of the effect of Ang II-induced increase in cytosolic and nuclear Ca^{2+} reached a plateau at 10^{-4} M (Fig. 4.7a). In addition, the EC_{50} of Ang II-induced increase in cytosolic and nuclear Ca^{2+} was far lower in VECs (4×10^{-14} M) when compared to the EC_{50} in VSMCs (near 4×10^{-11} M) (Fig. 4.7a). Thus, human VECs are more sensitive to Ang II compared to human VSMCs. In addition, the sensitivity to Ang II seem to depend in the cell type and this could be due to differences between cell types density of AT_1 and AT_2 receptors present at the plasma and nuclear envelop membranes.

Conclusion and Perspective

There is no doubt that Ang II and its AT_1 receptor are the most studied component of the cardiovascular system. Although the mechanism of action, as well as their implication in cardiovascular diseases, is well-documented, it is not surprising that the field of Ang II will continue to progress. The fact that an increase in Ang II is implicated in many cell disorders and remodeling demonstrates the importance of this octapeptide in physiology and pathology again. Its importance in the renin-angiotensin II-aldosterone system makes it difficult to generalize its actions. Its implication in the secretion of other cardiovascular active factors such as ET-1 should be better clarified. In addition, heterodimerization of AT_1-ET-1 receptors waits to be studied in depth. The most important aspect that should be pursued is to understand the role of AT_2 receptors in the regulation of the cardiovascular system. We should explore more in-depth the physiological antagonism of AT_2 receptors to AT_1 receptors.

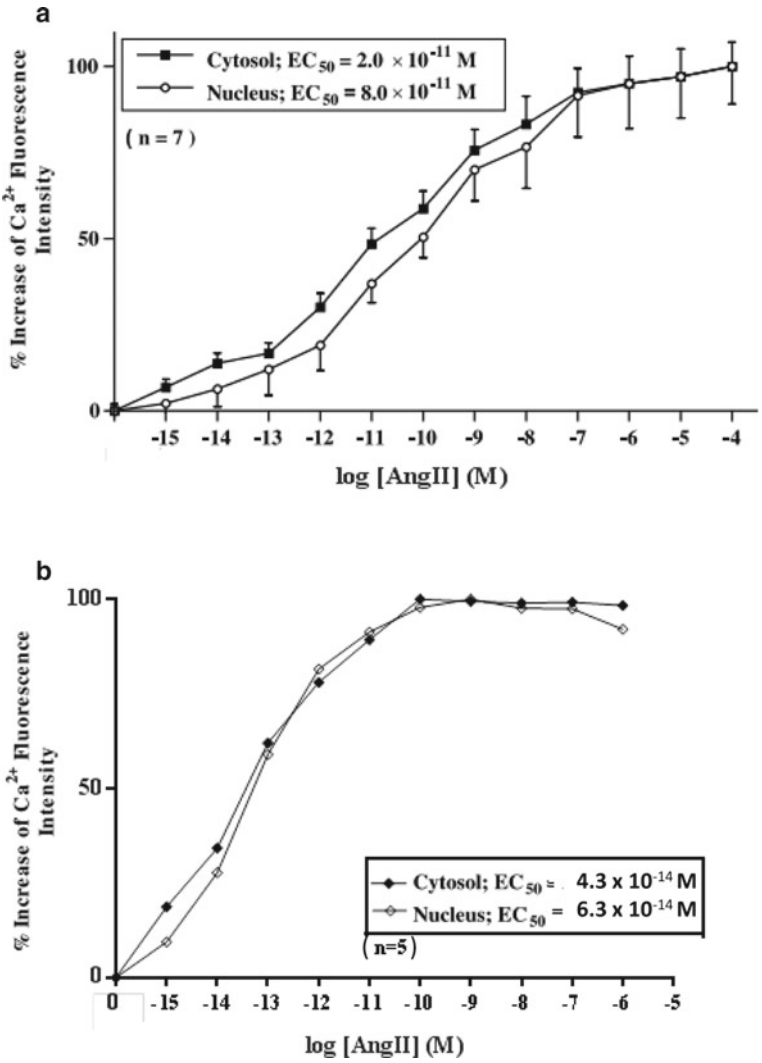

Fig. 4.7 Dose–response curves showing the effect of different concentrations of Ang II on cytosolic and nuclear calcium levels in human vascular smooth muscle cells (hVSMCs) (**a**) and vascular endothelial cells (hVECs) (**b**). As shown, the EC_{50} of Ang II is higher in hVSMCs (2×10^{-11} M and 8×10^{-11} M for the nucleus) (**a**) than in hVECs (4.3×10^{-14} M and 6.3×10^{-14} M for the nucleus) (**b**). Results are presented as mean ± SE (**a**) or ± SEM (**b**), and n is the number of cells of at least three different experiments. (Reproduced and modified with permission from Bkaily et al. 2003 (**a**) and from Kamal et al. 2017 (**b**))

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Chapter 5
Epigenetic miRNA Mediated Regulation of RAS in Cardiovascular Diseases

Aylin Caliskan, Samantha A. W. Crouch, and Seema Dangwal

Abstract The renin angiotensin system (RAS) is well-known for its function in blood pressure regulation and its association with numerous cardiovascular diseases (CVDs). Dysregulation of the RAS can result in hypertension, subsequently promoting cardiovascular disorders, including hypertrophy, cardiac fibrosis, and heart failure. During the Coronavirus disease 2019 (COVID-19) pandemic, further functions of the RAS came to attention, as it was associated with the viral entry. Moreover, the RAS has always been of great research interest due to its importance in physiology. Advances in research have revealed that in addition to the canonical RAS, several organs, for instance, the heart, appear to have their own local RAS. Furthermore, technical advances have led to the discovery of new RAS components and a greater understanding of their interactions and epigenetic regulation. Several mechanisms are associated with epigenetics, including histone modification, DNA methylation, and non-coding RNAs (ncRNAs) such as microRNAs (miRNAs). The role of epigenetic modifications and miRNAs has been of great research interest since miRNAs and their possible functions were discovered. In addition to established laboratory methods, new methods such as next-generation sequencing and bioinformatics provide the necessary tools for finding novel miRNAs with therapeutic value as biomarkers of disease or potential medication. Thus, we aim to give a brief overview of RAS-related miRNAs and their impact on CVDs.

Keywords Renin angiotensin system · miRNAs · Epigenetic regulation · Therapeutic targets · Heart failure · Hypertrophy · Fibrosis · Hypertension · Cardiovascular disease · COVID-19

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Introduction

The renin angiotensin system (RAS) is a widely studied homeostasis regulator that mainly regulates blood pressure through electrolyte balance, impacting multiple organs including the heart and vessels. The major pathophysiological effects due to disturbances in RAS lead to blood pressure irregularities, fibrosis, and inflammation in the cardiovascular system [1]. RAS can be divided into classical and local tissue-specific RAS [2, 3]. The Coronavirus disease 2019 (COVID-19) pandemic highlighted the pathophysiological importance of RAS due to the interaction of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [4].

Classical RAS: Mechanism of Action

Classical RAS has been described as an endocrine system with protective or deleterious physiological effects, for instance, anti- and pro-inflammatory effects [1–3, 5]. The protective components include the axis of angiotensin-converting enzyme 2 (ACE2), heptapeptide angiotensin (1-7) (Ang 1-7), and Mas receptor (MasR, short for MAS1 proto-oncogene G-protein-coupled receptor [6]), and the deleterious ones are angiotensin-converting enzyme (ACE), angiotensin II (Ang-II), and Ang-II type 1 receptor (AT_1R) [1] (Fig. 5.1).

In systemic RAS, the precursor of this regulatory cascade is the non-inhibitory plasma serpin, angiotensinogen (AGT), produced by the liver. The product of the AGT is the amino terminus decapeptide angiotensin I (1-10) (Ang-I), which is released after cleavage in the plasma by the specific aspartyl-protease, renin [2, 7]. Renin was detected as a key regulator of the RAS and is produced by juxtaglomerular cells in the kidney in response to changes in blood pressure and the volume and content of the extracellular fluid [8]. Therefore, this step is assumed as the rate-limiting step in this cascade [3]. Subsequently, Ang-I is cleaved by ACE, synthesized in the lung, to Ang-II, and binds to AT_1R. This activation is indicated by water intake and Na^+ perpetuation [8, 9] and is associated with vasoconstricting, fibrotic and inflammatory effects, and cancer progression [1]. Additionally, Ang-I and -II can also be cleaved by ACE2 to the nonapeptide angiotensin (1-9) (Ang 1-9) and Ang 1-7. The binding of Ang 1-7 to the MasR exerts protective effects like vasodilating, anti-fibrotic and anti-inflammatory effects [1]. The local tissue RAS regulates multiple tissues and organs and can be synergistically or independent of the classical RAS [3, 4].

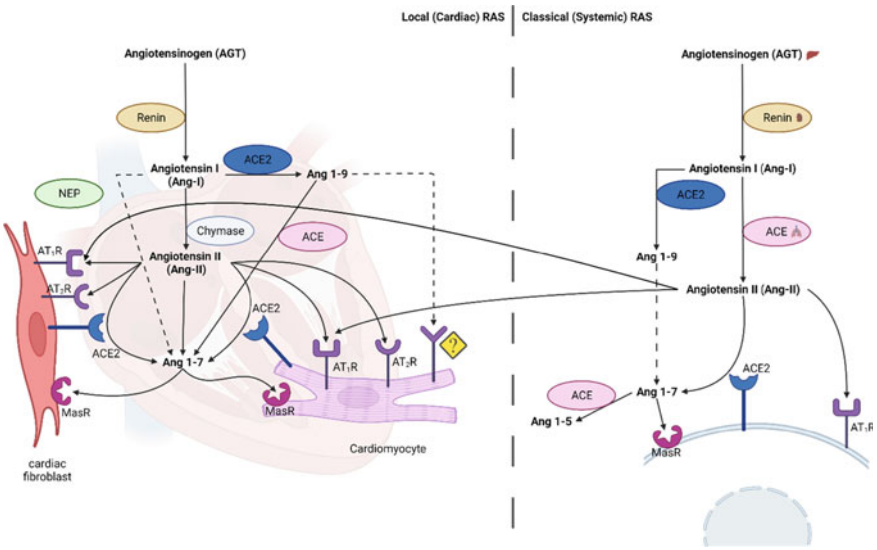

Fig. 5.1 Synergistic regulation of the cardiac (left) and the systemic (right) RAS mechanism. The figure depicts the most relevant elements of both systematic and cardiac RAS. Renin and angiotensinogen (AGT) form angiotensin I (Ang-I), which is subsequently cleaved to angiotensin II (Ang-II), binding to the vasoconstricting, pro-fibrotic, pro-hypertensive, and pro-inflammatory angiotensin II type 1 receptor (AT_1R). This mechanism is counteracted via ligands (e.g., Ang 1-7 via ACE2) binding to the cardioprotective receptors AT_2R and MasR. In the cardiac RAS, chymase cleaves Ang-I, which is done by ACE in the systemic RAS. Alternate pathways for generating Ang 1-7 include the cleavage of Ang-I by neprilysin (NEP) and of Ang 1-9 by ACE2. Ang 1-7 (binding to the cardioprotective Mas Receptor) and (membrane-bound) ACE2, ACE angiotensin-converting enzyme, ACE2 angiotensin-converting enzyme 2, AGT angiotensinogen, Ang-I angiotensin I, Ang-II angiotensin II, Ang 1-5 angiotensin (1-5), Ang 1-7 angiotensin (1-7), Ang 1-9 angiotensin (1-9), AT_1R Ang-II type 1 receptor, AT_2R Ang-II type 2 receptor, MasR Mas receptor, NEP neprilysin. Figure created with BioRender.com

The Role of Cardiac RAS in the Heart-Tissue

The ability of the cardiac cells to synthesize renin and AGT in the nuclei paves the way for independent regulation of RAS. The regulation of the cardiac RAS occurs over the cell signaling mechanisms such as paracrine, autocrine, and intracrine systems [10].

The downstream pathway of cardiac AGT to the Ang-I and Ang-I to Ang 1-7 by cleavage of ACE2 in the cardiac RAS is similar to the classical RAS (Fig. 5.1) [10].

Synthesis of Ang-II is regulated by cleavage of Ang-I by an alpha-form of chymase. It was previously reported that the alpha-form of chymase has a 20-fold higher catalytic activity to form Ang-II [10]. The binding of Ang-II on AT_1R or Ang-II type 2 receptor (AT_2R) on the cardiac fibroblast or cardiomyocyte can trigger certain effects in the cardiac system. The responses differ when the two receptors are activated. Activation of AT_1R is accompanied by vasoconstricting, fibrotic and

inflammatory effects. AT_2R, by activation, enhances the vasodilating and anti-fibrotic and anti-inflammatory effects [11]. The cleavage of Ang-II by ACE2 to Ang 1-7 is also related to the classical RAS. The acting of Ang 1-7 on MasR of the cardiac fibroblast and cardiomyocyte affects vasodilation and goes hand in hand with anti-fibrotic and anti-inflammatory effects [11]. Alternative pathways are a cleavage of Ang-I by neprilysin (NEP) to produce Ang 1-7 or Ang 1-9, which results from of Ang-I cleavage by ACE2 triggering an effect over an unknown receptor of the cardiomyocyte [10]. Trask and Ferrario concluded that the cardiac RAS is a synergistic system and enables the regulation of the physiological processes when these become imbalanced [10]. The cardiac RAS is supported by the circulating renin and Ang-I, which originates from the classic RAS.

For a healthy heart, this system must work in balance. Imbalance can result in cardiovascular diseases (CVDs), such as heart failure (HF) or atrial fibrillation (AF) [12, 13].

Epigenetics and RAS

The term "epigenetics" has been known since 1942 and was first used to describe how the phenotype of an organism is produced by its genotype during its development [14].

This definition has been redefined several times, reflecting the growing knowledge and research progress [14]. Today, several mechanisms are associated with epigenetics, such as histone modification, DNA methylation, and non-coding RNAs (ncRNAs) [14–17]. Epigenetic changes do not alter the DNA permanently but are a dynamic response to external influences and environmental factors [18]. Epigenetic changes that the individual lifestyle has caused can be reversible [19]. For instance, lifestyle changes such as abstaining from alcohol or reducing weight can reduce hypertension [19].

Permanently high blood pressure in hypertension can lead to several severe cardiovascular disorders, including HF [19]. As the RAS regulates blood pressure, it has been extensively studied to understand its function and regulation as well as to find possible treatments and therapies for its dysregulation [20].

Here, we will focus on epigenetic regulations of the RAS in cardiac disease with a particular focus on small non-coding RNAs: microRNAs (miRNAs/miRs). The role of miRNAs has been of great research interest since miRNAs and their possible functions were discovered. Despite most of the genome being transcribed into RNA, about 98% of the genome does not code for proteins. Therefore, it was assumed that two types of RNAs exist: RNAs with coding potential i.e. mRNA and RNAs without so-called ncRNAs [21], which were initially disregarded due to their perceived lack of function [16, 21]. Although these ncRNAs do not encode proteins, they are still important for biological processes as they are involved in fine-tuning genomic interactions with environmental factors [16]. If these regulatory RNA transcripts are dysregulated, they can affect the expression of functional

proteins and their physiological balance, resulting in pathologies such as cardiovascular disorders, including diabetes and HF [16]. MiRNAs are a subgroup of the short (<200 nucleotides) ncRNAs. They are very short (ca. 22 nucleotides), endogenous, single-stranded, and highly conserved [22]. By binding to protein-coding messenger RNA (mRNA) transcripts [16, 22] and thereby repressing gene translation by cutting the mRNA or blocking the mRNA [16], miRNAs can influence biological processes [22]. Due to the correlation of miRNA expression, stress and pathologic conditions [21, 22] and their presence in several body fluids, including blood and plasma [21], miRNAs are possible diagnostic markers and therapeutic targets [16, 23].

Epigenetic miRNA Regulation of RAS in Cardiovascular Diseases

Since miRNAs have garnered interest as biomarkers, therapeutic targets, and novel therapeutic approaches, several studies and trials have examined the role of miRNAs in diseases and their potential use as therapeutics [24].

For example, blood pressure is regulated by the RAS. Therefore, its components are therapeutic targets for treating hypertension. It has been revealed that the deregulation of miRNAs is also involved in hypertension and several cardiac diseases [25], including miRNAs that regulate the RAS [26]. Table 5.1 summarizes some of the miRNAs related to RAS components affecting CVDs. A graphical overview is shown in Fig. 5.2. These miRNAs and their role in the mentioned CVDs and the RAS will be discussed in the following.

Hypertension

Pulmonary arterial hypertension (PAH) is a subtype of pulmonary hypertension, a common disorder that is defined by high blood pressure in the mean pulmonary artery pressure (> 25 mm Hg) at rest [60]. The antiproliferative vasculopathy PAH mainly affects the small pulmonary arteries [60] and causes structural remodeling of the blood vessels due to an excessive cell proliferation combined with impaired apoptosis [61], leading to increased pulmonary vascular resistance [60], progressive right heart dysfunction [61], and HF [60]. The multifactorial disease systemic arterial hypertension (SAH) is associated with both genetic and epigenetic factors [62, 63] and is linked with the development of several other comorbidities, including diabetes [62] and other pathologies caused by high blood pressure. Furthermore, since chronic hypertension causes mechanical stress for the heart, the heart is trying to compensate, which leads to remodeling and left ventricular hypertrophy (LVH) [64], which can also favor the development of cardiac fibrosis [65] and can ultimately lead to HF [66].

Table 5.1 Overview of miRNAs interacting with RAS components in CVDs

RAS components	miRNA	Model	RAS related effect	References
Hypertension				
Renin	miR-181a	Patients with hypertension/BPH/2J mice	miR-181a negatively regulates renin	[27]
ACE	miR-136	Patients with primary hypertension	Inhibits ACE and decreases the expression of renin, Ang-II, aldosterone	[28]
ACE2	miR-421	Primary cardiac myofibroblasts/transformed cells	Downregulation of ACE2	[29]
ACE2, Ang (1-7), MasR, ACE	miR-143	Patients with hypertension	Negative regulation of ACE2/Ang-(1-7) signaling	[30]
Hypertension, hypertrophy				
ACE, ACE2, AGT, Ang-II, AT_1R, AT_2R	miR-483-3p	Vascular smooth muscle cells	Downregulation of ACE, ACE2, AGT and AT_2R miR483-3p is downregulated by Ang-II via AT_1R	[31]
Ang-II	miR-495	Mice	Ang-II induced expression of miR-410	[32]
Hypertension, hypertrophy, heart failure				
Ang 1-9, Ang-II, AT_1R	miR-129-3p	HEK293N cells	miR-129-3p mediates anti hypertrophic effects of Ang 1-9 Part of the Ang 1-9/AT_2R/miR-129-3p/PKA signaling axis	[33, 34]
Hypertension, heart failure				
ACE, Ang-II	miR-27a/27b	Rats/rat cardiomyocytes/mice	miR-27a/27b inhibit ACE expression	[35, 36]

(continued)

Table 5.1 (continued)

RAS components	miRNA	Model	RAS related effect	References
ACE, Ang-II	miR-145	Patients with hypertension/VSMCs/mice	Downregulation of ACE	[37]
Hypertension, hypertrophy, fibrosis				
Ang-II	miR-26a	Rats	Overexpression can inhibit Ang-II induced fibrogenesis	[38]
Ang-II, AT_1R	miR-29b	Cardiac fibroblasts/HEK293N/mice	Ang-II regulates miR-29b via AT_1R	[34, 39–42]
Hypertension, Fibrosis				
Ang-II	miR-let-7i	Mouse heart/cultured fibroblasts	Downregulated after Ang-II infusion, its downregulation is associated with fibrosis and cardiac inflammation	[43]
Hypertension, Hypertrophy, fibrosis, heart failure				
Ang-II, AT_1R	miR-132/miR-212	Rats/mice	Ang-II-induced expression, targets several Ang-II signaling genes	[34, 44, 45]
Ang-II, AT_1R, ACE2	miR-155	C57B1/6J mouse model/HUVECs	Ang-II induced cardiac fibrosis miR-155 regulates AT_1R	[31, 46–48]
Hypertrophy, heart failure				
AT_1R, Ang-II, ACE2	miR-208a/b	Rat/rat neonatal cardiomyocytes/H9c2 rat cells	Pro-hypertrophic miRNA Inhibits AT_1R	[49–51]
Hypertrophy, fibrosis, heart failure				
ACE2, Ang-II, ATG	miR-133a	Mice	Participates in the regulation of AT_1R	[52]
Fibrosis				
Ang-II, ACE, ACE2	miR-125b-3p	Ang-II treated cardiac fibroblasts	Regulated by Ang-II and regulating Ang-II via targeting ACE	[53–55]

(continued)

Table 5.1 (continued)

RAS components	miRNA	Model	RAS related effect	References
Ang-II	miR-146b	Rat cardiac ventricular fibroblasts	Regulated by Ang-II its targets are associated with cardiac fibrosis	[54]
Ang-II	miR-144	cardiac fibroblasts/transverse aortic constriction in mice	Regulated by Ang-II its targets are associated with cardiac fibrosis	[56]
Ang-II	miR-503	TAC mice/mice neonatal CFs	Ang-II-induced expression, its targets include Ang-II antagonists	[57, 58]
Ang-II	miR-22	Cardiac fibroblasts	Can reduce cardiac fibrosis by directly targeting TGFBR1 and attenuating Ang-II-induced TGF-β signaling	[59]

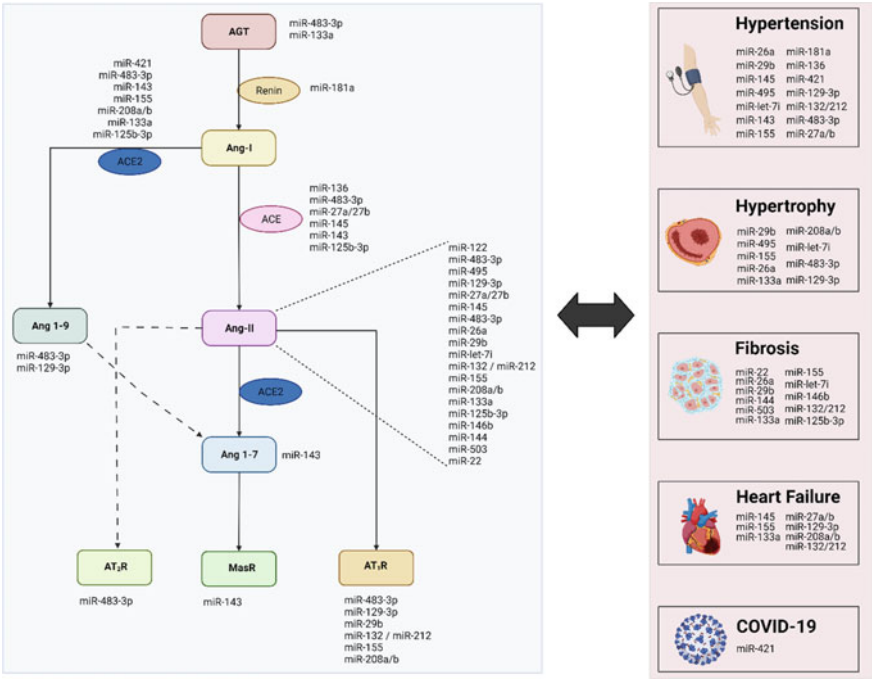

Fig. 5.2 A schematic overview of the RAS, how its components interact, and the miRNAs affecting the RAS. The miRNAs next to the RAS components either affect the respective component or are regulated by the component. Ang-II, in particular, can regulate various miRNAs that subsequently target other genes and affect other pathways. The red box on the right side gives a graphical overview of the miRNAs and diseases mentioned in the text. Figure created with BioRender.com

Chu et al. [28] investigated the role of miR-136 in essential hypertension. They discovered a negative correlation between miR-136 expression and ACE activity in patients with primary hypertension, indicating that the miRNA could inhibit the RAS-component ACE, which subsequently decreases the expression of Renin, Ang-II, and aldosterone (ALD) [28]. The downregulation of miR-136 in hypertensive patients is associated with elevated expression of RAS biomarkers and suggests its possible role as a biomarker for hypertension and its possible relevance for the progression of hypertension [28].

Another approach to finding therapeutically relevant miRNAs is by analyzing the sequence and the 3′-UTR of the components of interest. Due to the prediction that mRNAs of ACE, ACE2, angiotensinogen (AGT), and AT_2R all contain the target sequence of miR-483-3p, Kemp et al. evaluated the effect of the miRNA on these RAS components in vascular smooth muscle cells (VSMCs) [31]. They could prove the prediction and demonstrate that AGT is downregulated by miR-483-3p. The regulation of the four RAS components by miR-483-3p indicates that miR-483-3p might be a global regulator of RAS [31]. Correspondingly, miR-483-3p is down-regulated by Ang-II via the AT_1R receptor [31], activating the RAS. As VSMCs

are affected by Ang-II-mediated dysregulation in hypertension, vascular hypertrophy, and atherosclerosis, miR-483-3p might be a potential target for therapeutic intervention [31].

Besides miR-483-3p, miR-27a/27b, and miR-145 also inhibit the expression of ACE [30]. Both miRNAs also target putative binding sites in the three prime untranslated region of ACE transcripts [30]. By suppressing ACE, they inhibit the ACE/Ang-II/AT_1R-axis [30]. Additionally, the miR-27 family appears to be involved in hypertrophy and fibrosis, with miR-27a-3p being upregulated in cardiac hypertrophy and miR-27b having a potential anti-fibrotic role [35, 36].

In essential hypertension patients, miR-143 and miR-145 are dysregulated [30]. Both miRNAs, which are co-transcribed from the same gene [37], are important for maintaining vascular smooth muscle contractile differentiation [67] by activating Krüppel-like factor4 (Klf4) and Klf5 [30, 67] While miR-145 is involved in the downregulation of ACE, miR-143 appears to be involved in the negative regulation of ACE2 and thus ACE2/Ang-(1-7) signaling [30].

ACE2 expression is also downregulated by miR-421. Both miR-483-3p and miR-421 appear to decrease ACE2 expression via translational repression [30].

The RAS is also affected by Renin expression, which is also associated with several miRNAs.

Some of these miRNAs are involved in several heart pathogenies. For example, the miRNA miR-181a post-transcriptionally negatively regulates renin mRNA in vitro and is reduced in hypertensive patients [27]. Therefore, it has been associated with elevated blood pressure and RAS activity. However, as it appears to regulate the renal RAS via the sympathetic nerves, it might play a more complex role, highlighting the importance of miRNAs in cardiovascular research [27]. Furthermore, RAS components can affect miRNA expression, which can influence the RAS or RAS-related diseases [44] as well as subsequent pathways also involved in cardiovascular disorders.

Examples of RAS-regulated miRNAs are miR-132 and miR-212, which are regulated by the Ang-II-regulated cAMP response element binding protein and transcribed together [44]. Chronic administration of Ang-II in rats induced sustained hypertension, which resulted in hypertrophic and fibrotic changes of the heart as well as differentially expressed miRNAs, as a study by Eskildsen et al. [44] indicated. Eskildsen and colleagues noticed 50 differentially expressed miRNAs after ten days of continuous Ang-II administration, which had not been differentially expressed in acute hypertensive rats, who had only had 4hs of Ang-II [44].

Both miR-132/212 were significantly upregulated in the left ventricle and the kidney of Ang-II-induced hypertensive rats, and miR-131 was also upregulated in the plasma, indicating a regulatory function of miR-132/212 [44]. The team also repeated a similar experiment in mice. However, miR-132/212 was not significantly dysregulated in their mouse strain, which can be attributed to different Ang-II in the two species [44]. This highlights that possible differences in blood pressure regulation between species and different models need to be kept in mind.

Although it was initially assumed that miR-132/212 directly targets AT_1R, further analysis revealed that the two miRNAs have a fine-tuning function by affecting

several genes including the Ang-II-signaling-related genes, including AGTR1, cJUN, EGR1, JAK2, PKC, and SOD2, which are involved in hypertrophy and fibrosis [45].

An example for RAS-regulated miRNAs are miRNAs that are affected by Ang-II expression. For instance, Ang-II regulates miR-29b and miR-129 in a Gαq/11-signaling Mek1/Erk1/2-dependent manner in HEK293 cells via the AT_1R [34]. While miR-29b is preferentially expressed in fibroblasts, miR-129-3p is expressed in cardiac myocytes. Like miR-212, miR-129 is upregulated in the final stage of HF due to dilated cardiomyopathy [34]. After myocardial infarction, miR-29b inhibits collagen expression. In addition, miR-29b is also upregulated in dilated cardiomyopathy [34]. This also indicates the close relation between hypertension and other cardiovascular disorders such as HF and pathologies such as hypertrophy and fibrosis.

Myocardial Remodeling: Cardiac Hypertrophy and Fibrosis

High blood pressure can lead to myocardial remodeling [62], a stress response resulting in pathological changes in the heart [62, 68].

Cardiac Hypertrophy

A major response to stress stimuli, such as pressure or volume overload or a prior heart infarction [69], is cardiac hypertrophy [68], which describes several changes in the cardiomyocytes, including increased cell size [69] and results in thickening of the ventricular wall [68]. LVH is induced by the mechanical stress during SAH and results in a reduced volume of the cardiac chamber [62] and is associated with chronic RAS activation [70]. In addition, it has been shown that hypertrophic growth is associated with several cardiac pathologies [68, 69] and finally results in HF [69].

The RAS plays a central part in cardiovascular remodeling and hypertrophy [71] as it is involved in hypertrophic signaling, which is also negatively and positively modulated by miRNAs [68, 71]. The first pro-hypertrophic miRNA, miR-208a, was found in 2007 [16].

Since then, numerous new miRNAs have been found, and with ongoing research, the knowledge about miRNAs is constantly growing. Some of these miRNAs are regulated via the RAS. Ang-II, for instance, can increase the expression of miR-410 and miR-495, which are involved in the promotion of hypertrophy [62]. The angiotensin II receptor type 1 (AT_1R) is also stimulated by miRNAs, making the respective miRNAs particularly relevant for RAS-mediated cardiovascular effects [71].

Since hypertrophy is also a long-term effect of hypertension, many of the miRNAs involved in hypertension can also be associated with hypertrophy. One example is the Ang-II stimulated miR-483, which targets many components of the RAS, including AGT, the AT_2R, and the enzymes ACE and ACE2 [31].

Other miRNAs are involved in several pathogenies, such as miR-155, associated with inflammatory response, hypertrophy, and fibrosis. A C57Bl/6J mouse model comparing the effect of Ang-II infusion on miR-155$^{-/-}$ and wildtype mice demonstrated that miR-155 deletion alleviates Ang-II-induced cardiac fibrosis. Mice with miR-155 deletion had restricted inflammatory responses, improved cardiac function, and decreased heart size, as well as reduced myocardial fibrosis compared with their wildtype littermates. These results and in vitro experiments demonstrating that the overexpression of miR-155 in cardiac fibroblasts leads to increased fibroblast to myofibroblast transformation indicate a potential therapeutic use of miR-155 inhibition in treating cardiac fibrosis [46]. Upregulation of miR-155 is also associated with immune activation, viral infections, and cancer. Additionally, it is known that inflammatory cytokines such as TNF-α, TGF-β1, IL-1β, and INF-γ upregulate miR-155 expression [72].

Besides regulating inflammatory cytokines, miR-155 also regulates angiotensin II type 1 receptor expression [48] and could inhibit Ang-II induced hypertrophy by preventing its effects [47]. However, the components of the classical RAS are not the only RAS-related factors affecting CVDs. Ang 1-9, which is part of the non-canonical, cardioprotective RAS, can also affect cardiovascular health due to its anti-hypertrophic effects which have been shown in vitro and in vivo [33]. As part of the Ang 1-9/AT_2R/miR-129-3p/PKA signaling axis, miR-129-3p mediates the anti-hypertrophic effect of Ang 1-9 [33].

The miR-125 family participates both directly and indirectly in several cardiovascular and cerebrovascular disorders, including ischemia-reperfusion injury, myocardial ischemia, and HF, and is therefore of great clinical interest [73]. The expression of miR-125b has been connected to heart injury and remodeling and appears to rise significantly in patients with HF [73]. However, some of the miR-125 family members appear to have two sides: Regulating cardiac fibrosis and the transition of fibroblasts to myofibroblasts by targeting apelin [55], miR-125b can also protect cardiomyocytes by inhibiting inflammatory response and apoptosis [73]. In a mouse model, miR-125b deficiency was also connected to cardiac hypertrophy [53].

Cardiac Fibrosis

Cardiac fibroblasts are essential cells of the heart as they are involved in providing a structural scaffold for the heart muscle cells, the cardiomyocytes [74]. Upon heart injury, cardiac fibroblasts get activated, transform into their myofibroblast phenotype, create a profibrotic environment and contribute to the repair process by adding fibrotic scar tissue to maintain the structural integrity of the heart [68, 74]. In addition, persistent fibroblast activation leads to an accumulation of fibroblasts and subsequent cardiac remodeling [68]. Two forms of fibrosis are known, replacement fibrosis after injury, for instance after myocardial infarction, and fibrosis due to volume overload, e.g., in hypertension [74].

Members of the RAS can affect fibroblasts, and chronic RAS activation has been associated with cardiac fibrosis, among other disorders [70]. Ang-II, for instance,

triggers the over-expression of several miRNAs, which subsequently promote fibrosis [62]. Ang 1-7, which belongs to the "protective arm" of the RAS, counteracts this effect as it also inhibits fibrosis [71]. In cardiac fibroblasts, Ang-II upregulates miR-125b-3p and several other miRNAs, such as miR-132 and miR-146b [54]. In a neuroblastoma mouse model, miR-125b-3p was found to be regulated by p53; in Ang-II-treated cardiac fibroblasts, it appears to be regulated by Ang-II and regulating Ang-II via targeting ACE [54].

The Ang-II-mediated miR-133a, which is also involved in regulating cardiac hypertrophy [75], is directly linked to fibrosis as it targets collagen 1a1 (Col1A1) and connective tissue growth factor (CTGF) [16] as well as other pro-fibrotic factors such as SRF [76, 77] and RhoA [76]. In addition, miR-133a participates in the regulation of AT_1R and the RhoA/SRF/CTGF axis [78], and downregulation of miR-133a by Ang-II increases Col1A1 [75] and CTGF [79], promoting fibrosis. As CTGF inhibition can reverse fibrosis [80], CTGF inhibitors are an attractive therapeutic approach for treating fibrosis.

Col1A1 is also a target of miR-29b [75], a cardio-protective miRNA [42] that is a member of the miR-29 family, which is known for its involvement in fibrosis [39]. Upon Ang-II-stimulation, the miRNA is upregulated in HEK293N cells [34]. However, Ang-II seems to regulate miR-29b expression in a tissue-dependent manner. While Ang-II increases miR-29b expression in cardiac fibroblasts, it does not affect miR-29b expression in myocytes [34]. With many targets involved in fibrosis, such as matrix metalloproteinases, collagens, pentraxin 3, insulin-like growth factor 1, and leukemia inhibitory factor [42], miR-29b also exerts an anti-fibrotic function [42].

Other miRNAs mediated by Ang-II and impacting cardiac fibrosis are miR-29a, miR-let-7i, mir-122, miR-144, and miR-155. In spontaneously hypertensive rats, miR-26a is downregulated, associated with myocardial fibrosis, while miR-26a overexpression can inhibit myocardial fibrosis and Ang-II-induced fibrogenesis [38].

In mouse heart and cultured fibroblasts, miR-let-7i is downregulated after Ang-II infusion, while increased miR-let-7i attenuates Ang-II-induced fibrosis and cardiac inflammation by targeting interleukin-6 (IL-6) and collagens [43]. This indicates that miR-let-7i is an important regulator in Ang-II-induced cardiac inflammation and cardiac fibrosis and might be a target for therapeutic intervention [43].

Another promising therapeutic target is miR-122. MiR-122 might be a predictive biomarker indicating adventitial injury and a potential therapeutic target in vascular remodeling and vascular disorders [81]. In hypertensive rats with vascular injury, the levels of miR-122 were elevated in the aortic adventitia. Ang-II decreases the expression of sirtuin 6 (SIRT6), elabela (ELA), and ACE2, which can be rescued by inhibiting miR-122-5p. Additionally, the inhibition of miR-122-5p has anti-apoptotic, anti-oxidant pro-autophagic effects via activating SIRT6-ELA-ACE2 signaling.

A study performed by Lui et al. (2020) demonstrated that miR-144 expression is downregulated upon pathologic stimuli such as Ang-II administration to cardiac fibroblasts or transverse aortic constriction in mice [56]. Administration of miR-144, which directly targets cAMP response element-binding protein (CREB), affected the

cardiac fibroblast activation resulting in a decrease in proliferation and fibroblast-to-myofibroblast transformation [56].

In mice, miR-22 is downregulated after myocardial infarction (MI) which is accompanied by collagen deposition [59]. In cardiac fibroblasts, overexpression of miR-22 reduces collagen deposition and can negatively regulate Ang-II-induced cardiac fibrosis by abrogating Ang-II-induced collagen deposition [59]. Since miR-22 can reduce cardiac fibrosis by directly targeting TGFBR1 and attenuating Ang-II-induced TGF-β signaling, it is a possible therapeutic target [59].

Another potential therapeutic target is miR-503, which can promote Ang-II-induced cardiac fibrosis [57]. It targets the cardioprotective apelin, which is known for antagonizing the Ang-II-induced activations of CTGF and TGF-β [57]. Apelin inhibition by miR-503 is also involved in diabetic angiopathy, as it has been linked with enhancing inflammation and oxidative stress in high-glucose-induced injury of microvascular endothelial cells [58]. Furthermore, miR-503 is being investigated as a potential diagnostic marker or therapeutic target in cancer or CVDs [82].

Heart Failure

HF is the cause of death in more than a third of all cardiovascular disease related deaths [70] and has a 5-year survival rate of approximately 53% after diagnosis [83]. In addition, it is the end stage of several other CVDs [70], such as metabolic disorders and hypertension [16], and often a result of hypertrophy and fibrosis [16]. However, since the term "heart failure" is a rather general description of a complex disorder, it is often further classified by considering the underlying physiological impairment: (i) HF with reduced ejection fraction (HFrEF) and (ii) HF with preserved ejection fraction (HFpEF) [70].

HF with preserved ejection fraction (HFpEF) was erroneously assumed to be a pre-stage of HFrEF [70]. However, it is a separate disorder that requires its own therapies [70] and is associated with different comorbidities than HF with reduced ejection fraction (HFrEF). In HFpEF, which is often accompanied by non-cardiac comorbidities such as obesity, type2-diabetes, hypertension, and renal failure, the diastolic function is impaired [70, 84].

The other, equally prevalent HF syndrome, HF with reduced ejection fraction (HFrEF) is a systolic HF [16, 70] linked to coronary heart disease and MI [70, 84]. Chronic activation of the RAS [70] is regarded as one of the hallmarks of HF [70], and the RAS is involved in regulating several processes connected with HF, such as hypertension and inflammation. Several members of the RAS system and the counterregulatory-RAS system have been found to play a role in HF [20]. Elevated plasma levels of soluble ACE2 in HFrEF patients indicate that the counter-regulatory RAS might be involved in a mechanism for attenuating cardiovascular dysfunction [20] and indicates a possible protective function of ACE2 [20].

Mice lacking MRGD (the receptor for alamandine) present with left ventricular remodeling and severe dysfunction, indicating a possible role in HF [20]. As the

progression of HFpEF is attributed to cardiac remodeling due to hypertension [20], the counter-regulatory RAS and its components, including ACE2, Ang 1-7, Ang 1-5, are possible biomarkers for HF and potential therapeutic targets [20].

Since the first deregulated miRNAs were found in failing hearts of both humans and rodents [16], miRNAs have been of great interest in HF research [16, 49, 85, 86].

The AT_1R is inhibited by miR-208 and miR-155, which appears to play a role in HF [87]. In rats with pressure overload induced HF, the anti-miR to miR-208a attenuated cardiac remodeling [51], indicating a possible therapeutic value.

Since hypertension and its sequelae, such as LVH, can ultimately lead to HF, the hypertension-related miRNAs are also of interest in the treatment of HF [86]. Both miR-208a and miR-208b expression have been evaluated as biomarkers for cardiac function and HF [50]. Significantly increased miR-208b expression has also been linked to an increased risk of death within six months after acute myocardial infarction [50]. In the context of epigenetics of the RAS in CVDs, miRNAs such as miR-21, miR-155, miR-132, miR-29b, miR-126, and miR-212 that regulate both LVH and the RAS [62] are of special interest.

MiR-133a, which belongs to the same transcriptional unit as miR-1 [76], is especially mentionable since it not only participates in systemic arterial hypertension (SAH) and LVH but is also involved in vascular remodeling and fibrosis [88].

COVID-19 and the RAS

In December 2019, COVID-19 was first observed in Wuhan [89]. In January 2020, Huang and colleagues reported pneumonia cases of unknown cause observed in patients sharing a history of exposure and laboratory results indicating infection with a novel coronavirus [89], which is now known as SARS-CoV-2.

The course of the infection varies in patients and can range from asymptomatic to mild to severe and life-threatening symptoms and death [89, 90]. In addition, the severity of the disease appears to be correlated with age or/and underlying comorbidities such as CVDs, hypertension, or diabetes [91]. It became apparent that SARS-CoV-2 can also cause cardiovascular disorders, including arrhythmias, acute coronary syndrome, thromboembolism, and myocardial injury [91]. Additionally, survivors of COVID-19 can develop cardiovascular sequelae and other long-term health implications, including pulmonary, mental, and neurological health complications [92]. The symptoms of this often "long COVID" termed post-COVID-19 syndrome can range from extreme fatigue and chronic cough to dyspnea and CVCs like myocarditis [93].

Using next-generation sequencing methods, the viral genome was revealed soon after the first reported disease outbreaks [94]. The sequence similarity to the SARS-CoV of 2002–2003 helped unveil the cell entry mechanism [91]. The viral spike (S) protein interacts with the membrane-bound ACE2, which serves as a gateway for the endocytosis entry. In this context, the RAS was identified as an important pathway and is associated with cardiovascular complications (CVCs) and immune response [91, 93].

The ACE2 endocytosis entry mechanism by SARS-CoV-2 results in a reduction of the ACE2 on the cell surface, causing an imbalance in the cardiac RAS and subsequent CVCs [93, 95]. Besides ACE2, which appears to be the key regulator of COVID-19 [96], other RAS-related factors and miRNAs are dysregulated during infection, as blood samples of critically ill patients indicate [97]. The high mortalities were associated with a virus-driven cytokine storm in which the normal function of the cytokine-mediated regulation of the immune response is deregulated. This is described as a potentially fatal systemic inflammatory syndrome. Related to RAS, Ang-II increase dependent on the ACE 2 downregulation by SARS-CoV-2, resulting in an activating of AT_1R whereby the NF-*k*B pathway is activated [93, 98]. As miR-421 might affect ACE2 via post-transcriptional regulation, it might be a potential target for therapeutic modulation of ACE2 expression [29], as ACE2 is part of the cardioprotective ACE2/Ang (1-7)/MasR axis [30].

Medication

Blood pressure has long been of interest, judging by the first publication regarding the topic in 1733 [99]. Since the first attempts to influence hypertension via medication in 1900 [99], the drugs used and the knowledge regarding their modes of action have greatly improved.

Today several therapeutics targeting miRNAs and RAS components are known. They include angiotensin II receptor blockers (ARBs) such as telmisartan and angiotensin-converting enzyme inhibitors (ACEIs) such as enalapril [100]. Some of these drugs also affect miRNA expression; miR-132, for instance, can be regulated by ARBs [44]. ACE inhibitors are common medications to reduce blood pressure. ACE2 decreases blood pressure by converting Ang-II to Ang 1-7 [101]. Therefore, ACE2 activators [102], as well as recombinant human ACE2 (rhACE2), are being researched for their potential to alleviate hypertension [103]. Additionally, ACE2 could be an early biomarker for hypertension, just like its product Ang 1-7 [104, 105]. RAS-inhibiting alamandine has also been found to have anti-hypertensive effects [106]. The receptors AT_1R, MasR, and AT_2R are further of interest as therapeutic targets [20] as AT_1R blockers (ARB) can lead to an increase in Ang 1-7 [107]. As the deregulation of miRNAs is also involved in cardiac pathologies, miRNAs are of interest as biomarkers and therapeutic targets [22]. A possible strategy for inhibiting miRNAs is using so-called miRNA sponges or decoys, which absorb the miRNA via a Watson-Crick base-pair-dependent interaction, reducing the amount of active miRNA [22].

Another potential treatment is administering anti-miRNAs, antisense oligonucleotides that specifically inhibit a certain miRNA, to inhibit miRNAs with known pathological effects. A study by Hinkel et al. [86] demonstrated the beneficial effects of miR-132 inhibition in a porcine pressure-overload-induced HF model. The administration of anti-miR-132 attenuated myocardial hypertrophy, indicating the therapeutic value of anti-miR-132 and its possible clinical use [86].

The COVID-19 pandemic also highlighted the need to prevent and combat CVDs [108]. SARS-CoV-2 enters the cell by binding to ACE2, which can be upregulated by RAS-inhibitors [109]. Due to the higher mortality of COVID-19 patients who were also treated for hypertension [110], the question arose whether RAS-blockers affect the severity of the course of the disease [109–111]. The ACEI-COVID trial [109] and other studies [111], as well as meta-analyses [112, 113] were conducted to answer this question, and the results indicated no significant differences in the disease outcomes [109, 111–113], but was shown to be harmful to patients receiving the respective medications [92].

Due to the advances in technology, it is also possible to predict promising therapeutic targets and likely outcomes of treatments in silico, as a mathematical optimization framework by Breitenbach et al. [114] demonstrates. The authors created a network of a cardiomyocyte fitted to experimental data that is able to predict the network of inhibitions and activations following external stimuli such as the hypertrophic stimulus of Ang-II on AT_1R [114].

Promising models and trials such as those mentioned above and the ever-growing amount of available gene sequence and RNA sequencing data [115] as well as databases such as miRDB [116] indicate that the combination of bioinformatics and the progress in ncRNA research will lead to the discovery of new therapeutic targets and efficient treatments.

Conclusion

As the RAS plays a central role in blood pressure regulation, it is also indirectly involved in numerous pathogenies related to hypertension and its consequences, including debilitating cardiovascular complications [117]. Therefore, curing hypertension has been of great interest for more than a century [99]. The ongoing research has revealed that several epigenetic factors affect the RAS, ranging from lifestyle changes to a medical intervention targeting components of the RAS. These components include ACE2, which has recently garnered global attention due to its involvement in COVID-19 [91]. Technical advances have led to the discovery of miRNAs and their importance for the epigenetic regulation of the RAS [22]. Besides their function as biomarkers, miRNAs are also possible therapeutic targets [22]. While conventional hypertension therapy also affects miRNA expression [34], new promising therapeutic approaches that directly target specific miRNAs are being developed [86], indicating the importance and chances of miRNA research.

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Chapter 6
Epigenetics Changes in Renin Angiotensin System (RAS): Application of Biosensors for Monitoring These Changes

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Abstract The renin angiotensin system (RAS) is a hormonal system responsible for regulating blood pressure. Complex interplay of genetics and environmental factors in developing hypertension has attracted a lot of attention in recent years. Different approaches in genetic analysis has illustrated numerous genetic loci that contribute in hypertension. Some missing parts of heritability in hypertension might be explained by epigenetic changes. Expression of RAS genes changes due to alteration of DNA methylation. The consequence of this alteration could be the development of hypertension. DNA methylation is not the only epigenetic change in the RAS system. Other epigenetic modifications in the RAS system include histone modifications, nucleosome positioning, transcriptional control with DNA-binding proteins and non-coding RNAs, and translational control with microRNAs and RNA-binding proteins. These changes might be able to change the RAS function. Understanding and monitoring

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these changes would lead to better understanding of changeable and/or reversible alteration that would be of great value in diagnostic and therapeutic approaches. Biosensors are potential tools to detect and monitor changes. In this chapter, the epigenetic changes as well as available biosensors will be discussed.

Keywords Hypertension · Genetics · Epigenetics · Biosensor · RAS · Renin · Angiotensin

Abbreviations

ACE	Angiotensin converting enzyme
AGT	Angiotensinogen
AGTR1	Angiotensin II receptor type 1
CYP11B2	Aldosterone synthase gene
HTN	Hypertension
I/D	Insertion/deletion polymorphism
MAPKs	Mitogen-activated protein kinases
RAAS	Renin-angiotensin-aldosterone system
RAS	Renin angiotensin system
REN	RASS gene encoding renin
rS	Reference single nucleotide polymorphism identification
SNP	Single nucleotide polymorphism
SPR	Surface plasmon resonance
235 M/T	Substitution of threonine by methionine at position 235
1166A/C	Single nucleotide polymorphism of the Angiotensin II Type 1 Receptor Gene which is AC heterozygote at 1166 location

Introduction

The renin angiotensin system (RAS) is a hormonal system responsible for regulating blood pressure through fluid and electrolyte balance. Also, this system regulates systemic vascular resistance. Juxtaglomerular cells in the kidneys sense the reduced blood flow in the kidney and convert the precursor prorenin into renin (REN). Secreted renin into circulation converts the angiotensinogen (AGT) to angiotensin I. Then angiotensin-converting enzyme (ACE) converts Angiotensin I to angiotensin II. Angiotensin II carries out the vasoconstriction of blood vessels to narrow, leading to increased blood pressure. RAS regulates blood pressure and extracellular volume through different mechanisms. If the RAS system function is abnormal, blood pressure would be high that is called hypertension (HTN) [1]. More than 150 genes are

involved through different mechanisms in development of HTN and the genetic part is responsible for 30–50% of variety in HTN [2].

Polymorphisms in the RAS Genes and Hypertension

Individuals of the same species are not genetically similar. Their DNA sequences differ to a certain extent, which contributes to genetic diversity, in other words, genetic polymorphism. While human genetic polymorphism depends on multiple factors, one of the well-known associations is ethnicity [3]. With advancements in science and technology, researchers have been trying to identify the possible genetic etiologies for various non-communicable diseases. Identifying the RAS system and its genetic variation is one such best example. The RAAS is critical for blood pressure regulation. Genes encoding RAS components are thought to play a role in determining genetic predisposition to hypertension, and the association between RAAS gene polymorphism and pathogenesis of hypertension has been extensively studied across different ethnicities. The importance of RAS gene polymorphisms is due not only to the fact that its components play an essential role in the regulation of vascular homeostasis, but also to the role of angiotensin I converting enzyme (ACE) inhibitors in the therapeutic management of hypertension [4].

Xiaofeng Zhu et al. conducted a study in 2003 that included 192 African American and 153 European American families to assess the role of variations in genes that encode RAAS components in hypertension susceptibility. This study genotyped 25 single-nucleotide polymorphisms in 4 primary genes of the RAS: angiotensinogen (AGT), renin (REN), angiotensin I-converting enzyme (ACE), and the angiotensin II receptor, subtype 1 (AGTR1). Performing the family-based transmission/disequilibrium test with each single-nucleotide polymorphism and with the multilocus haplotypes showed that two individual single-nucleotide polymorphisms are significantly associated with hypertension among African Americans. This result persisted when both groups were combined. In African Americans, haplotype analysis for REN, AGTR1, and ACE confirmed the associations. In European Americans, there was consistent but less significant evidence. However, this study shows this biracial population sample that inter-individual variation in the RAAS genes contributes to hypertension risk [5]. In 2019, a study using 4150 Thais looked at the relationship between four single nucleotide polymorphisms in RAAS genes and hypertension, with one SNP per gene (i.e., rs1799752, rs699, rs5186, and rs1799998 for ACE, AGT, AGTR1, and CYP11B2 genes, respectively). However, this study found no link between four RAAS polymorphisms and hypertension, which could be attributed to low statistical power due to the small sample size [6]. A study in 2017 was done, and according to the genome-wide association studies (GWAS) Catalogue, there is no evidence regarding the direct connection of polymorphisms in the RAS genes and hypertension or any other cardiovascular disease [7].

However, according to the available literature, dozens of single nucleotide polymorphisms (SNPs) within RAAS genes have been reported to be significantly associated with essential hypertension [8, 9]. Therefore, few studies were conducted to see the association of multiple single nucleotide polymorphisms in RAAS genes and hypertension. Addressing this, Ji et al. [10] conducted a Case-control study in a group of Han Chinese people to examine the association of 41 single-nucleotide polymorphisms in 5 candidate genes of RAAS (REN, AGT, ACE, AGTR, and CYP11B2) with hypertension. This study discovered several genetic variants in the RAAS genes that were significantly associated with hypertension (rs3789678 and rs2493132 within AGT, rs4305 within ACE, rs275645 within AGTR1, rs3802230, and rs10086846 within CYP11B2) in the Han Chinese population [10].

Another case-control study was conducted to examine the relationship between three RAS gene polymorphisms (AGT 235M/T, ACE I/D, and AT1R 1166A/C) and essential hypertension in Burkina Faso in West Africa. The AGT 235M/T and AT1R 1166A/C polymorphisms had no association with the risk of developing hypertension, whereas the ACE I/D polymorphism had a strong association with the development of hypertension. Thus, the ACE gene DD genotype is involved in hypertension susceptibility and predicts hypertension risk in the Burkinabe population [11]. Mehri et al. [12] investigated the effects of three RAS genetic markers on the risk of hypertension: angiotensinogen (AGT) M235T, angiotensin-converting enzyme insertion/deletion (ACE I/D), and angiotensin II receptor 1 (AT1R) A1166C in the Tunisian population (North Africa). According to the findings of this study, these three RAAS gene polymorphisms were significantly associated with hypertension. Furthermore, when compared to healthy Tunisians, the TT genotype and the T allele of the AGT gene were strongly associated with HTA [12].

A Turkish population-targeted study looked at four RAAS gene polymorphisms: ACE (I/D), AGN T174M/M235T, and ATR1 A1166C in hypertensive and normotensive patients [13]. The findings confirmed the link between the ACE, AGN, and ATR1 gene polymorphisms and hypertension in the Turkish population, consistent with previous research in other white populations [13].

Epigenetics and Hypertension and RAS

In the last few decades, hypertension has been one of the significant risk factors for cardiovascular and cerebrovascular diseases. Environmental and genetic factors are effective in causing high blood pressure [14]. Some missing parts of heritability in hypertension might be explained by epigenetic changes. Expression of RAS genes might change due to alteration of DNA methylation. The consequence of this alteration could be the development of hypertension. DNA methylation is not the only epigenetic change in the RAS system. Other epigenetic modifications in the RAS system include histone modifications, nucleosome positioning, transcriptional control with DNA-binding proteins and non-coding RNAs, and translational control with microRNAs and RNA-binding proteins. These changes might be able

to change the RAS function [15]. RAS and specifically angiotensin II promote their function by acting on the angiotensin AT1-receptor (AT1R) [16]. AT1 is responsible for most of the known functions of the renin angiotensin system under physiological and pathophysiological conditions (such as vascular smooth muscle contraction, the proliferation of vascular smooth muscle cells, aldosterone release, and regulation of fluid-electrolyte balance) [17]. There are two types of AT1 receptors: AT1a and AT1b [18]. AT1b angiotensin receptor gene expression in the adrenal gland in the first week of life results in increased receptor expression with increased adrenal angiotensin response. AT1b expression is strongly dependent on promoter methylation [18]. Reports have suggested that AT1b may be involved in vasoconstriction at the level of junction (post-ganglion neurons), which supports the role of AT1b in the HTN program [18].

Nowadays, there is strong evidence that shows intrauterine stress causes high blood pressure. The phenomenon of fetal programming can be an effective model in mammalian species. Maternal water deprivation and protein deficiency are shown to increase expression of renin angiotensin system genes in the offspring. These findings indicate targets for environment–gene interactions in various hypertensive states and in essential hypertension [19]. A low maternal protein diet is associated with decreased methylation, which results in a deficiency of specific amino acids such as glycine [20]. In a research, in 2010, Ravi Goyal et al. demonstrated that for the developing fetal brain renin angiotensin system, maternal low protein diet leads to significant alterations in the mRNA and protein expression, with changes in DNA methylation and miRNA, key regulators of hypertension in adults [21]. Mothers who received a low protein diet would have offspring with not only increased expression of the AT1b receptor mRNA but also the corresponding protein in their adrenal. Expression of AT1 receptor has increased in sheep due to lack of protein in their mother's diet during pregnancy. Besides, the MLP rat adrenal is more responsive to angiotensin II (Ang II). They showed that these alterations occur very early in the life and last at least up to 12 weeks of age. The genes that change significantly at 12 weeks of age are including (angiotensinogen, renin, and AT1a receptor in kidney and AT1a and AT2 receptor in the adrenal). This change might be related to the early signs of developing hypertension [14]. Low protein diets also were evaluated in the pregnant rat as pathogenesis of hypertension which shows low birth weight and increased systolic and diastolic blood pressure during the first four weeks of life [22].

There has been an association between birth weight in neonates and blood pressure in their adulthood. This association has been observed in many parts of the world in both males and females. Mothers' poor nutrition during pregnancy causes diseases such as blood pressure. Its mechanism in various studies includes exposure to Glucocorticoid, kidneys role, and Renin angiotensin system. Fetus exposure to maternal Glucocorticoid plays a key role. The amount of 11-hydroxysteroid dehydrogenase type II (11-B-HSD II) is high in the placenta, and it metabolizes Cortisol to inactive Cortisone. 11-B-HSD II expression was lower in the placentas of fetuses whose mothers had a low-protein diet, so the fetus exposure to glucocorticoid would be more [23].

Blood pressure in this model is prevented by using ACE or ARB inhibitors at birth [24, 25]. Decreased renal nephrons were seen at autopsy in HTN patients and rats on a low embryonic protein diet [26, 27]. Changes in phenotypes may be due to the changes in the number or distribution of different cells or changes in gene expression [28]. With respect to epigenetic modifications of the RAS and its impact on kidney development, recent studies demonstrated that inhibition of histone deacetylase activity induces the expression of renin mRNA in renal juxtaglomerular cells. Renfang Song et al. demonstrated that lack of endogenous Ang II in AGT-deficient mice causes a decrease in ureteric bud branching. Exogenous Ang II up-regulates HDAC-1 and down-regulates acetylated histone H3 expression in the developing metanephros [29]. Furthermore, protein modifications also play essential roles in RAAS functions. For instance, casein kinase 2 phosphorylates the ACE on Ser1270 and regulates its retention in the endothelial cell plasma membrane. In contrast, the type 1A angiotensin II receptor is phosphorylated by G protein-coupled receptor kinases, and protein kinase C. Angiotensin II stimulates phosphorylation of mitogen-activated protein kinases (MAPKs) in proximal tubular cells and exerts broad effects on cellular signaling pathways [7].

Biosensors and Hypertension

Hypertension needs chronic outpatient follow-ups as well as regular in person visits and demands self-management skills from the patient [30]. Biosensors express enormous potentials in being impactful in different areas in hypertension. For example, Amaratunga et al. in 2020 using machine learning have shown that wearable biosensors are able to assess risk of developing hypertension with sensitivity higher than 80% positive predictive value higher than 90% [31]. Also, biosensors improve monitoring of hypertension at home [32]. There are two categories of biosensors in use for hypertension, cuff-based devices and cuff-less ones. Cuff-based devices are wrist watch-type blood pressure devices and also include finger monitors by which blood pressure and cardiac output can be measured [33]. Pressure sensors that monitor real time blood pressure and arterial pulsations in the postoperative arteries have contact modes and also non-contact modes. They are made of biodegradable materials wrapped around the artery to reduce risk of vessel trauma in follow ups and would not be needed to be removed [34]. Cuff-less devices for measuring blood pressure have been evaluated in clinical trials as well [35]. They have shown some promising aspects. However, they need more clinical validation [32]. Regarding renin angiotensin system, in a study in 2019, Salehabadi et al. evaluated Surface plasmon resonance (SPR) biosensor to monitor analyte-ligand interactions between new Angiotensin Converting Enzyme (ACE) inhibitors in five different medicinal plants and a specific immobilized ACE ligand on sensor chip. They illustrated that SPR has a great sensitivity in improving the drug discovery in ACE inhibitors [36]. Souza et al. used the genetically encoded Ca^{2+} biosensor GCaMP6 for monitoring of Ca^{2+}-signaling processes in the neurons of the paraventricular nucleus brain slices of

the mouse. They knock downed the paraventricular nucleus-prorenin receptor. This knockdown resulted in decline in hypertension. Decreasing in angiotensin II type 1 receptor-mediated Ca^{2+} activity have been shown as a part of the mechanism [37].

Biosensors have shown potential in detecting neurotransmitters in a real time manner by which we can study effect of antihypertensive medications on the brain activity [38]. However, use of biosensors in hypertension needs more clinical validation and is still in research [32].

Conclusion and Outline

RAS has been known as the hormonal system involved in developing HTN for decades. Yet, there are many areas needs to be explored. There are genetic differences among ethnicities. In addition, there are mounting evidences of epigenetic changes. These changes might be able to change the RAS function. There are wearable devices and new molecular techniques to monitor blood pressure and function of RAS. However, incorporation of these devices and techniques in clinical practice has been challenging both for physicians and for patients. Further studies are needed to develop the techniques as well as the devices.

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Chapter 7
Genetic Polymorphisms in the Renin-Angiotensin-Aldosterone System

Tomasz Rechciński

Abstract This chapter presents the evaluation of the impact of nonsynonymous single nucleotide polymorphisms (nsSNPs) in the renin-angiotensin-aldosterone system (RAAS) on patients' phenotypes not only in regard to arterial hypertension and its complications, but also in regard to other conditions from the fields of interest outside cardiovascular medicine. The impact of nsSNPs in panels for the genes of renin, angiotensinogen, angiotensin-converting enzyme, angiotensin receptor and aldosterone synthase is presented here together with a clinical picture of the investigated cohorts and the impact of nsSNPs on peptide-protein interactions. The first figure in the chapter presents—in a simplified mode—the location of the described genes in the human karyogram, and the second one—the geographical distribution of the probands who participated in the studies described here. A synopsis of the clinical context of the investigated nsSNPs is presented in the table. Genetic variability in nsSNPs of the RAAS is involved in the pathogenesis of arterial hypertension and its complications, and surprisingly also in the pathogenesis of conditions not associated with elevated blood pressure, like neoplasms or inflammatory processes.

Keywords Nonsynonymous SNP · Renin-angiotensin-aldosterone system · Genetic polymorphisms

Introduction

The renin-angiotensin-aldosterone system (RAAS) is strongly involved in the pathogenesis of arterial hypertension, as well as in the occurrence of its complications. The diversity of the courses of this disease and the acceleration of target organ damage may depend not only on the presence of concomitant diseases, on environmental factors including the style of life and the quality of health care, including adherence to medical therapy, but also to some extent on genetic factors. Rapid development

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of genetics shows that nonsynonymous single nucleotide polymorphisms (SNPs) are one of many types of abnormalities in the human genome, besides changes in epigenetic modulators (e.g. microRNA) or posttranscriptional modifications (e.g. methylation of DNA), which play a role in the shaping of the final picture of the disease. Although transcriptional errors which lead to a single nucleotide swap from a wild to a mutated variant usually have a spontaneous nature, they may be also inherited by descendants and be of importance even in foetal life.

In 2017 it was suggested that the role of polymorphisms in the RAAS assessed in various population studies was overestimated during the "candidate gene era" and that there is a need to reconsider the power of RAAS variants for individuals in the framework of "precision medicine" [1]. Surprisingly, in the review prepared by Ji et al., genetic variants of some genes related to the RAAS were not associated with cardiovascular diseases, but with thyroid function, schizophrenia, lead poisoning, fibrosis, angiotensin-converting-enzyme-induced cough or with the lipoprotein level. Additionally, some new technologies make it possible to use artificial intelligence for the prediction or assessment of the importance of SNPs in genes for the interactions of their products (protein-peptide interactions) and, potentially—for their altered function [2].

This chapter reviews critically the results of studies on the most investigated RAAS SNPs, published as original papers from 2017 to date.

The phenomenon of genetic polymorphism has been defined as a place in the sequence of DNA where there is variation in nucleotides, and the less common variant is present in at least one percent of the tested individuals. Although polymorphisms can be large in size and can involve long stretches of DNA—the most common form of polymorphism involves variation at a single base pair—the so called single nucleotide polymorphism [3]. The term synonymous SNPs denotes those polymorphisms which do not affect the protein sequence, whereas nonsynonymous SNPs change the amino acid sequence of protein or alter the level of expression of a gene. According to the data published in 2001, the number of polymorphisms in the human genome was estimated at 1.42 million, which provided an average density on available sequence of one SNP every 1.9 kilobases (kb) [4] Nevertheless, this list has since increased ultrarapidly, with, e.g., the Kaviar (Known VARiants) database currently mentioning 162 million single nucleotide variants [5]. The overwhelming majority of those variations are synonymous polymorphisms. The first figure in this chapter (Fig. 7.1) presents the location of described genes in the human karyogram.

Polymorphisms in the Gene for Renin (*REN*)

Human renin originates from preprorenin, composed of 406 amino acids. Analysis of its gene revealed that it consists of 10 exons and 9 introns, it spans roughly 11.7 kb and exists in a single copy in chromosome 1 [6]. Structural comparisons of the human renin gene and the pepsinogen gene (both enzymes belong to aspartyl proteinases)

suggest that they are descendants of a common ancestral gene [7] (Figs. 7.1 and 7.2; Table 7.1).

Since renin is associated with preeclampsia and eclampsia—genetic variability of its gene was a subject of studies with mothers and their offspring, as well as mothers

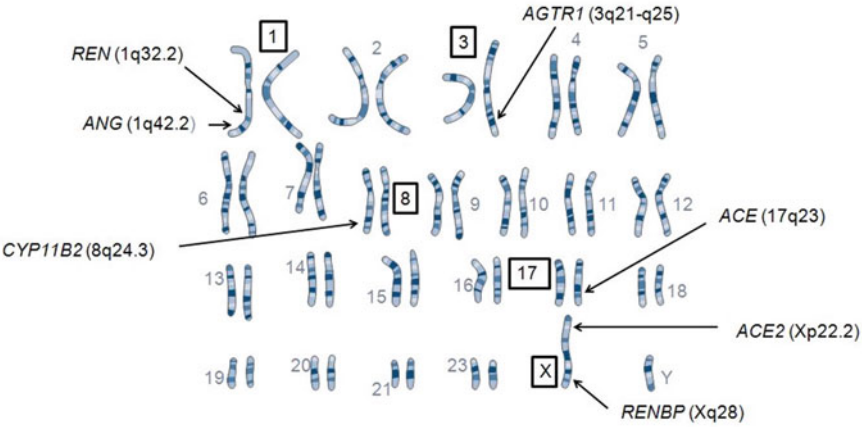

Fig. 7.1 The location of the genes involved in RAAS shown in a simplified way in the human karyogram. The numbers of chromosomes where RAAS genes are present are given in boxes. The abbreviations assigned to the names of the genes are followed by the location of genes according to the International System for Human Cytogenetic Nomenclature (ISHCN). p—the short arm of the chromosome, q—the long arm of the chromosome

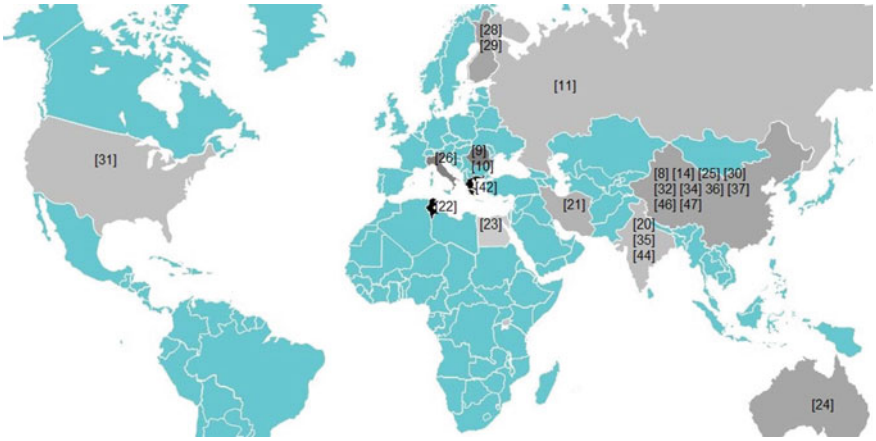

Fig. 7.2 The geographical distribution of probands who participated in the described studies—the highest number of studies mentioned in this chapter derives from China and India—10 and 3 respectively, 2 studies were conducted in both Finland and Romania, single studies originate from Australia, Egypt, Greece, Iran, Italy, Russia, Tunisia and the United States. The numbers in square brackets refer to the numerical order of references

Table 7.1 Synopsis of the clinical issues connected with nsSNPs in RAAS

Gene	Polymorphism	Investigated issue
REN	rs2368564	Preeclampsia, LV diastolic dysfunction, in-stent restenosis
	rs5707	preeclampsia/eclampsia
RENBP	rs78377269	BP response to sodium intake
AGT	rs11568020	Pro-HA, protection against HA
	rs1190025960	Survival with gastric and breast cancer
	rs2478544	Elevated BP in women
	rs267598410	Survival with gastric and breast cancer
	rs4762	Pro-HA, protection against HA, diabetic nephropathy, IHD, in-stent restenosis
	rs5050	Pro-HA, protection against HA
	rs699	Pro-HA, protection against HA, IHD, in-stent restenosis, diabetic nephropathy, ESRD, severity of symptoms in COVID-19
	rs7079	Blood lead level
ACE	rs1799752	Severity of symptoms in COVID-19
	rs4335	Risk of SCD in hemodialysed patients
	rs4340	Susceptibility to mycoplasmatic pneumonia in children
	rs4343	Risk of SCD in hemodialysed patients
	rs4353	Risk of SCD in hemodialysed patients
	rs9905945	Prognosis in sarcoidosis
ACE2	rs1514283	HA in women
	rs2074192	Severity of symptoms in COVID-19, LV hypertrophy
	rs2106809	LV hypertrophy
	rs4646155	HA in women, LV hypertrophy
	rs4646176	HA in women, LV hypertrophy
	rs6632677	AF in men
	rs879922	HA in women
AGTR1	rs12721297	Reduction of BP
	rs14922099	AF
	rs5186	Diabetic nephropathy, breast cancer
CYP11B2	rs1799998	Acute coronary syndromes
	rs542092383	Risk of HA
	rs73715282	Risk of HA
	rs7463212	Risk of HA

BP blood pressure; *ESRD* end-stage renal disease; *AF* atrial fibrillation; *HA* (*hypertensio arterialis*) arterial hypertension; *IHD* ischaemic heart disease; *LV* left ventricle; *SCD* sudden cardiac death

alone in this respect in Chinese and Romanian centers, respectively. Three SNPs were analyzed by Yu et al. in a study which included 347 preeclampsia/eclampsia patients and 700 controls. The foetal heterozygotic genotype rs5707 in the renin gene *REN* (AC) was significantly associated ($p = 0.004$) with an increased risk of preeclampsia/eclampsia when accompanied by the mother's body mass index $\geq$ 24 kg/m^2—odds ratio 2.75 (95% CI 1.5–5.06) [8]. In a study by Procopciuc et al., where 87 pregnant women with preeclampsia were compared with 130 controls, also SNPs rs2368564 (G83A) in *REN* with a heterozygotic form were significantly associated ($p = 0.009$) with late-onset preeclampsia [9]. The same polymorphism was found to be of importance in a study on adaptive morphological and functional response to essential hypertension, where carriers of G/G and A/G allele versus carriers of A/A had a 2.32-fold increased risk of developing left ventricular diastolic dysfunction ($p = 0.021$), although no effect of that polymorphism was found for left ventricular geometry, for the relative wall thickness or for left ventricular mass index [10]. The frequencies of minor and major alleles, as well as the frequencies of heterozygotes, major homozygotes, and minor homozygotes were found to be significantly different when 54 patients with in-stent restenosis after elective coronary angioplasty were compared with 59 patients with an uncomplicated course of interventional treatment of stable coronary artery disease ($p < 0.05$) [11].

Polymorphisms in the Gene for Renin-Binding Protein (*RENBP*)

The *RENBP* was found to be located in human chromosome X (Xq28 region), it spans about 10 kb and consists of 11 exons separated by 10 introns [12, 13]. The resequencing study conducted among the 1906 participants of the GenSalt study aimed to find SNP association with salt-sensitive arterial hypertension in a community from the Han Chinese population with a habitually high sodium-intake from rural areas [14]. Gene-based analyses were performed in selected 300 participants with the highest arterial pressure response to the sodium-intake (mean + 11.8 mmHg) and 300 ones with the lowest response (mean – 1.1 mmHg). Seven RAAS genes with a potential biological effect on blood pressure regulation were selected—the renin-binding protein (*RENBP*), angiotensin I converting enzyme 2 (*ACE2*) and angiotensin II type 1 receptor (*AGTR1*) were in this number. Within 50 single-nucleotide variants, only low-frequency, missense SNP in the *RENBP* gene described as rs78377269, present in the exonic region of chromosome X (C to A substitution in 153941584 position) reached statistical significance in a single marker analysis ($p = 0.03$) after adjustment for multiple testing when the studied subgroups were compared. Each copy of the minor allele corresponded with a 1.63 mmHg larger mean arterial pressure response to dietary sodium intervention and 2.21-fold increased odds of salt sensitivity (95% CI: 1.10, 4.42). The authors of that study do not exclude that the other SNPs tested in that study may explain in aggregate the phenomenon of salt-response hypertension.

Polymorphisms of the Gene for Angiotensinogen (*AGT*)

Human *AGT* is a single-copy gene located at the end of the long arm of chromosome 1 (1q42.2). It spans 12,063 base pairs (bp) between nucleotide 230,838,274 and nucleotide 230,850,336 and contains 5 exons and 4 introns. That gene encodes 485 amino acids. The 33-amino-acid signal peptide and more than half of the mature protein is coded by the second exon, and the code for the C-terminus of the protein is contained in exon 5 [15–17]. *AGT* expression is under developmental and hormonal controls in a cell type-specific manner [18].

In contrast to the studies described above, Goswami applied quite a different approach to genetic variability in the analysis of the influence of SNPs in the angiotensinogen gene (*AGT*) on protein–protein interactions and on their further clinical consequences [19]. That author validated 354 SNPs of *AGT* and determined their conservation degree in a 9-step scale. Using computational modeling, 3-dimensional structures of wild-type and mutant *AGT* variants were generated. The nature of each of the 485 aminoacids of this macromolecule was defined as exposed or as buried, depending on the extent to which it is hidden in the protein structure space; moreover, their functional or structural role for the protein was predicted. Three SNPs: rs1452925829, rs746613821 and rs1162645963 were identified as being highly destabilizing for the renin-angiotensinogen binding; they had also the strongest impact on the change of the interface area between renin and angiotensinogen. It was proved earlier that the majority of diseases associated with nonsynonymous SNPs are caused by the instability of the proteins. The highest scores (0.808–0.919) for the prediction of the structure, function and post-translational modifications of the human angiotensinogen were observed for rs1190025960, rs267598410 and rs1205199806, which lead to the following swaps of aminoacids in the indicated positions: G149R, R477G and L162P, respectively. Substitutions of aminoacids cause changes in some physical properties of angiotensinogen such as electrostatic potentials, nonpolar and polar solvation energy, mechanical energy and van der Waals forces. After *AGT* expression had been correlated with the survival of patients with four neoplasms, the author concluded that deregulation of angiotensinogen and des(angiotensin I)angiotensinogen is associated with survival outcomes in patients with gastric and breast cancer through their anti-angiogenic activity, as opposed to the pro-angiogenic action of angiotensin II with angiotensin II-receptor.

Returning from oncology to cardiovascular diseases, the next publication reestablishes the role of *AGT* variants and their haplotypes in the etiology of arterial hypertension [20]. The subject of interest in a study by Purkait et al. performed in Indian population were nine SNPs, both in 5′ untranslated regions (5′UTR), in the exon and in the intron regions of *AGT*. Four of them (rs11568020, rs5050—in promoter and rs4762, rs699—in exon 2) showed positive association with arterial hypertension ($p < 0.05$), when 256 hypertensive cases were compared with 158 normotensive controls. Additionally, from the pool of 13 haplotypes analysed in this study—3 with 2-4/9 minor alleles revealed a pro-hypertensive effect, and 1 with all major alleles—protective association with hypertension ($p < 0.01$). On the other hand, using multiple

logistic regression analysis, Khatami et al. found a significant association of the two SNPs mentioned in the previous study—rs4762 and rs699—with ischemic heart disease, when in a dominant model 148 Iranian patients with coronary artery disease (100% hypertensive as described in the patients' characteristics) were compared with 135 normotensive controls without coronary artery disease—OR 1.91, 95%CI 1.16–3.15; $p = 0.01$ and OR 1.8, 95%CI 1.1–2.93, $p = 0.01$ respectively [21].

The authors of one of the papers mentioned earlier compared the distribution of major and minor alleles of rs699 or rs4762 in 113 patients with coronary artery disease treated by means of percutaneous coronary intervention. The patients were divided into two groups: with or without in-stent restenosis. The latter group was subdivided into patients with restenosis within 12 months and those with restenosis more than 12 months after stenting. The researchers found that rs699 wild homozygotes were more prevalent in "the restenosis within 12 months group" ($p = 0.017$) and rs4762 mutated homozygotes—more prevalent in "the restenosis after 12 months group" ($p < 0.05$) [11]. Rs4762 and rs699 were also found among the SNPs which underwent analysis in order to verify a hypothesis about their linkage with diabetic nephropathy. Mutated alleles of these SNPs presented association with diabetic nephropathy with OR 10.25; $p = 0.001$ and OR 22.21 $p < 0.001$ respectively, in a comparison of 47 Tunisian type-2 diabetic patients with nephropathy and 189 without this complication [22].

A similar problem was investigated by El-Garawani et al. Their study involved Egyptian patients with two concomitant diseases: arterial hypertension and type 2 diabetes mellitus, who were enrolled in two groups: a group with end-stage renal disease (ESRD) requiring dialysis, and a group without ESRD. The study showed that the absence of a mutated allele for rs699 was significantly associated with lack of ESRD: OR 0.22; 95%CI 0.08–0.63, while rs4762 had no effect on that renal complication among the patients [23]. A cohort of 2872 white Australians from the Victorian Family Heart Study were enrolled in a program to test the association of systolic blood pressure with 88 SNPs in *AGT*, *AGTR1*, *REN* and the aldosterone synthase gene (*CYP11B2*). This study by Scurrah et al. revealed that in this group rs2468523 and rs2478544 at *AGT* presented sex-specific association (not in women), and the presence of every minor allele resulted in an increase in systolic blood pressure in men by 1.58 and 1.63 mmHg, respectively [24]. The next paper by Wu et al. presents the problem of occupational exposure to lead, of lead-related hypertension and its association with the rs7079 SNP [25]. Although the rs7079 nucleotide is located in the untranslated region of the gene, it is suggested that this DNA fragment is crucial for binding microRNA and that in this way it influences *AGT* transcription and translation, and subsequently its serum concentration. The authors compared the frequency of minor alleles and allele distribution between persons with blood lead level < 200 μg/L and those with blood lead level ≥ 400 μg/L. Heterozygotic variants (CA) and homozygotic variants with minor alleles (AA) of rs7079 appeared more frequently in lead-exposed than in unexposed individuals: 47.4% versus 33.3% $p = 0.02$. Apart from this, homozygotes with major alleles (CC) had significantly higher blood concentration of angiotensinogen than heterozygotes—$p = 0.01$. This may

be explained by the observation that the allele A decreased the binding between the miRNA and the angiotensinogen gene.

The ongoing COVID-19 pandemic caused an increased interest in the association of the SARS-CoV-2 virus and the RAAS. Cafiero et al. investigated six polymorphisms in the RAAS to assess their impact on the severity of symptoms during that infection. Fifty four symptomatic patients and 50 patients with an asymptomatic course of the infection underwent genotyping for rs699 and rs4762 (both in *ANG*) together with selected polymorphisms in *ACE* (also known as *ACE1*), *ACE2*, and *AGTR1*. The study of that group revealed that the haplotype with a wild variant present in rs699, a wild variant in rs2074192 in *ACE2* and a wild variant in rs1799752 in *ACE1* was significantly more frequent (36.7% vs. 6.27 $p < 0.00001$) in the asymptomatic group than in the symptomatic patients, whereas the haplotype with a mutated variant in rs699, a mutated variant in rs2074192 in *ACE2* and a mutated variant in rs1799752 in *ACE1* could be found only in the symptomatic group (0% vs. 15.08% $p = 0.000058$) [26].

Polymorphisms of the Gene for the Angiotensin-Converting Enzyme (*ACE*)

The human angiotensin-converting enzyme gene is located in the long arm of chromosome 17 (17q23), it contains 26 exons interrupted by 25 introns, and spans approximately 21 kb of DNA [27].

In recent years some studies on *ACE* focused on the association of its SNPs with inflammatory processes. It is reported in many studies that *ACE* has been associated with sarcoidosis. Although the group of Lahtela did not confirm this link in their own study on Finnish patients, they indicated an interesting trait in the association of rs9905945 with prognosis in this disease [28, 29]. Using the generally accepted criteria of the World Association of Sarcoidosis and Other Granulomas (WASOG), they divided 188 patients from pulmonary departments into two groups depending on the effects of treatment during a 2-year period: 89 patients in whom the disease resolved and 97 in whom it persisted. They discovered that the combination of rs9905945 in *ACE* and of previously reported HLA markers is helpful in predicting the course of this disease in the Finnish population more accurately than was previously known. The C phenotype was statistically more frequent in the group with better prognosis than in the group with poor prognosis (79.8 vs. 66% $p = 0.035$), and combining the frequencies of the C phenotype with two defined HLA markers revealed a strong association with resolved versus persistent disease prognosis—37.1% versus 11.3% $p < 0.001$, OR 4.61 (95%CI 2.15–9.86). Another interesting association of lung disease with *ACE* was tested in a population of Chinese children infected with *Mycoplasma pneumoniae* [30] Polymorphism within *ACE*—rs4340—was analysed together with SNPs within *IL-6* (interleukin-6 gene)—rs1800795, and within *NOS3* (nitric oxide synthase gene)—rs1799983 and two other genes.

The gene–gene interactions were tested using Multifactor Dimensionality Reduction (MDR) and cumulative genetic risk score approaches. The results of 715 blood samples (415 cases of mycoplasmatic pneumonia and 300 healthy controls) showed that a combination of a major allele in *ACE* (D) with a minor allele in *NOS3* (T) contribute to the genetic susceptibility of this germ-related pneumonia in children in China—$p = 1.86 \times 10^{-6}$, OR 3.44 (95% CI 2.014–5.888).

Quite a new association was described between three SNPs in the *ACE* gene and the risk of sudden cardiac death (SCD) during a 64-month follow-up in 1852 participants of the EVOLVE study (EValuation Of Cinacalcet Hydrochloride Therapy to Lower CardioVascular Events), who were from two ancestry group—European and African ones—and who were qualified for hemodialysis due to chronic kidney disease with secondary hyperparathyroidism [31]. Three correlated SNPs—rs4335, rs4343 and rs4353—in DNA from the European ancestry patients were associated with a 26–27% reduction of SCD [OR 0.74 (95%CI 0.56–0.99), $p = 0.004$; OR 0.74 (95%CI 0.55–0.99), $p = 0.04$; OR 0.73 (95%CI 0.54–0.98), $p = 0.036$ respectively] and only one SNP—rs4318—reduced the risk of SCD by 70% in the African ancestry patients [OR 0.3 (95%CI 0.1–0.85) $p = 0.03$]. Some ethnic differences in alleles distribution between European and African ancestry patients were found. In the former group—A is the minor allele in rs4335 and rs4343 with the frequency of 0.47 for both of them, whereas in the latter group it was G with the frequency of 0.23. For the third SNP—rs4353—the same G was a minor allele in both ancestry groups.

Three SNPs in the *ACE* gene were investigated among 125 genetic variants in a group of 1009 participants to evaluate the influence of the genetic variant on the peak oxygen uptake (VO_{2peak}) level during a cardiopulmonary exercise test (as a measure of cardiorespiratory fitness) in untrained people in China [32]. Only one of the 3 assessed SNPs—rs4295—was associated with VO_{2peak} (ß = 0.87; $p < 2.9 \times 10^{-4}$) and it was responsible for 1.1% of interindividual variance in VO_{2peak}.

Polymorphisms of the Gene for Angiotensin-Converting Enzyme 2 (*ACE2*)

The angiotensin-converting enzyme 2 gene is mapped on chromosome X (Xp22.2) with exon count—22, and it spans 112,671 bp [33].

The hypothesis that a SNP of the angiotensin-converting enzyme 2 gene (*ACE2*) may be associated with structural atrial fibrillation (AF) was verified in 300 patients with this most frequent supraventricular arrhythmia and 300 arrhythmia-free controls (the mean age in both groups was 67.6 ± 12.5 and 66.1 ± 12.5 respectively, $p = 0.133$). In those Chinese patients the prevalence of arterial hypertension, diabetes mellitus, coronary artery disease, heart failure and nicotine-addiction was similar in the compared groups. That study revealed that the C allele in rs6632677 was more frequent in males, and when males were analysed separately from females, the presence of the C allele increased significantly the risk of AF—OR 1.954 (95%

1.196–3.192) [34]. Additionally, an interaction was found between *ACE2* and *AGTR1* in Chinese patients with this arrhythmia in an MDR analysis in a 4-locus model (1 locus in *ACE2* and 3 loci in *AGTR1*). A 3-locus model with the same SNP in *ACE2* together with SNP in the troponin I-interacting kinase gene (*TNNI3K*) and in the calmodulin III gene (CALM3) was used by Kumar et al. for the risk prediction of hypertrophic and dilated cardiomyopathy (HCM and DCM). On the basis of the comparison of genotypes of 130 patients with HCM, of 161 patients with DCM, and of 236 controls, the authors concluded that these 3 polymorphisms significantly influence both the cardiomyopathy phenotypes. The 3-locus model predicted the risk of HCM and DCM with the prediction error 23.4% and 22.77% respectively, and $p = 0.03$ and $p = 0.04$ respectively [35].

Another large scale study (1024 hypertensive Chinese patients and 956 normotensive ones) focused on the influence of 34 SNPs of the *ACE2* gene. Interestingly, it was determined that five of those SNPs (rs1514283, rs4646155, rs4646176, rs2285666 and rs879922) were associated significantly with hypertension in women, but not in men. For example, in rs1514283 the CC genotype and the C allele were more frequent in hypertensive patients than in controls—2.2% versus 0.7% OR 4.209, 95%CI 1.633–10.851, $p < 0.05$ and 3.7% versus 2.2%, $p = 0.01$. That study reported for the first time that rs4646155 was associated with essential hypertension in women, and homozygotes with minor allele T were significantly more frequent in patients with hypertension than in controls—1.9% versus 0.7%, $p = 0.01$ OR 3.492 95%CI 1.324–9.212 [36]. Interestingly enough, in another study two of the above-mentioned SNPs (rs4646155 and rs4646176) together with rs2074192 and rs2106809 were investigated with regard to whether they could influence not only elevated blood pressure, but also susceptibility to hypertensive left ventricular hypertrophy in Chinese patients. These four SNPs were genotyped in 289 patients with arterial hypertension and left ventricular hypertrophy, and 358 controls without hypertrophy. The presence of minor allele T in rs2074192 and minor allele T in rs2106809 was significantly associated with left ventricular hypertrophy, with $p = 0.005$ for both and OR 2.094 95% CI 1.249–3.512; OR 2.029 95% CI 91.235–3.333 respectively [37].

Since angiotensin converting enzyme 2 interacts with spike glycoprotein of the host to facilitate the SARS-CoV-2 virus entry into the cells, Khalid et al., carried out an *in-silico* study to determine the effect of SNPs in the *ACE2* gene on the tertiary structure of this protein and the influence of these SNPs on binding the virus. Two variants in *ACE2* were identified as those which have an impact on the repulsion of ligands of the same negative charge (G405E, W461R), and the third was probably damaging to the protein (F588S). These findings may explain the differences in the course of the COVID-19 infection-related disease during the current pandemic [38].

Polymorphisms of the Gene for Angiotensin Receptor 1 (*AGTR1*)

AGTR1 was mapped to chromosome 3 and regionalized to 3q21–q25 [39, 40]. This gene consists of at least 5 exons and spans more than 55 kb of genomic DNA. The size of the exons ranges from 59 to 2014 bp. The amino acid sequence of human angiotensin II type 1 (AT1) receptor deduced from its base sequence has 359 amino acids, and it shows a high degree of sequence identity to animal (bovine or rat) angiotensin type 1 receptor sequences [41].

Many polymorphisms listed in this panel were described already in the studies cited previously in this chapter, since SNPs of the *AGTR1* gene were quite often studied together with SNPs of *ACE* or *AGT* genes. An exception to this rule is a study on the association of the rs5186 SNP (A1166C) with retinal vein occlusion; that study involved 69 patients and 82 controls. The minor allele C was significantly more frequent (17.4% vs. 1.2%, $p = 0.0001$) in individuals with such complications within ocular venous vasculature, which confirms earlier reports on the association of this SNP with other vascular events [42]. The rs5186 SNP was also studied in the aspect of its impact on the development of diabetic nephropathy and presented a positive association with this complication $p < 0.0001$. The same SNP was studied in the above-mentioned EVOLVE trial, too, and it presented a 31% increase of the risk of composite endpoints (defined as death, nonfatal myocardial infarction, unstable angina leading to hospitalization, aggravation of heart failure or an event related to peripheral artery disease) in patients qualified for dialysis—OR 1.31 (95%CI 1.15–1.49), $p = 4.4 \times 10^{-5}$. The molecular importance of this SNP may be explained by the fact that AGTR1 is co-expressed with miR-155 in many tissues and the latter molecule represses the expression of *AGTR1* only in the presence of the major allele A, but not in the presence of the minor allele C [43]. Additionally, as the EVOLVE study proved, the minor allele C was found more frequently in patients of European ancestry (28%) than in patients of African ancestry (5%) [31].

Another *AGTR1* polymorphism—rs1492099—was studied in the aspect of structural atrial fibrillation (AF) in Chinese population in a group of 300 patients with this arrhythmia and 300 controls [34]. The authors revealed a higher frequency of the minor allele A in the AF-group than in controls: 14.2% versus 8.8%, $p = 0.004$ with a 72.7% increase of the risk of arrhythmia for the minor allele—OR 1.727 (95%CI 1.154–2.487).

Of the four SNPs in the *AGTR1* gene tested in the Victorian Family Study—rs12721297, rs385338, rs1800766 and rs275649—only the first one presented an association with decreased values of systolic blood pressure with ß(SE) – 2.54 (0.76) mmHg [24]. Finally, as was mentioned for *ACE*, some SNPs of the *AGTR* gene play a critical role in breast cancer. One hundred and sixty one women in a North India breast cancer cohort were compared with 152 healthy women, among others in respect of *AGTR1*—rs5186 (A1166C) and ACE insertion/deletion (I/D) polymorphism. The results of the analysis suggest a significant association of the two tested polymorphisms with the risk of breast cancer: individuals harboring the

AC or CC genotype in *AGTR1* together with the DD genotype for *ACE* I/D polymorphism—present an increased risk of breast cancer with OR 258 (95% CI 34.2–1944.4, $p < 0.001$) [44].

The Polymorphisms of the Gene for Aldosterone Synthase

An important rating enzyme in the process of the synthesis of aldosterone from cholesterol is aldosterone synthase (CYP11B2), and genetic variability in its gene is crucial for the blood concentration of this hormone. Aldosterone synthase is a member of the cytochrome P450 family of enzymes. The CYP11B2 gene (*CYP11B2*) was mapped to chromosome 8 (8q24.3) and it contains 9 exons and 8 introns [45].

One of the best investigated SNPs of the *CYP11B2* gene is the rs1799998 (T344C), but Qian et al. in their study added some new aspects to this knowledge after researching 96 adult patients with chronic kidney disease. They confirmed not only that homozygotes with major alleles (TT) have a significantly higher aldosterone concentration when compared with homozygotes with minor alleles (CC)—247.5 ± 93.6 pg/mL versus 190.0 ± 81.7 pg/mL, $p = 0.036$, but also that the median annual decline of the estimated glomerular filtration rate during a 1.5-year observation was significantly higher in the TT group (5.2 ± 16.1%) than in the CC group (− 32.8 ± 82.5%) with $p = 0.011$ [46]. Additionally, those authors aimed to assess the impact of T344C on the incidence of cardiovascular events defined as ST elevation myocardial infarction, non-ST elevation myocardial infarction and unstable angina, in this group. The distribution of cardiovascular events was significantly different from random, and it was highest in the TT group—25%, lowest in the CC group (0%), and intermediate in the CT group—7.8% with $p = 0.033$. Less known polymorphisms of *CYP11B2* were a subject of investigation in 1024 patients with essential hypertension and 956 normotensive controls [47]. Of the 7 SNPs selected for this study, only one—rs542092383—was found to increase the risk of essential hypertension—OR 3.48 (95%CI 1.407–8.597), $p = 0.004$. An interesting result was obtained also for the next two SNPs—rs73715282 and rs7463212 after adding them to the haplotype analysis with rs542092383. When major alleles in rs73715282 and rs7463212 were accompanied by a minor allele in rs542092383 (which was observed in 1.1% of patients with essential hypertension and in 0.2% normotensive controls), the OR reached 5.729 (95%CI 1.889–17.371), with p values < 0.0005.

Final Remarks

Some everyday-practice clinicians may be disappointed by a very weak impact of SNPs on the phenotype in some descriptions from this chapter, e.g. significant differences in allele frequency between various study groups like 3.7% versus 2.2%. It is important to be aware, however, that such is the nature of SNPs, and a stronger

association with clinical manifestations is possible only when a higher number of harmful SNPs coexist in an analyzed individual. So in the case of the need of public health care to identify individuals at high risk of a given disease it was proposed to consider inclusion of the so-called polygenic scores in this procedure. Such scores are already determined for the five most common diseases like coronary artery disease, atrial fibrillation, type 2 diabetes mellitus, inflammatory bowel disease and breast cancer [48]. Their advantage over the assessment based on clinical risk factors relies on the possibility to assess them directly after birth, so that primary prevention could be started at the earliest stages of the disease. Essential hypertension as a complex disease will be probably even more difficult to predict in childhood on the basis of genetic tests than the already studied diseases. A list of nsSNPs suggested for inclusion in such a score is provided in (Table 7.1).

Of course, this presentation of selected papers has some limitations: first of all, its focus was only on nonsynonymous SNPs. Other polymorphisms like an insertion/deletion polymorphism or the polymorphism of variable number of tandem repeats were virtually ignored, although they also belong in genetic studies of proteins involved in the RAAS[49, 50]. Apart from this, also epigenetic changes—such as DNA methylation, histone modifications or translational control with microRNA—may alter the RAAS function. Furthermore, to some extent also enzymatic post-translational modifications of proteins play a role in the function of RAAS, although predisposition to protein destabilisation depends also on nonsynonymous SNPs, as was explained by Goswami [19]. The next limitation of this review is that the patient samples are not ethnically homogeneous. We cannot automatically transfer data on genetic test results, for example, from Southeast Asia to Central Europe. The distribution of the origin of probands from individual populations is shown in the second figure (Fig. 7.2).

To summarize, individual susceptibility to hypertension and its associations with genetic variability still remain a subject of investigations, which involve also the proteins and enzymes not analyzed in this review. Generally, it should be emphasized that many regions of the genome are still unexplored and require further investigation. Undoubtedly, knowledge about the importance of the underestimated regions will be deepened. Especially, the noncoding regions of the described genes deserve detailed research, since, as Singh reports, they constitute 35 times more genetic material than the coding regions [51].

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Chapter 8
Mitochondria: A Key Protagonist of the Renin Angiotensin System

Rini Varghese and Anuradha Majumdar

Abstract Mitochondria are tiny organelles in the cells that produce energy. Apart from cellular metabolism, they play an essential role in other cellular activities. Damaged mitochondria have been connected to the pathophysiology of a variety of kidney and cardiac ailments. Angiotensin II (ATII) contributes to oxidative stress by inducing the production of mitochondrial reactive oxygen species (ROS) in endothelial cells, resulting in mitochondrial damage and a loss in energy metabolism. Overstimulation of the renin angiotensin system is linked to changes in mitochondrial structure and function. As a result, these small cell organelles have been shown to play an important part in the renin angiotensin system, kidney and cardiac function, and overall health. The book chapter thus aims to focus on the role of mitochondria in the renin angiotensin system thereby highlighting the association between the two. We intend to uncover the link between mitochondria, ROS, oxidative stress, mitochondrial function and its relevance in the renin angiotensin system.

Keywords Mitochondria · Angiotensin II · Renin angiotensin system · Oxidative stress · Mitochondrial biogenesis

Introduction

Mitochondria are small cell organelles that are known to serve a pivotal role in living cells. These double membrane cell organelles produce energy in the form of adenosine triphosphate (ATP) and are so appropriately referred to as the cell's "power house". Apart from being the source of energy, mitochondria are involved in several other important cellular functions namely storage of calcium for signalling, arbitrating cell survival, proliferation, growth and death, maintaining the redox state in the cells and generation of heat [1]. They are abundant in the major organs requiring high amount of energy viz. heart, kidney, liver, brain and muscles. In the recent past,

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mitochondrial biogenesis and dysfunction has been linked to the progression of multiple diseases such as hypertension, diabetes, and diseases of heart and kidney. Damaged mitochondria have been correlated to the pathogenesis of a variety of cardiovascular illnesses. Moreover, there has been growing evidence that prove the significant role of mitochondria in the development of many diseases.

The renin angiotensin system (RAS) also termed as renin–angiotensin–aldosterone system is the physiological system that regulates blood volume and blood pressure. The functioning of RAS system consists of the involvement of renin, angiotensin II (ATII) and aldosterone which works unanimously to elevate arterial blood pressure and maintain salt-water balance. The RAS is perceived to be the system consisting of circulating or endocrine system (tissue-to-tissue), cell-to-cell or paracrine and intracrine i.e., intracellular and/or nuclear system. Reactive oxygen species (ROS) generation have also been linked to renal and cardiovascular disorders, with ATII contributing to oxidative stress in both conditions. Mitochondrial malfunction and damage are also caused by oxidative stress affecting the overall health of mitochondria. As a result, there is a strong relationship between the RAS system, reactive oxygen species (ROS), oxidative stress, mitochondrial function, and cardiovascular and kidney illnesses.

RAS and Its Effect on Cardiovascular System and Kidney

The RAS system involves several important components. Angiotensinogen is a precursor protein produced in the liver and it is broken down by renin and angiotensin converting enzyme (ACE) which bring about the release of several hormones namely angiotensin I (ATI), angiotensin II (ATII), angiotensin 1–7 and angiotensin 1–9. ATII plays an essential role in RAS system and it mainly functions by acting on angiotensin II type 1 receptor (AT1R) and angiotensin II type 2 receptor (AT2R). Both ATIR and AT2R are a type of G-protein coupled receptors. ATII is substantially associated with pathogenesis of vascular remodelling. Binding of ATII to AT1R leads to physiological changes such as sodium reabsorption, acid–base homeostasis, constriction of blood vessels, synthesis of aldosterone, release of catecholamines and activation of central sympathetic nervous system. Activation of AT2R leads to sodium excretion and vasodilation [2]. The enzymatic cascades in RAS system can be activated in two ways: classic and non-classic RAS. The classic RAS is majorly mediated by ATII and its receptors AT1R and AT2R. The non-classic RAS involves angiotensin 1–7 which is a peptide produced by proteolysis of ATII by the enzyme ACE2. The action of angiotensin 1–7 is mediated by Mas receptor which is a G-protein coupled receptor. The receptor has strong affinity for angiotensin 1–7 and low affinity for ATII [3, 4].

RAS system is closely associated with cardiac ailments such as cardiac failure and atrial fibrillation. In the heart, ATII stimulates fibroblast proliferation and synthesis of collagen. This leads to development of fibrosis and cardiac hypertrophy. It has been identified to bring about fat accumulation and give rise to electrophysiological

changes in the heart [5]. This epicardial fat accumulation can induce atrial fibrillation via multiple pathophysiological mechanisms such as infiltration of epicardial adipocytes in atrial myocardium and release of inflammatory mediators [6, 7].

Moreover, production of ATII locally can lead to production of collagen and fibronectin and induce inflammatory changes. ATII thus can stimulate the release of inflammatory molecules such as TNF-α and IL-6 and secretion of matrix metalloproteinases which in turn can contribute to atrial fibrillation. The production of ATII locally in the heart has been linked to a rise in expression of collagen I, collagen III and fibronectin in the myocardium [8]. Additionally, ATII is responsible for aldosterone production. This in turn can stimulate the production of ATII locally in the heart along with collagen [9, 10]. In a study carried out by Zankov et al., in the atrial myocytes of guinea pig, ATII was found to have electrical cardiac remodelling effects by reducing the atrial effective refractory period and increasing the action potential duration [11].

ATII also stimulate the generation of ROS, superoxide ions and hydrogen peroxide by activating NADPH oxidase (NOX) [12]. In the heart when ATII binds to AT1R it leads to activation of NOX. In the kidneys, ATII increases blood flow and glomerular filtration rate (GFR) and the activation of Mas receptor also leads to an increase in GFR and renal blood flow and sodium excretion [13, 14]. Studies have shown that when ATII was administered continuously for several weeks, it caused kidney injury and hypertension. Administration of ATII within the cells led to expression of NF-κB, TNF-α and TGF-β1 and induced the production of free radicals in the nucleus of proximal tubule cells [15]. Thus ATII contributes to the occurrence of renal injury and hypertension. Also, ATII and the signalling pathways are activated in the proximal tubules of the kidney in the condition of renal ischemia and reperfusion which further contributes to renal injury.

Mitochondria and Mitochondrial Biogenesis

Mitochondria are energy-producing organelles that perform crucial cellular functions, and injury to them can impair a variety of tissue functions. Mitochondrial failure, especially in high-energy-demanding organs, could have far-reaching ramifications for overall cellular processes. Molecular oxygen is converted to superoxide anion when electrons become entangled in the electron transport chain (ETC). In the presence of antioxidants, mitochondrial superoxide dismutase instantly diminishes this oxidising agent forming hydrogen peroxide (H_2O_2), which then is turned to water [16]. When mitochondrial oxidative phosphorylation is hindered or antioxidant defence signalling is repressed, the formation of mitochondrial ROS increases, resulting in oxidative stress.

Mitochondrial biogenesis is the mechanism through which cells create new mitochondria by increasing the number of copies of their mitochondrial DNA. In mammalian cells, this mechanism is required because proteins are created via the process of gene transcription and translation from the genetic material in the cells

[17]. Mitochondrial biogenesis happens on a regular basis in normal conditions to furnish a sound source of energy by ensuring that there is an adequate number of functional mitochondria to compensate for the defective mitochondria that are removed. Mitochondrial biogenesis can also be triggered by increased energy needs, physical activity, or diseases that disrupt energy metabolism [18]. Multifaceted stimuli may trigger multiple signalling pathways, but to perform biogenesis programmes, every pathway culminate on a single collection of transcription and respiratory factors [19]. A signalling cascade involving peroxisome proliferator-activated receptor coactivator 1 (PGC-1α) and transcription factors initiates mitochondrial biogenesis in the nucleus [20, 21]. The two respiratory factors associated with mitochondrial biogenesis are nuclear respiratory factor 1 (NRF1) and 2 (NRF2). PGC-1α operates as a dominant player of mitochondrial biogenesis, boosting the gene transcription involved in mitochondrial DNA replication and transcription. It particularly activates the mitochondrial transcription factor A (Tfam), by increasing the expression and activity of the respiratory factors NRF1 and NRF2. Thus an increased expression of Tfam increases mitochondrial biogenesis. This is via the synthesis of mitochondrial RNA implicated in the process. NRF1 and NRF2, in addition to Tfam, control genes associated with mitochondrial nuclear-encoded proteins [20]. The transcription factor Tfam is also involved in the regulation of mitochondrial DNA transcription and replication as well as homeostasis [22–25].

Nuclear factor erythroid 2-like 2 (Nrf2; gene name NFE2L2) is an important transcription factor involved in mitochondrial biogenesis regulation. Nrf2 can stimulate mitochondrial biogenesis either in a direct way by raising NRF1 gene expression or in an indirect way by boosting PGC-1 gene expression [26]. PGC-1α expression in the organs like heart is well understood to be periodically controlled in response to environmental triggers including temperature, physical exercise, and dietary status [27]. During the mitochondrial biogenesis process that takes place in the cardiac cells, the nicotinamide adenine dinucleotide (NAD)-dependent deacetylase sirtuin 1 (SIRT1) targets PGC-1α. AMP-activated protein kinase (AMPK) also promotes PGC-1α via SIRT1 deacetylation by regulating NAD + levels [28]. This signalling mechanism involving AMPK, SIRT1 and PGC-1α may also contribute in brain mitochondrial biogenesis.

Mitochondrial bioenergetics is another important aspect of mitochondria. Mitochondrial bioenergetics is the oxidative phosphorylation mechanism that produces ATP. The mitochondrial membranes, both inner and outer, the intermembrane space, the cristae, and the matrix make up each mitochondrion. The intermembrane space is the space that lies in between the mitochondria's inner and outer membranes. It is necessary for the transfer of protons across mitochondrial membranes during the process of oxidative phosphorylation that takes place in the inner mitochondrial membrane [29].

During Krebs cycle, NADH and FADH2 are generated in the mitochondrial matrix. When the oxidative phosphorylation process takes place, NADH and FADH2 transmit electrons to the ETC complexes I through V located in cristae.

NADH electrons are transported to complex I known as NADH-coenzyme Q reductase or NADH-dehydrogenase and then to complex III that is coenzyme Q-cytochrome c reductase via ubiquinone which is an electron carrier related to the inner membrane. It is then transferred to complex IV or cytochrome c oxidase via cytochrome c. ETC complex II also known as succinate-coenzyme Q reductase in the Krebs cycle, creates FADH2 and transmits electrons to ubiquinone and complexes III and IV. As a result of electron transfer mechanisms, complexes I, III, and IV take protons from the mitochondrial matrix and send them into the intermembrane area [30]. The proton motive force generated by electron transport across the inner mitochondrial membrane then supplies energy for complex V or ATP synthase to drive ATP synthesis via ADP phosphorylation. To preserve the right amount of oxygen, efficient electron transfer via the ETC enzyme complexes, and a balanced mitochondrial inner membrane potential is required which is also crucial for ATP generation. A variety of mechanisms thus drive the workflow of mitochondrial oxidative phosphorylation and bioenergetics. Bioenergetic dysfunction can lead to a reduction in ATP production on the one hand and increased ROS production on the other, resulting in mitochondrial oxidative stress.

RAS and Oxidative Stress

The reactive oxygen species (ROS) are basically the byproducts of oxygen generated during its metabolism. The ROS incudes various chemical molecules such as hydrogen peroxide, super oxide ions and reactive nitrogen species. There are several mechanisms by which the RAS system, especially through the activation of ATII can lead to generation of ROS. One such mechanism has already been mentioned above when ATII binds to AT1R, it leads to activation of NOX and other free radicals. NOX mediated generation of ROS is the dominant source of production of ROS and oxidative stress in cardiovascular system. It is involved in microvascular wall remodelling in arterial hypertension [31]. These generated ROS can be highly injurious and toxic to the body. ATII can also induce ROS by inhibiting the anti-oxidant enzymes like superoxide dismutase (SOD) and catalase. These are supported by multiple evidences and studies carried out in the in-vivo models and on cultured cardiac cells. In a study carried out by Lijnen et. al., the treatment of rat cardiac fibroblast cells with ATII curbed the activity of anti-oxidant enzymes and when rats were treated with inhibitors of RAS, the activities of these enzymes were improved [32, 33]. In another study conducted by Iram et. al, ATII activation brought down the anti-oxidant activity of the enzyme catalase in mice and cardiac myocytes [34]. Another study carried out by Rey et. al., demonstrated that in ATII infused hypertensive rats, NOX subunit expression and activity was increased and administration of a NOX inhibitor reduced vascular generation of superoxide anion [35].

Some of the other producers of ROS include xanthine oxidase (XO), endothelial nitric oxide synthase (eNOS) and cyclooxygenase (COX) [36]. XO majorly contributes to the generation of ROS in cardiac failure and ischemia–reperfusion

injury. eNOS can generate ROS and nitric oxide in conditions of hypertensive diseases. COX is an enzyme that metabolises arachidonic acid from membrane-bound phospholipids to bioactive prostaglandins and thromboxane A2 and it has been identified as major source of ROS in hypertension.

Crosstalk Between Mitochondria and RAS

Recently a link between RAS and mitochondria has been known and mitochondria were identified to play a significant role in RAS. The angiotensin receptors AT1R and AT2R were initially recognized to be present on the plasma membrane [37, 38]. However, lately the receptors have been found in the mitochondrial membrane which is suggestive of a crosstalk between RAS and mitochondria. The role of ATII in the induction of ROS has long been recognized. However, its role in ROS generation locally has only recently been established [39]. ATII causes mitochondrial dysfunction, which promotes ROS production in the mitochondria through a protein kinase C (PKC)-dependent pathway [40]. Electrons generated in excess are delivered to the respiratory chain of the mitochondria, where they are transformed to super oxides, as a result of which there is a rise in mitochondrial ROS. This affects the process of Krebs cycle, resulting in a decrease in energy, which is reversed when ATII is inhibited by angiotensin receptor blockers (ARBs) [41]. ATII via activation of free radicals in the mitochondria inhibits mitochondrial electron transport chain and decreases mitochondrial membrane potential.

It was observed that when rodents were treated with ATII, they exhibited a reduction in the number of mitochondrial DNA. The respiratory capacity of mitochondria was also diminished. This is particularly mediated by decreased expression of respiratory chain complex expression, specifically I and III and increased DNA removal, resulting in mitochondrial impairments [42]. Altogether, an excessive stimulation of the RAS pathway alters mitochondrial morphology and biogenesis. Mitochondrial NOX 4 has been proposed to play a role in mitochondrial function in addition to the action of ATII guided by NOX in plasma membrane. NOX 4 silencing reduced mitochondrial ROS synthesis, whereas over expression led to mitochondrial dysfunction and decreased biogenesis. This was validated by the presence of localized mitochondrial NOX 4. Surprisingly, excessive activation of NOX 4 distinctively enhanced mitochondrial ROS generation, thereby increasing overall ROS production via a positive feedback loop [2, 43].

According to a recent study, ATII stimulates mitochondrial ROS production in vascular smooth muscle cells. The study also reported the effect of ATII and its oxidative effects in the rat aorta [44]. ATII stimulates mitochondrial superoxide production in bovine aortic endothelial cells as a result of vascular NOX activation. A link between ATII-related ROS production and mitochondrial activity was discovered in a study that found that antioxidants hinder the regulatory effects of ATII on the AP-1 signalling pathway [45]. ATII increases mitochondrial ROS production in endothelial cells which leads to stimulation of redox-sensitive NF-κB. NF-κB then stimulates

the release of vascular cell adhesion molecule-1 which is a crucial molecule in the development of atherosclerotic lesion [46].

Mitochondria and Cardiovascular Diseases

It has been suggested that dysfunction and excessive ROS production associated with mitochondria play a role in the occurrence of arterial hypertension. In hypertension, mitochondrial abnormalities are clearly observed [15]. These changes include a decreased mitochondrial mass and density, resulting in impaired energy production and accelerated ROS formation due to mitochondrial respiratory chain instability [47]. SIRT3 uses intracellular metabolites like NAD + and acetyl-CoA to modify mitochondrial activity in response to nutrition supply [48]. It stimulates mitochondrial SOD2, resulting in significant mitochondrial antioxidant activity that decreases with age. Notably, these molecular changes coincide with an increase in the prevalence of hypertension.

Corroborations suggests that aside from their impact on the peripheral vascular system, the primary modulation of systemic circulatory function may also be aided by mitochondria-derived ROS [49].

Experimental studies in the organs like heart, kidneys, and blood vessels subjected to hypertension have documented mitochondrial anomalies and malfunction in the last few decades. Renin-induced hypertension in rats, for example, is linked to abnormalities in the mitochondrial structure and apoptosis in the heart [50, 51]. In a study carried out in mice induced with hypertension, the kidneys showed an increase in ROS generation, whereas altered mitochondrial bioenergetics and antioxidant activity was observed in renal cells from spontaneously hypertensive rats [52]. Renovascular hypertension in pigs resulted in damage to mitochondria in the tubular cells of kidney, and cardiomyocytes, which ultimately can lead to dysfunction of kidney and heart. Obesity-induced hypertension in pigs resulted in mitochondrial changes in the arteries of kidney and heart, impairing endothelial function in the blood vessels [53–55]. These findings add to the increasing data that defective mitochondria are instrumental in the pathogenesis of hypertension-induced organ damage.

Mounting evidence indicating mitochondrial participation in cell signalling pathways through the conversion of intracellular calcium reserves, formation of reactive oxygen species (ROS), and interaction of NO with mitochondrial processes like respiration and biogenesis has reignited interest in mitochondrial physiology. Furthermore, the revelation that stress in cells whether acute or chronic, causes mitochondrial dysfunction, has reshaped their importance in the pathophysiology of diseases. Mitochondrial malfunction triggers signalling cascades that result in cell necrosis and apoptosis, and eventually organ failure. Disturbances in mitochondrial Ca^{2+}, ATP, or ROS metabolism characterize the bulk of many diseases apart from cardiovascular diseases namely obesity, diabetes as well as disorders of the brain [56].

Mitochondrial function is extremely important in the development of CVD. Importantly, because of the myocardium's high energy requirements, cardiomyocytes have

a greater percentage of mitochondria as compared to other types of cell. In addition, inherited dysfunction in mitochondrial oxidative phosphorylation system is becoming more recognised. Mitochondrial diseases appeared to have been tied to impairments in the respiratory chain process connected to mitochondrial DNA mutations [57]. Several mitochondrial DNA mutations have been noted in patients as the source of derangements and disorders of enzymes of oxidative phosphorylation. Diseases caused by familial mitochondrial DNA alterations are not highly prevalent than those caused by nuclear DNA errors. This could be related to the fact that mitochondria have multiple genome copies, and also the fact that mutated genes are associated with normal genes during the series of activities of mitochondrial fusion. Myocardial ischemia–reperfusion injury also lead to excess of Ca^{2+} in mitochondria, which causes unrestrained ROS production and the opening of the mitochondrial pore resulting in apoptosis. As a result, drugs that reduce excessive mitochondrial Ca^{2+} reducing ROS generation in mitochondria, and improve mitochondrial energy production could all be therapeutic targets for the disorders described [56].

Researchers have been particularly interested in mitochondrial dysfunction that is brought about by oxidative damage and its association to disease development since mitochondria are the principal source of oxidative stress in the cells. Oxidative damage to mitochondrial DNA has been attributed to several types of cancer, ageing, hypertension, and cardiovascular disease [58]. Damage to the mitochondrial membrane from oxidants can result in membrane depolarization and oxidative phosphorylation uncoupling, due to alterations in respiration process within the cells. Alterations in mitochondrial transcription levels and protein synthesis have also been reported. Injury to mitochondria affects the cell's ability to create energy. It disrupts the reduction and oxidation signalling, and hence affects an array of key physiological functions mediated by mitochondria. ROS generated in the ETC, have been identified as NF-κB activation intermediate messenger. The activation of NF-κB is strongly inhibited in cells lacking mitochondria. As a result, the overall equilibrium of mitochondrial ROS generation stimuli and the resulting debris of organelle damage might eventually alter the functionality of the cells. The resulting cellular response to increasing oxidative stress may thus contribute to CVD. As a result, in patients with cardiovascular conditions, mitochondrial damage appears to be an effective measure of mitochondrial dysfunction.

Mitochondria Targeted Treatment

Understanding the role of mitochondria and its relevance in RAS system and renal and cardiovascular diseases makes it crucial to find therapies that are targeted towards preventing mitochondrial damage or improving overall mitochondrial health.

Various therapies have emerged which utilizes CoQ10 or ubiquinone to provide antioxidant benefits in chronic cardiac and renal diseases with an aim to enhance mitochondrial health [59]. In a study conducted by McLachlan et. al., a combination of MitoQ10, a mitochondria specific anti-oxidant and low-dose ARB losartan

provided additive therapeutic benefit. It significantly attenuated the development of hypertension and reduced left ventricular hypertrophy in spontaneously hypertensive rats. In addition to this, MitoQ10 mediated a direct antihypertrophic effect on rat cardiomyocytes in vitro. MitoQ10 therefore exhibited a great potential to be used as a therapeutic agent along with antihypertensive drugs. When compared to control or solo therapies, this combined therapy showed considerable additive haemodynamic improvement, antihypertrophic, and antifibrotic effect. Only diastolic blood pressure did not exhibit an additive reduction when coupled with single medication, implying that MitoQ10 and losartan have a same diastolic pressure antihypertensive mechanism [60].

As previously mentioned, various ROS sources, including NOX, XO, uncoupled nitric oxide synthase (NOS), and the mitochondrial ETC, play a role in the development of cardiovascular disease [37]. The specific contributions to the underlying disease pathways of these various ROS are unknown due to the complicated interrelationship between them. ROS can further induce the production of ROS. Thus mitochondria can be initially triggered by NOX which guides to ROS generation potentially resulting in ROS production feed-forward cycle [37, 61].

Endothelial dysfunction is a precursor to the development of cardiovascular disorders (CVD). This disease is characterised by a decrease in the bioavailability of the vasodilator nitric oxide (NO) which is mostly owing to an increased degradation of NO induced by its reactivity with ROS. Although multiple factors, such as increase in glucose levels, hyperinsulinemia, and increased levels of lipids, all promote endothelial dysfunction, increased oxidative stress appears to be a major factor. Drugs utilised in clinical practise today, such as anti-hypertensive agents, ARBs and anti-hyperlipidemic drugs protect many organs via anti-oxidative stress mechanisms in addition to their original pharmacological capabilities. Furthermore, antioxidant-rich compounds like vitamin C, E have been employed to alleviate the oxidative stress linked to CVD. Clinical trial outcomes using agents that work against oxidative damage in individuals with CVD are conflicting, which might be due to poor research design or target selection. Controlling mitochondrial respiration and production of ROS while simultaneously shielding mitochondria from oxidative stress can be accomplished by targeting certain antioxidants and NO donors to mitochondria.

By conjugating antioxidants to the triphenylphosphonium (TPP) molecule, a diverse variety of antioxidants could be tailored to mitochondria. TPP conjugated ubiquinone derivatives, tocopherol, lipoic acid spin traps, and ebselen are among them [62–64]. Given the importance of lipid peroxidation in mitochondrial oxidative damage and the tight association of alkyl TPP conjugates with the inner membrane of the mitochondria, research has tended to focus on antioxidants that are efficient against lipid peroxidation. MitoQ, which is the specific variant of ubiquinol, in particular has been thoroughly investigated and is the most well-known member of the family [64]. MitoQ is swiftly picked up by isolated mitochondria as a result of the membrane potential of mitochondria, and approximately all of the accumulated MitoQ adheres to the inner membrane's facing the matrix, within mitochondria. In the respiratory chain, complex II reduces mitoQ to the active ubiquinol antioxidant. Because the reduced form of MitoQ is not oxidised by complex III, it cannot reinstate

the process of respiration in the mitochondria that are in short of coenzyme Q. As a result, all of MitoQ's actions are most likely resulting in the build-up of the antioxidant ubiquinol form. Notably, the ubiquinol form of MitoQ gets oxidised to the ubiquinone form when it serves as an antioxidant. Complex II then rapidly reduces this ubiquinone molecule, restoring its antioxidant activity. MitoQ is regarded to be an excellent source of antioxidant against lipid peroxidation because it is commonly carried in to the inner membrane. Additionally, its structure allows its antioxidant component, ubiquinol to travel far within the membrane. Damage caused by peroxynitrite has also been demonstrated to be protected by MitoQ [65]. It can also react with oxygen however; its interaction with H_2O_2 is minimal when compared with other ubiquinols. MitoQ thus appears to meet the majority of the requirements for a targeted antioxidant delivery in isolated mitochondria [66].

Conclusions

The inter relationship between RAS, mitochondria, generation of ROS and its effect on cardiovascular function is summarized in Fig. 8.1. Mitochondrial dysfunction and oxidative stress, which are frequently connected to inflammation, are important mechanisms that underpin a wide range of chronic illnesses. Expanding therapeutics to minimize oxidative stress and mitochondrial damage has the potential to inhibit or drastically decline metabolic changes in a variety of disorders, such as diabetes and obesity. Knowing the underlying processes that contribute to these derangements is crucial for developing effective obesity, insulin resistance, and diabetes preventive and therapeutic techniques. As previously stated, the RAS system and ATII is closely connected to the genesis and pathophysiology of a number of illnesses, in part via forming ROS. More research is needed, however, to completely comprehend the signalling processes initiated by ATII that lead to ROS production and mitochondrial abnormalities.

Furthermore, studies that combine network pharmacology and bioinformatics indicating connection across multiple organs in a disease condition is crucial for understanding the complicated interaction between ROS, mitochondria, and the RAS system. Additionally, substantial number of clinical studies involving ARBs or ACE inhibitors that target mitochondrial abnormalities and ROS are required as specific and safe RAS inhibitors are potentially available. These studies will help to translate cell and animal research into therapeutic practice. Furthermore, ROS signalling could be a promising target for future therapeutic approaches aiming at suppressing RAS.

Treatments to protect mitochondrial health and reduce oxidative stress in cardiovascular disease have made tremendous advances in recent years. Many of these prospective treatments show significant promise in vitro, and converting success in preclinical animal research into therapeutics for ROS mediated cardiomyopathies in human patients is one of the most difficult difficulties we have presently.

The grasp of several crucial issues will have to be extended. Mitochondria are the primary producers of energy in the cells along with ROS. Mitochondrial ROS is

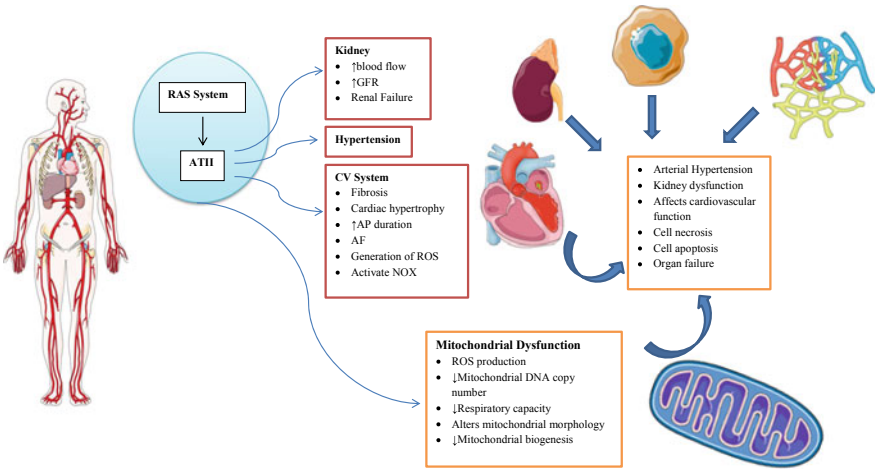

Fig. 8.1 Illustrates an overview on the impact of RAS system on mitochondria and disease manifestations. RAS, renin angiotensin system; ATII, angiotensin II; GFR, glomerular filtration rate; CV, cardiovascular; AP, action potential; AF, atrial fibrillation; NOX, NADPH oxidase; ROS, reactive oxygen species

involved in cell signalling, but they could potentially be oxidative stress mediators. According to the research, ATII increases mitochondrial ROS production, resulting in a decrease in mitochondrial energy metabolism. As a result, ATII inhibition reduces mitochondrial ROS production, improving mitochondrial ETC efficiency. This ultimately protects the mitochondrial structure. In rat models of diseases like hypertension and ageing, this appears to be one of the pathways causing the positive benefits of Ang-II inhibition. If these discoveries can be replicated in humans, it will open up a whole new world of possibilities for RAS blockage as a treatment modality.

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Chapter 9
The Renin Angiotensin System at the Time of COVID-19

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Abstract The renin angiotensin system RAS is one of the most complex regulatory circuits in our body, e.g. responsible for the regulation of blood pressure. The components of the RAS represent the target of pharmacological interventions for the treatment of various cardiovascular diseases. In the context of the coronavirus disease 2019 (COVID-19) pandemic, triggered by severe acute respiratory syndrome coronavirus type 2 (SARS-CoV-2) the RAS has currently come into even sharper focus. The background for this is that SARS-CoV-2 can bind to the RAS component angiotensin-converting enzyme 2 (ACE2) and thus penetrate host cells. We highlight here the discovery of the RAS and its components, but especially the interactions of SARS-CoV-2 with the RAS, the influence of lifestyle diseases, and what to consider for cardiovascular disease management in COVID-19 patients.

Keywords RAS · SARS-CoV-2 · COVID-19 · ACE2 · Lifestyle disease · Cardiovascular diseases

Some Background on RAS

The renin angiotensin system (RAS) is arguably one of the most studied physiological regulatory systems, and its pharmacological inhibitors are among the most prescribed drugs worldwide, particularly the antihypertrophic acting angiotensin-converting enzyme (ACE)-inhibitors and angiotensin II type 1 receptor (AT1R) antagonists. Often the hormone aldosterone is also named and then abbreviated as RAAS. Basically, the RAS is a regulatory circuit with various hormones and enzymes that primarily regulate the body's volume balance as well as blood pressure. The RAS is not only well studied, but also known for a comparatively long time.

A brief history of the discovery of the RAS: The first observations in this context date back to 1836, when Richard Bright related left ventricular hypertrophy to

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increased resistance in small blood vessels [1]. About 40 years later, in 1872 F. A. Mahomed set the stage for many further developments in the field by using a primitive sphygmograph for the first time and describing high blood pressure and also linked that to left ventricular hypertrophy [2]. Shortly before the turn of the century, it was finally Riva-Rocci who, in 1896, described an indirect sphygmomanometer for measuring arterial pressure in patients, thus considerably simplifying the measurement and making serial examinations possible [3]. A possible link between pathological changes in the kidney and the development of arterial hypertension was already postulated at that time. Tigerstedt and Bergman from the Karolinska Institute identified the presence of a factor in renal extracts of rabbits that directly affects blood pressure and which, because of its origin, was named renin [4], the first component of the later RAS. Based on their research, they realized that the association of kidney disease and cardiac hypertrophy was caused by the release of a vasoactive substance from the kidney that induced the contraction of blood vessels. However, the substance itself has not yet been identified at that time. After many years of various unreliable animal models in which the kidney function was experimentally manipulated, finally, the experiments of Goldblatt et al. in 1934 led to the successful discovery of the active polypeptide renin [5]. Goldblatt used a silver clip to partially constrict the renal artery of dogs—the technique was subsequently called Goldblatt technique/model—and he already proposed the existence of a vasoconstrictor factor. In 1936, two groups in parallel in Buenos Aires, Argentina, led by Dr. Bernardo Houssay, and in Indianapolis, USA led by Dr. Irvine H. Page used the Goldblatt technique to identify the factor secreted by the ischemic kidney [6, 7]. Again, numerous animal experiments in different laboratories were necessary to fit the new findings into an overall picture. All these experiments concluded that renin from the kidney acts enzymatically on an inactive plasma precursor protein, thus creating a new pressure factor. This substance could be isolated from the blood and was first given different names, hypertensin in Buenos Aires and angiotonin in Indianapolis. Only after almost 20 years, both sides agreed to a uniform name: angiotensin [8]. The RAS has been discovered! Shortly before, the biologically active angiotensin octapeptide could be synthesized for the first time in Dr. Page's laboratory [9].

Timing and mechanism of the RAS: It is not our intention to describe the mechanism and all involved components of the RAS in detail and fully comprehensive, there are many good reviews to this topic, e.g. [10, 11] and other chapters within this book. Rather, we want to give a general overview of the RAS and its chronological sequence for an adequate understanding of the following article.

The RAS cascade can be well explained with renin as the starting point. The protease renin is released from specialized parts of the kidney tissue, the juxtaglomerular apparatus. This happens, for example, when the blood pressure drops (e.g. in the case of renal artery stenosis) or the loss of electrolytes and water (and thus blood volume) occurs. Subsequently, renin cleaves angiotensinogen synthesized in the liver to generate the decapeptide angiotensin I (Ang I) which is then further cleaved by ACE to form the octapeptide angiotensin-II (Ang II). Ang II represents the physiologically active component of the RAS and mediates both direct and indirect effects. First, Ang II directly leads to a constriction of small blood vessels (vasoconstriction)

via stimulation of the vascular expressed AT1R, which leads to an increase in blood pressure. Via direct AT1R stimulation in the kidney, Ang II regulates renal sodium and water reabsorption and consequently blood pressure. Indirectly, Ang II mediates effects through the release of the hormone aldosterone from the adrenal gland and antidiuretic hormone (ADH or vasopressin) from the pituitary gland to regulate electrolyte and water balance. In addition, Ang II, together with other hormones in the central nervous system, leads to a demand for electrolytes and thus triggers the sensation of thirst [10, 11]. In 2000, ACE2, a homolog of ACE, was identified [12, 13], which was increasingly associated with the effects of severe acute respiratory syndrome coronavirus type 2 (SARS-Cov-2) during the pandemic. We discuss this in more detail later in this chapter and a simplified schematic of the RAS and its interaction with SARS-CoV-2 is summarized in Fig. 9.1. ACE2 is predominantly expressed in the kidney and heart [14] and uses both Ang I and Ang II as substrates. Ang II is directly converted by ACE2 to Ang 1–7, which has vasodilator effects. Ang I is first degraded by ACE2 to Ang 1–9 and then by ACE to Ang 1–7 and Ang 1–5. Therefore, ACE2 plays important role in the regulation of the RAS [15]. Ang II binds specifically to two G protein-coupled receptors on the cell surface, with quite a different signal transduction and opposite effects. The aforementioned AT1R, which mediates most of the physiological effects of Ang-II, and the angiotensin II type 2 receptor (AT2R). The AT1R mediates vasoconstriction and inflammatory effects and the AT2R vasodilatation and anti-inflammatory effects, and both receptors are correspondingly expressed in the kidney, adrenal gland, and brain. In addition to the classical AT1R and AT2R, another angiotensin receptor has been identified [16], the Mas receptor (MasR), which is activated by Ang 1–7 [17] and mediates vasodilation. Excessive activation of the RAS is avoided by a negative feedback loop. Thus, high blood pressure and high levels of Ang II, as well as aldosterone, inhibit the release of renin. In addition to the circuit described above, in which various organs are involved, all components of the RAS could be also found e.g. in the brain, the vasculature, and the kidney, which form a local RAS [15].

RAS inhibition: The RAS is the target of several drugs, mainly for the treatment of high blood pressure. Ang II in particular is the target of pharmacological inhibition. There are two different strategies. Inhibition of ACE by ACE inhibitors (ACE-I) prevents the formation of Ang II, or angiotensin II receptor blockers (ARB), also called AT1R antagonists, prevents the action of Ang II by blocking its receptor. However, other components of the RAS are also pharmacological targets. We discuss them in more detail in Sect. 9.4.

COVID-19 in the Context of RAS

Since the end of 2019, the RAS and, in particular, ACE2 is gaining much interest in the context of the coronavirus disease 2019 (COVID-19) pandemic. This pandemic is caused by SARS-CoV-2 [18], a beta coronavirus that also includes SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV). Coronaviruses have a

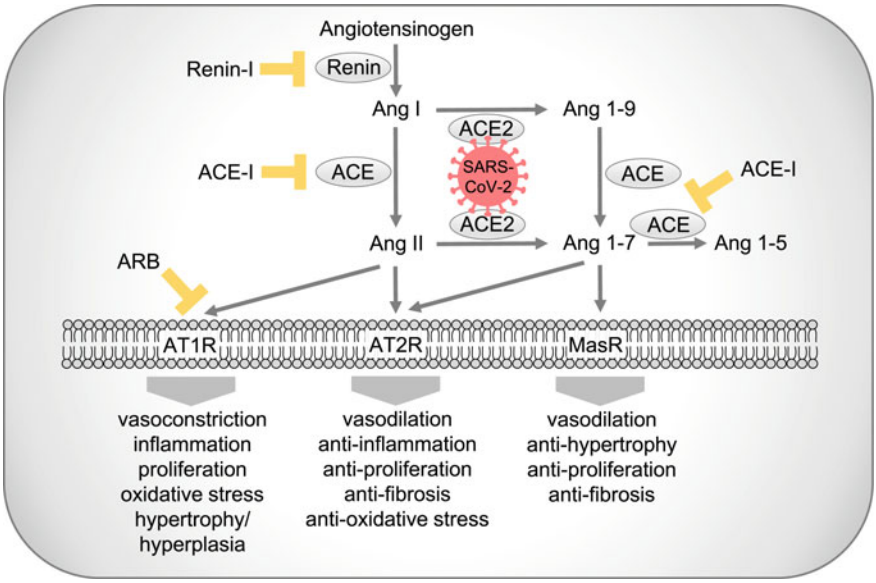

Fig. 9.1 Simplified schematic of the RAS and its interaction with SARS-CoV-2. ACE = angiotensin-converting enzyme, ACE-I = angiotensin-converting enzyme inhibitors, ARB = angiontensin II receptor blockers, Ang = Angiotensin, AT1R = angiotensin II type 1 receptor, AT2R = angiotensin I type 1 receptor, MasR = Mas receptor, SARS-CoV-2 = severe acute respiratory syndrome coronavirus type 2, Renin-I = renin inhibitors

single-stranded RNA genome with positive polarity and a length of about 30 kilobases, the largest known genome of any RNA virus. Coronaviruses are widespread among mammals and birds; due to the possibility of homologous recombination, they can relatively easily expand the host range [19]. They mainly cause mild colds in humans but can sometimes cause severe pneumonia and acute respiratory distress syndrome (ARDS). During the first SARS pandemic (SARS-CoV-1) in 2002 and 2003, ACE2 was found to be a necessary receptor for entry into human cells [20]. According to the World Health Organisation (WHO), during the SARS-CoV-1 pandemic, there were 774 deaths worldwide, and it was found that the virus was probably transmitted from bats to humans as a zoonosis. MERS-CoV was first detected in patients in Arabia in April 2012 [21]. So far, more than 2400 laboratory-confirmed cases (including more than 800 deaths) have been reported to the WHO. As with SARS-CoV-1 and SARS-CoV-2, MERS-CoV is a zoonotic pathogen, and dromedaries are considered to be the reservoir. The SARS-CoV-2 pandemic has caused several million deaths worldwide, with the first cases reported in Wuhan in China [22]. Investigations continue into the exact origin of the SARS-CoV-2 virus.

Intensive research has shown that SARS-CoV-2 and related coronaviruses are characterized by spike proteins on their surface. SARS-CoV-2 (like SARS-CoV and HCoV-NL63) uses the transmembrane enzyme ACE2 as a receptor to enter host cells. Scientific studies have shown that SARS-CoV-2 has a significantly higher

binding affinity to ACE2 than SARS-CoV-1, and this is considered one of the possible explanations for the higher transmission rate. Cell entry requires additional cofactors that couple the virus to the host cell's ACE2 receptor. This causes the spike protein to change its conformation and the viral genome to enter and replicate in human cells. An important cofactor is the cellular protease TMPRSS2 [23]. This enzyme forms a complex with the ACE2 receptor, and it has been found that ACE2 and TMPRSS2 have a high co-expression rate in the upper respiratory tract [24]. It was shown that the main route of transmission for SARS-CoV-2 is the ingestion of virus-containing particles via the respiratory tract. However, high ACE2 expression is also found in the intestine, in vascular cells, in the kidneys, and the heart muscle.

Since the beginning of the spread of SARS-CoV-2, it has been observed that the viruses have multiple mutations with polymorphic nucleotide positions that lead to amino acid exchanges. Relatively frequently, these changes affect the spike protein. Based on mutation analyses, the viruses are classified into variants. These changes in the pathogen genome can be associated with altered properties of the pathogen, e.g. higher transmissibility, an altered immune response, or a more severe course of the disease. At the pathogen level, this may be associated with a change in the spike protein region towards a more open conformation, favoring binding to the ACE2 receptor protein of target cells [25].

SARS-CoV-2 frequently causes respiratory infections. Pneumonia may develop, usually in the second week of illness, and may progress to ARDS, requiring ventilation. ARDS is a massive reaction of the lungs to various damaging factors and is the most severe form of acute pneumonia. Irrespective of the triggering noxious agent, a cascade of pathophysiological reactions occur in three phases, culminating in global respiratory insufficiency. The RAS is involved in the pathogenesis of ARDS. The RAS influences mechanisms such as vascular permeability, vascular tone, fibroblast activity, and alveolar epithelial cell survival. In ARDS, epithelial damage occurs, and the ability to produce ACE2 appears to be severely impaired, leading to dominant ACE activity during ARDS [26]. It has also been shown that there is an association between reduced ACE2 activity in patients with ARDS and the occurrence of an exaggerated inflammatory response [27]. However, other organ systems are frequently affected in addition to the lungs, resulting in a broad spectrum of sometimes severe extrapulmonary manifestations. Underlying pathomechanisms include cytolysis, meaning direct damage to host cells by the replicating virus. Furthermore, a dysregulated, exuberant immune response can lead to a life-threatening cytokine storm [28]. In addition, organ-specific inflammatory reactions and endothelial damage occur, which are associated with a dysregulation of the renin angiotensin system and can lead, for example, to thromboembolic complications [29, 30].

Association of ACE2, Lifestyle Diseases, and SARS-CoV-2

Among the typical lifestyle diseases of the Western world, which have arisen as a result of material and ideational influences within the modern society, are diabetes

mellitus, obesity, and hypertension. According to the WHO, they are among the Top-10 leading causes of death worldwide. 600 million people are affected by obesity, 1.5 million patients annually die from diabetes mellitus, the number of hypertensive patients worldwide is arising and was 1.13 trillion, already in 2015 [31–33]. All of these diseases summarized as "metabolic syndrome", have in common that they exert a significant influence on the innate, but primarily adaptive immune defense [34, 35] Thus, diabetes, obesity, and hypertension are considered as risk factors for both, infection with and progression of COVID-19 disease [36, 37]. This is of particular importance as this combination of metabolic disorders and compromised immune defenses leads to a significant increase in mortality associated with COVID-19 disease [37]. Likewise, ACE2 plays an essential role in this context [38].

Transmembrane ACE2 is required for penetration of the SARS-CoV-2 virus into e.g. cardiomyocytes, pneumocytes, and vascular cells. In diabetes, the dysregulation of ACE2 due to recurrent hyperglycemia leads to increased insulin resistance and consequent accumulation of body fat [38]. In a vicious cycle, obesity, in turn, triggers persistent low-grade inflammation in adipose tissue with at least elevated levels of interleukin (IL)-6, IL-8, IL-15, tumor necrosis factor (TNF)-α, and leptin. Leptin, a proteohormone, for example, influences the modulation of the innate and adaptive immune response by regulating a.o. T-cells, monocytes, and dendritic cells [39]. Thus, the circle of diabetes, obesity, and inflammation closes.

Anti-inflammatory aspects on the other hand are associated with another member of the RAS-family, Ang 1–7 via binding to MasR. Cytokine release and tissue fibrosis are significantly reduced under the influence of Ang 1–7. However, the imbalance of the ACE2/Ang 1–7/MasR axis impairs these protective effects, resulting in the aggravation of diseases and pathological processes [40]. In this context recent publications could show, that the insulin effect is much improved in the presence of Ang 1–7, moreover, the opposite effects of Ang 2 occurred in a diminished form [41, 42].

Other clinical characteristics, same associated with ACE2 downregulation and the corresponding pathological processes as described above, are older age, vascular disease, and arterial hypertension. Worth mentioning in this context are thiazide diuretics, which are considered to be an important substance class in the drug therapy of arterial hypertension. Thiazide diuretics influence the members of the RAS due to volume depletion [43]. In a dose-dependent manner, the sodium–potassium exchange is modified, which in turn affects insulin secretion and favors pro-diabetic metabolism. Carriers of certain genetic ACE polymorphisms bear different risks in this respect. Carriers of the ACE 4656 C allele and ACE 4656 GG homozygotes belong to a class with a particularly high risk of developing diabetes in context with thiazide diuretics [45].

However, the SARS-CoV-2 virus itself also leads to ACE2 deficiency [46]. Thus, in patients with COVID-19 disease and metabolic disorders, the described, pre-existing systemic inflammatory reaction is further potentiated due to the "cytokine storm" triggered by SARS-CoV-2, which ultimately leads to further deterioration of organ function and vascular damage.

As an aside, the combination of smoldering inflammation and arterial hypoxia in the presence of a previously existing, underlying obstructive pulmonary disease

or/and COVID-19 results in further functional impairment of the respiratory musculature and corresponding hypoxia. In this context is particularly noteworthy, that malnutrition in terms of trace elements and vitamins, such as in obesity, plays a fundamental role in immunity and the regulation of immune responses. Zinc deficiency for example causes the release of cytokines whereas vitamin C and D have protective, anti-oxidative effects on cells and inhibit pro-inflammatory factors [39]. For vitamin D in particular, a regulatory effect of ACE members, in general, is also described with a positive effect in acute respiratory failure [47].

Overall, the level of ACE2 expression is affected in a multifactorial manner in the presence of metabolic disorders. Simultaneous infection with SARS-CoV-2 further potentiates the resulting pathological effects. Thus, in the future, early therapy and broad prevention of typical lifestyle diseases are urgently needed to limit the severity and consequences of COVID-19.

Treatment of Cardiovascular Diseases Considering COVID-19

The RAS is one of the most important starting points in the treatment of cardiovascular diseases. It plays an important role in the therapy of coronary artery diseases, arterial hypertension, and chronic heart failure. The drugs that intervene in the RAS system belong to internationally recommended therapy schemes and are used regularly [48–51]. The currently recommended treatment proposals are summarized in Table 9.1.

In the presented drug treatment concepts, the RAS blockers each form an important pillar. In the last few years, different substance classes have been developed which intervene at different points in the RAS system. The following list in Table 9.2 provides an overview of the substance groups that can be used.

Table 9.1 Therapy of cardiovascular diseases

Coronary artery disease	Arterial hypertension	Chronic heart failure
Beta-blockers	ACE-I/ARB	Beta-blockers
ACE-I/ARB	Calcium-channel antagonist	ACE-I/ARB
Nitrates	(a) Dihydropuridine	Loop diuretics for fluid retention
Calcium-channel antagonist	(b) Verapamil/Diltiazem	Ivabradine
Ivabradine	Beta-blockers	digitalis glycosides
Ranolazin	Diuretics	MRA
Trimetazidine	Possibly:	ARNI
	+ Alpha-blocker	$SGLT_2$ inhibitor
	+ Spironolactone/Amiloride	

Overview of the therapy schemes for selected cardiovascular diseases
ACE-I = angiotensin-converting enzyme inhibitors, ARB = angiontensin II receptor blockers, ARNI = angiotensin receptor-neprilysin inhibitor, MRA = mineralocorticoid receptor antagonists, SGLT2 = sodium-glucose co-transporter 2

Table 9.2 RAS inhibitors

Active ingredient	Approach in RAS
ACE-I	ACE (not ACE2)
ARB	Prevent binding of Ang II to AT1R
Renin inhibitor	Block the active center of renin
Mineralocorticoid receptor antagonists	Block the aldosterone receptor directly or inhibit the incorporation of epithelial sodium channels

Active ingredients to block the RAS
ACE = angiotensin-converting enzyme, ACE-I = angiotensin-converting enzyme inhibitors, Ang II = angiotensin II, ARB = angiotensin II receptor blocker, AT1R = angiotensin II type 1 receptor

The therapy of cardiovascular diseases is influenced in different areas in the context of the COVID-19 pandemic. Above all the discussion about the use of RAS blockers, but also how to deal with COVID-19 positive or suspicious patients who are admitted with an acute coronary syndrome [52]. At the beginning of the pandemic, there were many reports about possible risk factors, including pre-existing illnesses, for infection with COVID-19. Arterial hypertension in particular often seemed to correlate with severe disease, the need for intensive care therapy, the need for ventilation, or increased mortality. This was accompanied by the suspicion that drug therapy for previous illnesses, including the use of RAS blockers, could also be involved in the serious course of the disease [53]. Studies before the COVID-19 pandemic, which examined risk factors for pneumonia, pointed in a similar direction. The identified risk factors for pneumonia in patients >60 years include lifestyle factors such as alcohol consumption, arterial hypertension, and heart disease [54]. As a result, the majority of patients with lower respiratory tract infections bring drug therapy for cardiovascular comorbidities with them. Because ACE2 is the binding receptor for the spike protein of SARS-CoV-2 [20], it was initially assumed that the risk of infectivity increases under RAS blocker therapy. This theory is based on results from animal experiments that the blockade of the Ang II/AT1R axis leads to an increased upregulation of ACE2 via a feedback mechanism. This hypothesis was initially supported by the observations of increased morbidity and an increased number of complicated disease courses in patients suffering from COVID-19 who had arterial hypertension as a pre-existing disease [55]. Reports from two particularly affected countries (China and Italy) could not, however, further substantiate the hypothesis [55]. In the course of the study, it was shown that infection with SARS-CoV-2 and the spike protein of SARS-CoV-2 lead to suppressed expression of ACE2. In the mouse model, after the injection of SARS-CoV-2 spike protein with a consecutive reduction of ACE2, severe acute lung failure was observed, which could be weakened by blocking the RAS [56]. Contrary to the initial theories in the pandemic, RAS blockers can thus be considered to have a protective function with regard to acute lung failure [55]. In a small study, increased plasma levels of angiotensin II were detected in COVID-19 positive patients, which correlated with the absolute viral load and the degree of lung damage [57]. In preclinical studies, the

application of recombinant ACE2 appears to halt the process of lung damage caused by a viral infection [58] and has been shown to reduce the angiotensin II level [59].

Overall, it is not recommended to interrupt therapy with ACE-I or ARB if it is indicated [55]. If this important pillar in the therapy of coronary artery disease, arterial hypertension, and chronic heart failure patients is withheld or discontinued, a significant increase in cardiovascular complications can be expected [48–51, 55]. In addition, it could be shown that the Ang II/AT1R path supports lung failure, whereas an increased occurrence of ACE2 has a protective effect on avoidable lung damage. This also further supports the thesis of maintaining an existing RAS blockade [55].

Concluding Remarks and Future Perspective

Taken together, the RAS is pivotal not only for blood pressure regulation but also plays a critical role in COVID-19 pathogenesis within the vascular system. ACE2 is found throughout almost all organ systems in mammals and the binding of the SARS-CoV-2 virus on ACE2 might explain the symphony of COVID-19 symptoms but might also explain the detrimental effects seen in so-called post-acute or long-COVID patients [60, 61]. Contrary to initial reports, RAS blockade in COVID-19 patients is safe and could be even beneficial for patients with cardiovascular risk factors [62, 63]. It is tempting to speculate that AT1R blockade may be more protective via the upregulation of ACE2 as traditional ACE inhibition. However, both drug regimens suppress excessive cytokine production such as IL-6 synthesis via the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway and prevent reactive oxygen species production in the vasculature thereby protecting from SARS-CoV-2 detrimental vascular effects [64, 65].

Moreover, the direct IL-6 blockade has been shown in several clinical trials (e.g. the TOCIVID-19 trial in Italy) to prevent a severe disease progression [66–68]. Thus, RAS inhibition in the early phase and anti-IL-6 therapy by tocilizumab in the later phase could help to prevent COVID-19 progression in patients. Based on our patho-physiological understanding (see Fig. 9.1) it is worth speculating that selective ACE2 inhibition will be protective in COVID-19 patients but needs further evaluation.

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Part II
Pathophysiological Implications of Renin Angiotensin System

Chapter 10
A Ying-Yang Perspective on the Renin Angiotensin System in Cardiovascular Disease

Sarfaraz Ahmad and Carlos M. Ferrario

Abstract The renin angiotensin system (RAS) plays a critical role in the regulation of the homeostatic control of arterial pressure, body fluids, and cardiovascular adjustments to metabolic needs. Angiotensin II (Ang II) is considered to be the main effector molecule of the RAS contributing to the adverse cardiac, vascular, and renal organ remodeling in the development and progression of the cardiovascular disease (CVD) through the activation of specific Ang II type 1 receptor (AT_1R). The endocrine action of circulating Ang II in blood pressure regulation have been extensively documented. The biochemical pathways leading to the generation of the biologically active angiotensins result from the metabolic processing of angiotensinogen, a 425 amino acid protein synthetized primarily by the liver. According to the classical pathway, Ang II is generated by sequential cleavage of angiotensinogen to angiotensin I (Ang I) by renal renin. Ang I is then cleaved into Ang II primarily by angiotensin converting enzyme (ACE) in circulation and by chymase in the tissues. The complexity of biochemical cascade leading to the production of Ang II, the vasodilator peptide angiotensin-(1-7) [Ang-(1-7)], and other biologically active peptides has now been expanded by the identification of shorter forms of the angiotensinogen substrate that upstream of Ang I are processed by non-renin dependent mechanisms. This chapter will detail the biochemical physiology of angiotensin-(1-12) [Ang-(1-12)] and its function as an endogenous source for Ang II generation. Collectively, the discovery of Ang-(1-12) offers an opportunity to unravel how intracellular synthesis of angiotensins proceeds through different biochemical mechanisms.

Keywords Renin angiotensin system · Renin · Angiotensin-(1-12) · Angiotensin I · Angiotensin II · Cardiovascular disease · Angiotensin converting enzyme · Chymase · RAS inhibitors · Monoclonal antibody therapy

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Introduction

The renin angiotensin system (RAS) [also called as renin–angiotensin–aldosterone system, RAAS] constitutes a critical hormonal system that regulate multiple physiological pathways affecting the long-term control of tissue perfusion, fluid balance, and almost all other physiological functions [1–5]. The biochemical physiology of the classical RAS represents one of the most extensively studied endocrine system that early on was considered to be an atypical endocrine system restricted to participating in blood pressure (BP) regulation and body electrolyte balance. Scientific advances attained with the explosion of molecular biology approaches permitted the identification of RAS genes and proteins in tissues other than the kidneys and the enunciation of hypothesis in which the local production of the angiotensins permitted these peptides to function as intracrine (intracellular), autocrine (on the cell surface) and paracrine (adjacent cells) regulators of cell-to-cell communication and cellular function [5, 6]. Despite the significant advancements attained in the characterization of the biochemical physiology of the RAS, the precise contribution of angiotensins in the pathogenesis of cardiac and vascular diseases, type 2 diabetes, and chronic kidney disease remains to be fully resolved [7–9].

CVD is one of the leading causes of death worldwide that can largely be mitigated by a healthy lifestyle, medications, or both. In general terms, CVD refers to conditions affecting the heart, blood vessels (veins and arteries), and the kidneys. The biologically active peptides of the RAS have very diverse pathophysiological actions and are present in almost all tissues of the body [6]. Activation of the RAS and alteration in RAS components have been correlated with CVD in both experimental animals and clinical conditions. A robust literature demonstrate that the RAS is regulated differently in the circulation and at cellular/tissues levels. In this review chapter, we discuss the complementary, interconnected and interdependent functions of circulating and tissue/intracellular RAS in the pathophysiology of CVD.

Current Prospect of RAS in CVD (Systemic *vs* Tissue)

As outlined in Fig. 10.1, earlier the RAS was recognized as a simply classical linear pathway in circulation to generate Ang II by a sequential process of the liver-derived angiotensinogen protein into angiotensin I (Ang I, a decapeptide) by the action of renin enzyme (secreted by kidney into circulation), which in turn is acted upon by angiotensin converting enzyme (ACE, a dipeptidyl carboxypeptidase that cleaves two amino acid from C-terminal) to generate angiotensin II (Ang II, an octapeptide). Ang II is further cleaved by a carboxypeptidase ACE homologue, angiotensin converting enzyme 2 (ACE2, a carboxypeptidase removing only a single amino acid from the peptide C-terminus) [10] into angiotensin-(1-7) [Ang-(1-7), a heptapeptide] which possesses novel biological functions that are distinct from Ang II. The pathophysiology of RAS comprises three distinct counteracting axes in cardiovascular

system, which counteract each other in terms of vascular function. The activation of ACE/Ang II/Ang II type 1 receptor (AT_1R) pressor arm responsible for deleterious effects of Ang II (including vasoconstriction, endothelial dysfunction, inflammation, and fibrosis), whereas the ACE2/Ang-(1-7)/Mas-R depressor/protective arm opposes the Ang II molecular and cellular effects of Ang II in CVD [11]. Ang II also binds a second receptor, Ang II type 2 receptor (AT_2R) that firmly established as a protective arm (ACE/Ang II/AT_2R) in RAS by eliciting functional antagonism to AT_1R [12, 13]. Largely accepted that these three arms play a coordinated role in regulating cardiovascular functions [8, 14–17].

Most all the components of the circulating RAS are also detected in the tissues and even in single cells of the cardiovascular system including cardiac cells (cardiomyocytes and fibroblasts), endothelial cells and vascular smooth muscle cells [9, 18–20]. It is still controversial whether RAS components are exclusively synthesize intracellularly or some components are internalized from the circulation [21]. However, in either case, their presence in the tissues and intracellular environment contributes to homeostatic regulation of cardiovascular function. Robust literatures show that the receptors for Ang II (AT_1R and AT_2R) and Ang-(1-7) (Mas-R) are expressed in

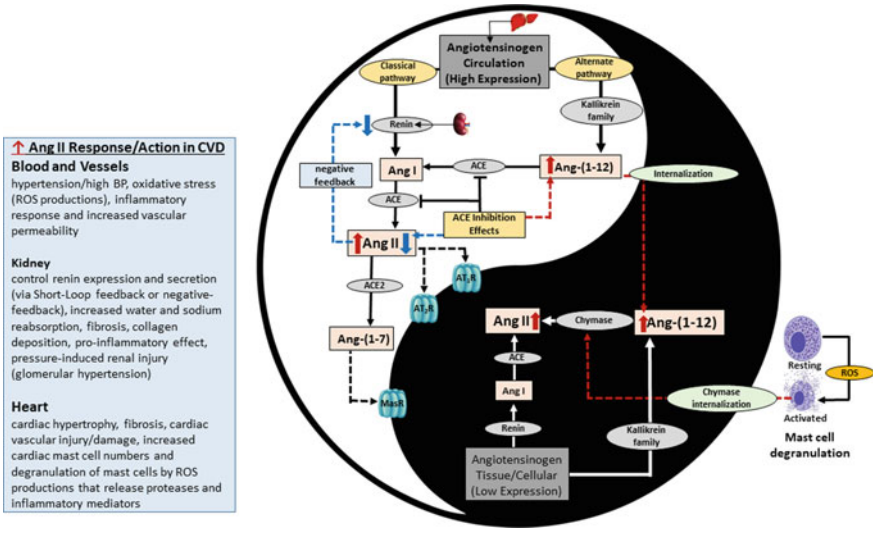

Fig. 10.1 Prospect of circulating and tissue RAS. Angiotensinogen (high expression in circulation and low expression in tissue/cell) is primarily metabolized into angiotensin peptides via the classical pathway (renin-dependent) and an alternate pathway (renin-independent). The formation of Ang II in circulation depends on ACE and in tissues/cells on chymase. Angiotensin-(1-12) is generated from angiotensinogen by a kallikrein like enzyme and serves as an alternate substrate for Ang II generation. High levels of circulating Ang II may directly inhibit renin expression and secretion from the kidney mediated by an AT_1R via negative feedback mechanism. Both Ang-(1-12) and chymase are internalized into cells from the extracellular environment. Inhibition of ACE may increase the Ang-(1-12) level in circulation (alternate pathway), tissue/cellular accumulation of Ang-(1-12) internalization and increase tissue burden of Ang II

cardiovascular tissues [22–26]. In addition to AT_1R, AT_2R and Mas-R, specific receptors for (pro)renin and renin [(P)RR] has been also identified and widely expressed in various tissues. This finding suggest that circulating RAS components may influence the RAS level in cardiovascular tissues [27, 28]. The renin mRNA concentration in normal hearts are close to or below detectable limit suggesting that normally renin is not synthesized in cardiac tissue and might be internalized from the circulation. However, several fold increases in renin and angiotensinogen mRNA have been reported in the heart of experimental animals after myocardial infarction [29].

RAS has emerged as one of the essential link in the progression of the cardiovascular pathophysiological changes [24, 30, 31]. Chronic activation of the RAS (both circulating and tissue level) promotes cardiovascular damage and this effects is antagonized by components of the counter-regulatory RAS [24]. The principal mechanism(s) of an activated RAS include the upregulation of the Ang II signaling pathways, Ang II-mediated generation of reactive oxygen species, and triggering of pro-inflammatory cytokines. These changes stimulate endothelial dysfunction, cell hypertrophy and adverse cardiac and vascular remodeling [32]. As described above, the two biologically active angiotensin peptides [Ang II and Ang-(1-7)] act oppositely. Ang II elicits vasoconstriction and Ang-(1-7) acts as a vasodilator in circulation. Whereas in tissues, Ang II exerts pro-fibrotic effects and Ang-(1-7) acts as an anti-fibrotic agent through distinct signaling pathways. The physiological actions of Ang II and Ang-(1-7) are triggered through the binding of the G protein-coupled receptors (GPCR) AT_1R, AT_2R and Mas-R, respectively [33].

It is widely accepted that circulating RAS components influence the cardiovascular system. Ang II is considered the major final mediator of the RAS pathway implicated in CVD (such as CHD, CAD and heart failure). Our and other studies show that the level of Ang II in rat heart tissue are several times higher than those measured simultaneously in plasma [34]. The tissue level of Ang II generating components (angiotensinogen, renin and ACE enzyme) are very low in normal cardiac tissues [35–37]. From experiments entailing bilateral nephrectomy, most if not all renin detected in the cardiac tissue originates exclusively from the kidney [21]. Increased expression of RAS components in cardiac tissues were reported in CVD suggesting that either the RAS peptides are up-taken from circulation into the cardiac tissues through receptors or synthesis occurs intracellular in the cardiac cells parallel to the circulation [21, 38, 39]. Cellular internalization of RAS peptides, proteins and receptors (AT_1R and Mas-R) have been documented earlier [39–42]. Unlike AT_1R, AT_2R does not undergo internalization alone. AT_2R internalization was specifically observed only in the presence of AT_1R [43].

Ang II Generating Substrates and Enzymes in CVD

There is generally agreement that Ang II is generated from angiotensinogen via two different pathways, 1) the classical [renin dependent] canonical pathway; and 2) the alternate non-canonical pathway [renin-independent]. As illustrated in Fig. 10.1, in

the classical pathway Ang II generation in circulation is mainly depending on the sequential hydrolytic actions of renin on angiotensinogen and the secondary conversion of Ang I into Ang II by ACE. ACE is present as a membrane bound form in endothelial cells, epithelial cells and also present as a soluble form in blood and other body fluids. In hypertensive patients, an increase in ACE levels have been detected [44, 45]. Chymase (a chymotrypsin-like serine protease) is another enzyme that also generate Ang II very efficiently from Ang I substrate; the catalytic activity of chymase is 20-fold higher compared to ACE [46]. Although, the chymase protein has been detected in the range of picogram (pg) to nanogram (ng) per mL in human serum of normal and hypertensive patients by ELISA, a direct pressor activity of chymase has not been demonstrated. A beneficial effect of an orally active chymase inhibitor in reversing adverse cardiac remodeling in patients with left ventricular dysfunction (LVD) after acute myocardial infarction (MI) paves the way for a resurgence of interest in understanding the function of the Ang-(1-12)/chymase axis in human cardiovascular disease [47, 48]. Although chymase is not directly involved in the functional regulation of blood pressure, it plays a critical role in structural remodeling of the cardiovascular system [49–52]. This conclusion is in keeping with recent studies of novel chymase inhibitors in 3 randomized, single-center, phase 1 studies in healthy male volunteers [48], in patients with LVD after acute MI [53], and patients treated with fulacimstat after acute ST-segment-elevation myocardial infarction (STEMI) [47] or diabetic kidney disease [54]. The lack of direct blood pressure effect is to be expected as chymase functions as a cellular protease [55]. The inability of chymase-mediated Ang II generation in blood may be due to the presence of high concentration of endogenous natural protease inhibitors (such as α2-macroglobulin, α1-antitypsin, α1-antichymotrypsin and eglin) in the human plasma [56–59].

A growing number of studies suggest that Ang II formation in the tissues is primarily mediated by chymase and is independent of ACE [34, 60–65]. In tissues, chymase, stored in secretory granules of mast cells (MCs) as a fully active enzyme, has no protease activity as long as the enzyme is confined within the MC. Degranulation of MC lead to massive release of chymase into extracellular environment and might have a major impact on CVD development and progression [55, 66–68]. Oxidative stress-induced MC activation and degranulation plays an important role in pathogenesis of cardiovascular tissues [69]. Accumulating evidences suggest that the binding of Ang II to AT_1R stimulates the signaling cascade to produce reactive oxygen species (ROS) such as the superoxide anion and hydrogen peroxide in the cytoplasm and mitochondria of cardiomyocytes [70–72]. Intracellular ROS are implicated in MC activation and degranulation to release various proteases (including chymase) and inflammatory mediators [73–76]. Internalization of chymase from extracellular space into adult rat cardiomyocytes and intracellular expression of chymase in the human atrial cardiomyocytes obtained from cardiac patients undergoing cardiac surgery for primary control of atrial fibrillation have been demonstrated [41, 60]. MCs arise from the hematopoietic stem cells in bone marrow, travel through the blood and mature within vascularized tissues [77–79]. A long-lived MCs reside in low numbers between the cardiomyocytes in heart tissue and other tissues [80]. Several

fold increase in cardiac MC number has been reported in an ischemia–reperfusion model in dogs, chronically stressed and diseased hearts [69, 81, 82].

In the classic RAS cascade model, Ang I is believed to be the only precursor peptide for Ang II generation both in blood and tissues. The discovery and identification of angiotensin-(1-12) [Ang-(1-12)] as an intermediate precursor peptide that is derived from the angiotensinogen protein changes the whole concept of Ang II generation. In 2006, Nagata and colleagues [83] discovered the Ang-(1-12) peptide in plasma and tissues of a strain of Japanese Wistar rats. Recently, we documented for the first time the presence of high circulating levels of Ang-(1-12) in the blood of normal and primary hypertensive patients [84]. Further, our study shows that the level of Ang-(1-12) in human plasma was 12 to 66-fold higher in hypertensive subjects when compared to the Ang I level reported in normal human male subjects (25–143 pg/mL plasma) [85]. Compared to Ang I substrate, the presence of such a high level of Ang-(1-12) in the human plasma can easily to be interpreted to serve as a source of Ang II generation in the circulation. Earlier, we had demonstrated that Ang-(1-12) is metabolized sequentially first into Ang I and then to Ang II by ACE and directly into Ang II by chymase [60–65, 86].

Current and Proposed Therapeutic Aspect of CVD

RAS inhibitors [e.g. ACE inhibitors (ACEi) and/or AT_1R blockers (ARBs)] are currently favored to treat patients with hypertension based on their cardio-renal protective effects, besides their direct effects on reducing blood pressure. Retrospective analysis of clinical events by us [87−90] and others [91, 92] show a non-superior efficacy of RAS blockers over other antihypertensive agents in terms of reducing the risk of CVD, HF and cardiac tissue remodeling. Quantification of a lifetime residual risk of cardiovascular events in hypertensive patients, several order of magnitude greater than the risk reduction [87, 93–95], is puzzling given the strength of the research implicating the complex nature of RAS in CVD. The non-superiority effects of the ACEi and ARBs over other agents may be due to the inability of these agents to reach the intracellular sites where Ang II influences cardiovascular function and the existence of ACE-independent non-canonical alternate pathway of Ang II formation from Ang-(1-12) substrate.

Literature questioning the effectiveness of RAS inhibitors to reduce cardiovascular events beyond an optimistic 30% continues to await acknowledgement [92, 96, 97]. Well-articulated arguments of a substantial “residual CVD risk” in atherosclerosis has gained favor with cardiologists; however, hypertension specialists and basic science investigators are less willing to accept treatment limitations in reducing hypertension-related CVD events with current agents (ACEi and ARBs). To address this significant treatment gap and improve outcomes, we have turned our attention to non-canonical Ang II-forming pathways, wherein the Ang-(1-12) serves as a main substrate for Ang II-formation in humans [55, 98]. Compared to Ang I, several fold higher level of Ang-(1-12) was detected in the blood of hypertensive patients and

rat tissues [34, 83–85, 99, 100]. The research to date, including 36 peer-reviewed publications by us and others [34, 60–64, 77, 90, 92, 97, 98, 101–111], strengthens the idea that the Ang-(1-12) is the predominant renin-independent substrate both in humans and animals by which Ang II contributes to hypertension and cardiovascular remodeling and should be a target for the development of innovative therapies using highly specific conventional antibodies and small molecule nanobodies.

Monoclonal antibodies (mAbs) are now a central component in the successful treatment of human diseases [112, 113]. Their arrival brought about a therapeutic revolution due to their capacity to target specific molecular components. Relevant to cardiovascular disease, a human mAb targeting IL-1β (canakinumab; Novartis Inc.) showed a 15% reduced risk of clinical events in patients with previous myocardial infarction [114–119]. To-date, the potential for mAbs as a therapeutic tool for the treatment of hypertensive-mediated vascular disease has not been investigated. We have successfully generated a highly specific mAb against the C-terminus of human Ang-(1-12) sequence and tested in a humanized model of hypertensive rats [120]. We are the first to demonstrate that this human Ang-(1-12) mAb by itself decreases the arterial pressure in transgenic hypertensive rats suggests that circulating Ang-(1-12) serves an endogenous Ang II-forming substrate that may contributes to blood pressure regulation. In this study, we demonstrated that the mAb stable in rat serum for a seven-day period completely blocks the cleavage sites of chymase and ACE on Ang-(1-12) to generate Ang II. Our investigations are in progress to explore the potential abilities of Ang-(1-12) mAb alone or combined with other antihypertensive medications to control the BP, improve the cardiac function and reduce/prevent CVD progression Fig. 10.2. We posit the idea the circulating Ang-(1-12) captured/neutralized by a specific mAb will not internalized into the cells thus reducing the tissue/cellular accumulation and tissue Ang II.

Another potential novel therapeutics agents, nanobodies (Nbs) have gained traction as a source to combat variety of human diseases including against cancer and COVID-19 [121, 122]. Scientist have developed various Nbs (injectable or inhaled) to neutralize the COVID-19 infection [123–125]. Compared to conventional antibody (mAb IgG, size 150 kDa), the Nbs are much smaller size (~15 kDa), offer many desirable features including rapid targeting, better cellular penetration, less immunotoxicity, easy cloning, high capability to distinguish and neutralize specific antigen. Nbs against Ang-(1-12) can be engineered and produced at low cost in prokaryotic or eukaryotic host organisms to treat the CVD. Work is in progress to develop cell-penetrating Nbs against the Ang-(1-12). The advantage of using the Nbs to neutralize systemic, autocrine, paracrine and intracrine Ang II actions by blocking the chymase as well as ACE cleavage sites of Ang-(1-12).

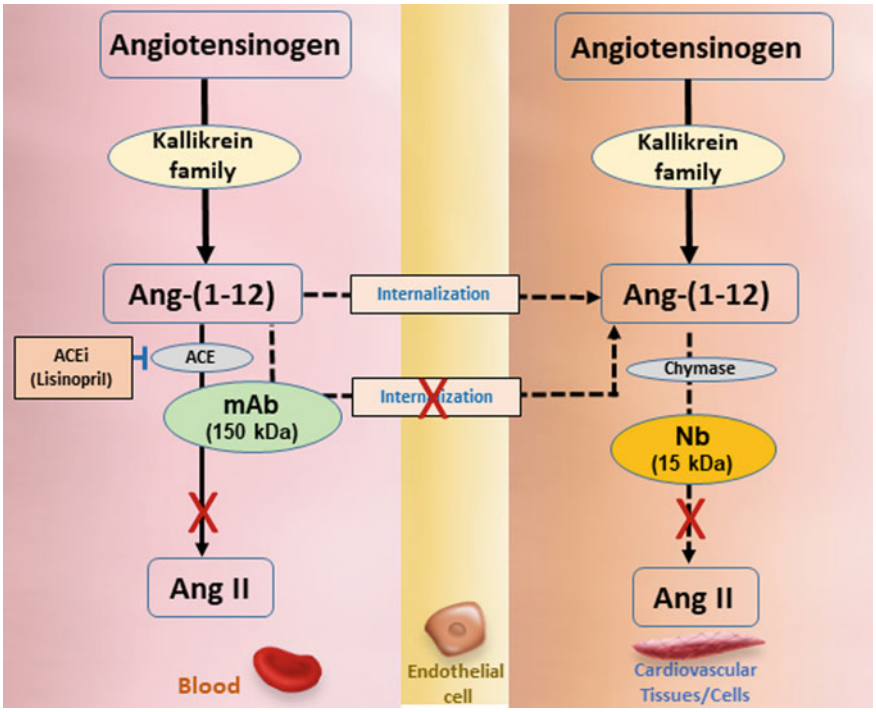

Fig. 10.2 Therapeutic approach to control the blood pressure and prevent/reduce CVD using the mAb alone or in combination with an ACE inhibitor (lisinopril). The binding of mAb to circulating Ang-(1-12) will block the Ang II formation in circulation (by ACE), prevent the internalization of Ang-(1-12) in tissues/cells and reduce the tissue burden of Ang II. Further, work is in progress to develop cell-penetrating nanobodies (Nbs) to neutralize the intracellular Ang-(1-12) substrate and prevent Ang II-formation by chymase

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Chapter 11
Central Control of Sympathetic and Renin Angiotensin System in the Development of Hypertension

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Abstract There is now ample evidence to suggest that many diseases are accompanied by chronic elevations of central sympathetic and renin angiotensin systems and a possibility exists that these two distinct systems interact. The present article will specifically focus on (1) central sympathetic system (2) central sympathetic system in the control of blood pressure (3) modulation of central sympathetic system by various neuropeptides 4) central renin angiotensin system (4) interaction between the central sympathetic and renin angiotensin system and finally, (5) a concept that the central mechanisms play a pivotal role in developing hypertension. We believe that renin angiotensin has a stimulatory influence on the sympathetic system, and the central renin angiotensin system may augment catecholamine outflow working on the presynaptic facilitation of sympathetic nerves. It is reasonable to believe that central receptor blockage will be ideal for antihypertensive drugs. Thus, a study on the central receptors of renin angiotensin may be of interest for developing drugs as a newer therapeutic intervention during heightened sympathetic flow. These findings from animal and human studies will be discussed and integrated to provide a concise update on literature reviews and provide insight into the ongoing controversies in these critical areas of hypertension.

Keywords Renin angiotensin systems · Central sympathetic · Hypertension · Catecholamine

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Introduction

It is now well accepted that the tonic discharge of the sympathetic nerves contributes to the resting vasomotor tone and through baroreceptor reflex, blood pressure homeostasis is maintained. Interestingly, the sympathetic nervous system not only plays a significant role in blood pressure control, but it is intimately related to renal control and metabolism [1]. Thus, increased blood pressure is a combination of events directly related to often higher sympathetic tone. Over the past three decades key regulatory sites within the brain that govern the sympathetic flow have been mapped and identified and these areas are of intense investigation. The question arises as to what triggers the heightened activity of the sympathetic system? The specific area of the brain, such as the intermediolateral cell column, rostral ventrolateral medulla, nucleus tractus solitarius, paraventricular nucleus of the hypothalamus is affected by glutamate, angiotensin II, neuropeptide Y, CCK, and other agents. It will be essential to understand the central sympathetic control and consequences of central sympathetic overactivity if we target the basic mechanisms of increased blood pressure [1]. For more information, the readers are referred to an excellent review article published earlier [2]. In recent years, the role of the renin angiotensin system has also been implicated in the heightened central sympathetic system and release of norepinephrine. Angiotensin receptors have been shown to modulate the sympathetic tone and thus, the present review will dissect these two central systems so that interaction at the end may provide some insight into the mechanism involved in the development of hypertension.

Central Sympathetic System in the Control of Blood Pressure

While it has been known for some time that neurons present within the brain plays a vital role in regulating sympathetic activity for maintaining arterial pressure, their exact location and distribution are known only recently. Enormous progress has been made in identifying forebrain and medullary structures which control cardiovascular function. In this regard, attention has focused on neurons in dorsomedial and ventrolateral medullary reticular formation and hypothalamic structures. In addition, several catecholamine-containing centers have been identified within the brain including ventrolateral pons, the locus coeruleus and number of hypothalamic nuclei particularly the paraventricular nucleus and median preoptic nucleus. Several reports strongly suggest that catecholaminergic neurons within these centers are ultimately responsible for the increased sympathetic nerve function associated with hypertension [1]. To date, the most important catecholamine neurons and pathways appear to be localized within the ventrolateral and dorsomedial medullary reticular formation, an area that includes the region of the nucleus of the solitary tract and dorsal motor nucleus of the vagus and the paraventricular nucleus of the hypothalamus. There are

several studies to confirm that these areas are directly related to the development of hypertension.

Even though the pathogenesis of essential hypertension is yet to be completely understood, it is at least known that essential hypertension is a multifactorial disorder. A combination of genetic and environmental factors contributes to the pathogenesis of hypertension. Genetic studies, involving more than 1 million subjects, have identified at least 300 loci associated with hypertension [3]. Even though a response to antihypertensive medications has been found to be altered with some genetic variants, it is still not obvious how beneficial these hypertension genes are in clinical practice [3, 4]. Numerous other factors have been implicated in increasing blood pressure and the development of hypertension, including age, race, family history, obesity, high alcohol intake, smoking, low socioeconomic status, psychosocial stressors, sedentary lifestyle, high sodium intake, and low potassium [5, 6]. In addition, recently the role of sympathetic nervous system overactivity and brain renin angiotensin system (RAS) is being studied extensively. These seem to hold a vital part of how essential hypertension develops.

The sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS) are the main components of the autonomic nervous system (ANS). These systems have components in the central nervous system (CNS), the brain and spinal cord, and in the peripheral nervous system, nerves and ganglia. The SNS mediates numerous physiologic functions in response to stressful stimuli, either physiological or psychosocial [7]. Therefore, the SNS response is referred to as the "Fight or Flight" response. On the other hand, the PNS response is the "Rest and Digest" response. This is because the PNS mediates physiologic functions related to feeding and rest. The central nervous system (CNS) areas that regulate the sympathetic nervous system (SNS) is a complex network of neurons that integrate sensory signals from various inputs. These signals are due to external stressors either physiological or psychological. As a result, these regions process the sensory signals, and an appropriate response is conducted that controls the blood pressure (BP). There are two pathways where the brain senses the changes in the blood pressure: an indirect neurogenic signal from baroreceptors presents on the vasculature and a direct humoral signal on the brain. A baroreceptor is a mechanical receptor that detects any change in the blood volume or pressure and relates it through afferent fibers that terminate in the nucleus of the tractus solitarius (NTS). NTS is the main center in the brainstem for baroreflex regulation. It has efferent fibers that provide excitatory signals to the caudal ventrolateral medulla (CVLM). These signals regulate the rate of GABAergic signals from CVLM to the rostral ventrolateral medulla (RVLM). The RVLM, in turn, alters the sympathetic fibers, which are implicated in the control of BP by its effects on various organs in the body. On the other hand, the humoral signals affect circumventricular organs (CVOs), which are brain areas in the forebrain that lack the blood–brain barrier. These regions contain angiotensin II (ANG II) and osmotic receptors which mediate a constant check on the BP, blood volume, and extracellular osmolality.

The paraventricular nucleus (PVN) is a primary area in the hypothalamus for which it receives inputs from the NTS in the brainstem and the CVOs in the forebrain. Therefore, it has a crucial role in adjusting the interaction between those centers.

Furthermore, efferent neurons extend from PVC to intermediolateral cell column (ILM) of the spinal cord or indirectly through the RVLM. ILM is an area in the spinal cord composed of pre-ganglionic neurons of the SNS which relays the signals to post-ganglionic neurons and then to different body organs.

Apart from the well-known sympathetic areas in the brain, there are several places where stress-induced changes are implicated. Amygdala is the primary area in the diencephalon that responds to various psychological stressors. It receives signals from the medial prefrontal cortex and hippocampus, implicated in the memories learned from previous stress encounter and provides rapid action. The diencephalon region in the brain affects a cardiovascular fight or flight response. It has fibers from the dorsomedial hypothalamus to RVLM and then to ILM, affecting the SNS. The SNS and PNS help in maintaining blood pressure relatively stable. This is done through various reflexes, and the baroreflex is the most important. The question arises as to the precise role of the sympathetic nervous system in the pathogenesis of essential hypertension.

The role of the central sympathetic nervous system in controlling blood pressure was previously thought to be relevant only in the short term [7]. Baroreflexes and chemoreflexes regulate this short-term control of blood pressure. The role of the central SNS in the long-term control of blood pressure was not appreciated before. This is because chronic elevation or decline in blood pressure caused adaptation of these reflexes [7]. Therefore, the central SNS was not one of the factors suspected to be implicated in the pathogenesis and progression of essential hypertension. Nowadays, it is recognized that the central SNS and its chronic overactivity is one of the most vital factors implicated in the pathogenesis and progression of essential hypertension [7–10]. This role was demonstrated in various human and animal studies. The development of new tools to help study the SNS played a vital role in these discoveries.

The first tools used to study the SNS activity were sympathetic nerve traffic recording and plasma catecholamine measurement [11]. Sympathetic nerve traffic recording, or microneurography, provided a method for measuring efferent sympathetic nerve fibers activity in the periphery [12]. However, the measurement of plasma catecholamine levels had major limitations. Understanding the role of SNS in a particular organ pathology requires the ability to measure SNS activity in that specific organ. This was not possible initially, as the measurement of plasma catecholamine levels provides a global, rather than organ-specific, indication of SNS activity. Moreover, plasma catecholamine levels are affected by the rate of catecholamine clearance from plasma [13]. In addition, microneurography is only used for peripheral measurements of nerve traffic, such as in skeletal muscles and skin, as it is not possible to use in other organs [14]. Therefore, it was necessary to find a way that can overcome all these limitations. This was possible with developing regional measurement of norepinephrine spillover [13]. This method provided an index of sympathetic activity in a specific organ by measuring the rate of release of norepinephrine from a specific organ into the plasma. These methods were essential in facilitating further research that unraveled the SNS and its role in various disease processes.

Neurogenic hypertension is elevated blood pressure that is caused by SNS overactivity. The hallmark of neurogenic hypertension is increased sympathetic nerve activity, which interacts in a positive feedback loop with angiotensin II, inflammation, vascular dysfunction and hypoperfusion [15]. At least half of all cases of essential hypertension are due to neurogenic hypertension. The basis for this estimation has come from the number of untreated essential hypertension patients with evidence of SNS overactivity, as well as antiadrenergic agents treated essential hypertension patients who achieved significant decrease in blood pressure [14].

As indicated early, various studies in animal models and humans demonstrated that essential hypertension is associated with SNS overactivity [8, 14]. In adult spontaneously hypertensive rats (SHR), increased sympathetic nerve activity (SNA) was observed compared to normotensive Wistar-Kyoto (WKY) rats [15]. Single fiber SNA recordings from the kidneys of SHR were also increased compared to WKY rats [16]. In patients with essential hypertension, microneurography from skeletal muscle's blood vessels shows a two to three times increase in sympathetic nerve traffic compared to normotensive patients [17]. The readers should be aware of the fact that the increased sympathetic tone does not work in isolation in hypertension. In fact, the renin angiotensin system also plays an important role both in the periphery and in the brain and is involved in concert in the development of hypertension [18, 19]. We will address this part later in this article. In obese and lean patients with essential hypertension, microneurography shows increased muscle sympathetic nerve activity (MSNA) [20]. Muscle sympathetic nerve activity is also increased in patients with metabolic syndrome associated with elevated and normal blood pressure [21]. In addition, norepinephrine spillover from the heart and the kidney is also increased in patients with essential hypertension [22]. Consequently, this association between essential hypertension and SNS overactivity was the trigger for further investigation of the role of SNS in essential hypertension. Later, numerous studies demonstrated that SNS overactivity plays a crucial role in the pathogenesis and progression of essential hypertension [8, 23]. In SHR, early measurement of SNA during the prehypertensive phase showed that it was increased compared to WKY [24, 25]. In addition, the development of hypertension in SHR was found to be preventable by early sympathectomy [26]. By using regional norepinephrine spillover and microneurography, studies have shown SNA to be significantly elevated in young patients with established essential hypertension as well as borderline hypertension [23, 27, 28]. In young adults, the risk of later development of high blood pressure was found to be higher in those with high resting heart rate compared to controls with normal resting heart rates [23]. In another study, over twenty years of follow-up, the development of hypertension was predictable by arterial plasma epinephrine [23]. Therefore, the results are interpreted to mean that SNS overactivity indeed plays a role in the development and pathogenesis of essential hypertension. Additionally, in patients with essential hypertension, MSNA was found to increase proportionally with blood pressure [29, 30]. This was observed in young, middle-aged, and elderly subjects whose blood pressure ranges from normotensive to severely hypertensive. However, in patients with secondary hypertension, MSNA was not elevated at all [29]. Therefore, it is likely that SNS overactivity plays a role only in the state of essential

hypertension and not in secondary hypertension, indicating how SNS overactivity contributes to the progression of essential hypertension. It has also been demonstrated that SNS overactivity is associated with the development and progression of end-organ damage in hypertensive patients [23, 31].

Even though SNS overactivity is one of the major causes of essential hypertension, little is known about the mechanisms implicated in inducing this increased SNS activity. In this regard, the interaction between SNS activity and various factors has been studied and provided explanations for excess SNS activity in essential hypertension [7, 9, 31]. The brain renin angiotensin system (RAS), microglial cells and astrocytes, reactive oxygen species (ROS) and oxidative stress, and pro-inflammatory cytokines have all been shown to contribute to augmenting SNS activity. Additionally, sensitization of hypertensive response by various stimuli might be another factor that increases SNS activity. Further understanding of mechanisms implicated in augmenting SNS activity might lead to revolutionary new treatment and management options for essential hypertension.

Modulation of Central Sympathetic System by Neuropeptide Y

Immunohistochemistry has proven a powerful technique and is used for localizing neuropeptides in many areas of the brain. A significant advance has been the technique of double or even triple staining of two or three antigens using secondary antibodies labelled with different fluorophores in the same sections. One can then visualize and photograph these sections by switching between filter combinations. Since catecholamines are frequently co-localized with active peptides, which have biological importance, we earlier tested [1] peptides in hypertensive animals using immunohistochemistry and in vivo microdialysis procedures. To focus on one such peptide, neuropeptide Y (NPY), the release of norepinephrine from the paraventricular nucleus of spontaneously hypertensive rats was monitored using in vivo microdialysis technique. It may be pointed out that the hypothalamus is rich in neuropeptide Y-containing fibers and therefore it is possible that a functional interaction between NPY and norepinephrine may exist. Within the hypothalamus norepinephrine levels in the hypertensive were markedly elevated compared with control animals. Interestingly, these hypertensive animals demonstrated no changes in norepinephrine after exposure to neuropeptide Y, whereas decreases of more than 50% of norepinephrine levels were seen in control animals. The density of neuropeptide Y receptors was decreased in the hypothalamus of hypertensive rats. It is reasonable to believe that heightened sympathetic activity may be precipitated due to a defect in the neuropeptide Y receptor acting centrally. This could be due to an intrinsic problem or a down-regulation in the presence of heightened neuropeptide level. Such a change may lead to increased sympathetic activity believed to be responsible for increased blood pressure.

Central Renin–Angiotensin–Aldosterone System (RAAS)

The Renin angiotensin system or renin–angiotensin–aldosterone system plays a vital role in regulating arterial blood pressure, fluids, plasma salts (Na + concentration), and electrolytes of the body [6]. The Renin angiotensin system is a complicated system composed of receptors, enzymes, and peptides; that play a crucial function in regulating the renal blood pressure, influencing the cardiac output and arterial pressure. Furthermore, the Renin-Angiotensin Aldosterone system (RAAS) is present in different tissues and organs throughout the body, including the brain, heart, kidney, adipose, skeletal muscle, and adrenal gland [6, 7, 32, 33]. We will address the most critical two relevant sites from these different sites (for our review), the Kidneys and the Brain.

The kidneys are one of the most critical organs facilitating this system's function. The juxtaglomerular cells (JGC) are present within the kidney. These are modified afferent arteriole smooth muscle cells capable of synthesizing, secreting, and storing Renin, the active form of prorenin. Prorenin is an enzyme that cleaves angiotensinogen, a precursor secreted by the liver, into angiotensin I. Multiple stimuli can trigger the synthesis and release of Renin when they interact with the JGCs. These include sympathetic nervous system stimulation through adrenergic receptors (beta 1), decreased kidney perfusion pressure, and decreased sodium delivery to macula densa cells (part of the distal convoluted tubules). Angiotensin-converting enzyme (ACE) is another important enzyme that participates in this process. It is secreted by the endothelial cells of the lungs. This enzyme converts the dipeptide angiotensin I (Ang I) into the octapeptide angiotensin II (Ang II). Angiotensin II acts directly on blood vessels, stimulating vasoconstriction, and the adrenal gland to release aldosterone. The aldosterone, in turn, acts on the kidneys and stimulates salt (NaCl) & water reabsorption and excretion of potassium K+, thus moving the blood pressure up again [6].

The second site where the renin angiotensin system can be found is within the brain. It consists of two separate pathways called the peripheral (forebrain pathway) and central angiotensinogen pathways. The peripheral pathway is the main pathway because it is composed of the circumventricular organs (CVOs) that are neighboring the third and fourth ventricles. Still, most of the brain zones do not have access to the peripheral renin angiotensin system (RAS), because the blood–brain barrier (BBB) does not allow the peripheral RAS from entering most of the brain regions [6]. That makes the need for cerebral RAS crucial. The circumventricular organs (CVOs) which are neuroendocrine structures are formed of fenestrated capillaries to permit the peripheral access of the renin angiotensin system (RAS) to the forebrain. Moreover, the central angiotensinogen system pathway in the brain is composed of the hypothalamus and medulla, where the main angiotensin is synthesized. These two systems (the central & peripheral) in the brain participate remarkably in the balance

of the cardiovascular system, by regulating the blood pressure through vasoconstriction and vasodilation, as angiotensin I receptor (AT1R) responsible for vasoconstriction and angiotensin II receptor (AT2R) responsible for vasodilation [6, 34–37], respectively.

Angiotensin II can bind to the angiotensin type 1 and angiotensin type 2 receptors [6]. When Ang II binds to AT1R in the brain it will cause vasoconstriction, enhance sodium reabsorption, and promote the secretion of aldosterone and arginine vasopressin (AVP) from the adrenal and pituitary glands, respectively, as well as increase central sympathetic outflow. The result is an increase in the blood pressure. In contrast, when Ang II binds to AT2R, it will induce vasodilation, decrease sodium reabsorption, apoptosis, cellular proliferation, decrease arginine vasopressin (AVP) secretion, and decrease sympathetic outflow thus decreasing the blood pressure even further [6]. It is generally known that angiotensin II is cleaved by aminopeptidase A (AMN A) to angiotensin III, which is converted further by aminopeptidase N (AMN N) into angiotensin IV. Angiotensin II can be also converted by ACE 2 into angiotensin 1–7 (Ang 1–7). Equivalent to Ang II, Ang III plays an essential role in regulating blood pressure by binding to AT1R. Recent studies have shown that Ang III in the brain is more important than Ang II, in the central regulation of hypertension and vasopressin release [6]. Ang III has affinity for AT1R and AT2R receptors that is comparable to that of Ang II and may have a significant role in disorders characterized by Na^+ and fluid retention, such as hypertension and congestive heart failure. Angiotensin IV binds to angiotensin type 4 receptor (AT4R) and plays an essential role in learning memory, cognitive function, decreasing neurons apoptosis, and pro-inflammatory cytokine release. Angiotensin 1–7 binds to the MasR and antagonizes the harmful effects of Ang II. Both ACE 2 and Ang 1–7 play a defensive and protective role for the cardiovascular system [6, 34–37].

Variation in blood pressure and progression of hypertension has been observed in clinical and in animal models related to the sex differences [32, 35]. For the sex difference in humans, many epidemiological and observational studies have shown that men have higher blood pressure than women despite other factors like ethnicity, race, or regions. However, talking about sex differences in animals, one study was done to compare the mean arterial pressure (MAP) in the early sixties on males and female dogs. The study showed that the highest MAP readings were in male dogs compared to female dogs. Moreover, this study showed that this higher reading in blood pressure is not limited to mammalian animals only, but also in one type of birds. Female hormones have been found to have a direct preventive effect on the RAS system in the kidneys, Heart, CNS, and blood vessels against Hypertension development [38]. Female hormones, particularly the Estrogen influences the expression of Ang 1–7 and MasR in the brain, which reflects on blood pressure readings by low readings than males and slower the development of hypertension through vasodilation, decreasing heart hypertrophy, and decreased firing of the sympathetic nervous system (SNS) [6, 38]. Moreover, Estrogen hormonal therapy for postmenopausal women has been demonstrated to reduce Ang II AT1R receptor binding and mRNA inside the hypothalamus of female rats by decreasing the Na + reabsorption in kidneys and

decreasing vasoconstriction in blood vessels [6, 38]. This might explain why female hormones are cardio protective and have slower progression of hypertension.

Brain RAS has also been well established and contributes to the various effects of this system on hypertension. One way by which brain RAS is activated is through sodium loading [39], which is opposite to renal RAAS response. In the kidney, sodium depletion (and captopril treatment) was found to correspond with increased expression of renal renin mRNA. However, in the brain, it corresponded with lower brain renin mRNA expression [6, 18]. Studies found a higher expression of brain renin mRNA in the hypothalamus of rats on a high-salt diet. In addition, the expression of both ACE mRNA and AT1 receptor mRNA was high in rats under similar salt-loaded conditions. In the kidneys, sodium loading decreased renal renin mRNA (inferred from the article) expression. Therefore, driving plasma renin activity (which reflects the activity of renal Renin) and serum aldosterone concentrations to be lower than average. This data demonstrates the varying response of the RAS depending on the location where it is synthesized. The changes in brain RAS activity caused by the sodium load are proposed to be mediated by epithelial sodium channels. This is supported by the fact that osmolar changes of the cerebrospinal fluid would not lead to a similar effect as sodium changes [18]. Also, another clue is that the use of Epithelial sodium channel blockers (ENaC blockers) eliminated the effects of increased brain RAS on blood pressure [6, 34–37].

Interaction Between Central Sympathetic and Renin Angiotensin System

A significant concept regarding the development of hypertension is that abnormal renal excretory function is a part of the initiation and development of primary hypertension. The renal body fluid feedback mechanism works through extracellular volume (sodium and water) homeostasis, whereby the kidneys respond to changes in arterial pressure by altering urinary sodium and water excretion. Such long-term maintenance of sodium and water balance by the kidneys is believed to be the primary control of arterial pressure. If there is an increase in arterial pressure it will lead to an increased urinary sodium and water excretion via the pressure natriuresis mechanism, and the arterial pressure goes to normal. Thus, factors that decrease renal excretory function and alter sodium and water balance by the kidneys lead to an increase in arterial pressure. Increased renal sympathetic nerve activity (RSNA) decreases renal excretory function. The renal effects of increased RSNA include increased renal tubular sodium reabsorption leading to renal sodium retention; decreased renal blood flow and glomerular filtration rate with renal vasoconstriction and increased renal vascular resistance. These result in an increased renin release. Increased RSNA is a critically important factor contributing to this renal excretory dysfunction in hypertension. It has been shown earlier that single-fiber RSNA in spontaneously hypertensive rats is higher than in normotensive Wistar-Kyoto rats [15, 16, 24].

Several studies showed [1, 2, 6, 36] that angiotensin I and II injected into the central nervous system elevated blood pressure. In fact, the chronic subcutaneous infusion of Ang II caused rapid and marked neuronal activation in various parts of the brain such as, the nucleus of the solitary tract, paraventricular nucleus, and supraoptic nucleus. In vitro study demonstrated that Ang II facilitated the potassium-evoked NE release in the rabbit hypothalamus in a concentration-dependent manner and was affected by its inhibitor. The result indicated that there is an increase in NE release. This might be mediated through presynaptic facilitatory receptors attached to angiotensin on noradrenergic nerve terminals. It was also shown that Ang II increased NE release from rat parietal cortex, and that the effect was blocked by saralasin, but not by the Ca channel blocker. Moreover, the facilitative action of Ang II on NE release is significant in the hypothalamus of spontaneously hypertensive rats compared to normotensive rats. In a microdialysis study [34] it was shown early that intracerebroventricular administration of 100 ng of Ang II increased blood pressure and NE release in the anterior hypothalamus of conscious rats, which was antagonized by the Ang II receptor blocker. Others also reported that Ang II led to significant dose-dependent increases of NE release in the paraventricular nucleus [6, 34–37].

Although angiotensin plays a significant role in the control of blood pressure, it was pointed out early that the effect of the angiotensin type 1 (AT1) receptors is quite different from the angiotensin type 2 (AT2) and that these receptors may have an opposite role on blood pressure regulation [34–37]. In this regard, it may be pointed out that neuronal AT1 receptors might have a pivotal role in NE neuromodulation, and that evoked NE neuromodulation might involve AT1 receptor-mediated rapid NE release. Furthermore, AT1 receptor-mediated enhanced neuromodulation might involve the Ras-Raf-MAP kinase cascade and lead to an increase in NE transporter [37]. On the other hand, neuronal AT2 receptors might signal via a Gi-protein. Earlier it has been demonstrated that AT1 receptor binding in the caudal ventrolateral medulla and dorsomedial medulla was increased in spontaneously hypertensive rats compared with normotensive rats. In general, the facilitative effect of Ang II on NE release might be an important factor in the excitation of sympathetic tone in the central nervous system. The possibility exists that central Ang III may have also similar effect on the sympathetic activity. Thus, further studies are needed to assess more thoroughly the precise roles of the different types of neuropeptides and Ang II receptors regulating central sympathetic nerve activity in hypertension. While more research may address these issues, it is logical to believe that centrally acting renin angiotensin receptor antagonists should decrease sympathetic outflow and can be seen as potent antihypertensive agents.

Conclusion

We believe that the central renin angiotensin system may augment norepinephrine outflow working on the presynaptic facilitation of the sympathetic system. Thus, our discussion on both central sympathetic and renin angiotensin systems strengthens the

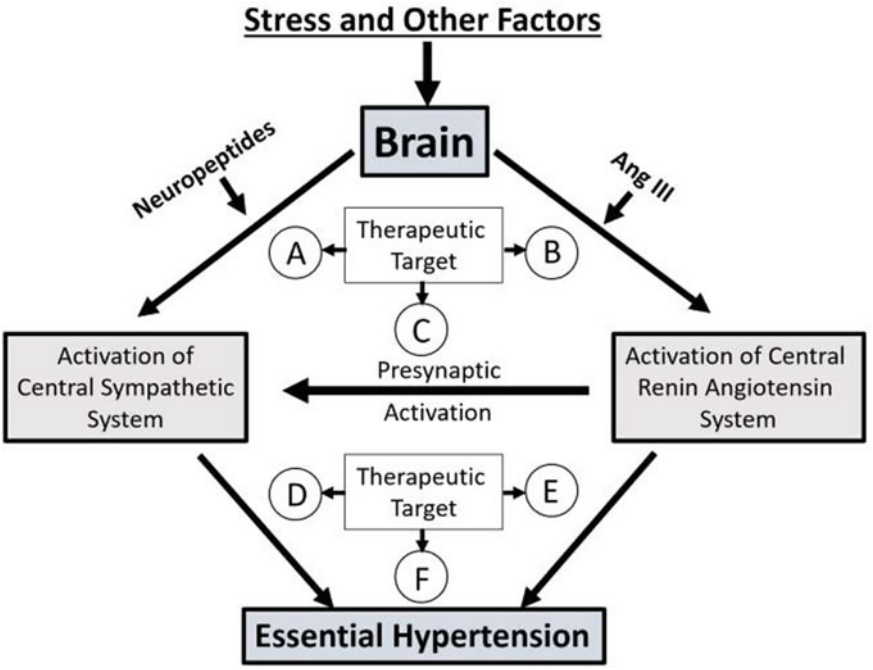

Fig. 11.1 Shows the possible origin of essential hypertension involving both the sympathetic and renin angiotensin systems. The target areas of therapeutic intervention have been indicated as A to F. Although the exact etiology of essential hypertensin is unknown, the predisposing factors are related to stress and several other factors such as obesity, insulin resistance, high alcohol intake, high salt intake, sedentary lifestyle, low potassium, or calcium intake. It is felt that these factors trigger neuropeptide release including ANG III derived from amino peptidase in the brain. An activated central renin angiotensin system can further activate the sympathetic system through presynaptic facilitation and ultimately the development of hypertension

fact that angiotensin- converting enzyme inhibitors, angiotensin-receptor blockers and direct renin inhibitors should paly significant role in combating the central effect of hypertension. Although the clinical significance of the modulation of central sympathetic out flow is not very well understood, earlier studies have indicated that neuropeptide Y, vasopressin, glutamate, CCK may also have a pivotal effect on the modulation of increased blood pressure [40, 41]. Future research on these areas is highly warranted to have any meaningful conclusion and promote newer therapeutic intervention (Fig. 11.1).

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Chapter 12
Role Renin Angiotensin System in Hypertension

Prithiviraj Nagarajan

Abstract Hypertension, which affects more than one billion people, is the major modifiable risk factor for cardiovascular disease death. Although the pathophysiology of hypertension is not entirely understood, disruption of the renin angiotensin system (RAS), which includes the systemic and brain RAS, has been identified as one of the key causes of numerous forms of hypertension. As a result, developing a solid understanding of the fundamental science of RAS and the underlying processes of the signalling pathways associated with RAS may aid in the development of new therapeutic targets for the treatment of patients with cardiovascular and renal illnesses. Four kinds of angiotensin II receptors (AT1RAT4R) have been found, with AT1R playing an essential function in vasoconstriction and receiving the most attention. It has been discovered in numerous areas of the brain, and its distribution is closely related to that of angiotensin-like immunoreactivity in nerve terminals. The impact of AT1R includes the activation of various media and signalling pathways, the most prominent of which are the AT1R/JAK/STAT and Ras/Raf/MAPK pathways. Furthermore, the impact of ATR1 is linked to the regulation of the nuclear factor light-chain enhancer of activated B cells (NFB) and cyclic AMP response element-binding (CREB) pathways. Their action mechanisms are associated with proinflammatory and sympathetic excitatory effects. At1R is involved in nearly every type of hypertension, including spontaneous hypertension, obesity-induced hypertension, renovascular hypertension, diabetic hypertension, L-NAME-induced hypertension, stress-induced hypertension, and angiotensin II-induced hypertension. Acute and chronic central AT1R inhibition are the two forms of central AT1R blockade. The latter is possible by chemical blockage or genetic modification. This chapter study aimed to emphasize the prevalence, functions, interactions, and modulation methods of central AT1R to treat a variety of clinical disorders. The discovery of angiotensin-derived peptides and the creation of AT2R agonists may give a more comprehensive understanding of RAS and innovative treatment methods.

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Keyword Renin angiotensin system · Angiotensin II · AT-1R · Hypertension

Angiotensinogen

AGT is the only known renin angiotensin system substrate that produces all downstream angiotensin peptides. Multiple animal models, including a global AGT-deficient mouse model and a human AGT and renin transgenic mouse model, have shown that AGT modulates blood pressure [1–3]. AGT was also linked to atherosclerosis using a transgenic mouse model producing both human angiotensinogen (Agt) and renin genes. Two recent investigations have shown that AGT modulates blood pressure and contributes to atherosclerosis through Ang II-mediated pathways [4, 5]. To repopulate the modified Agt in vivo, these investigations employed a variety of genetic alterations, including AGT hypomorphic mice, bone marrow transplantation, a hepatocyte-specific AGT-deficient mouse model, and adeno-associated viral infection. These findings show that hepatocyte-derived AGT is the primary source of AGT that regulates blood pressure and promotes atherosclerosis. A pharmaceutical method based on antisense oligonucleotides has also opened the way to directly targeting AGT to prevent hypertension and atherosclerosis [6].

Renin is the most important enzyme in the renin angiotensin system. It is also the only enzyme that can break down AGT, so it is so important to have it. Renin could be a good target for stopping the renin angiotensin cascade and improving heart problems caused by Ang II [7, 8]. Reduced blood pressure and atherosclerosis are seen in animal models when renin is blocked [9–12]. Unfortunately, renin inhibitors haven't worked better than well-known ACE inhibitors or AT1 receptor blockers for people with heart problems [13]. There have been some bad results in human studies of blocking renin, but that hasn't stopped researchers from figuring out how renin affects cardiovascular diseases. This is how it works: Renin is made and released by cells in your kidney that are close to the tubules. Renal denervation, which aims to cut down on sympathetic nerve activity, has been getting much attention; some conflicting findings need to be looked into more [14–18]. In pigs who took part in a recent study, the kidney-brain-heart axis and plasma renin activity changed significantly. This suggests that the renal renin angiotensin system is at work in lowering blood pressure and improving cardiovascular function [19, 20].

Angiotensin and Dysfunction

As the primary bioactive peptide of the renin angiotensin system, there are several molecular approaches to how Ang II contributes to various cardiovascular physiological, and pathological processes. We present a brief overview of the disorders listed below, recently reported in ATVB. Most of these trials employed continuous subcutaneous Ang II infusion [21, 22]. Ang II causes cardiac dysfunction in various

ways, including hypertrophy, arrhythmias, and ventricular function failure [23, 24]. Basigin is a multifunctional transmembrane glycoprotein. Genetic deficiency of basigin in a mouse model of transverse aortic constriction resulted in reduced cardiac hypertrophy, fibrosis, and heart failure [24]. Smooth muscle stromal-mal interaction molecule 1, lacking an endoplasmic reticulum Ca^{2+} sensor, also inhibits Ang II-induced cardiac hypertrophy [25, 26]. These data support the notion that renin angiotensin inhibition is necessary to reduce cardiac dysfunction.

RAS—Hypertension

Hypertension affects more than a quarter of the adult population in developed countries, posing a serious health risk 26. Hypertension is associated with several clinical disorders, including renal failure, heart failure, stroke, and myocardial infarction [27]. Additionally, its pathophysiology is unknown. Previous research has revealed that genetics may aid in modifying arterial blood pressure through molecular pathways and identify possible therapeutic targets for hypertension [28]. Disruption of the renin angiotensin system (RAS), both systemic and cerebral, has been identified as a key cause of hypertension. The RAS is a hormonal system responsible for fluid balance and blood pressure regulation. Renin is a hormone released by granular cells in the kidney's juxtaglomerular apparatus [29]. It is the medium through which angiotensinogen is converted from the liver to angiotensin I. The conversion to angiotensin II is subsequently mediated by the angiotensin-converting enzyme (ACE) (ANG II). ANG II is a vasoactive peptide that causes blood capillaries to become smaller by activating G-proteins inside them, which causes blood pressure to rise, which is why ANG II is important. Additionally, ANG II stimulates the adrenal cortex's release of aldosterone, resulting in salt and water retention in the kidneys and increased blood pressure. Angiotensin receptors are a subclass of G protein-coupled receptors activated by ANG II binding [30].

There are four distinct ANG II receptors (AT1R–AT4R). The most extensively researched of these receptors, AT1R, has been identified in the heart, lung, kidney, brain, blood vessels, and adrenal cortex, while AT2R has been implicated in vascular development. It was believed to be straightforward when the peripheral RAS was initially found, as detailed above. Recent research, however, has proven that the RAS is more complicated [31, 32]. There have been many novel mechanisms found that govern the function of AT1R, one of which is the function of AT1R after ANG II binding. It is suggested that activation of AT-2R in response to ANG II binding may serve as a counterbalance to AT-1R function and protect against brain damage [33]. As part from the RAS, all components of the brain RAS have been discovered, and their roles have been intensively investigated during the last 30 years.

The pathophysiology of essential hypertension and other types of hypertension is extremely complex and not fully elucidated yet, with the RAS contributing only as part of a multifactorial pathophysiological mechanism [34]. Furthermore, the RAS may play an entirely different role depending on hypertension [35]. nAt1R and AT2R

are expressed in various brain areas, and their distribution is related to angiotensin-like immunoreactivity in nerve terminals. Through the AT1R, ANG II regulates fluid homeostasis and autonomic pathways that govern the cardiovascular and neuroendocrine systems in the brain. The AT-2R is involved in cardiovascular and behavioral functions in the brain [36]. In contrast to AT1 and AT2 receptors, AT4 receptors are not broadly distributed throughout the brain [37, 38]. The existence of angiotensin receptors in the brain suggests that they perform various critical physiological activities. This chapter talks about AT1 receptors and how they work, intending to find out how they communicate with each other.

Central AT1R in Different Types of Hypertension

Diabetes Hypertension

Diabetes increases the risk of high blood pressure and other cardiovascular problems by predisposing the arteries to atherosclerosis, which causes the hardening of the arteries. The development and severity of a range of diabetes complications, such as diabetic eye disease and renal disease, are influenced by hypertension, which is influenced by diabetes. Insulin-mediated vasodilation is a critical element in developing impaired vasodilation in diabetics [39]. When AT1R is expressed at a high level, the biological efficacy of ANG II is regulated. This biological effectiveness is influenced by various factors, including ANG II, blood glucose, insulin, ROS, low-density lipoprotein (LDL), and diabetes [40]. ANG II has been demonstrated to inhibit insulin signalling via the AT1 and AT2 receptors, resulting in insulin resistance and affecting the pathophysiology of diabetes and its complications [41]. It was discovered by Van Linthout et al. that high-density lipoprotein (HDL) reduced AT1R gene expression and had vasoprotective effects on the aortas of STZ-induced diabetic mice in vivo as well as HAECs in vitro [42]. When there is a pathological condition such as hypertension, vascular injury, or inflammation, AT2R levels have been elevated, and studies have shown that AT2R is involved in the control of particular cardiovascular processes such as vasodilation and diuresis [43, 44]. In contrast to AT1R, AT2R promotes cell differentiation and proliferation while inhibiting the activity of AT1R; consequently, it is crucial in preventing tissue remodeling and the development of illness [45]. First, it was found that NIDD M had abnormal AT2R levels and high levels of iNOS. This is because it activates AT2R, stops NO-induced ANGII-induced contraction, and increases vasodilation through NO [46].

Spontaneous Hypertension

ANG II increases vasopressin release, reduces baroreflex sensitivity, and stimulates sympathetic pathways through activating AT1R in cardio regulatory hypothalamic and brain stem nuclei [47]. AT1R expression and activity were increased in the

subfornical organ, nucleus tractus solitarius, median preoptic nucleus, the dorsal motor nucleus of the vagus, and the paraventricular nucleus in SHRs [48]. Notably, pharmacological or genetic AT1R inhibition reduces blood pressure in SHRs [49]. Injecting AT1R or ACE antagonists into SHRs reduces arterial pressure [50]. AT1R or Angiotengen antisense oligonucleotides lower arterial pressure in SHRs but not in normal controls [51]. Sun et al. developed an in vitro neural cell culture model from prehypertensive SHRs to examine the mechanisms behind AT1R-mediated increases in ANG II activity in the prehypertensive SHR brain [52]. According to a prior study, the physiological response of ANG II operating in the brain included the modulation of certain neuronal pathways in the brain [53]. According to the findings, inhibiting PI3 kinase decreased the heightened chronotropic effect of ANG II in SHR neurons, suggesting that this enzyme may be solely responsible for ANG II's chronotropic activity [52]. ANG II activates AT1R in SHR neurons through the signalling pathway, causing an increase in the firing rate. This signalling pathway simultaneously needs CaMKII and PKCa activation in SHR neurons [54]. AT1R seems to be linked with the PI3 kinase signalling pathway in SHR neurons, contributing to the heightened chronotropic response to ANG II found in these neurons [55]. AT1R expression in neurons from the SHR hypothalamus or brain stem was 2–4 times higher than in neurons from normotensive rats. This is consistent with an increase in ANG II-mediated neuromodulatory effects in SHR neurons, including a Ras/Raf/MAPK pathway [56] and an increase in AT1R expression. Sun et al. found a link between an enhanced chronotropic response to ANG II and another PI3 kinase signalling pathway in the SHR [52]. The PI3K signalling in SHR neurons was distinct from other neurons, perhaps because of a hyperactive brain angiotensin system (HAAS). In vivo studies suggest that ANG II stimulates PI3 kinase activity in the brain stem and hypothalamus, two brain areas important in cardiovascular regulation [57]. In SHRs alone, inhibiting PI3 kinase in the RVLM increases neuronal excitation, decreases baseline arterial blood pressure, and diminishes ANGII-induced blood pressure spikes [58]. There has been a huge drop in the expression of the PI3K regulatory subunit, called p85. This may be because the catalytic component of PI3K, called [59], is more active in the brains of SHR people.

Stress Causes Hypertension

Chronic hypertension may be caused by frequent exposure to a stressful situation or a chronic stress state with increased blood pressure [60]. Genetic predisposition may have a role in the hypertensive response to emotional stress. To better understand the genetic and physiological underpinnings of rat stress arterial hypertension, the NISAG strain was developed. In adult rats (NISAG rats), Epstein et al. explored the impact of ultralow dose antibodies against ANG II and their receptors on genetic stress-induced arterial hypertension [61]. Antibodies targeting the C-terminal region of the angiotensin II receptor were shown to have the largest blood pressure-lowering

effect, lowering systolic blood pressure by 16.400.62 mm Hg. Affinity-purified antibodies against ANG II have a faster antihypertensive effect. Two hours after these antibodies were given, the systolic blood pressure decreased by 12.805.49 mm Hg [61]. More research is needed in the future to confirm these findings.

L-NAME-induced Hypertension

The replacement of one or both of the terminal guanidino (G or w) nitrogen (s) in L-Arginine analogues makes them NOS inhibitors [62]. NwnitroLarginine (LNA) or its esterified version, NwnitroLarginine methyl ester (LNAME), is a nonselective inhibitor that is one of the most frequently utilised Larginine substituents [63]. LNAME affects the cardiovascular system, including inhibiting acetylcholine-induced relaxation and increasing induced arterial blood pressure. According to Miguel-Carrasco et al. [64], L-Carnitine (LC) caused a considerable, although not total, decrease in blood pressure in LNAME-treated rats. While LC therapy was reversible, LNAME administration raised plasma levels and gene expression of IL1, IL6, and TNF in the hearts of rats [64]. Although LC therapy decreased IL1, IL6, and TNF in hypertensive rats, hypertension did not affect plasma ACE activity. Finally, protein and mRNA expression of ACE and AT1R rose in the hearts of rats given LNAME, and LC reversed this trend.

As a consequence of the partial inactivation of RAS caused by LC, the generation and impact of ANG II decreased. When both the AT1R inhibitor and the AT2R increase, AT2R mediates ANG II-induced vasodilation [65]. ANG II/AT2R-driven vasodilation involves nitric oxide. Pathways involving nitric oxide (NO) and cyclic guanosine monophosphate (cGMP). Savoia et al. demonstrated that ANG II produced vasodilation in stroke-prone spontaneously hypertensive rats (SHRSP) treated with valsartan, which was reversed by LNAME, demonstrating the importance of nitric oxide (NO) in this process [66]. In the presence of AT1R antagonists, their tests demonstrated that valsartan raised eNOS expression and NO concentration in SHRSP twofold. They concluded that ANG II enhances vasodilation through NOS/NOmediated pathways via AT2R. Another study discovered that while norepinephrine causes contraction, ANG II only causes concentration-dependent relaxation in the aorta of SHR, who had been taking losartan for a long time [67]. Relaxation is inhibited by the AT2R selective blockers PD123319 and LNAME. After AT1R blockage, AT2R mRNA rose considerably, while ANG II merely boosted NO generation in the treated SHR aorta. According to these findings, long-term blocking of the AT1R is linked to the opposite vasomotor response of ANG II to NO production through the AT2R in hypertensive rats.

Renovascular Hypertension

Renal vascular hypertension is a sickness characterized by hypertension induced by constriction of the kidney's blood supply artery. Macular dentin cells sense low perfusion pressure through the glomerular device, which causes the production of renin, which causes the conversion of ANG to angiotensin I. Angiotensin I then reaches the lungs and is altered to ANG II by ACE. ANG II causes the release of aldosterone, which causes water retention, salt retention, and a lack of potassium. An increase in blood volume produces a rise in blood pressure, which contributes to the development of hypertension. RAS inhibitors commonly treat ANG-dependent hypertension and congestive heart failure [68]. Standard hypertension treatments include ACE inhibition and AT1R blockade. Because polymorphisms in the AT1Rencoding gene are connected with hypertension in human and hypertension animal models, it is rational to expect that AT1R is a relevant target for hypertension treatment [69]. Because ACE inhibitors may slow the course of kidney disease, their usage has grown in patients with hypertension and renal insufficiency to avoid progression to endstage renal failure, necessitating dialysis or kidney transplantation. AT1R antagonists have also been shown in studies to be equally effective as ACE inhibitors in reducing renal disease development. Nakaya et al. investigated the structural and ultrastructural alterations of the rat kidney in the early stages of hypertension, and the effects of ACE inhibitors and AT1R antagonists were compared [70]. They also looked at how these medicines affected the mRNA expression of AT receptor subtypes in the kidney. They discovered that ACEI inhibitors and AT1R antagonists had no meaningful effect on renal alterations in hypertensive rats. Notably, ACE inhibitors and AT1R antagonists elicit a transitory reduction in kidney AT2 mRNA expression. Katovich and colleagues revealed that using AT1R antisense (AT1R AS) in adult hypertensive rats efficiently decreased BP to control values and reversed the pathophysiological impact of hypertension on renal blood vessels [71]. In addition to its function in normalizing hypertension and vascular pathophysiology, this gene therapy approach may significantly decrease hypertension patients' non-compliance with traditional treatment. Zhi et al. found that treating rats with renal vascular hypertension with AT1R antagonist losartan averted alterations in kidney structure and function and greatly decreased the frequency and titer of AT1R autoantibodies [72].

Obesity-induced Hypertension

Inflammation and dyslipidemia are hallmarks of obesity, which also predisposes to hypertension, diabetes, and cardiovascular disease. A cardiovascular homeostasis regulator, ANG II, maybe a drug target for obesity-related metabolic disorders [73]. Adipose tissue fat cells and inflammasomes release inflammatory mediators when challenged by high-fat diets. These chemicals enter the circulation and have systemic effects [74]. Obesity in the CNS increases the risk of type 2 diabetes. Adipose tissue

dysfunction increases proinflammatory mediator release, affecting insulin signalling and pancreatic cell function. According to Cole et al. [75], the AT1R blocker valsartan significantly reduced the harmful effects of a high-fat diet in rats. MCP1, interferon, IL6, IL12, and inducible nitric oxide synthase (iNOS) diminish systemic insulin sensitivity and dominate MCP1 negative expression in wild type C57BL/6J mice or db/db mice [76]. According to Cole et al., the Western diet raised MCP1, IFN, IL12, and iNOS expression in mice's blood and adipose tissue, affecting glucose tolerance and insulin sensitivity. Treatment with Valsartan repaired these changes [75]. The Western-style diet increased AT1R expression in fat cells, which was reversed by valsartan medication. The valsartan-treated animals on Western diets had smaller fat cells, confirming previous findings that AT1R improves fat cell function by promoting the formation of smaller, more metabolically efficient fat cells. Angiotensin receptor blockers may target 12LO platelets, as shown by reduced expression of 12LO platelets in the fat cells of valsartan-treated rats.

ANG II Causes Hypertension

Due to ANG II, RAS's main effector molecule can raise blood pressure, negatively affecting different body parts' vasculature. Atherosclerosis and vascular dysfunction may be linked to ANG II's propensity to produce proinflammatory, prothrombotic, and prooxidative phenotypes [77]. ANG II activates AT1R on white blood cells, platelets, endothelial cells, and vascular smooth muscle. ANG II has been linked to ischemic and hemorrhagic strokes. In ischemia/reperfusion (I/R) animals, AT1R blockers minimize brain inflammation and tissue damage [78]. Mice lacking AT1R or angiotensinogen had fewer ischemic lesions after cerebral I/R, whereas renin/angiotensinogen transgenic mice had an abnormally strong brain injury response (mediated by AT1R) [79–81]. In ANG II-induced hypertension and ANG II-accelerated atherosclerosis, blood cell-related AT1R plays a role in inflammation and vascular dysfunction/injury [82]. Nagai et al. revealed that genetic abnormalities in AT1aR/can protect normal blood pressure and ANG II hypertensive mice from I/R-induced brain injury, microvascular inflammation, and tissue damage 83. On the other hand, the AT1aR/mice showed unique roles in the blood vessel wall and blood cell-associated recruitment of AT1aR and brain damage response to I/R [83]. In both normal and ANG II hypertensive rats, AT1aR, connected to the blood vessel wall, appears to increase leukocyte and platelet recruitment in the cerebral microvasculature. Acute (24-h) exposure to high ANGII levels has been shown to stimulate leukocyte and platelet adhesion in capillary venules through AT1R-dependent Pselectin pathways [84]. Using ANGII may cause high blood pressure, BBB failure, and cerebral edema, which may be avoided by first using AT1r antagonists [85]. ANG II causes BBB failure, resulting in a rise in blood pressure, directly reliant on AT1R endothelial cell activity [86]. This impaired BBB function was linked to increased inflammation in cerebral microcirculation. They also showed that AT1R was implicated in the ANGII-mediated microvascular response and immune cells,

RANTES, and P-selectin. Thus, ANG II-mediated hypertension may enhance the risk of ischemic stroke and its severity. A prolonged ANG II infusion affected cerebral microcirculation by increasing leukocyte and platelet recruitment in small capillary veins while lowering BBB permeability [87]. Angiotensin II (ANG II) raises blood pressure and draws blood cells. AT1R on blood vessel walls causes the BBB to break down.

Conclusions

The ANG II-mediated AT1R response reveals RAS involvement in the aetiology of clinical hypertension, diabetes, chronic renal illness, and heart failure. Ang II inhibitors like ACE inhibitors and ARBs are commonly used to treat various illnesses. These drugs' effects on blood pressure and mortality from MI, heart failure, atherosclerosis, stroke, diabetes, peripheral vascular disease, chronic renal disease, and stroke are well-known. Despite the well-known molecular mechanisms of the ANG II-mediated effect in physiological and pathological conditions, there are still fresh molecular pathways for research. A variety of pathological disorders may be treated using the central AT1R prevalence, functions, interactions, and modulation techniques identified in this research. The discovery of angiotensin-derived peptides and the creation of AT2R agonists have changed the way researchers view RAS and lead to novel treatment approaches. Understanding the RAS and its signalling pathways, especially in human rather than animal models, may help develop new therapeutic targets for patients with cardiovascular and renal illnesses. The renin angiotensin system was discovered nearly half a century ago, yet it continues to draw study in many domains. However, it also points out that there are still a lot of unknowns and mysteries about this hormone system that need to be looked into more.

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Chapter 13
Comparing the Influence of Angiotensin II and TGFβ_1 on Cardiac Fibroblasts; Myofibroblast Plasticity and Resistance to Apoptosis

Besher Abual'anaz, Sunil G. Rattan, and Ian M. C. Dixon

Abstract Heart disease with attendant cardiac fibrosis carries a major societal burden in developed countries. Recent work underscores the high diversity of cardiac fibroblasts and myofibroblasts in both the healthy heart and diseased heart, and the long-term presence of activated myofibroblasts in the infarct scar. Angiotensin II and Transforming Growth Factor β_1 (TGFβ_1) have an impact on cardiac fibroblasts and cardiac fibrosis. Both factors serve as fibroblast activators and are implicitly involved in the pathogenesis of heart disease, but their influence of elevated fibroblast resistance to apoptosis and thus their contribution to senescence of cardiac fibroblasts is relatively understudied. The myofibroblastic phenotype incorporates αSMA to stress fibres with attendant contractility and are hypersecretory for extracellular matrix (ECM) components. These cells facilitate both acute wound healing (infarct site) and chronic cardiac fibrosis. Quiescent fibroblasts are associated with normal myocardial tissue and provide relatively slow turnover of the ECM. Following their activation, cardiac myofibroblasts (unlike dermal myofibroblasts) do not always revert to their quiescent phenotype, and they may resist entry to the apoptotic phase following acute wound healing. Angiotensin II has been shown to participate in contributing to the apoptosis resistant phenotype. The literature reveals that fibroblasts may halt on the precipice of apoptosis and acquire what is commonly referred to as a senescent phenotype. Within this broad description, these cells may then persist at the site of damage by resisting apoptotic cell death, and present with specific phenotypes, which may go on to contribute to chronic cardiac fibrosis. Thus an understanding of mechanisms that enable myofibroblasts to evade apoptosis is required, be it based on signalling or in the microenvironment via matrix-specific clues. While most work highlights angiotensin II and TGFβ_1 as contributors to fibroblast activation far less is

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known about mechanisms governing novel recent findings of myofibroblast deactivation (reverting to their quiescent phenotype or even a less differentiated progenitor) as well as their differentiation to adipocytes, chondrocytes and osteocytes. Thus major knowledge gaps exist, and its solution may lie in the identification of specific proteins in angiotensin or TGFβ_1 signalling to allow for phenotype manipulation and apoptotic removal of profibrotic cells. The current review will provide an overview of work highlighting novel information pertaining to angiotensin II and TGFβ_1 which mediate resistance to fibroblast apoptosis, fibroblast activation and deactivation.

Keywords Cardiac fibroblast · Myofibroblast · Angiotensin II · Extracellular matrix · TGFβ_1 · Mechanical stress · Extracellular matrix · Cell senescence · Myofibroblast apoptosis · Cellular plasticity

Introduction: Fibroblasts, Myofibroblasts and Cardiac Fibrosis

There is a high incidence of death secondary to heart disease in North America, the economic and societal burden being higher than any other disease, including cancer [1]. Following mechanical dissociation or ischemic injury with parenchymal tissue damage, damaged tissues undergo wound healing, which itself relies upon timely engagement of fibroblast activation, healing, scar formation and removal of myofibroblasts following healing. Fibroblasts from various organs have been known to display wide diversity, topographic differentiation and positional "memory" for more than a decade [2], and it is likely that cardiac fibroblasts are unique in their behaviour and function when compared to their counterparts in skin, kidney, or corneal cells [3]. Fibroblasts are classically defined as mesenchymal cells present in interstitial spaces between parenchymal cells. In the heart, fibroblasts reside in the atria, ventricles, coronary arteries and valves (as valvular interstitial cells), and recent work has revealed an each fibroblast sub-population may express unique transcriptional profile [3]. While there are no exclusive markers to identify fibroblasts, generally these cells express TCF-21, discoidin domain receptor 2 (DDR2), vimentin and low levels of PDGFRα. Cardiac fibroblasts are involved in multiple aspects of cardiac function in both the healthy [4] and damaged or diseased heart via remodeling of the ECM [5]. For example expansion and remodeling of the extracellular matrix (ECM) following myocardial infarction (MI) is essential for wound healing to prevent left ventricular rupture. Normal wound healing in most tissues is typified by fibroblast activation (to myofibroblasts) [6] and rapid scar formation followed by a period of myofibroblast apoptosis—wherein the resident activated myofibroblasts are removed from the scarred tissue. In dermal wound healing, the healed scar is characterized by occupation of quiescent fibroblasts and these cells preside over a continuous and relatively slow matrix turnover for years and decades following wound healing [7]. On the other hand, wound healing after myocardial infarction in the heart frequently may differ,

with the persistent occupation of the cardiac infarct scar with activated cardiac myofibroblasts [8, 9]. This is a harbinger of global cardiac fibrosis as apoptosis-resistant myofibroblasts may contribute to ongoing attendant excessive synthesis and deposition of ECM and matrix-associated proteins, including fibrillar collagens type I and III [8, 9], periostin [10, 11], and the cell-associated fibronectin ED-A splice variant [12, 13]. Long term residence of senescent myofibroblasts may exert a spillover effect to noninfarcted cardiac tissues, including the right ventricle [7]. The extended presence of cardiac myofibroblasts in the infarct scar may be related to prosurvival signaling that delays their removal by apoptosis [7], and the chronically damaged myocardium may contain several subsets of fibroblast phenotypes, including ECM-producing myofibroblasts, cells that are αSMA-negative (promoting angiogenesis) and senescent fibroblasts [3]. Thus following the activation of fibroblasts after cardiac damage, the latter group of cells in chronic fibrosis or prolonged wound healing are a potential target for therapeutic intervention.

The activated myofibroblast phenotype does not represent a differentiation event per se, but rather is fibroblast activation event, with de novo formation of αSMA incorporating stress fibres, ED-A fibronectin, and periostin as well as fibrillar collagens I and III secretion [4]. These cells are typically associated with acute wound healing of infarcted myocardium, or chronic fibrosis of the cardiac interstitium, while quiescent fibroblasts are associated with normal myocardial tissue and provide relatively slow turnover of the ECM. While the resolution of the idea that cardiac myofibroblasts contribute to chronic cardiac fibrosis has been a step forward in our understanding of mechanisms for cardiac fibrosis and heart failure, the lack of availability of specific drugs to treat cardiac fibrosis per se perpetuates this serious clinical problem. In this context, a major knowledge gap exists about which paracrine growth factors contribute to fibroblast activation (there are many and the list of identified and putative activators grows each month), and more importantly to the resistance of cardiac myofibroblasts to apoptosis [7, 14]. Our lab has contributed to the literature dealing with cardiac myofibroblast deactivation, in that SKI may accomplish its anti-fibrotic effects by suppressing R-Smad function [15, 16]. While cardiac fibrosis is causal to heart failure [17–19], our understanding of what maintains chronic presence of cardiac myofibroblasts and related cells within the infarct scar is poor, and the need to zero in on the key mechanisms for chronic fibrosis is paramount. For example, we and others believe that periostin (POSTN) is an important fibroblast activator [14, 20, 21], and while this is an interesting development, we suggest that the research of signalling pathways that govern the deactivation of myofibroblasts (fibroblast cellular plasticity) and those that signal resistance of myofibroblasts to apoptosis (myofibroblast apoptosis) are areas that will bear fruit in terms of making therapeutic inroads to novel treatments of cardiac fibrosis. Thus, while the current trend to identify what triggers fibroblast activation is but a single part of the whole solution, in a concerted effort to improve outcomes for the cardiac patient suffering cardiac fibrosis eg, the post-MI patient [22].

Myofibroblast Plasticity—The Extracellular Matrix Niche, Biomechanical Input and SKI

Very little is known about myofibroblast deactivation compared to the bulk of literature generated in the past 10 years to address fibroblast activation—the former is a clear knowledge gap in our understanding of normal cardiac wound healing and possibly, the pathogenesis of cardiac fibrosis. To this end, there is a recent wealth of literature that describes possibility of signalling by biomolecules within the matrix itself, that may contribute to the control of fibroblast phenotype. An emerging concept in cardiac physiology and pathophysiology is that the ECM itself is a dynamic tissue component first, providing structural support and second, contributing to signalling events in healthy heart, during cardiac injury, and in heart disease remodeling [23]. Clearly, the ECM may contain matrix-bound signaling molecules or matrikines that may govern the phenotypic fate of fibroblasts and thus a recent shift of focus has fallen on the characterization of what may constitute a "healthy matrix". The ECM is relatively complex and includes relatively insoluble structural proteins including fibrillar collagens I and III, vitronectin, laminin and fibronectin [24], matricellular proteins including matrikines such as thrombospondin, osteopontin, and periostin [14, 18] and exosomes, which package TGF- β_1, microRNAs, and other cytokines. A major knowledge gap as to how do cardiac fibroblasts interact with the cardiac ECM, and how does this interaction change in healthy versus diseased or fibrosed myocardium. Part of the answer to this question may lie in the fact that fibroblasts express integrins and β -catenin to facilitate cell–matrix or cell–cell interaction [24], and integrins may cluster at the site of these contacts [25]. β-catenin activation is associated with elevation of p53 in lung fibroblasts, and its loss or pharmacologic inhibition is associated with reduced secretion of ECM by cardiac or lung fibroblasts respectively [26, 27]. Thus the ECM functions as a store for signaling proteins to fibroblasts within the ECM and in particular, matrikine proteins that provide clues for fibroblast phenotype regulation.

Biomechanical Input and Regulation of Fibroblast Phenotype

Most recent findings support the idea that resident quiescent fibroblasts are the main contributors to wound healing in damaged tissues, including the heart [28–30], but the question of whether activated cardiac myofibroblasts may deactivate (or even de-differentiate to mesenchymal precursor cells) in response to matrix-borne clues or suppression of cytokine signalling is relatively understudied. While many experimentalists continue to use common plastic culture plates for two dimensional propogation and study of fibroblasts, it is well known that these cells are especially sensitive to mechanical input [31]. Further, it has long been known that different organs house unique populations of fibroblasts, and an increasing documentation of their diversity is now found in the current literature, including several forms of

myofibroblasts (senescent, pro-fibrotic and pro-angiogenic) in healed myocardium [2, 3]. Thus, rather than displaying a strictly defined phenotype, fibroblasts exist on a phenotypic continuum with quiescent fibroblasts, pre-activated proto-myofibroblasts and finally pro-fibrotic myofibroblasts (or activated fibroblasts) as definitive phenotype forms within the spectrum [4, 32]. While quiescent fibroblasts are motile and maintain tissue homeostasis via slow turnover of extracellular matrix components, activated fibroblasts incorporate αSMA into their stress fibres and are contractile and hyper-secretory for fibrillar collagens and other structural matrix components [33]. The biomechanical microenvironment in the heart is different from virtually any other organ, and subject fibroblasts to repetitive mechanical loading with every contraction/relaxation cycle and we suggest that cardiac fibroblasts may be partially activated in vivo. This notwithstanding, cardiac fibroblast activation to following a pathological stimulus or in response to abnormal matrix found in fibrosed heart, may represent a tipping point for the evolution of cardiac fibrosis.

TGFβ_1, Smads and SKI in Fibroblast Plasticity

It is well known that cardiac fibrosis is driven, in part, by TGFβ$_1$/Smad signalling [34–38], in resident cardiac fibroblasts [39] however cardiac fibrosis per se has no druggable targets for translatable cures [6]. While TGFβ$_1$ is a common cytokine stimulus associated with fibroblast activation, a druggable target to quell this driver of fibrosis has remained an elusive therapeutic goal due to its ubiquitous use by different cell types and also in the signaling complexity associated with Smads and other effector pathways. TGFβ$_1$/Smad signalling in the myocardium and other tissues has been complemented by the discovery of members of the SKI family of proteins, including SNO, SNOn and SKI [40]. This superfamily of negative regulators of TGFβ$_1$/Smad signalling function by binding to activated nucleus-bound Smad binding elements on DNA target strands, allowing the Smads to bind and then inhibiting Smad signaling at the site via the disrupting bridge model. The importance of R-Smads and associated Smad signal proteins, and their own unique fibrotic effects may extend beyond one direct pathway for fibrosis and thus the existence of cooperative profibrotic signaling may exist at the cellular level, with Smads as the signalling nexus. SKI is known to play a role as a negative regulator of TGFβ$_1$ associated proteins including Smads, as well as signaling proteins from other pathways, including Hippo [40, 41]. Our previous work indicates that overexpression of SKI may decrease secretion of ECM components from cardiac myofibroblasts via SKI-mediated deactivation of myofibroblasts—which is an much understudied area of fibroblast plasticity [15]. As cardiac fibrosis is continues to be associated with high patient mortality and absence of treatment for this condition, investigation of this reverse plasticity of fibroblasts is critical to the development of translatable therapeutic approaches [6]. Thus as SKI directly deactivates cardiac myofibroblasts to fibroblasts, SKI's repressor function is a putative candidate for treatment of cardiac fibrosis [16].

Myofibroblast Evasion of Apoptosis—The Role of Angiotensin II

Cardiac fibrosis is a lethal outcome of systemic activation of normal quiescent fibroblasts to activated myofibroblasts. Gabbiani and colleagues first put forward the suggestion that, at least in dermal tissues, the majority of myofibroblasts enter apoptosis upon the completion of dermal wound healing [42]. Despite this time-honoured finding and for more than three decades, reports of the persistence of myofibroblasts in fibrotic tissues, including heart, has raised a central pointed question—how are myofibroblasts able to survive for extended periods in tissues that already display hypertrophic scarring and overt fibrosis? There is a growing recent awareness of fibroblast plasticity as well as fibroblast diversity in heart, as mentioned above, and these findings have paved the way for investigation of the persistence of myofibroblasts in fibrotic tissues. It is likely that the signalling for myofibroblast persistence is derived from a number of sources including matrix-borne biomechanical signals from the altered ECM microenvironment itself in combination with growth factors or cytokine signals—to produce a unique signalling environment that facilitates a net pro-survival signal. The novel hypothesis that the combined matricryptic and growth factor signal for myofibroblast survival in fibrosed tissues has only recently been put forward [7]. This hypothesis states that myofibroblasts in fibrosed tissues are not resistant to apoptosis, but rather, are primed for it by the simultaneous activation of cellular death signalling pathways, and that they will only proceed to apoptosis when the survival pathways are inhibited. Well-known pro-survival growth factors for dermal myofibroblasts include TGFβ_1 and platelet derived growth factor (PDGF) [7] and it is possible that cardiac fibroblasts share this response. We have recently demonstrated that cardiac myofibroblasts taken from chronically healed myocardial infarction in rat model of congestive heart failure express significantly high levels of PDGF receptor α(PDGFR α) [43]. While the cardiac myofibroblast is actively carrying out wound healing functions it follows that pro-survival mechanisms and signals are in place to support its role in healing—this hypothesis is supported by early work in this area [44, 45]. Hinz and Largares have recently suggested that myofibroblasts become "addicted" to pro-survival growth factor signalling during the inflammatory phase of wound healing and that in normal tissue, and the loss of these signals may herald myofibroblast apoptosis [7]. They also suggest that the changes that underpin the shift from normal wound healing to pathological organ fibrosis is due to signalling that prevents the termination of normal myofibroblast wound healing. In the heart, what are the pro-apoptotic signals that prime the myofibroblast for apoptotic removal from the site of infarction or other damage? In this regard, angiotensin II has recently been shown to prime the fibroblast for apoptosis (and reduction of cellular readiness to enter autophagy) in experiments using cultured neonatal rat cardiac fibroblasts [46]. Thus angiotensin II might oppose the actions of TGFβ_1 and PDGF, insofar as it may counter the strong pro-survivorship signal. Further, recent studies from our lab indicate that chronic SKI overexpression promotes autophagy in primary cardiac myofibroblasts [47]. In this case, angiotensin II and the SKI transcription factor may serve a

putative cooperative role to prime the cardiac myofibroblast for cell death. How then does the biomechanical matrix microenvironment of healed cardiac infarct (seen in post-myocardial infarction) provide the myofibroblast with a pro-survival signal that is sufficiently robust to allow the cell to survive being primed for death? The answer may lie in the fibrosed ECM, and in that context its important to acknowledge that the myofibroblasts themselves create the specific biomechanical conditions within the fibrosed ECM that are required. A recent review of the topic reveals that matrix stiffening following pathological fibrosis may confer specific signals for myofibroblast survivorship, via integrin-mediated transduction to the myofibroblast nucleus [7]. Specifically this extracellular myofibroblast survival factor may rely upon FAK activation via β1 integrin [44, 48, 49] and downstream PI3K-AKT [50, 51] which may phosphorylate and inhibit BAD [44, 52, 53], thereby inhibiting apoptosis at the crossover point between extrinsic and intrinsic apoptosis pathways. Finally, recent evidence using in vitro cultures both neonatal rat cardiac fibroblasts and human ventricular myofibroblasts supports the hypothesis that angiotensin II may play an expanded role in allowing myofibroblasts to evade apoptosis by the activation of the prosurvival AT1 ERK1/2 RSK1 pathway [54]. Clearly, further investigation of the precise effect of angiotensin II in vivo is required to resolve what signalling pathways dominate in cardiac myofibroblasts, and thus resolve whether angiotensin II either primes these cells for apoptotic death, or whether it signals for their evasion of apoptosis. This notwithstanding the stiffening of the fibrosed ECM remains the most important prosurvival stimulus in the fibrosing myocardium.

Conclusions

Pathological wound healing in heart following cardiac damage is frequently associated with overt cardiac fibrosis and heart failure. Unlike normal wound healing wherein resident myofibroblast undergo apoptotic removal from the site of healing, cardiac myofibroblasts that participate in the pathogenesis of cardiac fibrosis are first primed for apoptosis but then also receive powerful prosurvival signal from extracellular sources, the most important of which is the stiffened fibrosed ECM itself. In this way cardiac myofibroblasts continue to survive in the fibrosed tissue and continue to lay down matrix components, setting up a vicious cycle of additional fibrosis. While the net cellular result of the multiple signals provided by angiotensin II eg, is angiotensin II more important in priming the cell for apoptosis, or is it more important for signalling the evasion of apoptosis, remains to be determined. Nonetheless, investigation of the precise extracellular signaling that mediates the evasion of apoptosis may provide a specific clue for the development of small-molecule agents for the treatment of cardiac fibrosis.

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Chapter 14
Role of Renin Angiotensin System in the Pathophysiology of Coronary Heart Disease: Advancements in Diagnosis, Therapy and Preventive Strategies

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Abstract The incidence of coronary heart disease (CHD) has been escalating over the years, and epidemiological data suggests that CHD causes nearly 17.9 million deaths worldwide annually. Myocardial infarction and stroke resulting from CHD not only remain the major reasons of morbidity and premature death, but also cause high rate of absenteeism from work and prolonged hospitalisation that pose high economic burdens on the healthcare systems globally. The renin angiotensin system (RAS) has been the focus of attention in the pathogenesis of CHD, and modulators of RAS such as angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers are clinically used for treating cardiovascular disease (CVDs). The RAS is also involved in causing pathological changes in endothelial lining of arterial blood vessels, promoting inflammation, and increasing oxidative stress, that collectively make the coronary arteries more vulnerable to plaque deposition and vascular injury by interfering with various signalling pathways and transcription factors which regulate gene expression. The goals of this chapter are to provide an update on the current knowledge about pathophysiology of CHD, biomarkers used in its diagnosis, and treatment of CHD with pharmaceuticals, and management of CVDs with affordable and cost-effective phytotherapies and nutraceuticals. The involvement of miRNAs as future possible targets for the prevention of CHD progression will also be addressed.

Keywords Coronary heart disease · Atherosclerosis · Biomarkers · Renin angiotensin system · Treatment options

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Abbreviations

CVDs	Cardiovascular diseases
RAAS	Renin–angiotensin–aldosterone system
ROS	Reactive oxygen species
NO	Nitric Oxide
TNF-α	Tumor necrosis factor-α
LDL	Low-density lipoprotein
CD36	Cluster of differentiation 36
miRNA	MicroRNAs
NADPH Oxidase	Nicotinamide adenine dinucleotide phosphate oxidase
MAPK	Mitogen activated protein kinase
VCAM-1	Vascular cell adhesion molecule-1

Introduction

Hypertension, myocardial infarction, atherosclerosis, arrhythmias and valvular heart disease, coagulopathies, and stroke, collectively known as cardiovascular diseases, contribute greatly to the mortality, morbidity, and economic burden of illness in all countries of the world. According to WHO estimates, CVDs cause nearly 17.9 million global deaths annually [1]. The heart is a highly vascular organ surrounded by the extensive network of exterior major and minor coronary arterial blood vessels that are responsible for fulfilling the high oxygen demand and energy supply to the cardiac tissues and cells. Since neither oxygen nor energy supply materials present in the blood can diffuse through the cardiac endothelium to the thick walls of the heart from within the heart chambers, therefore the exterior network of coronary arteries plays a pivotal role to meet the oxygen and nutrient needs of the heart [2]. In view of these observations, any pathophysiological interruption in the coronary blood supply to the heart is the major risk factor for heart attack or sudden cardiac death. Coronary artery disease (CAD), or ischemic heart disease (IHD), is also known as coronary heart disease (CHD). According to the global epidemiological data, around 126 million people died of CHD-related disorders from 1990 to 2017 [3, 4]. CHD is characterized by fatty plaque deposition in the endothelium lining of the coronary blood vessels caused by atherosclerosis in the coronary arteries around the heart leading to hypoxia and curtailed nutrient supply to the myocardium which in turn leads to progressive narrowing and stiffening of coronary arteries and chronic heart failure [5]. The multiple array of risk factors associated with the development and progression of CHD are genetics [6], advancing age [7], gender [8], environmental toxicants [9], anxiety and psychosocial [10], poor dietary habits and fatty foods [11], geospatial location, smoking and sedentary lifestyle, and pre-existing comorbidities such as obesity and diabetes [12, 13]. CHD is associated with various symptomatic outcomes such as angina pectoris, reduced cardiac output, myocardial

infarction, shortness of breath, low energy in the body, and sometimes sudden cardiac death [14]. Atherosclerotic progression is complex and is accompanied by fluctuating lipid metabolism, inflammatory responses in the arterial blood vessels, and endothelial lining disruption causing plaque formation [15]. The renin–angiotensin–aldosterone system (RAAS) plays a critical role in the physiological homeostasis of blood volume, blood pressure, and Na+ /water balance to regulate vascular hypotension and hypovolemia. The physiological role of RAAS is attributed to its paracrine, autocrine, and intracrine functions [16]. The RAAS is also involved in the regulation of inflammatory and oxidative stress process leading to cardiovascular diseases [15]. In addition, the RAAS plays a key role in the progression of CHD by its interaction with angiotensin II which acts at the vascular cell through direct and indirect pathways [17] as well as by up-regulation of reactive oxygen species (ROS) and simultaneously down-regulation of endothelial nitric oxide (NO) [18]. The RAAS sends signals to inflammatory mediators, thereby promoting the generation of cytokines like tumor necrosis factor-α, interleukin 6 (IL-6) and cyclo-oxygenase 2 [15]. The current strategies for treatment of CHD caused by the overreactive RAAS are faced with many limitations due to many drug-related side effects [19] and gut dysbiosis [20]. Hence new safe and effective treatment pharmacotherapies are needed to target the RAAS and the development of molecular biomarkers for slowing down the progression of CHD at the early stages.

Pathophysiological Mechanisms of CHD

The coronary vessels are made up of three layers, namely Tunica adventitia, Tunica medium, and Tunica intima, which run from outside to inside. A layer of endothelial cells covers the intima. When endothelium layer is disrupted (ageing, hypertension, altered blood sugar & lipid dysfunction) [21], fatty materials such as cholesterol particles, lipoproteins, monocytes and platelets begin to deposit. LDLs can pass through damaged endothelial barrier due to assistance of cytokines, TNF-α and interferon-γ and then is oxidised in the sequence of occurrence. Oxidized LDL helps attract vasculo-adhesive molecules (VCAM-1 and P,E-selectins) which in turn attracts leukocytes. This oxidized LDL is taken up by matured monocytes called macrophages, resulting in production of foamy cells due to expression of lectin type oxidized LDL receptor 1, CD36 and various scavenger receptors. These foam cells along with leukocytes (mainly T-lymphocytes and mast cells) lead to generation of ROS and promote inflammation. Foam cells divide and multiply, forming fatty streaks, which are lesions. These lesions signal smooth muscle cells, which then congregate over foam cells, generating and depositing components like proteoglycans and collagen, eventually forming a plaque. This causes a narrowing of the coronary artery called focal stenosis. Finally, plaque calcifies due to inflammatory activities of metallic metalloproteinases, resulting in formation of a large lesion with necrotic substance and a rich fatty core which can further cause blood clot formation over that plaque which might lead to thrombosis. In future it might rupture causing

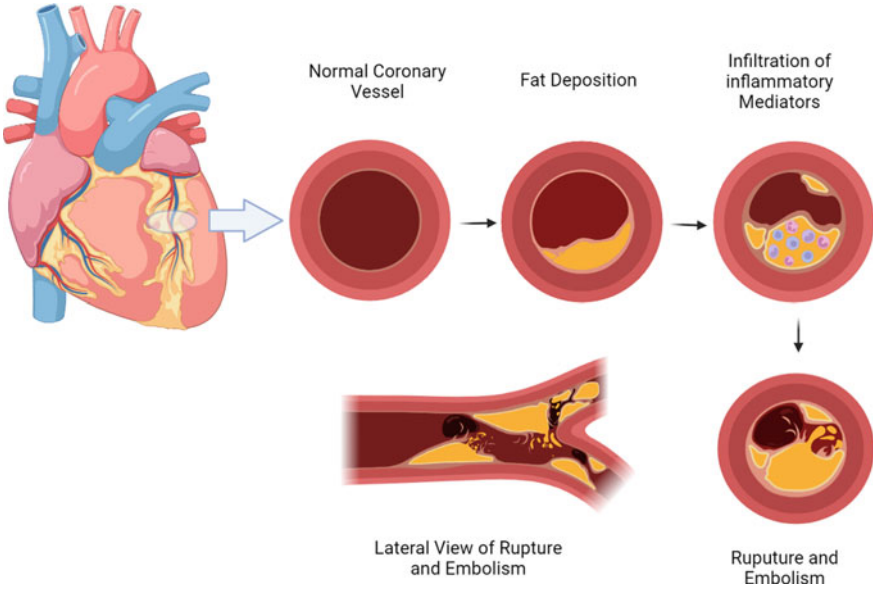

Fig. 14.1 Steps involved in the formation and progression of atherosclerotic plaques in the coronary blood vessels

embolization. It also renders vasculo-protective effect of NO inactive [15, 22–26] (Fig. 14.1).

As stenosis progresses, symptoms become more prominent leading to classification of disorders based on occurrence and type of symptoms [27–29] as depicted in Fig. 14.2.

Biomarkers Used in Diagnosis of CHD

Blood work analysis is ubiquitous in the healthcare system for the diagnosis of many diseases. Likewise, several key biomarkers have been developed and are used in the diagnosis of CHD and cardiovascular diseases. Specific biomarkers are needed for the diagnosis and determining the extent and severity of disease progression. The specific biomarkers must be reliable, quantifiable, and accurate to diagnose the disease. Upon analysis, they should provide a clear picture to the clinician to efficiently diagnose the disease and to underscore various therapeutic options. Some CHD biomarkers depicted in Fig. 14.3 may overlap with other CVDs.

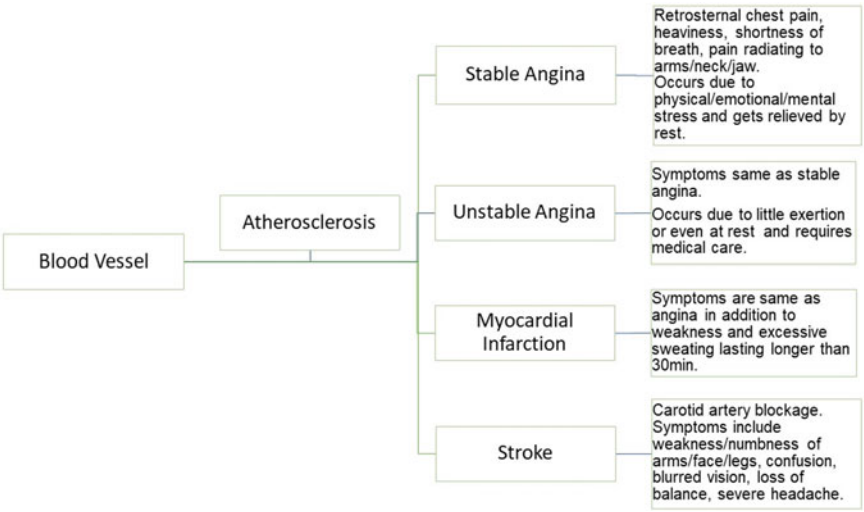

Fig. 14.2 Diagrammatic representation of the progression of CHD and related symptoms

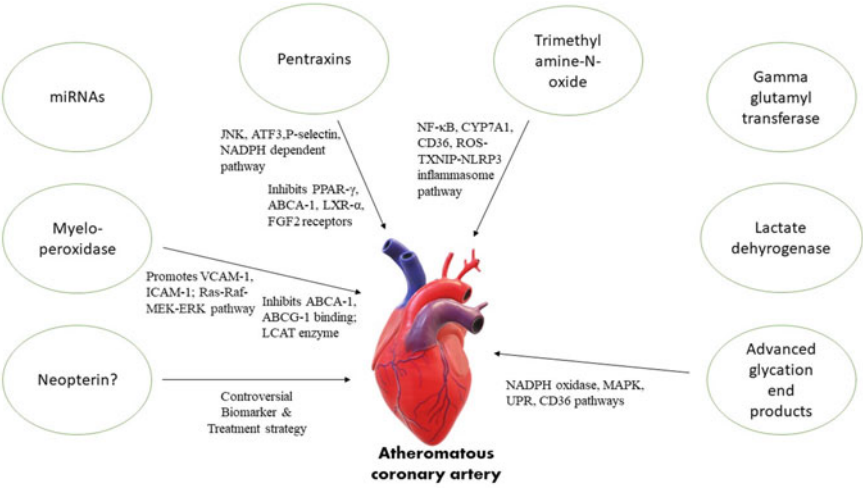

Fig. 14.3 Diagnostic biomarkers of CHD

MiRNAs

Small non-coding RNA work after the cell transcription level, regulating gene expression, by identification of a faulty mRNA and thereby leading to their downregulation [30]. In recent years there has been an overwhelming amount of evidence that miRNAs play an important role in cardiovascular diseases. These are single stranded

Table 14.1 miRNAs involved in the pathogenesis of CHD

miRNA	Role	References
miR-21	Cell proliferation by apoptosis inhibition Inhibits PPARα and activates activator protein-1 allowing monocyte binding leading to inflammation Increases oxidative stress by down-regulation of superoxide dismutase 2	[31–33]
miR-92a	Downregulation causes increased eNOS expression Targets Kruppel 2 and 4, increasing atherosclerotic predisposition Activates endothelial cell using oxidized LDL and again targets Kruppel 2 and 4	[34]
miR-106a-5p	Overexpressed in heart disease patients Promotes ox-LDL apoptosis and oxidative stress	[35]
miR-17-5p	Overexpressed in heart disease patients ER stress response and apoptosis of cells	[36]
miR-451a	Downregulated in atherosclerotic patients	[37]
hsa-miR-1-3p	Upregulated in cardiomyocytes in patients suffering from arrhythmogenic right ventricular cardiomyopathy (ARVC) and may lead to myocardial infarction	[38]
hsa-miR-21-5p	Upregulated in cardiac fibroblasts in patients suffering from ARVC and can also lead to myocardial infarction	[39]
hsa-miR-206	Overexpression leads to downregulation of Cx43 which leads to abnormal heart rate and shortened life span in mice	[40]

RNAs that are about 20–25 nucleotides long with major functions of gene silencing and post transcriptional regulation of gene expression. Most miRNAs present in our system have been transcribed from DNA sequences into primary miRNAs which are processed into precursor miRNAs and then into mature miRNAs. The miRNAs usually interact with the 3' UTR region of their target mRNA but they have also been reported to interact with 5' UTRs and some promoter regions. The interaction of a particular miRNA with its target is dependent on factors like abundance of miRNA, affinity of miRNA-mRNA interactions, subcellular location of miRNAs and the abundance of target mRNAs. They have been identified as potential biomarkers known to act via vascular smooth muscle cells and cardiovascular homeostatic pathways. A list of miRNAs associated with progression of atherosclerosis and thereby in CHD have been tabulated in Table 14.1.

Advanced Glycation End Products (AGEs)

Researchers have classified AGEs as high serum levels of methylglyoxal hydroimidazolone, N_{ε}-(carboxymethyl)lysine, MGH1, 3-deoxyglucosone hydroimidazolone, N_{ε}-(carboxyethyl)lysine, pentosidine and glyoxal hydroimidazolone and small

amounts of two oxidative products (methionine sulfoxide & 2-aminoadipic acid) which co-relates to the magnitude of coronary atherosclerosis in patients with type 2 diabetes [41–43]. But high AGE levels have also been observed in non-diabetic CHD patients [44] thus making AGEs a useful biomarker to address severity of CHD progression. Studies show how AGEs affect vascular endothelium, inflammation and oxidative stress thus increasing burden of CHD in this population. AGEs have been known to act via their receptors called RAGE present on vascular smooth muscles cells causing their proliferation and further calcification; endothelium via NADPH oxidase pathway, MAPK pathway and the Unfolded Protein Response (UPR) pathway which signal pro-inflammatory mediators; and platelets via NADPH oxidase and CD36 scavenger receptors increasing thromboxane A2 activity which in turn cause endothelial dysfunction [45]. Although latest studies indicate that AGEs are not co-related to CHD severity since CHD parameters did not yield statistically significant results in patients with diabetes mellitus but instead glycated haemoglobin shows more promising results when measured in diabetic and non-diabetic population [46] which makes this biomarker somewhat questionable.

Trimethylamine-N-Oxide (TMAO)

Trimethylamine (TMA) is synthesized by gut bacteria in vivo from numerous precursors, primarily choline, carnitine, butyrobetaine, and phosphatidylcholine from consumed meals (red meat, fish, eggs and dairy products) is the source of TMAO catalysed by flavin-containing monooxygenase [47, 48]. TMAO levels in body has been directly corelated with atherosclerotic heart disease [49–51]. Studies conducted using TMAO supplementation have shown an increase in macrophage scavenger receptors that are involved in pathogenesis of atherosclerosis confirming the role of oxidative stress [52]. It downregulates the expression of cholesterol 7 alpha-hydroxylase (CYP7A1) enzyme necessary for bile acid synthesis and cholesterol breakdown along with upregulation of scavenger receptor A and CD36 [53] which causes reverse cholesterol transport inhibition, variation in bile acid (synthesis, size, composition and transporters), aggravating inflammation of fat tissues, changing macrophage characteristics and worsening of already impaired glycemic tolerance [52, 54–56]. It also activates NF-κB inflammatory signalling pathway and ROS-TXNIP-NLRP3 inflammasome signalling pathway thereby increasing vasculitis [57, 58]. TMAO also has potential to cause platelet hyperactivity and thrombosis through release of intracellular Ca^{2+} ion stores [59] which can result in a fatal atherothrombotic event. But there are still many limitations to studies carried out and they need to be optimized to obtain reliable results.

Gamma-Glutamyl Transferase (GGT)

An extracellular enzyme and major cellular antioxidant present on top of membranes of highly absorptive or secretory cells. It is concentrated in organs such as kidneys, intestine, epididymis, pancreas, spleen, brain, and heart. It catalyses transfer of gamma-glutamyl moieties of glutathione getting converted to cysteinyl-glycine and thereby working as an antioxidant [60, 61]. Elevated GGT levels have been associated with various cardiovascular diseases including CHD [61]. Higher GGT levels, even within normal range, is known to cause dyslipidaemia, increase oxidative stress even when adjusted for other environmental factors such as alcohol (known oxidative stress precursor) and even co-relates to low-level inflammation all of which make cardiomyocytes as well as endothelial cells more susceptible to CHD burden. This increased oxidative stress leads to formation of ROS which upregulates GGT expression through Ras, Phosphoinositide 3-Kinase, p38 MAPK and Extracellular-signal Regulated Kinase signalling pathways. Another plausible mechanism attributed to it is generation of ox-LDL due to superoxide anion produced by end-product cysteinyl-glycine [62, 63]. Higher GGT levels have also been associated with development of insulin resistance which in turn leads to endothelial disruption [63].

Pentraxins (PXT)

The pentraxin family is known to be involved as humoral immunity acting as first line of immune defence in acute phase reactions, is further bifurcated into short and long pentraxins yielding important atheromatous biomarkers of CHD that is C-reactive protein (CRP) and pentraxin 3 respectively [64]. Of these, more attention is given to PTX3 since it is independent and expressed early in endothelial vasculature inflammation relative to CRP hence it is considered as a much better and reliable biomarker for CHD [65, 66]. It is acted upon by inflammatory mediators such as interleukin 1β and TNF-α thereby upregulating PTX3 production in endothelial cells starting a cascade of inflammatory, complementary and vascular remodelling pathways and supresses NO production due to granule membrane protein 140 and matrix metalloproteinases. Inhibition of cholesterol elimination pathways liver X receptor alpha (LXRα), peroxisome proliferator-activated receptor-γ (PPARγ), and ATP-binding membrane cassette transporter A-1 (ABCA1) causes ox-LDL to accumulate in the body [67]. It has been known to decrease NO synthesis and increases vasculitis by interaction with P-selectin and/or activation of c-Jun N-terminal kinase (JNK) and activating transcription factor 3 (ATF3) pathways which further reacts with matrix metalloproteinases leading to vascular dysfunction. It is also known to produce ROS by decreasing eNOS expression in reaction with lysophosphatidic acid and thereby activating the NADPH dependent pathway. It also blocks activity of fibroblast growth factor 2 (FGF2) by binding to it and thereby limiting its beneficial effects such as tissue repair, cell growth, tumor growth, wound healing, and angiogenesis [68].

Myeloperoxidase (MPO)

An inflammatory molecule involved in formation of atherosclerotic plaque and its destabilization thus increasing its chances for rupture by formation of various ox-LDL molecule types which then acts via intercellular adhesion molecule-1 and VCAM-1, decreasing eNOS expression, hence decreased NO production which causes endothelial insult. This is synergistic with modified HDL formation which in turn recruits VCAM-1 that causes inflammation [69]. This modified HDL is known to contribute to plaque instability via smooth muscle cell multiplication and migration involving Ras-Raf-MEK-ERK pathway [70]. Conversely if MPO activation is inhibited unstable fibrous plaques can be converted into a more stable plaque [71] which may partly delay further fatal consequences.

Neopterin

An inflammatory biomarker generated during a range of conditions. Not only is it found elevated in the general population with susceptibility of CHD, but also in various other conditions with may exacerbate CHD progression such as obesity [72]. Since this biomarker is controversial, review by Gieseg et al. may provide a solution to this issue which considers neopterin and total neopterin (neopterin+ 7,8-dihydroneopterin) to be evaluated simultaneously which would provide a more clear picture about the extent of inflammation and oxidative stress [73].

Lactate Dehydrogenase (LDH)

This is unique biomarker compared to other biomarkers as it is inversely related to CHD with specific focus on LDH-1 and LDH-2 due to its expression in cardiac cells [74]. This particular biomarker still needs to be studied to prove its accuracy and effectiveness in CHD diagnosis.

Impact of Renin–Angiotensin–Aldosterone System in the Pathophysiology of CHD

Figure 14.4 shows that RAAS-induced endothelial dysfunction along with oxidative stress and pro-inflammatory properties are collectively associated with the development of CHD. Renin production from the kidney is activated when salt-water imbalance occurs in the body, which in turn triggers a cascade of events leading to the formation of angiotensin II. Through its action on angiotensin type I receptors, angiotensin II regulates salt-water balance, blood volume, and blood pressure.

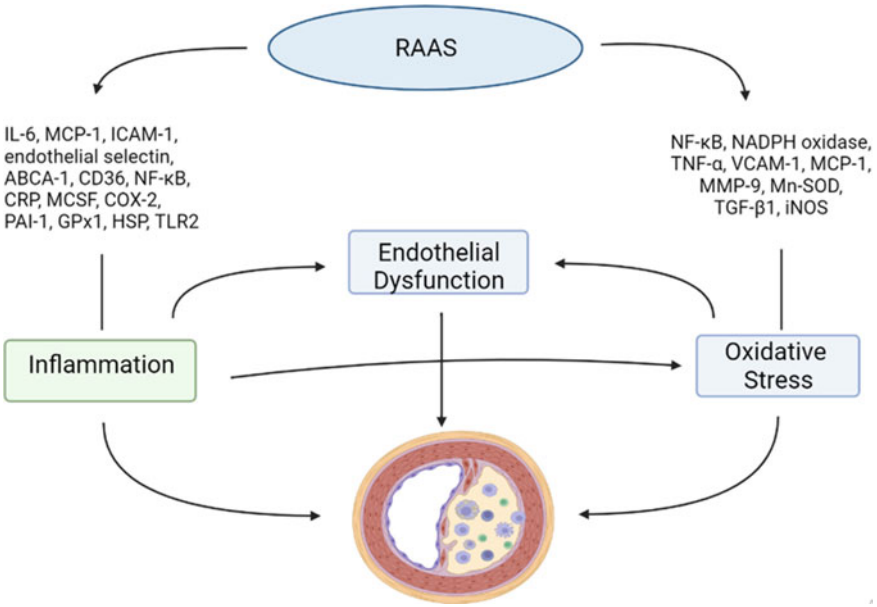

Fig. 14.4 Diagrammatic representation in the involvement of RAAS in endothelial dysfunction and pathological progression of CHD

However, overactivation of RAAS also produces harmful consequences such as cardiac reconfiguration, stiffness of arteries, and thromboembolism due to promotion of oxidative stress, pro-inflammatory mediators, fibrosis and cell apoptosis [75]. It is known to cause oxidative stress via production of ROS as well as other inflammatory factors by activating angiotensin type 1 receptors. The precursor of renin called prorenin has been observed to have higher plasma levels when attributed to the progression of CHD. The mechanism of action of this molecule is known to act via (pro)renin receptor activating MAPK ERK1/2, heat shock protein 27 and p38 pathways which in turn leads to increase in DNA, transforming growth factor beta 1, cyclo-oxygenase 2, plasminogen activator inhibitor 1, fibronectin and collagen 1 which coincides with CHD developmental pathways. Although there was a very weak co-relation of between CHD and non-CHD volunteers the higher cut-off value was determined at values greater than 1100 pg/mL with a 94% positive accuracy for developing CHD [76]. This is the only study to have been conducted in last two decades and thus needs to be further evaluated by other studies to determine its accuracy. Plasma renin activity has also been known to be elevated in CHD independent of the other co-existing risk factors thus indicating that it can be measured and co-related accurately to CHD severity [77]. Angiotensin II levels have been associated with endothelial disruption, inflammation, and plaque susceptibility all of which are classic CHD progression variables. It increases oxidant/antioxidant ratio thus activating NADPH oxidase pathway for generation of ROS [18].

RAAS system also plays a role in ROS generation thereby increasing oxidative stress via activation of protein kinase B, JAK/STAT, MAPK, and various other pathways that help in activation and multiplication of endothelial cells, and cell death which in turn promotes atheroma formation and progression [78, 79].

On the contrary to ACE 1, ACE 2 has been found to exert cardioprotective role with its high expression on the coronary endothelium. Its importance in cardiology arose from the discovery of cDNA library for heart failure and their inhibitory actions on Angiotensin I and Angiotensin II to Ang-(1–9) and Ang-(1–7), respectively, thereby inhibiting their activity with Ang-(1–7) having vasodilatory effects as opposed to Angiotensin II, acting through the g-protein coupled receptor based Mas receptor making the ACE2-Ang-(1–7)-Mas axis a central point for evaluation of CHD [78, 80].

Recent Advances in the Pharmacotherapy and Preventive Strategies of CHD

The current therapeutic strategies are based on attenuating the progression of CHD. However, these treatment interventions are also associated with undesirable side effects of pharmacotherapies. Thus, newer comprehensive treatment options for CHD are needed to block the RAAS pathways with minimal side effects.

Synthetic Drugs

Table 14.2 shows the current drug treatments of CHD, which mainly focus on the prevention of plaque build-up in arteries by lowering blood lipid levels, reduce endothelial inflammation, and improve blood flow in coronary blood vessels.

Phytotherapies

Phytotherapies are plant-derived bioactive macromolecules which are known to prevent diseases. They are isolated from fruits, vegetables, medicinal and aromatic plants. Many plants like *Brassica oleracea* and isolated phytochemical compounds such as soy isoflavones, resveratrol, and colchicine help to reduce cardiovascular diseases and act as anti-ischemic, hypolipidemic, antioxidant, anti-inflammatory agents as well as inhibitors of platelet aggregation [89] (Table 14.3).

Table 14.2 Current pharmaceutical therapies in CHD

Class of agents	Role	References
Cholesterol-modifying medications		
HMG-CoA inhibitors (Statins)	Statins are considered the first line of choice among cholesterol-lowering drugs, Rosuvastatin and atorvastatin are more effective than simvastatin according to lipid profile, Rosuvastatin is more effective than atorvastatin based on increase in HDL levels	[81, 82]
Fibric acid derivatives (Fibrates)	Effective for modifying atherogenic dyslipidaemia, and lowering of serum triglycerides Reduces mortality and morbidity associated with CVD, used as primary prevention of CVD	[83]
Bile acids-binding resins (Bile-acid sequestrants)	Can be used as first choice for single cholesterol-lowering therapy Combined therapy along with fibric acid or HMG-CoA reductase inhibitors Depletes endogenous bile acid pool, thus increasing bile acid synthesis from cholesterol, and lower LDL-C	[81, 84]
Ischemia relievers		
Beta-blockers (Propranolol, Metoprolol, Atenolol, Pindolol)	Decrease heart rate and metabolic requirements for the myocardium and prevent ischemia Act through multiple pathways, affecting chronotropy and inotropy, providing anti-ischemic effects and hence inhibiting renin release Show better prognosis, and hence are useful to improve long-term therapy in older patients with CAD	[85–87]
Calcium-channel blockers (Amlodipine, Nicardipine, Verapamil, Diltiazem)	Used for coronary vasospasm along with beta blockers therapy Relieve symptoms only by increasing myocardial supply and decreasing myocardial demand of oxygen	[86]
Nitrates (Glyceryl trinitrate, Isosorbide dinitrate)	Administered as sublingual & spray formulations of nitroglycerin Increase myocardial oxygen supply, hence are used for immediate relieve of angina by relieving the symptoms Also given in combination with beta-blockers if beta-blockers are inefficient alone	[86]

(continued)

Table 14.2 (continued)

Class of agents	Role	References
Anti-thrombotic agents		
Antiplatelet agents (Abciximab, Ticlopidine, Aspirin, Tirofiban)	Inhibit cyclo-oxygenase responsible to produce prostaglandins to alter platelet function Include aspirin, sulfinpyrazone, and NSAIDs. Thienopyridines, which also comes under antiplatelet drugs are prodrugs activated by CYP450 They irreversibly block the P2Y12 receptor, which leads to activation of glycoprotein IIb/IIIa receptors which are involved in strong and irreversible platelet aggregation Ticlopidine was the first thienopyridine which was replaced by clopidogrel Prasugrel is also a currently developed drug which is undergoing Phase III trials in patients with percutaneous coronary intervention	[86]
Anti-coagulant agents (Apixaban, Clopidogrel, Warfarin)	Stroke and MI are reduced using high-intensity and moderate-intensity oral anti-coagulants Used for CHD as they help to dissolve the formed clot	[88]

Dietary Supplements

Micronutrients include many vitamins and minerals such as selenium, zinc, copper, cobalt, chromium, and manganese which act as co-factors in the metabolic pathways that regulate inflammation and oxidative stress, and consequently reduce the risk of cardiovascular diseases. They are also involved in capturing the reactive oxygen species. Vitamin A, C, E and beta-carotene function as antioxidants and help in reducing the amount of free radicals and prevent oxidative damage to macromolecules like low-density lipoproteins [102] (Table 14.4).

Other Treatment Strategies

These are treatment strategies that do not fit the above classification but have shown certain promising results on attenuating the CHD severity. They are either repurposed synthetic drugs, natural treatments from traditional systems of medicine or various other actives/substances that can act on the same pathway as disease or a different pathway altogether thereby acting on the disease directly or indirectly.

Decreased ApoB/ApoA1 Ratio

Exercise plays an important role in cardiac restoration by lowering CVD related death, disability, and hospital readmissions while also reducing mental stress thus keeping a check on CHD risk factors [109]. Aerobic exercise training has

Table 14.3 Phytotherapies for the prevention of CHD: preclinical and clinical evidence

Phyto-chemicals	Dose	Mechanism of action	References
Polyphenols			
Flavonoids (Soy isoflavone)	Around 5 mg/kg protein	Utility in many cardiovascular diseases Genistein (tyrosine kinase inhibitor prevents ox-LDL formation) and daidzein which are soy isoflavones are present with weak proestrogenic activity which act via estrogen receptors and decrease serum cholesterol concentrations	[90]
Stilbenes (Resveratrol) (Rat model)	Standard dose: 20 mg/kg/day Preclinical studies: 50 mg/kg/day	Protective action mediated by the antioxidant enzyme heme oxygenase-1 and by NO Protects cardiac cell apoptosis and upregulates vascular endothelial growth factor expression Preclinical studies suggest high dose to prevent expansion of necrotic area and improve cardiac function Clinical studies suggested increase of adiponectin and rection of thrombogenic plasminogen activator inhibitor type 1, it acts as cardioprotective	[91, 92]
Phenolic acids (Curcumin) (Rat model)	Preclinical studies: 1.66 curcumin/kg Clinical studies: 500 mg/kg	Prevents aortic fatty streak, reduces proinflammatory cytokine release, collagen synthesis and fibrosis and cardiomyocyte apoptosis Decreases lactate dehydrogenase release in the coronary vessels through activation of JAK2/STAT3 Clinical studies shows that 500 mg/day intake decreased total cholesterol & serum lipid peroxides and increased HDL cholesterol	[91]
Anthocyanins (Kanglexin) (Male mice model)	20, 40 mg/kg per day, intragastric gavage	Anthraquinone compound used for alleviating ischemic heart disease Prevents MI-induced cardiac dysfunction and cardiac damage partly through cardiomyocyte pyroptosis and attenuating NLRP3(nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3)	[93]

(continued)

Table 14.3 (continued)

Phyto-chemicals	Dose	Mechanism of action	References
Alkaloids (Colchicine)	0.5 mg/day	Binds to tubulin, thus interfering with its polymerization, altering microtubule dynamics, and finally disrupting mitosis Inhibits the movement of WBCs (neutrophils by E-selectin expression inhibition) and other inflammatory cells, thereby limiting the inflammatory response to the formed urate crystals Inhibits L-selectin expression on endothelial lining and NLRP3 inflammasome and increases cAMP production to release prostaglandin E2 with simultaneous decrease in thromboxane A2, leukotriene B4 and cyclo-oxygenase 2	[94, 95]
Betalains (Red beetroot)	50 mg betalain/betacyanin	Decreases concentration of total cholesterol, triglyceride, and LDL Decreases intercellular and vascular cell adhesion molecules along with interleukin 6, TNF-α and endothelial-leukocyte adhesion molecule 1 Used as supplementation on atherosclerotic risk factors in CAD patients	[96, 97]
Phytosterols	315 mg/day	Inhibit cholesterol absorption, reduce LDL cholesterol concentration Lower other pro-atherogenic serum lipids and lipoproteins thus reducing atherosclerosis	[98]
Sulphur compounds (Allicin)	40 mg thrice daily for 12 weeks	Decreases homocysteine levels (homocysteine is important risk factor for CAD) Improves impaired endothelial function with hyperhomocysteinemia Decreases total cholesterol and triglyceride level	[99–101]

shown to improve endothelial function to enhance the shape and function of coronary arteries, compensate for improved blood flow to coronary arteries, promote creation of coronary auxiliary circulation, and help stabilize coronary artery clots thereby improving circulation and minimizing the occurrence of new disease alterations [110]. Various studies as reviewed Muscella et al. have reported benefits of workouts on decelerating CHD progression by upregulating ApoB/ApoA1 ratio thus reversing HDL & LDL concentrations back to normal by affecting reverse cholesterol transport and HDL cholesterol maturation & composition [111]. Reverse cholesterol

Table 14.4 Influence of dietary supplements in the prevention of CHD

Dietary supplements	Dose	Role	References
Antioxidants			
Beta-carotene	50 mg (Every other day)	Most abundant form of provitamin A and effective source for supplementation The Alpha-Tocopherol Beta-Carotene (ATBC) trial found that long term supplementation shows no risk of major cardiac events	[103]
Calcium	Varies from 800 to 2000 mg	Possible natriuretic effect, calcium supplementation shows anti-hypertensive effect	
Chromium	Varies from 200−600 μg	Decreases cholesterol levels Prevents lipid peroxidation	
Vitamin E	Preventing cardiac outcomes: Varies from 50−800 IU For hyper-lipidaemia: 500 IU	Alpha-tocopherol—most abundant form of Vitamin E in body Decrease in major coronary by lowering blood pressure and increasing HDL concentrations	[102, 103]
Coenzyme Q10	120 mg	Ubiquitous electron carrier, involved in generation of ATP in electron transport chain Reduces LDL peroxidation Increases HDL concentrations	[104]
Magnesium	Varies from 15 mmol−40 mmol	Decreases total cholesterol, triglyceride, LDL, VLDL levels and GI absorption of fats	[105]
Zinc	–	Maintains coherence of endothelial lining, inhibits NF-κB by zinc pyrithione formation thus reducing inflammation and apoptosis	[106]
Dietary foods			
Policosanol	Varies from 5−80 mg	Rich in long chain fatty acids: upregulates LDL processing and downregulates 3-hydroxy-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, and decreases cholesterol levels	[103]
Gugul	Varies from 100–6000 mg	Gugulsterones, the bioactive component can be used in both sterone-rich form known as gugulipid or as isolated form antagonist of bile acid receptors and increases LDL receptor reuptake and receptor binding sites Used as anti-inflammatory, hypolipidemic and anti-coagulant	

(continued)

Table 14.4 (continued)

Dietary supplements	Dose	Role	References
Artichoke extract	1800 mg	Decreases cholesterol biosynthesis by acetate to mevalonate shift in the 14-carbon chain structure Lowers cholesterol levels (decreases total cholesterol& LDL) and inhibits ROS generation in the endothelium	[103, 107]
Garlic	For hyperlipidaemia: 10−1350 mg 10−1350 mg	Anti-coagulant, inhibits cholesterol synthesis Reduces total LDL, total cholesterol, triglyceride with an effect on HDL and creatine kinase Protects eNOS from degrading by protein kinase B signalling pathway thus protecting endothelial lining from oxLDL insult Slows progression of necrotic plaque area from foam cells	[103, 108]

transport (RCT) inhibits generation of foam cells and progression of atherosclerotic plaque by transporting superfluous cholesterol from periphery to the liver for hepatic elimination [112]. But adipose as energy source is utilized only when exercise is carried out in mild to moderate amounts and this is supposed to be associated with elevated catecholamine responsiveness [111]. With advancements in newer technologies, monitoring of exercise has become quite easier and hence this can be used to our advantage to monitor CHD patients [113].

TMAO Inhibitor

As previously described about the role of TMAO in exacerbating CHD, 3,3-dimethyl-1-butanol (DMB), a choline analogue, has been shown to inhibit the first step of TMAO production which is trimethylamine (TMA) production by gut microbiota from choline by antagonizing TMA lyase enzyme. Preclinical dose in mouse model was calculated as 1% v/v. The uniqueness of this treatment approach is that it selectively inhibits TMAO production in a non-lethal manner thereby conserving the bacterial species involved and slowing CHD progression [114].

Sumatriptan

A 5-HT 1B/1D receptor agonist used to relieve headache and exerts anti-inflammatory properties. Administration of 0.3 mg/kg decreases lipid oxidation, tumor necrosis factor concentration, creatine kinase-MB levels, lactate dehydrogenase levels, Nf-κB' protein production and myocardial tissue injury. It increases

eNOS expression [115]. But research also has proved it as a contraindication due to its vasoconstrictive properties [116] thus making it debatable in terms of its effectiveness as a treatment strategy for CHD.

Neopterin

Contrary to misconception that neopterin is an inflammatory biomarker, it has recently come to limelight that neopterin can inhibit the progression of CHD making it very controversial as a biomarker as well as a treatment strategy. Latest studies on neopterin provide promising results on inhibition of vasculitis [117] which prevents further cascade of atherogenesis like foam cell formation and proliferation. It has also been shown to supress various cell adhesion molecules such as intercellular cell adhesion molecule-1, VCAM-1 and monocyte chemotactic protein-1 which further supports the above result of vasculitis inhibition along with ox-LDL transport inhibition and prevention of foam cell formation [118].

Guan-Xin-Shu-Tong Capsules (GXSTC)

A Traditional Chinese Medicine composed of *Salvia Miltiorrhiza* (Danshen), *Concretio Silicea Bambusae* (Tianzhuhuang), *Flos Caryophylli* (Dingxiang), *Fructus Choerospondiatis* (Guangzao) and *Borneolum* (Bingpian) is used to treat coronary heart disease. Mechanism is still unclear, but it has shown to restore impaired cardiac function and reduce myocardial infarction area. It regulates the contents of creatine kinase, lactate dehydrogenase and aspartate transaminase and metabolic disorders of endogenous components [119].

Future Prospects

Pharmaceuticals used for treating CHD mainly act by controlling symptoms or reducing the risk of any subsequent infarction. The scar tissue after infarction reduces the efficiency of heart resulting in higher chances for subsequent attacks. As CHD-related mortality rates continue to rise, the future for CHD prevention and management looks towards a new horizon of holistic approach which would include dietary modifications, major lifestyle changes and treatments with fewer side effects leaning towards the new age of natural or biologically derived products. Along with these, novel drug delivery mechanisms are being explored mainly in the domain of nanotechnology in order to provide drugs with better target approach and fewer or no side effects [120]. Thus, more research is required to find treatments specific to CHD which can reduce risk to benefit ratio. Newer treatment strategies targeting gut microflora, genetics of the disease or other potential targets involved in CHD exacerbation pathways are required. Cardiac cell cycle regulating miRNAs are the

future of miRNA therapy for cardiac disease treatment. A completely mature human heart has a much lower regenerative capacity than neonatal human heart therefore, identifying targets which are focused on tissue repair will provide viable therapeutic targets [121] or miRNAs that are able to focus on cardiac repair after injury are the future of miRNA therapy in coronary heart diseases [122].

Concluding Remarks

The multifactorial development of CHD and role of RAAS system in the participation and progression of CHD are well understood till date and it is important to address the issue from an early stage of life. A more preventive approach rather than end-stage management approach, which can result into fatal consequences, is required to prevent RAAS system involvement in pathogenesis of CHD. Thus, novel preventive strategies must be incorporated into daily lifestyle schedule from an early age which in the long run would be beneficial and protective from CHD development or progression considering the individual maintains an overall health fitness regimen. The new age of genetics and epigenetics are coming at the forefront for early diagnosis and treatment induction. As a result, natural supplements would be advantageous in terms of patient compliance, as well as in reducing burden of continual monitoring of synthetic drug administration under medical supervision. As a result, novel treatments would address current difficulties, reduce dangers, and ensure a long-term healthy lifestyle.

Conflict of Interest The authors declare no conflict of interest.

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Chapter 15
Renin Angiotensin System in the Pathophysiology of Diabetic Cardiomyopathy in Type 2 Diabetes

Karina P. Gomes, Anshul S. Jadli, and Vaibhav B. Patel

Abstract Type 2 diabetes mellitus is associated with a high risk of heart failure, in part because of its potential to induce impaired cardiac function even in the absence of coronary artery disease and hypertension. The pathogenesis of diabetic cardiomyopathy is not yet fully understood, although it is known that alterations in the cardiac substrate metabolism and energy play a significant role in the development and progression of this condition. Among these abnormalities, increased fatty acid storage and utilization, associated with reduced glucose oxidation and altered mitochondrial oxidative phosphorylation stand out. One of the key connections linking diabetes and the remarkably prevalence of cardiomyopathy is the renin angiotensin system (RAS) hyperactivation. RAS has been described for its crucial involvement in the generation and progression of diabetic cardiovascular complications, as its exacerbated activation supports mechanisms that lead to cardiomyocyte death and myocardial fibrosis. The importance of RAS blockage in the prevention of diabetic cardiomyopathy exhibits the fundamental role that RAS plays in the onset and development of this pathology. Angiotensin-converting enzyme inhibitors and blockers of angiotensin II actions denote the first-line therapy for primary and secondary prevention of cardiovascular disease in type 2 diabetic. Recent studies have revealed new features of RAS and, consequently, new therapeutic potential against diabetic cardiomyopathy. In this chapter, a description of the main mechanisms involved in the correlation between excessive activation of the RAS and type 2 diabetes is presented, with attention on mechanisms associated to pathogenesis and progression of diabetic cardiomyopathy.

Keywords Diabetic cardiomyopathy · Renin angiotensin system · Metabolic dysfunction · Cardiac remodeling · Therapeutic actions

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Introduction

Diabetes mellitus has emerged as a critical public health concern and represents a significant threat to the global population. It is estimated that by 2040 almost 700 million people will be affected if no effective prevention methods are adopted [1]. Type 2 diabetes is a chronic metabolic disorder represented by insulin resistance and hyperglycemia, also characterizing one of the greatest risks for the development of heart failure [2]. Patients with type 2 diabetes are 2–5 times more likely to develop heart failure than non-diabetic and age-matched patients, independent of other medical conditions. It suggests an intrinsic and specific mechanism that generates pathological cardiac remodeling in this population, currently known as diabetic cardiomyopathy [3].

Diabetic cardiomyopathy is characterized by the impaired cardiac structure and function that happens when other cardiac risk factors, such as coronary artery disease, hypertension, and valvular disease are not present [4]. It was first described more than forty years ago, with hyperglycemia and abnormal insulin signaling in the heart comprising key roles in its genesis and progression [5]. The diabetic heart is mainly characterized by diastolic dysfunction with preserved ejection fraction, changes caused by the pathological cardiac remodeling. Increased perivascular and interstitial fibrosis, in addition to left ventricle hypertrophy, are structural features commonly observed in diabetic hearts [6]. The underlying pathogenic mechanisms of diabetic cardiomyopathy include but are not limited to abnormal extracellular matrix deposition, enhance in inflammation and oxidative stress associated with mitochondrial dysfunction, and alterations in energy production and cardiac metabolic profile [7].

One of the key connections linking diabetes and the remarkably prevalence of cardiomyopathy is the renin angiotensin system (RAS) hyperactivation. RAS has been described for its crucial involvement in the generation and progression of diabetic cardiovascular complications, as its exacerbated activation supports mechanisms that lead to cardiomyocyte death and myocardial fibrosis [6–9]. In this chapter, a description of the main mechanisms involved in the correlation between excessive activation of the RAS and type 2 diabetes is presented, with attention on mechanisms associated to pathogenesis and progression of diabetic cardiomyopathy.

Diabetes-Induced Activation of the Renin Angiotensin System

Overview of the Renin Angiotensin System

The RAS has a extensive and substantial history that began in 1892 with the discovery of renin [10]. Over the past 130 years, many other elements of this intricate and vital system, in conjunction with angiotensinogen, angiotensin-converting enzyme

(ACE) isoforms, angiotensin peptides, and their associated receptors, have been described. Concurrently, pharmacological approaches have also emerged to regulate this system at several different stages. RAS performs key physiological functions in fluid and electrolyte regulation, systemic vascular resistance, blood pressure, and cardiovascular homeostasis [7]. Notwithstanding, its hyperactivation may induce oxidative stress, inflammation, and endothelial dysfunction, leading to many problems such as hypertension, kidney disease, and heart failure. Thus, ACE inhibitors and angiotensin II (Ang II) type 1 (AT1) receptor blockers have been at the forefront of the clinical management of various cardiovascular diseases. Angiotensinogen is the precursor of the various RAS family peptides, which are generated by tightly regulated biochemical processes (Fig. 15.1). Although the liver is the primary systemic source of angiotensinogen, it is also expressed in various other tissues. To date, five main bioactive angiotensin peptides have been studied: Ang II, Angiotensin-(1–7) (Ang-(1–7)), Angiotensin-(1–9) (Ang-(1–9)) and Alamandine [11, 12]. Ang II is the most studied peptide of the RAS family and is considered the effector peptide of the RAS. Besides to its well knows renal and vascular effects, Ang II also has direct actions at the cellular level, affecting cell inflammation, differentiation and survival [13–15].

To generate the octapeptide, Ang II, Angiotensinogen is cleaved into the decapeptide Angiotensin I (Ang I) by Renin. Ang I is further processed by ACE, a key dipeptidyl carboxypeptidase, leading to Ang II production. To mediate its physiological functions, Ang II binds to two main G-protein coupled receptors (AT1 and AT2 receptors) (Fig. 15.1). Ang II can be further converted to Angiotensin III (Ang III) through the aminopeptidase A. Ang III binds to the AT1 receptor with a lower affinity than Ang II and to the AT2 receptor with a higher affinity than Ang II [16]. To form the heptapeptide Ang-(1–7), Ang II is cleaved by angiotensin-converting enzyme type 2 (ACE2), an ACE isoform first described in 2000, and then binds to its receptor, the Mas receptor [17]. With less efficiency, ACE2 is also able to cleave Ang I into Ang-(1–9), which can be further cleaved into Ang-(1–7) by ACE or by Neutral Endopeptidase or Neprilysin (NEP) [18]. Furthermore, Ang-(1–7) can be formed directly from Ang I, through the action of the enzymes NEP, Timet Oligopeptidase (TOP) and Prolyl Endopeptidase (PEP). Although effects of Ang-(1–7) are thought to be primarily mediated via the activation of Mas receptor, Ang-(1–7) can also bind with lower affinity to the AT2 receptor and the MrgD receptor (Fig. 15.1) [19]. Recently, Alamandine, a heptapeptide analogous to Ang-(1–7) and formed by its decarboxylation, was shown to bind with and activate MrgD, which led to the inclusion of MrgD receptor the RAS family [20]. Alamandine may also be produced by the decarboxylation of Ang II to Angiotensin A (Ang A), which is later cleaved by ACE2 [20].

ACE is well known for its action in converting Ang I to Ang II, and although Ang II can bind to both AT1 and AT2 receptors, increased ACE activity pointedly conduct to higher activation of AT1 receptor. AT1 receptor hyperactivation and increased ACE levels are well recognized for their vasoconstrictor effects, in addition to cell death and inflammation [21, 22]. Whereas the activation of AT2, Mas, and MrgD receptors are known primarily for its role in vasodilation and cell survival through their

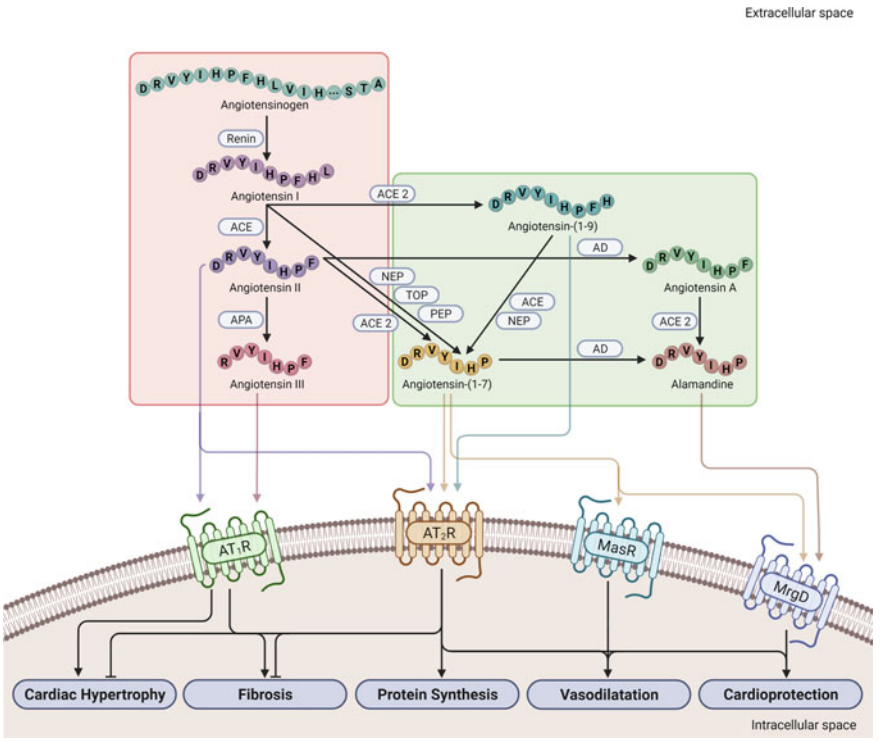

Fig. 15.1 Classical and counter-regulatory renin angiotensin pathways. In the renin angiotensin system, angiotensinogen is processed by renin to form Angiotensin I. Angiotensin I can be cleaved by angiotensin-converting enzyme (ACE) to generate Angiotensin II, which can bind to the Angiotensin II type 1 receptor (AT1R) and Angiotensin II type 2 receptor (AT2R). Angiotensin II can be further processed by aminopeptidase A (APA) to form Angiotensin III, which also binds to the AT1R. In addition, Angiotensin I can be processed by ACE2 and neprilysin (NEP) to respectively produce Angiotensin-(1–9) and Angiotensin-(1–7). AT2R can also be activated by Angiotensin-(1–9), while Angiotensin-(1–7) binds to the proto-oncogene Mas receptor (MasR). Angiotensin-(1–7) can additionally be produced from Angiotensin II cleavage by ACE2 and be further metabolized to Alamandine. Occasionally, Angiotensin II may be cleaved by aspartate decarboxylase (AD) to produce angiotensin A, which can be converted to Alamandine by ACE2. Alamandin binding to the Mas-related G protein-coupled receptor member D (MrgD) promotes similar effects to those reported for Angiotensin-(1–7). Hyperactivation of AT1 receptor signaling are well recognized for their role in inducing cardiac hypertrophy and fibrosis, whereas the activation of AT2, Mas, and MrgD receptors are known primarily for its role in vasodilation and cell survival through their cardioprotective properties. Figure created using Biorender.com

anti-inflammatory and antioxidant properties [22, 23]. Despite binding with greater affinity to the Mas receptor, Ang-(1–7) can also bind to AT2 and MrgD receptors. Emerging evidence suggests that the signaling pathways of Mas, MrgD, and AT2 receptors are inter-dependent. The activation of the AT2 receptor has been shown to increase the expression of ACE2, an enzyme primarily involved in Ang-(1–7) formation. Moreover, elimination of AT2 receptor results in a marked reduction in

the levels of Mas receptor and ACE2, suggesting a potential role of AT2 receptor signaling in the regulation of ACE2/Ang-(1–7)/MasR signaling [24]. Notably, Mas and AT2 receptors activation can directly antagonize the AT1 receptor through a heterodimer formation, leading to decreased signaling pathway of the primary Ang II receptor [25]. Additionally, ACE2 significantly increases the formation of these heterodimers, facilitating the ACE2/Ang-(1–7) actions. On the contrary, the activation of the AT1 receptor negatively regulates ACE2 levels, impeding the ACE2/Ang-(1–7) actions [18]. The complex nature of these actions substantiates the synergistic interaction between RAS receptors and enzymes to preserve an intricate balance.

Ang-(1–7) has been at the center of various seminal preclinical and clinical research works, primarily due to its cardio and vasculoprotective effects that are mediated by antagonizing the deleterious actions of Ang II on the cardiovascular system. In this regard, various shreds of evidence point to the concept of a RAS formed by two antagonistic axes. (i) a classic axis, where the primary mediator is Ang II, activation of which leads to vasoconstriction, inflammation, oxidative stress, apoptosis, and cell proliferation; (ii) an alternative axis, where the primary mediator is Ang-(1–7), activation of which leads to vasodilation, anti-inflammatory, antioxidant, anti-apoptotic, and angiogenic actions [26–29].

In addition to its systemic effects, known for key outcomes on blood pressure, fluid and electrolyte balance [30, 31], RAS elements have also been detected in several tissues, including heart, kidney, brain, nerve fibers, reproductive organs, blood vessels, liver, skeletal muscle, and pancreas [3]. The demonstration of components of the RAS in these tissues has established the concept of a tissue or local RAS, whose regulation is believed to be independent of the systemic RAS.

Renin Angiotensin System in Type II Diabetes

Studies of RAS in diabetes have shown a tissue-specific activation of this system mainly in the kidneys, pancreas, heart, vasculature, and adipose tissue [32]. Furthermore, Ang II has been shown to play a key role in the development of insulin resistance at the cellular level, mainly via increased oxidative stress and altered insulin signaling [33, 34]. Physical inactivity is a major contributor to obesity, and together they are known to be crucial pitfalls for the progression of insulin resistance and, consequently, type 2 diabetes. The reduction in insulin sensitivity causes a continuous burden on the impaired pancreatic islet β-cells. At a certain point, insulin secretion is no longer sufficient to support normoglycemia, and glucose levels start to rise to the prediabetic stage, leading to impairment of glucose tolerance and/or changes in fasting glucose [34]. β-cell failure precedes the progress of type 2 diabetes, and the decline of β-cell power, as well as the loss of operational β-cell mass, are the main predictors for the development of this condition [3, 33].

It is noticeable that insulin displays deleterious effects on the functional and structural characteristics of islet cells by generating Ang II-mediated oxidative stress. Via AT1 receptor, Ang-II interferes with the effects of insulin on skeletal muscle and

vascular tissue, through disruption of insulin signaling via phosphatidylinositol 3-kinase, and its downstream protein kinase B (Akt) signaling pathway [9, 35]. In animal models, Ang II reduces blood flow to pancreatic islets, leading to decreased insulin deliver from β-cells. Hence, RAS inhibition increases pancreatic blood flow, improving islet's function [36, 37]. In fact, both in vitro and in vivo assessments indicate that RAS activation leads to inflammation, oxidative stress, fibrosis in pancreatic islets and defective insulin secretion. Meanwhile, RAS repression with AT1 receptor blockers or ACE inhibitors increases systemic glucose tolerance, and improves islets morphology and function [38–40]. Despite substantial data obtained from pre-clinical research, there is still a lack of corroboration of these findings in humans subjects. Collectively, the available evidence suggests that the hyperactivated RAS in pancreatic islets may be involved in compromised insulin secretion, and RAS inhibition can be a feasible approach to improve insulin secretion in individuals with a high potential to have type 2 diabetes.

RAS activation from both systemic and cardiac tissue also plays a key direct contribution in the development and progression of diabetic cardiomyopathy. A recent study showed systemic activation of RAS in response to hyperglycemia, which was associated with increased vascular resistance and, consequently, increased blood pressure [32]. The hyperactivation of RAS in diabetes is strongly supported by the effects of AT1 receptor antagonism that results in a more significant decrease in blood pressure in hyperglycemic conditions, when compared to normoglycemic conditions [33]. In fact, Ang II directly stimulates oxidative stress by increasing nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. Furthermore, RAS hyperactivation activates the mTOR-S6K1 pathway, leading to systemic and cardiac insulin resistance [18]. Concurrently, the hyperactivation of AT1 receptor signaling in the heart reflects in exacerbated proinflammatory immune responses [5]. These changes further lead to the activation of hypertrophic and profibrotic pathways that result in cardiac remodeling associated with cardiac fibrosis, diastolic dysfunction, and ultimately, heart failure.

Metabolic Disturbances in the Diabetic Heart

Normal Cardiac Energetics

Over the course of an average human life, 200 million liters of blood are pumped by the heart in about 2−3 billion heartbeats. This high workload requires a high consumption of adenosine triphosphate (ATP), which can reach up 30 kg of ATP per day [41]. Accordingly, the heart becomes the most energy-demanding organ in the human body, and even small changes in energy availability can quickly lead to changes in cardiac function [42]. The high energy demand also requires an uninterrupted generation of ATP that depends on the continuous supply of substrates and oxygen, in addition to a functional oxidative phosphorylation, since this efficient

metabolic pathway produces almost all the ATP used by the heart [43]. Roughly 70% of the ATP produced in the myocardium is used in the cardiac contractions, while the remnant is used for ion pumps, such as sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA). Albeit the heart has the ability to change its substrate preference according to its needs, the two main energy substrates used are fatty acids and glucose [44].

Abnormal Cardiac Fatty Acid Uptake and Utilization in Diabetes

The preference of the heart for fatty acids as energetic substrates is amplified in type 2 diabetes. This is a result of the increased plasma fatty acid levels due to increased lipolysis from insufficient insulin action and triglycerides production in the liver, in addition to inefficient adipocyte triglycerides storage (Fig. 15.2). The intensified delivery of fatty acids then elevates fatty acid uptake and triglycerides stores in cardiomyocytes [45]. As a result, fatty acid abundance in the cardiac muscle activates peroxisome proliferator-activated receptor (PPAR)-α, which heightens the dependence on fatty acids through the upregulation of its uptake, storage, and β-oxidation, while the glucose utilization is suppressed [46]. In addition, PPAR-α acts in the regulation of key factors that lead to fatty acid β-oxidation, and therefore is considered an essential regulator in the homeostatic control of energy balance. Marked PPAR-α activity has been shown in animal models of diabetic cardiomyopathy, and hearts from PPAR-α knockout mice do not change when they become insulin resistant [47]. In humans with type 2 diabetes, increased cardiac fatty acid oxidation has been described to be two times higher when compared with healthy individuals [48], and aberrant fatty acid metabolism has been associated with adverse cardiac damage.

Exaggerated fatty acid retention is highly toxic to cardiac cells, and fatty accumulation in the heart is correlated with cardiac dysfunction in type 2 diabetes. Myocardial triglycerides levels have been shown to be increased by up two-fold in type 2 diabetic patients, with greater triglyceride content associated with impaired left ventricular systolic and diastolic function [48, 49]. The increase in fatty acids within the heart is proposed to also elevate mitochondrial reactive oxygen species by augmenting β-oxidation and electron transport chain activity [50]. Elevated levels of reactive oxygen species are consecutively involved in cardiac hypertrophy and inefficient excitation–contraction coupling due to uncoupled oxidative phosphorylation and mitochondrial dysfunction [51, 52]. Additionally, elevated lipid intermediary metabolites can unbalance signaling, inducing cardiomyocyte apoptosis through cytochrome c pathway. These circumstances determine the lipotoxicity involved in the development of diabetic cardiomyopathy. Considering its greater oxygen demand—approximately 86% higher than glucose—increased fatty acid metabolism eventually leads to cardiac inefficiency [53].

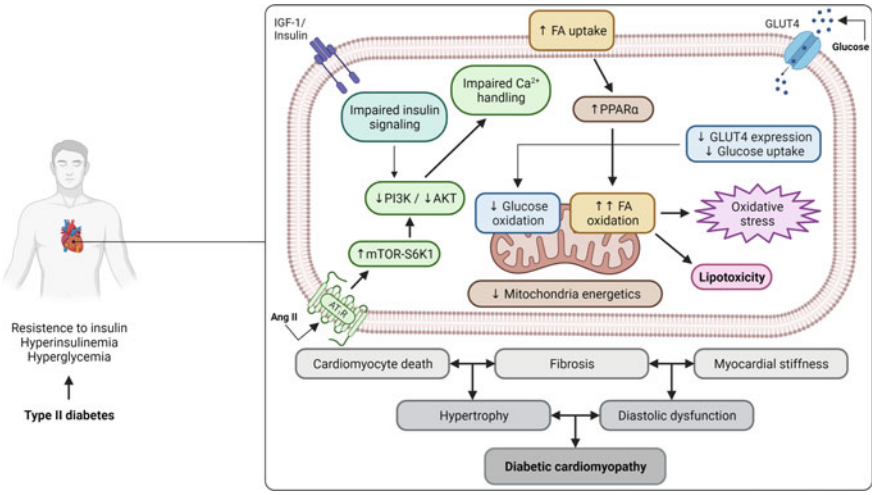

Fig. 15.2 Molecular mechanisms involved in the development of diabetic cardiomyopathy. Resistance to the metabolic actions of insulin, compensatory hyperinsulinemia, and the progression of hyperglycemia promoted by type II diabetes result in several effects at the cardiac level. The mobilization of free fatty acids and activation of the renin angiotensin system can cause lipotoxicity, mitochondrial dysfunction and oxidative stress that result in impaired insulin signaling with changes in calcium metabolism and cardiomyocyte death. These abnormalities further induce fibrosis and myocardial stiffness, and eventually that result in hypertrophy, diastolic dysfunction and diabetic cardiomyopathy. IRS-1: insulin receptor substrate 1; AT1R: Angiotensin II type 1 receptor; mTOR: mechanistic target of rapamycin; S6K1: S6 kinase 1; PI3K: phosphatidylinositol 3-kinase; AKT: protein kinase B; FA: fatty acids; PPAR-α: peroxisome proliferator-activated receptor-α; GLUT4: insulin-regulated glucose transporter type 4. Figure created using Biorender.com

Defective Cardiac Glucose Uptake and Utilization in Diabetes

Although systemic hyperglycemia still leads to suitable glucose uptake in some tissues, myocardial glucose oxidation is decreased by 30 to 40% in type 2 diabetic patients [54]. This mainly refers to fatty acid accumulation in the heart. Lower glycolysis is followed by reduction in the pentose phosphate pathway flux and simultaneous increase in polyol, hexosamine biosynthetic and glycogenic pathways, which may contribute to the cardiac damage [55, 56]. Hyperglycemia plus the resulting end-products of the altered glucose dislocation facilitate several features of diabetic cardiomyopathy (Fig. 15.2). Hyperglycemia increases myocardium glucose levels that may glycate proteins to generate advanced glycation end-products (AGEs) in a non-enzymatic manner. AGEs elevate reactive oxygen species, and can interact and damage macromolecules such as the ryanodine receptor (inducing contractile impairment), SERCA (causing diastolic impairment) and collagen (generating myocardial fibrosis and stiffness) [57, 58]. The elevation in polyol flux and the reduced pentose phosphate pathway activity decreases NADPH levels, enhancing oxidative stress and damage [55, 56]. Increased hexosamine biosynthetic pathway flux elevates the

production of glycosaminoglycans, and that can also assist to impaired SERCA activity, matrix remodeling and cardiac fibrosis [59].

Cardiac Mitochondrial Oxidative Phosphorylation Impairment in Diabetes

Cardiac mitochondria show a decrease in rates of oxidative phosphorylation, along with higher uncoupling of respiration and impaired ATP production in type 2 diabetes. This happen even with the confirmation of higher cellular anaplerosis [60] and lower transportation of the Krebs cycle end-products FADH2 and NADH to the electron transport chain. This reveals that elements related to the Krebs cycle narrow oxidative phosphorylation. In fact, preclinical and clinical studies show a coherent depletion in the levels and catalytic activity of hexosamine biosynthetic pathway complexes in type 2 diabetes, and this depletion has been linked to oxidative stress and myocardial impairment [61, 62]. In animal models, reduced oxidative phosphorylation also results from free fatty acid-mediated upregulation of uncoupling proteins that uncouple mitochondria, reductions in mitochondrial quantity, size, and complexity [63, 64]. In type 2 diabetic patients, attenuations in mitochondrial size respond to compromised mitochondrial fusion with reduced mitofusin-2 levels [65].

Mitochondrial dysfunction is related to impaired cardiac structure and function in type 2 diabetes. In animals and humans with type 2 diabetes, depletion in oxidative phosphorylation is evidenced by reduction in myocardial phosphocreatine and ATP content. Pronounced energetic deficiency due to reduced electron transport chain activities impairs diastolic function and exercise capacity [66, 67]. Furthermore, in atrial trabeculae extracted from surgical type 2 diabetic patients, lower oxidative phosphorylation is related with compromised contractility [68]. As mitochondrial dysfunction may lead to systolic and diastolic impairment through its potential to restrict, respectively, ATP supply to myofibrils and SERCA, it also increases reactive oxygen species generation, which is involved in cardiac remodeling [69, 70]. Enhanced reactive oxygen species generation follow the abundance of electrons distributed to the electron transport chain, in response to the higher fatty acid oxidation.

Cardiac Remodeling in Diabetic Cardiomyopathy

The progression of diabetic cardiomyopathy is characterized by a short physiological adaptation to metabolic challenges, that gradually culminates in deteriorating alterations that the myocardium is unable to repair, ultimately leading to irreversible pathological remodeling [71]. Hyperglycemia and hyperlipidemia with progressive increasing of their substrates in the heart cause structural and functional changes by

various mechanisms. These changes, which establish themselves slowly, begin with diastolic dysfunction, accompanied by impaired left ventricular systolic function. This heart failure is related with altered myocardial energy metabolism with gradual myocardial hypertrophy and fibrosis, leading to early death if not diagnosed and treated promptly [72].

Cardiac remodeling can be understood as a physiological adaptation to a prolonged stressful stimulus that increases the workload of the myocardium or as a response to reduced myocardial contractility or changes in the composition of myocardial tissue associated with heart disease. Thus, changes in left ventricular mass and/or volume represent only a phenotype common to various pathological processes [73]. Although cardiac remodeling appears to be an adaptive process, many studies conducted in patients with cardiovascular disease have shown a poor association between left ventricular hypertrophy and clinical outcome. For example, in a large prospective study, cardiovascular risk was directly correlated with increasing ventricular mass [74]. In addition, left ventricular hypertrophy was associated with higher mortality in some patient groups, such as the elderly [75], and is considered a step in the progression to dilated cardiomyopathy and, therefore, heart failure [76]. Thus, left ventricular hypertrophy is considered the main predictor of morbidity and mortality in cardiovascular diseases. In obese and diabetic individuals, left ventricular hypertrophy has been systematically and consistently observed [73]. It is important to note that while left ventricular hypertrophy associated with systemic arterial hypertension or myocardial infarction can be attenuated or reversed as a function of the pharmacological treatment of these diseases, there are still no defined therapeutic strategies to treat cardiac remodeling associated with type II diabetes mellitus.

Ang II, in addition to its classic actions, stimulates cell hypertrophy and increases collagen synthesis and deposition by cardiac fibroblasts, leading to cardiac remodeling [77]. In the absence of any intervention that causes illness in mice, selective overexpression of the AT1 receptor in hearts induces left ventricular hypertrophy [78]. This experimental demonstration clarifies the hypertrophic role of Ang II on the heart. Ang II also activates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme involved in the formation of reactive oxygen species. The oxidative stress generated by the excessive formation of reactive oxygen species, with a decrease in antioxidant defense, brings serious complications to cardiac cells, resulting in structural changes and compromising cardiac contractility [79]. Interestingly, when cardiomyocytes are stimulated in vitro with glucose, mimicking diabetes, there is an increase in intracellular production of Ang II [80]. Therefore, hyperglycemia can induce left ventricular hypertrophy via activation of the local intracardiac RAS.

Renin Angiotensin System Modulation as a Treatment of Diabetic Cardiomyopathy

Since the insulin advent in 1921, refinement in diabetes diagnosis and treatment have remarkably decreased mortality from acute metabolic emergencies. However, chronic complications, including cardiovascular disease, remain as the leading cause of morbidity and mortality among patients with type 2 diabetes. The importance of RAS antagonism in the prevention of diabetic cardiomyopathy illustrated the fundamental role that RAS plays in the onset and development of this pathology. ACE inhibitors and blockers of Ang II actions denote the first line therapy for primary and secondary prevention of cardiovascular disease in diabetic patients. The latest studies have revealed new aspects of RAS and, consequently, new potential therapeutic targets that could be used against diabetic cardiomyopathy in the near future.

AT1 Receptor Antagonists

Treatment with olmesartan, an AT1 receptor antagonist, in an animal model of type II diabetes induced by a high-fat diet, attenuated cardiac inflammation and interstitial and perivascular fibrosis. The mechanism of improved cardiac function by olmesartan was attributed to the inhibition of apoptosis signal regulation kinase-1 (ASK1), an enzyme involved in cell death and hypertrophy, showing the participation of Ang II in the activation of these mechanisms [81]. Another work showed that when candesartan was associated with an oral hypoglycemic agent in an animal model of obesity, *db/db* mice (harbors mutation in leptin receptor), there was an improvement in fibrosis and cardiac inflammation. These effects of candesartan have been associated with improved cardiac antioxidant defense [82], which is in complete agreement with the literature on the contribution of reactive oxygen species to Ang II actions [79].

A study with increasing doses of irbesartan showed a dose-dependent effect on the reversal of left ventricular hypertrophy in diet-induced type II diabetes rats. Improvements in diastolic and systolic dysfunctions were also observed on echocardiographic measurements and histological analysis of myocardial interstitial fibrosis. In this case, treatment with irbesartan inhibited the protein kinase D pathway, a kinase involved in the regulation of cell survival, differentiation, proliferation, and migration [83]. Finally, losartan was used in a normotensive animal model of diet-induced obesity. Losartan treatment not only reversed left ventricular hypertrophy but also partially improved insulin resistance and lipid and glycemic profiles. The beneficial cardiac effects were associated with reduced activity of several mitogen-activated protein kinases (MAPK) [84]. All studies with AT1 receptor antagonists showed a beneficial effect on left ventricular hypertrophy. However, these effects were not always independent of blood pressure control (since some animals become hypertensive) or improvement in insulin resistance. In addition to its contribution to the induction

of cardiac remodeling, Ang II is also intimately involved in the pathophysiology of metabolic alterations.

Angiotensin-Converting Enzyme Inhibitors

As ACE is a significant source of Ang II generation, ACE inhibition consistently leads to a reduction in systemic Ang II levels. Although the use of ACE inhibitors is indicated for the treatment of left ventricular hypertrophy, ACE inhibitors are also used for the clinical management of heart failure, coronary artery disease, and renal failure. Furthermore, it has also been suggested that ACEi are able to reduce the incidence of diabetes [85]. In *ob/ob* mice (lacking functional leptin signaling), 20 weeks with temocapril reduced cardiac hypertrophy. Moreover, temocapril also reversed the obesity-associated loss of cardiac contractility. Notably, temocapril did not affect the blood glucose or insulin levels, suggesting the direct effect of temocapril on hearts with minimal effects on systemic metabolic abnormalities. Importantly, plasminogen activator inhibitor type 1 (PAI-1), the main fibrinolysis factor, was also involved in the effect of temocapril on cardiac hypertrophy [86]. Cardioprotective effects of ACE inhibition were also validated in another preclinical model of obesity and type II diabetes, the Zucker rats (harbors mutation in the leptin receptor gene). Perindopril treatment of Zucker rates led to a reversal of myocardial hypertrophy and cardiac extracellular matrix remodeling, which was accompanied by a decrease in PAI-1. However, these effects were thought to depend on blood pressure reduction, as the ACE inhibition also reduced markedly increased blood pressure in the Zucker rats [87].

Two separate studies with captopril using *ob/ob* mice showed that the treatment did not reverse the increase in cardiac weight or changes in myocyte contractility in vitro. However, when dyslipidemia and arterial hypertension were co-induced in obese animals, the reduction in myocyte contractility was more severe, and, in this case, the ACE inhibitor significantly reversed it [88]. Initiation of captopril treatment four weeks before the induction of obesity, captopril showed more potent cardioprotective effects, resulting in reduced cardiac hypertrophy and improved metabolic parameters in *ob/ob* mice [89]. Various preclinical investigations suggest that the cardioprotective effects of ACE inhibition against diabetic cardiomyopathy are at their peak when the ACE inhibitors are administered early and chronically.

Renin Inhibition

Among the therapeutic agents that act on the RAS, the most recently introduced class is renin inhibitors, of which aliskiren is the only available representative. In addition to being an antihypertensive, aliskiren is also cardioprotective. Aliskiren treatment for 3 months reduced diabetic nephropathy in *db/db* mice, which was related with

decreased urinary albumin excretion, glomerulosclerosis, and suppressed profibrotic and proinflammatory cytokines synthesis [90]. Consistently, aliskiren also prevented cardiac fibrosis, and myocardial inflammation in *db/db* mice [91]. Cardioprotective effects of aliskiren are considered independent of hemodynamic improvement, as hydralazine, a vasodilator, did not produce similar prevention of left ventricular hypertrophy in type 2 diabetic animals [91].

ACE2/Ang-(1-7)/Mas Axis Activation

ACE2 and Ang-(1–7) are potent negative regulators of the ACE/Ang II/AT1 receptor axis of the RAS. Ang-(1–7) is a biologically active heptapeptide generated by proteolytic cleavage of either Ang I or Ang II by the actions of several endopeptidases and carboxypeptidases, including ACE and ACE2, respectively [92]. Ang-(1–7) antagonizes Ang II/AT1 receptor axis and exhibits vasodilatory, anti-fibrotic, antiproliferative, and antihypertrophic properties [17, 93]. While the loss of ACE2 exacerbates diabetic cardiomyopathy [94], enhancing ACE2 has a protective effect on Ang II-induced cardiac dysfunction [95] and streptozotocin-induced diabetic cardiomyopathy [96]. These reports suggested the role of the ACE2/Ang-(1–7)/Mas receptor axis as a potential target for the development of novel treatment for diabetic cardiomyopathy. Notably, a critical research report suggests that the majority of cardioprotective effects of ACE2 are mediated via activation of Ang-(1–7)/Mas receptor signaling, signifying the importance of Ang-(1–7) as a potential therapeutic agent in various cardiovascular disease [97, 98].

Overexpression of ACE2 in a rat model of diabetic cardiomyopathy demonstrated markedly reduced myocyte hypertrophy, myocardial fibrosis, and improved left ventricle remodeling and function [96]. The underlying mechanism of protective effects encompassed ACE2 overexpression-mediated inhibition of collagen deposition by converting Ang II to Ang-(1–7) and by increasing MMP-2 activity. Moreover, the administration of A779, a Mas receptor antagonist, in the group injected with the adenoviral ACE2 (Ad-ACE2) overexpression exhibited increased collagen protein expression in response to Mas receptor inhibition, indicating a key role of Ang-(1–7)/Mas receptor signaling in negative regulation of cardiac collagen synthesis. Significantly, ACE2 overexpression exhibited superior cardioprotective potential compared to losartan, an angiotensin receptor blocker (ARB), as evident by reduced left ventricular end-diastolic diameter (LVEDD) and collagen expression along with increased left ventricular ejection fraction (LVEF).

Ang-(1–7) exhibited protective effects against diabetic cardiomyopathy by attenuation of pathological characteristics such as cardiac hypertrophy and lipotoxicity, adipose inflammation, myocardial oxidative stress, and upregulation of adipose triglyceride lipase (Fig. 15.3). In a *db/db* murine model of diabetic cardiomyopathy, Ang-(1–7) alleviated diastolic dysfunction and proved to be a promising therapeutic target [99]. The right ventricular (RV) fibrosis, a distinctive feature of

diabetic cardiomyopathy, was ameliorated by Ang-(1–7) treatment in streptozotocin-induced diabetic cardiomyopathy in rats [100]. In diabetic rats, administration of Ang-(1–7) reduced cardiac fibrosis and dysfunction via a intricate interaction of AT2 receptor and Mas receptor for consequent downregulation of ACE expression and activity along with AT1 receptor expression. This complex interaction facilitated upregulation of ACE2 activity, as well as increased expression of AT2 receptor and SERCA2a. As the high dose of Ang-(1–7) was found to be superior to perindopril in mitigating cardiac remodeling, the study suggested the clinical potential of Ang-(1–7) as a novel treatment for diabetic cardiomyopathy [100]. Along with its prominent vasodilatory, anti-proliferative, and anti-fibrotic properties, Ang-(1–7) treatment resulted in a significant decrease in dyslipidemia, a major predisposing factor of cardiac dysfunction in type 2 diabetes. Ang-(1–7) alleviated left ventricle hypertrophy, cardiac fibrosis, and improved endothelial function, thus attenuating diabetic cardiomyopathy in streptozotocin-induced type 1 diabetic rats with cardiomyopathy [101].

Pharmacological agents alone or in combination with Ang-(1–7) were investigated to identify therapeutic potential in diabetic cardiomyopathy through modulation of ACE2/Ang-(1–7)/Mas receptor axis of RAS. A study using *db/db* murine model of diabetic cardiomyopathy revealed that majority of cardioprotective effects of azilsartan, an AT1 receptor blocker, were mediated by activation of ACE2/Ang-(1–7)/Mas receptor signaling cascade. Azilsartan significantly abrogated downregulation of ACE2 and Mas receptor in *db/db* mice and reduced pathological cardiac remodeling, oxidative stress, cardiac fibrosis, thus alleviated diabetic cardiomyopathy in *db/db* mice by modulating ACE2/Ang-(1–7)/ Mas receptor pathway [102]. In a preclinical study, administration of Ang-(1–7) dose-dependently alleviated left ventricle remodelling and cardiac dysfunction in diabetic rats by reducing cardiac hypertrophy, myocardial fibrosis, and apoptosis, effects which were mediate via activation of both the Mas and AT2 receptors. The underlying mechanism of cardioprotection involved upregulation of ACE2 activity and increased Ang 1–9 levels, resulting in reduced oxidative stress, collagen synthesis and attenuated inflammatory cytokine expression, TGFβ1 expression, and ERK1/2 and p38-MAPK phosphorylation. Combination of Ang-(1–7) and perindopril, an ACE inhibitor, demonstrated superior cardioprotective effect than single therapy, suggesting synergistic effects of blockade of ACE/Ang II/AT1 receptor pathway and activation of Ang-(1–7)/Mas receptor pathway [103]. Hence, various preclinical studies provide strong scientific evidence suggesting that Ang-(1–7) alleviates pathological processes such as cardiac remodeling, inflammation and oxidative stress involved in the onset and progression of diabetic cardiomyopathy; enhancing Ang-(1–7) actions may provide a promising therapeutic approach for the treatment of diabetic cardiomyopathy (Fig. 15.3).

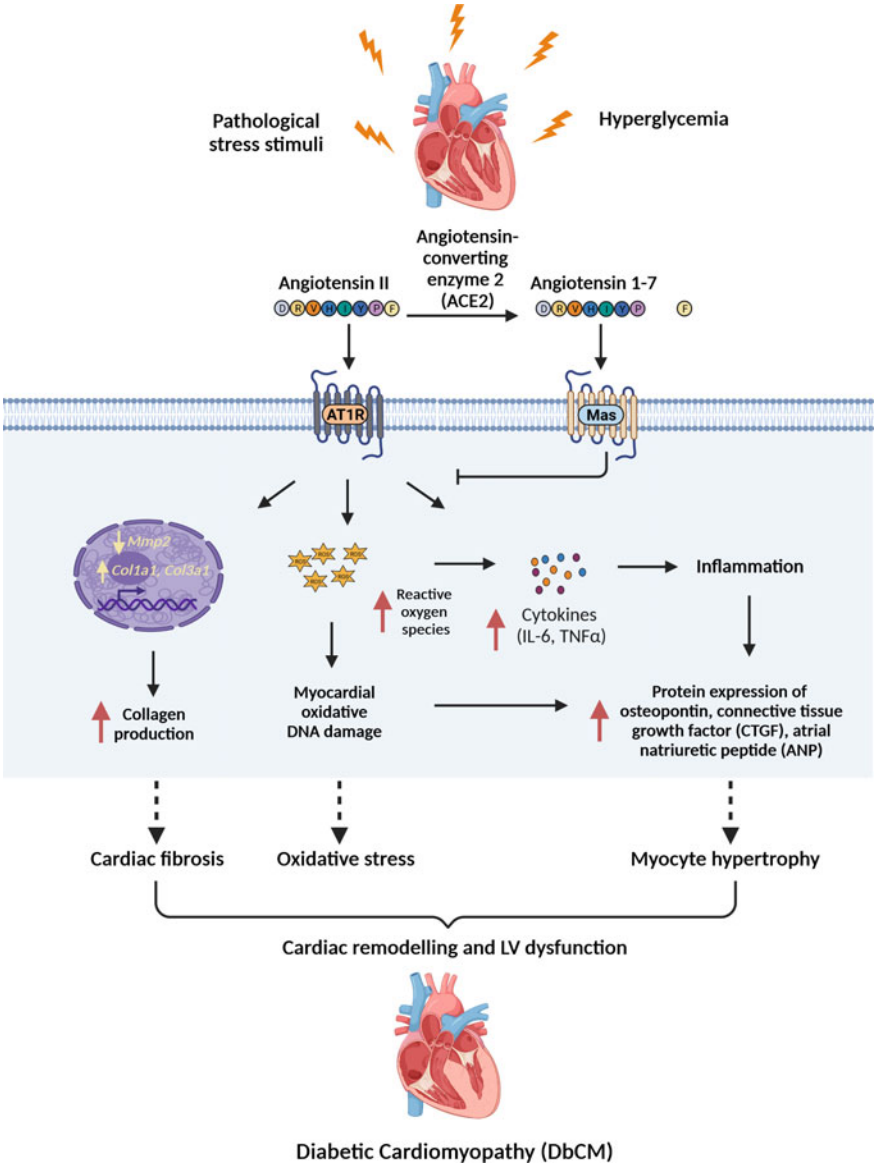

Fig. 15.3 Potential role of Ang 1–7 as a therapeutic agent in diabetic cardiomyopathy. Schematic representation of protective effects mediated by Ang 1–7 shows attenuation of hallmarks of diabetic cardiomyopathy i.e. cardiac fibrosis, inflammation, hypertrophy, and cardiac remodeling. Ang 1–7 exerts beneficial effects via binding to Mas receptor and antagonizes Ang II/AT1R axis of the renin angiotensin system. Under obesity and hyperglycemia-induced pathological conditions, activation of Ang II/AT1R axis potentiates increase in expression of genes (Col1a1, Col131, Mmp2) and proteins (OPN, CTGF, ANP) associated with cardiac fibrosis and hypertrophy. Pathological stress stimuli increase oxidative stress via ROS generation and induce inflammation by proinflammatory cytokines (IL-6, TNF-α, MCP-1). Ang 1–7 suppresses inflammation, oxidative stress, and cardiac remodeling, thus preserving cardiac function. Figure created using Biorender.com

Conclusion

Resistance to the metabolic actions of insulin, compensatory hyperinsulinemia, and the progression of hyperglycemia promoted by type II diabetes activate RAS resulting in several effects at the cardiac level, such as loss of cardiomyocytes by apoptosis, increased proliferation of fibroblasts with fibrosis, and hypertrophy. The resulting heart failure intensifies neurohormonal responses and the activity of RAS, which is related to the progression of diabetic cardiomyopathy, generating a vicious cycle. Diabetes leads to other changes, such as a downregulation of the ACE2 and an increased expression of AT1 receptors in cardiomyocytes, which is associated with a decreased left ventricular systolic pressure and increased in diastolic pressure. It also demonstrates that the ACE/Ang II/AT1 receptor pathway is activated in diabetic cardiomyopathy, while the ACE2/Ang-(1–7)/Mas receptor is not. Therefore, the goal of RAS modulation-based therapies goes beyond the inhibition of Ang II deleterious effects, but also aims to enhance the actions and activity of potentially helpful pathways, by ACE2 replenishing strategies, Ang-(1–7) administration, and Mas receptor agonists.

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Chapter 16
Renin Angiotensin System and Obesity-Related Organ Damage

Isabel Galceran and Anna Oliveras

Abstract Obesity is a growing global disease that results in serious health risks. Obesity is a known cause of arterial hypertension, and it is also responsible for structural and functional changes in the heart, arterial vessels and kidneys, among others. Thus, obese people have a higher prevalence of left ventricle hypertrophy and heart failure, as well as arterial stiffness. In kidneys, obesity is responsible for glomerular hyperfiltration and proteinuria, which could lastly produce focal and segmentary glomerulosclerosis and renal function decline. On the other hand, the adipocyte dysfunction observed in obesity causes an abnormal activation of cytokines and pro-inflammatory factors, insulin resistance, and the renin angiotensin system (RAS) upregulation. Adipose tissue has the RAS peptides necessary for the local production of angiotensin II, in addition to the systemic RAS. The over-activity of the RAS in obese individuals leads to an increase in blood pressure levels and to structural and functional changes in several organs, such as the heart, arterial vessels or the kidneys. Normalization of both elevated blood pressure and these pathological obesity-related organ changes observed after weighting loss by bariatric surgery appears to be mainly mediated by the RAS system. In this chapter, the relationships between the RAS and obesity-derived organ damage and hypertension and their modifications after BS are explored.

Keywords Renin angiotensin system · Aldosterone · Angiotensin II · Obesity · Bariatric surgery · Organ damage

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Renin Angiotensin System Changes in Obesity

Obesity is a chronic multifactorial disease that has become a serious worldwide health problem due to its rapid increase in developed countries in recent decades. Obesity prevention and treatment is also a permanent challenge for the medical community.

Obesity is defined as a body mass index (BMI) $\geq$ 30 kg/m^2 and morbid obesity (MO) as a BMI $\geq$ 40 kg/m^2. The prevalence of obesity is estimated at nearly 500 million people in the world [1, 2]. In the adult population of the United States, the prevalence of obesity has almost tripled in the last decades (13.4% in 1960–1962 versus 35.1% in 2009–2010) [3]. In Spain, the ENRICA study places the prevalence of obesity in adult population at 22.9% (24.4% in men and 21.4% in women) according to BMI, and around 36% (32% in men and 39% in women) if central obesity is considered (waist circumference > 102 cm in men or > 88 cm in women) [4].

Many studies have observed an increase of plasma concentration of the different components of the of the renin angiotensin system (RAS) (plasma renin activity [PRA], angiotensin I, angiotensin II [Ang II], aldosterone and angiotensinogen [Agt]) in obese patients in comparison to people with normal BMI [5]. Local production of Ang II is facilitated by adipose tissue, which has all the necessary RAS peptides [6]. For this reason, obese patients, with a higher proportion of adipocytes compared to population with normal weight, have a higher concentration of the RAS components. In addition, obesity is related to adipocyte dysfunction that leads to insulin resistance and dysfunction of RAS activation [7, 8].

La Sala et al. [5] described a relationship between PRA and insulin resistance and plasma ferritin concentration, both parameters related to patients' inflammatory status.

Body weight and the proportion of adiposity is directly influenced by the amount of Ang II, as shown in experimental studies [8–10]. In addition, the adipocyte dysfunction associated with obesity has also been associated with an abnormal activation of cytokines and pro-inflammatory factors (elevation of pro-inflammatory cytokines such as interleukine-6, C-reactive protein or TNF-alpha) [11]. Therefore, it is postulated that an increase in RAS activation in obesity could be related to a significant inflammatory state of the patient [12].

All of the above leads to a vicious circle between obesity, RAS activation and inflammatory pathways, that results in an increased cardiovascular risk in obese patients.

Role of the Renin Angiotensin System in Obesity-Related Hypertension

High blood pressure is one of the most important cardiovascular risk factors in general population. The relationship between human obesity and hypertension was

first described by Vague in 1956 [13]. In obese subjects, hypertension is approximately 6 times more frequent than in lean subjects [14]. Increased body mass, hyperinsulinemia and sympathetic over-activity, among others, have been pointed as the main responsible factors. However, the role of RAS in obesity-related hypertension is gaining importance in recent years. Experimental and clinical studies have shown that adipocytes size is increased in obese individuals [15]. In addition, it is known that abnormal body fat distribution, such as excess visceral adipose tissue, is a main factor for morbidity. Therefore, visceral adiposity reportedly plays a major role in the occurrence of hypertension and other cardiovascular risk factors in obese humans and in animal models. As aforementioned, the obesity leads to an increase in the production of both the RAS components and proinflammatory adipokines. Thus, visceral adipose tissue is a source of numerous adipokines, most of which are considered proinflammatory. In parallel, plasma aldosterone concentration is positively correlated with the amount of visceral adipose tissue, but unrelated to PRA. Despite sodium retention, RAS activation occurs in obese subjects and, accordingly, the classical RAS has been found to be over-activated during the adipose tissue enlargement [16]. Moreover, several studies suggest that adipose tissue secretes adipokines which in turn stimulate aldosterone release from adrenal cells, so-called aldosterone-releasing factors [17]. As previously mentioned, individuals with visceral obesity often have mild-to-moderate increases in PRA, Agt, angiotensin converting enzyme (ACE) activity, Ang II and plasma aldosterone concentration, but these increases were not found in patients with peripheral obesity [18]. Moreover, Goodfriend et al. demonstrated that plasma aldosterone concentration is positively correlated with the amount of visceral adipose tissue, independent of the PRA level [19]. Indeed, adipose tissue expresses all the RAS components that play an important role in the adipogenesis, lipid and glucose metabolism regulation in an auto/paracrine manner. Thus, adipose tissue excretes aldosterone-releasing factors, thereby stimulating aldosterone secretion independently of the systemic RAS, and aldosterone/mineralocorticoid receptor (MR) activation plays a key role in the development of hypertension and organ damage in obesity.

The production of Ang II is linked to hypertension and several inflammatory diseases including cardiovascular and kidney diseases, dyslipidemia and glucose intolerance. In this line, increased levels of Ang II and aldosterone, in part resulting from adipose tissue dysfunction, not only induce sodium retention and sympathoexcitation, but may also impair the insulin-associated microvascular function and modulate arterial stiffening, ultimately resulting in elevated blood pressure [20] (Fig. 16.1).

On the other hand, adipose tissue expresses also ACE2, and both adipocytes and pre-adipocytes express angiotensin receptors including Ang II receptors type 1 (AT1) and type 2 (AT2) as well as Ang IV and Ang(1–7) receptors (MasR). There is a synergistic contribution of AT1 and AT2 in mediating the in vivo effect of Ang II on adipose tissue development [21]. Pro(renin) receptor may be involved in the pathogenesis of the obesity by increasing the local production of Ang II in adipose tissue as well as triggering signal transduction independently of Ang II [16]. In general, Agt is produced mainly in the liver; however, Agt secretion by

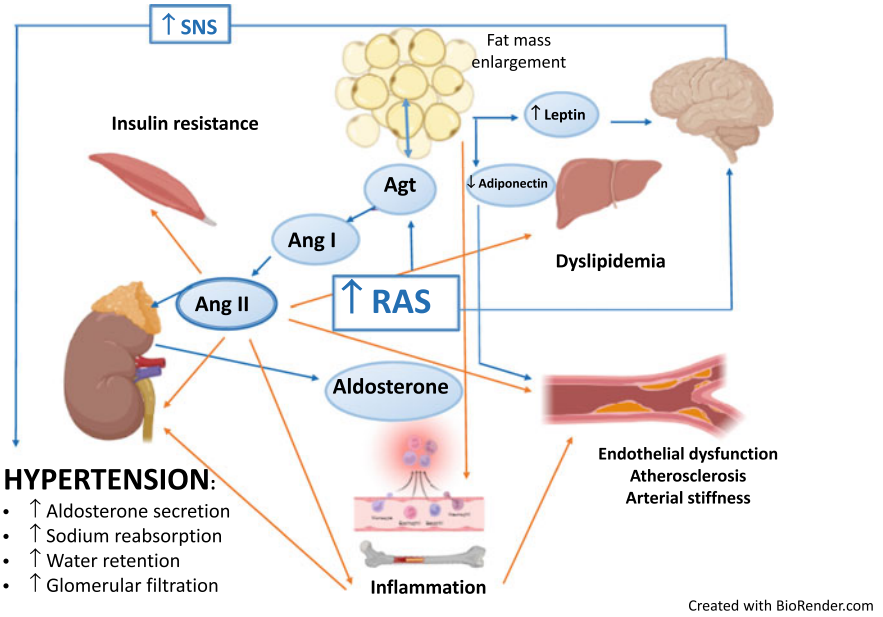

Fig. 16.1 Mechanisms of obesity-induced hypertension. Adipocytes release different components of the RAS as well as adipokines. Its action on the kidney, liver, brain, blood vessels and muscles, and indirect activation of the SNS, will cause alterations that will eventually lead to hypertension. Agt: angiotensinogen; Ang: angiotensin; RAS: renin angiotensin system; SNS: sympathetic nervous system

adipose tissue is substantially augmented in obese subjects. Although Yiannikouris et al. [22] suggested, according to their work, that adipose tissue serves as a major source of Ang II in the development of obesity-related hypertension, it is yet to be determined whether adipocyte-derived Agt or Ang II has a major influence on blood pressure regulation in obesity. In fact, animal studies have shown that adipose-derived Agt contributes to circulating RAS, kidney, and blood pressure regulation. Further, mice overexpressing Agt have high blood pressure and increased adiposity characterized by inflammation, adipocyte hypertrophy, and insulin resistance, which can be reversed at least in part by RAS inhibition [23]. The adipose tissue RAS not only has the classic pathway of Ang II generation catalyzed by ACE but also by cathepsins and chymase [24]. Moreover, it is known that abnormal regulation of the RAS such as enhanced renal AT1R function and reduced ACE2 activity contributes to obesity-related hypertension. In experimental models it is shown that long-term AT2R activation shifts the opposing arms of RAS and contributes to natriuresis and blood pressure reduction in obese animals, thus highlighting the importance of AT2R as a target for treating obesity-related hypertension [25] (Fig. 16.2).

Overall, adipose tissue contributes to increased circulating levels of Ang II and aldosterone and potentially impairs the metabolism of Ang II to Ang1–7. Whether adipocyte-derived Agt is predominantly converted to Ang peptides within

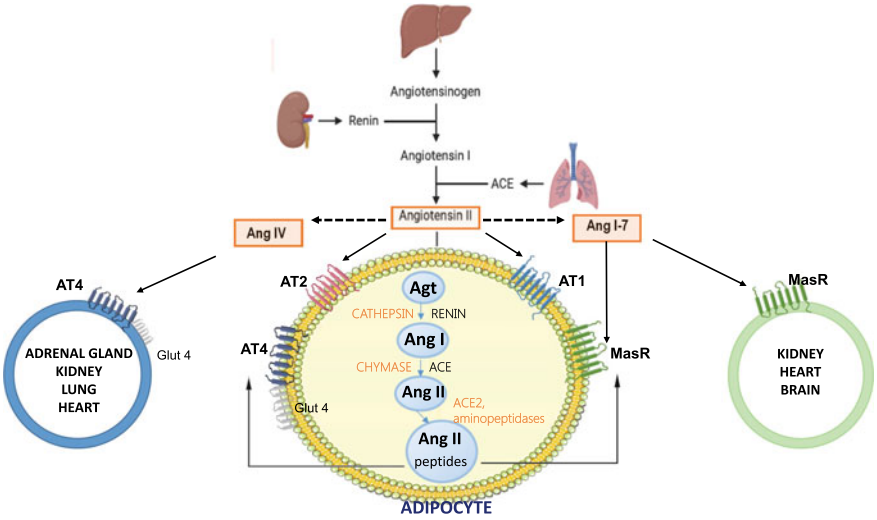

Fig. 16.2 The renin angiotensin system (RAS). The precursor Agt is converted to Ang I through renin, which is cleaved into Ang II by the ACE. Ang II is typically the effector hormone of the system and regulates blood pressure acting as a vasoconstrictor. AT1 and AT2 are the two receptors which mediate the actions of Ang II, usually exerting opposing effects. The novel RAS comprises angiotensin peptides derived from Ang II, which include Ang IV and Ang 1–7, the latter produced by the action of ACE2. The receptors for Ang IV and Ang (1–7) are AT4 and Mas, respectively. Apart from renin and ACE found in the bloodstream and tissues, cathepsins and chymase catalyze the production of Ang II locally in various tissues, including adipocytes. ACE: angiotensin-converting enzyme; Agt: angiotensinogen; Ang: angiotensin; Glut: glucose transporter; MasR: Mas receptor

adipose tissue or by systemic RAS components, and how RAS dysregulation affects perivascular adipose tissue anti-contractility is not fully elucidated. Nevertheless, the involvement of adipose tissue in obesity-associated RAS over-activity stresses the importance of weight loss as antihypertensive strategy in hypertensive obese individuals [26]. In theory, by intervening in the regulation of both extracellular volume and vascular tone, ACE inhibitors, angiotensin receptor blockers, and mineralocorticoid receptor antagonists could be of great value in the treatment of obesity-associated hypertension.

Long-term hypertension involves cardiovascular and renal disorders, which lead to greater complexity of its pathophysiology. This, together with the fact that patients with obesity-associated hypertension often receive antihypertensive agents that modify the pathways linking obesity and hypertension, favours research in this field in both thin and obese individuals, with mild hypertension of relatively short duration, preferably before and after weight loss.

Furthermore, research on the role of Ang 1–7 in sodium homeostasis and the effect of its central actions on blood pressure, as well as on the effect of its administration on different blood pressure mechanisms, i.e., sodium balance, sympathetic nerve activity, and micro- and macrovascular function, in lean as compared to obese

individuals, could contribute to a better understanding of these pathways. According to that, possible treatments with Mas receptor agonists in humans could have a promising therapeutic potential [26].

In addition, the development of salt-sensitive hypertension is favoured by the sodium retention due to impaired renal-pressure natriuresis observed in obese individuals. It appears that the kidneys physical compression by visceral fat, as well as the activation of the sympathetic nervous system, in addition to the already mentioned activation of RAS and aldosterone MR system, are possible mechanisms for this impaired renal-pressure natriuresis. Obese subjects often exhibit hyperaldosteronism, with increased salt sensitivity of blood pressure. Therefore, in obese subjects, both salt sensitivity of blood pressure, enhanced by obesity-related metabolic disorders including aldosterone excess and increased dietary sodium intake, are closely related to the incidence of hypertension. Some salt sensitivity-related gene variants, such as *G-protein–coupled receptor kinase type 4 (GRK4), A486V, CYP11β-2, ACE,* and *SLC12A3* variants, affect the risk of obesity, and together with salt intake, its combination is possibly associated with the development of hypertension in obese subjects [7].

All of these studies point to different mechanisms that lead to obesity-related hypertension, with RAS activation being one of the most relevant, if not the most relevant. But it should be emphasized that it is not only systemic RAS but especially RAS derived from adipose tissue that plays the most important role.

Role of the Renin Angiotensin System in Obesity-Related Organ Damage

Obese subjects often have not only a major prevalence of hypertension but also of related cardiovascular and renal diseases, and this fact has become a serious worldwide health problem [27]. Obesity causes chronic inflammation that contributes to atherosclerosis. As seen, the pathophysiologic mechanisms in obesity that contribute to inflammation and atherosclerosis include activation of adipokines/cytokines and increases in aldosterone in the circulation. Increased aldosterone in the circulation not only expands the blood volume but also promotes platelet aggregation, vascular endothelial dysfunction, thrombosis, and fibrosis, and these mechanisms are the basis for future organ damage [28].

Patients with morbid obesity have a higher prevalence of organ damage than normal weight patients and hypertension plays a major role in its development. These alterations of the target organs consist mainly of changes in the structure and function of the heart, vessels and kidneys [29].

Visceral adiposity is deeply involved in the development of hypertension and related organ damage, probably related to an imbalance of pro- and anti-inflammatory adipokines, aldosterone excess, and glucose metabolism impairment, among other factors [7].

With regard to obesity-related heart disease, overweight clearly increases the risk of atrial fibrillation, myocardial infarction, heart failure, sudden death and decreased survival [30]. Morphologic left ventricular alterations have been described in patients with morbid obesity, with a 56% of left ventricular hypertrophy being reported from a meta-analysis of 22 studies including 5486 obese individuals [31]. This is in accordance with our reported cohort of obese subjects in which more than half were found to have pathological relative wall thickness, another measurement of left ventricular morphologic alteration [32]. Even in normotensive obese subjects, it has been reported that left ventricular hypertrophy has a prevalence of around 13% [30, 33]. As regards left ventricular systolic function, there are contradictory reports on ejection fraction in obese patients. Thus, different groups have reported depressed, normal or even supernormal ejection fraction values [34]. We also reported that global longitudinal strain, another indicator of systolic function, was pathological in 62% of patients [32]. Recently, it has been emphasized that activation of RAS in obesity causes amplification of inflammation and structural remodeling, thus inducing cardiac and vascular damage, as well as other structural alterations leading to cardiac dysfunction [35, 36].

Arterial stiffening, which is both a consequence of greater mean arterial pressure and a cause of increased pulse pressure, is commonly observed in obese individuals, then being able to precede elevations in systolic blood pressure and incident hypertension [20]. Therefore, obesity also has a significant impact on vascular structure and function and it has been noted that RAS plays a potential role in these alterations. As stated above, it is well known that obesity promotes increased PRA, plasma Agt and ACE activity, resulting in elevated plasma levels of Ang II. Angiotensin II is a profibrotic, proinflammatory, and prooxidant factor that can induce vascular smooth muscle cell proliferation, thus contributing to vascular remodeling and obesity-associated endothelial dysfunction. In the context of obesity, RAS activation has been found not only at the systemic level, but also at the local level [37]. It is recognized that local RAS in both vascular and perivascular adipose tissue plays an important role in endothelial dysfunction, contributing significantly to arterial stiffness in human and animal models of obesity. The cross talk observed between angiotensin and aldosterone underscores the importance of mineralocorticoid receptors in modulation of insulin resistance, decreased bioavailability of nitric oxide, endothelial dysfunction, and arterial stiffness. In addition, both innate and adaptive immunity are involved in this local tissue activation of RAS [38]. Systemic and cardiovascular insulin resistance and related arterial stiffness in the setting of obesity are, in large part, due to the interactions between diet and activation of both systemic and adipose tissue RAS. Increased production of Ang II in the vasculature as a result of a high-fructose diet underscores the importance of activation of the tissue RAS in favouring remodelate and stiffness. Ang II and aldosterone act together to promote cardiovascular remodelling and stiffness in states of insulin resistance and obesity, and the critical role of activation of cell-specific vascular mineralocorticoid receptors in this setting, especially in females, is highlighted by recent studies [39].

Remarkably, several experimental studies have shown that for both endothelial dysfunction and vascular remodeling, treatment with RAS blockers has succeeded

in reversing these alterations. In obese hypertensive individuals, ACE inhibitors and angiotensin receptor blockers are more potent than other antihypertensive drugs in reducing pulse-wave velocity and augmentation index, which are markers of arterial stiffness, illustrating the significance of AngII-induced vascular remodeling in obesity [20]. This ability of RAS inhibitors to reduce arterial stiffness seems to be independent of its ability to reduce blood pressure [26].

Similarly, aldosterone increases arterial stiffness through actions that may be partly exerted by mineralocorticoid receptors on smooth muscle cells and, again, do not necessarily depend on blood pressure. In addition, aldosterone was found to enhance some of the hypertrophic effects of Ang II on cultured smooth muscle cells. Despite some promising experimental studies, the role of the Ang1–7/Mas axis in modulating obesity-related arterial stiffness needs to be further investigated [20].

Taking together, targeting endothelial function and arterial stiffness by modulating tissue RAS could be an attractive therapeutic strategy to reduce the cardiovascular and renal complications associated with obesity.

Kidneys are also targets of obesity-induced damage. Pathologic changes in the kidney include the development of focal segmental glomerulosclerosis and glomerulomegaly. This translates in glomerular hyperfiltration and proteinuria. At long-term follow-up, increased BMI has been linked to a loss of renal function as well as increased risk of end stage renal disease. An excess of albuminuria and proteinuria is found in obese population, with a higher prevalence in those with diabetes [40].

RAS is known to be one of the mechanisms that link obesity-derived hypertension and renal function. As we have seen, excessive adiposity increases blood pressure, which in turn is one of the main drivers of cardiovascular and kidney diseases. The abnormal tubular sodium reabsorption associated to obesity-related kidney damage is one of the triggers of hypertension. This mechanism is derived from kidney compression by visceral, perirenal and sinus fat, as well as by the increased renal sympathetic nerve activity, both of them contributing to RAS activation [41]. Mineralocorticoid receptors blockade has shown to attenuate glomerular hyperfiltration in obese animal models, therefore suggesting that renal vasodilation in obesity can derive, at least partly, from the activation of such receptors. Antagonism of the RAS with ACE inhibitors or Ang II receptor blockers also partially attenuates the progression of kidney injury in patients with obesity, hypertension and type 2 diabetes mellitus.

In conclusion, RAS seems to be one of the main pathways that link obesity with its derived organ damage.

Renin Angiotensin System Modifications After Bariatric Surgery

Bariatric surgery (BS) is an effective method to achieve weight loss, with the maximum loss observed after 12 months of the intervention. For this reason, nowadays BS is the best treatment for patients with MO. BS is highly cost-effective to

achieving weight reduction and long-term maintenance, and improves comorbidities and quality of life in these patients [42]. Among the different types of BS, both gastric bypass and laparoscopic sleeve gastrectomy have shown satisfactory results in weight loss [43]. Multiple studies have linked the weight loss achieved one year after BS (regardless of the type of surgery) to the plasma reduction of the different components of the RAS (PRA, angiotensin I, Ang II, aldosterone and ACE2 activity) and an increase of angiotensin 1–7 and ACE/ACE2 ratio [32, 44, 45]. In addition, a reduction in plasmatic inflammatory factors (such as ferritin, C-reactive protein or interleukin-6), plasma leptin, and urinary cortisol one year after BS has also been described [5, 32]. The reduction of several RAS components and inflammatory factors after BS has been linked to an overall decrease in insulin resistance [5, 11, 46]. La Sala et al. [5] observed that this downregulation of systemic RAS and decreased plasma ferritin after BS occurred in hypertensive patients, but not in normotensive ones.

Interestingly, it has been observed that higher levels of PRA before BS predict a persistence of hypertension after surgery in patients with MO and hypertension [5].

Finally, the downregulation of RAS after BS has been linked to a further decrease in office systolic and diastolic blood pressure, 24 h-ambulatory blood pressure, BMI, waist circumference and percentage of body fat [47].

Blood Pressure Changes After Bariatric Surgery. Role of the Renin Angiotensin System

Several studies have shown a BP decrease with an improvement and even normalization of BP levels[48–50], although some study observed this BP decrease in patients with baseline hypertension, but not in those with normal BP values [51]. As for the possible underlying mechanisms, several groups have shown different changes in RAS in obese individuals after losing weight. Dall'Asta et al. [44] described in obese individuals a decrease on BP in hypertensive subjects, with a concordant decrease in PRA and supine aldosterone levels, not observed in normotensive patients. A few decades ago it was proved that weight loss is accompanied by reductions in PRA and aldosterone, irrespective of sodium intake, and this affects the decline in BP in obese patients [52]. Interestingly, high levels of PRA, ACE, aldosterone, and insulin with sodium retention and potassium loss were found in patients with visceral obesity, but all of these tended to disappear upon weight reduction and were not found in patients with peripheral obesity [18].

Obesity-Related Organ Damage Changes After Bariatric Surgery. Role of the Renin Angiotensin System

The effects of BS on cardiac structure and function have been recorded in several studies [53, 54] and compiled in a systematic review and meta-analysis [55], concluding that this therapeutic approach results on regression of left ventricle (LV) hypertrophy, improvement of LV geometry and diastolic function, and reduction of left atrial size. These changes have been documented to occur independently of improvement in obesity-related co-morbidities, including obstructive sleep apnea [56]. Going further, our group [32] found a statistically significant correlation between main changes in cardiac structure after BS and PRA, ACE and ACE/ACE2 ratio but not with plasma aldosterone levels, although an overall tendency to decrease was observed. These results suggest that RAS may link cardiac hypertrophy in obese patients with its improvement after weight loss.

Some reports have shown that weight loss, either by lifestyle intervention or by BS, results in a reduction in arterial stiffness. Thus, several studies have reported a significant decrease in pulse wave velocity or augmentation index after the intervention [57–60].

Although the mechanisms that link RAS system and vascular damage are not fully elucidated, it is relevant the recent finding that ACE and ACE2 are independent factors for changes in arterial stiffness parameters in the short-time follow-up after BS, although remarkably aldosterone and PRA significantly modify later on [61].

As for what happens to the kidneys, morbid obesity increases intraglomerular pressure and glomerular surface, resulting in hyperfiltration and albuminuria. Weight loss after BS is known to lead to improvements in obesity-related proteinuria, albuminuria, and hyperfiltration by reverting these obesity-induced changes [62–65]. Moreover, it has been shown that BS is associated with a long-term protection against end-stage renal disease and stage 4 chronic kidney disease [66, 67]. Of note, greater weight loss but not the mechanism through which weight loss is achieved appears to be an independent predictor of reduced chronic kidney disease risk [68]. Several mechanisms may be involved in the known obesity-related glomerulopathy, one of which includes the increased sodium reabsorption that takes place in obesity. Contributing factors include RAS activation due to renal compression by visceral, perirenal and renal sinus fat, or increased renal sympathetic nerve activity, as well as increased levels of anti-natriuretic hormones, such as Ang II and aldosterone [41]. Indeed, mild elevations in Ang II levels in obesity are largely driven by increased renal renin secretion due to all these mechanisms. On the other hand, elevated concentrations of plasma aldosterone in obesity might partly be a result of Ang II stimulation of adrenal aldosterone secretion. However, adipocyte-derived factors such as leptin may also stimulate the adrenal gland to produce aldosterone, therefore underlying the increased plasma aldosterone concentration observed in these individuals, as well as its decrease after BS. Going in depth to the mechanisms of reducing albuminuria in obese patients with diabetes after BS [69], we have to look at what happens to the podocytes and the mechanical stress on them. Impaired renal perfusion in

obese individuals activates the juxtaglomerular apparatus leading to release of renin, aldosterone, and Ang II. Ang II favors efferent arteriole vasoconstriction resulting in glomerular hypertension, and also inhibits the expression of nephrin, a protein vital for maintaining the slit diaphragm. Due to the glomerular hypertension promoted by capillary wall stress, podocytes develop a maladaptative response, leading to alterations in podocyte differentiation and adherence that may in turn produce a leakage of albumin. Moreover, infusion with glucagon-like peptide-1 in humans has been shown to lead to a rapid decrease in plasma Ang II concentration, thus suggesting that the effect glucagon-like peptide-1 has on the kidneys could be primarily through the inhibition of RAS activity. In parallel, inflammation is likely to have also an important role in the development of albuminuria in obesity patients and its reduction after BS [70].

Improvement in organ damage obesity-related after BS and possible mechanisms are represented in Fig. 16.3.

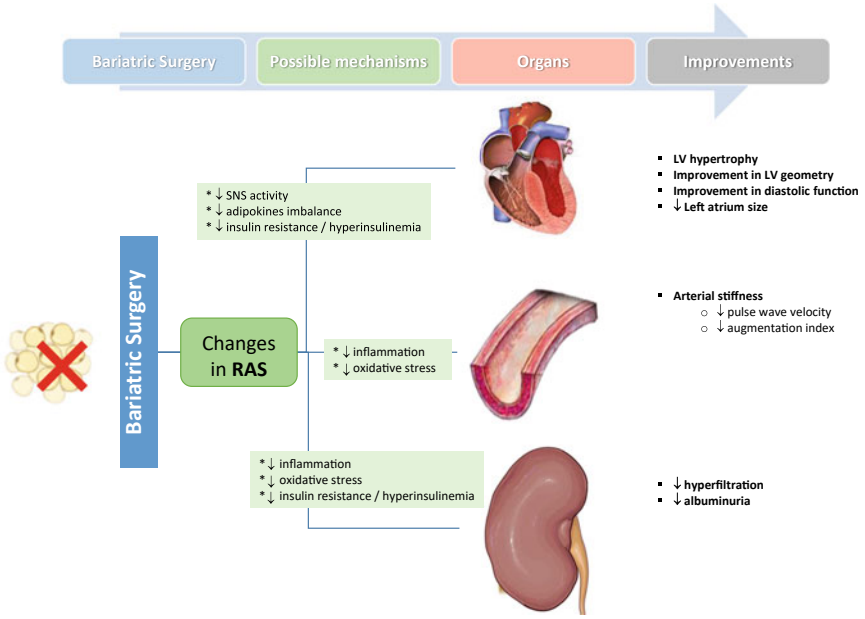

Fig. 16.3 Effects of bariatric surgery on main organs and its mechanisms. After bariatric surgery, several mechanisms, mainly changes in renin angiotensin system but also other mechanisms such as changes in inflammation, oxidative stress, adipokines balance or insulin resistance, are responsible for the improvement of heart, vessels or rein alterations obesity-related. RAS: renin angiotensin system; SNS: sympathetic nervous system

Conclusions

In summary, obesity is a growing global disease that results in serious health risks. Adipocyte dysfunction gives rise not only to an abnormal activation of cytokines and pro-inflammatory factors, as well as to insulin resistance, but also to an increase of the different components of the RAS. Indeed, adipose tissue has the RAS peptides necessary for the local production of Ang II, in addition to its known systemic activation. The overactivity of the RAS, alone or together with the mentioned mechanisms as inflammation or insulin resistance, leads to both arterial hypertension and structural and functional changes in several organs, such as heart, vessels or kidney. Normalization of blood pressure and these pathological organ changes after weight loss by BS appears to be mainly mediated by the RAS system.

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Chapter 17
Renin Angiotensin System in Aging and Regeneration

Neha Rawal, Anupam Mittal, and Madhu Khullar

Abstract Age is an independent risk factor for developing various diseases related to the cardiovascular system, kidney, nervous system, diabetes etc. Renin Angiotensin System (RAS) is the main regulator of normal physiology, body fluid homeostasis, normal organ development and cardiovascular functions. Blockage in the RAS system has been shown to induce longevity and to prevent the age-related reduction in the multiple organ functions. RAS has deleterious effects in the acceleration of age-related phenotypes through the over activation of Angiotensin-II receptor type 1 (ATR-1). This promotes excessive cellular growth, inflammation and oxidative damage which leads to ageing. Other pathway is anti-inflammatory and counter-regulatory, which involves Angiotensin-II receptor type 2/Angiotensin converting enzyme/Angiotensin 1-7/Mas receptor or ATR2/ACE2/Ang1-7/MasR axis. This chapter focuses on the mechanisms of involvement of RAS in age-related diseases and the therapeutic strategies from an interdisciplinary clinical perspective.

Keywords Renin Angiotensin (RAS) System · Angiotensin-II · Regeneration · Aging

Introduction

With advances in biological and interdisciplinary research, we are getting a better insight into the molecular mechanisms that govern self-repair potential, regeneration, and aging. Such an intimate understanding of the associated master regulators will guide us to the road of longevity and will improve the quality of life [1]. Renin Angiotensin System (RAS) is one such key player. It is involved in classical hemodynamic homeostasis and blood pressure regulation, but it exerts diverse roles in

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the other vital physiological processes. It is essential for early embryonic development supported by evidence that showed that the deficiency of Angiotensin-II (Ang II) and ATR1a and ATR1b in mice resulted in abnormal kidney development [2]. RAS modulates growth processes involved in forming the organized architecture of a fully differentiated kidney from a primitive renal tissue [3]. On the other hand, its overactivity leads to the cardiovascular pathologies and other ageing-related diseases.

Ang-II is an essential player of RAS and is associated with aging [4]. Ang-II is generated through the action of ACE on Angiotensin-I (Ang-I, which in turn, is produced by the action of renin on angiotensinogen. Ang-II mediates its acute physiological effects through active binding to its pharmacologically and functionally distinct G-protein coupled receptors (GPCRs); ATR1 and ATR2. ATR1 has two isoforms in rodents, i.e., ATR1a and ATR1b. While in humans, there is a single gene of ATR1, encoding a single isoform.

ATR1a is the murine homolog of the ATR1 receptor of humans, and studies have revealed that the significant effects of RAS are mediated by ATR1 receptors [5]. ATR1 and ATR2 have sequence similarity to approximately 95% and almost similar affinity to Ang-II, but they have counter-regulatory effects. ATR1 is greatly expressed in most tissues, including the brain, heart, kidney in an adult body. At the same time, ATR2 expression is nearly limited to foetal tissues and decreases significantly to low levels in adult tissues [6]. ATR1 leads to vasoconstriction and the production of reactive oxygen species (ROS), stimulates inflammation and extracellular matrix (ECM) remodelling, and accelerates various organs aging processes (Fig. 17.1). On the contrary, ATR2 prevents aging by reducing collagen deposition, fibrosis, vasodilation, and exerts anti-inflammatory and anti-oxidative effects [7].

ACE2-Ang-(1-7)-MasR axis is responsible for vaso-protective effects. Ang-II is metabolized by Angiotensin-converting enzyme 2 (ACE-2) to produce heptapeptide Ang-(1-7). Besides this significant pathway of Ang-(1-7) formation, it is also produced by the cleavage of Ang-I by some endogenous peptidase like Prolyl endopeptidase, neutral endopeptidase, and oligopeptidase [8]. Ang-(1-7) acts on another kind of seven transmembrane GPCR, Mas Receptor. The physiological effects of Ang-(1-7) binding to MasR is like the effects mediated by Ang-II binding to the ATR-2. Both pathways counteract the ATR1 pathway which has a detrimental impact on the cardiovascular system [9].

Prorenin receptor (PRR) is the third major component of the RAS system, which binds to renin and its precursor prorenin. Activation of this receptor leads to the conversion of angiotensinogen to Ang-I and the downstream conversion of Ang-I to Ang-II.

This chapter reviews the current understanding of RAS components involved in the progression of aging-related diseases, their mechanism of action, therapeutic strategies to delay or suppress the onset of associated diseases, and a brief discussion of future perspectives.

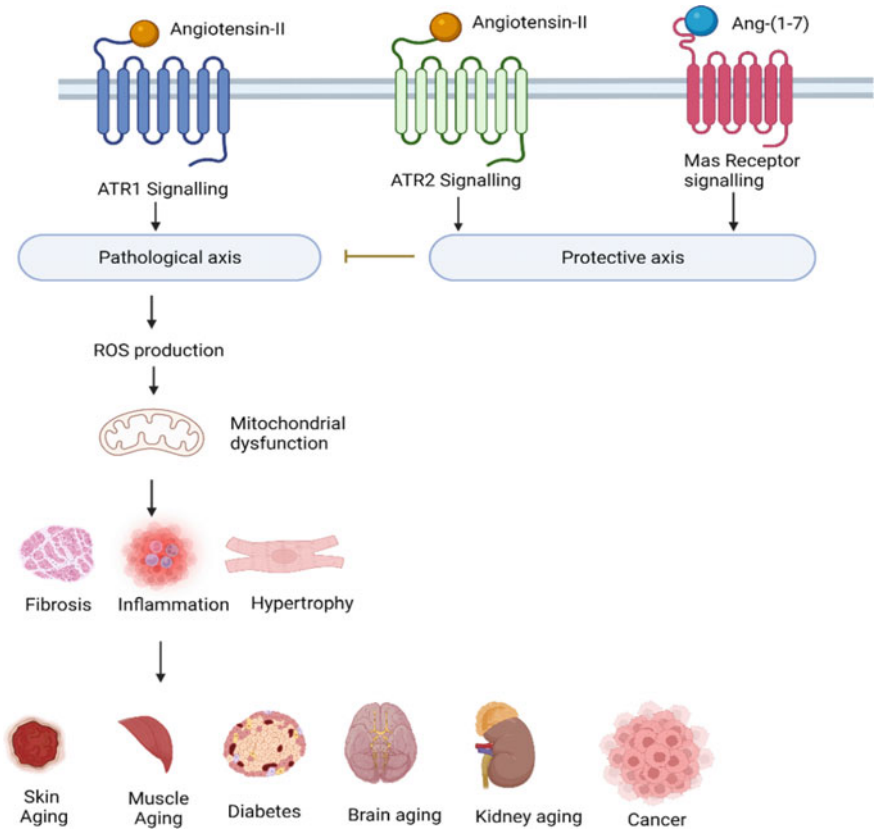

Fig. 17.1 RAS components in aging phenotypes. ATR1 stimulation manifests the pathological axis of RAS via ROS production mainly due to mitochondrial dysfunction. This leads to the phenotypes like tissue fibrosis, hypertrophy, inflammation and oxidative stress, which are known to impede the multi-organ functions and accelerates their aging. These effects are counterbalanced by Vaso-protective axis of RAS involving ATR2 and Mas Receptors

RAS in Aging

Local RAS components have been identified in the heart, kidney, brain, etc. They are expressed in a tissue-specific manner and work independently or closely with circulating levels of Ang-II and Renin [4]. Circulating RAS expression has been shown to decrease while the tissue-specific expression of Ang-II and other RAS effectors increase in the aging process in humans [10].

RAS in Vascular Aging

First evidence of the correlation between vascular dysfunction and aging came from experiments done by Minamino et al. They reported the role of small G-proteins (Ras) in inducing senescence and inflammation in human atherosclerotic lesions in vascular smooth muscle cells [11]. In the current scenario, this area has been explored extensively, and now it is well known that Ang-II promotes vascular stiffness and senescence.

Vascular senescence is mediated by an increase in ROS production due to activation of Mitogen-Activated Protein Kinase (MAPK) and other transcription factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), Activator protein 1 (AP-1) [12]. Moreover, ATR1 receptor activation leads to inhibition of cyclin dependent kinases (CDKs) complex by upregulation of p53, p21, and other cell cycle regulators. Ang-II acts on ATR1 and works with aldosterone via mineralocorticoid receptors to activate Ras/ NF-κB, AP-1/p53/p21 signalling pathway, which has a synergistic action on vascular senescence [12].

RAS in Cardiovascular Aging

RAS plays a pivotal role in vasoconstriction, vascular hypertrophy, therefore, it promotes the dysfunction of endothelium and leads to the pathogenesis of cardiovascular diseases [13]. Most of the effects of introducing RAS blockers like angiotensin receptor blocker (ARB) result in reduced occurrence of hypertension in predisposed as well as high-risk individuals [14]. In one elegant experiment, the administration of ARB doubled the lifespan of hypertensive rats due to improved cardiac and vascular functions, extending the life of stroke-prone rats to almost 30 days [15]. Moreover, RAS induces atherosclerosis by vascular remodelling. Stimulation of ATR1 leads to cardiac hypertrophy, fibrosis and changes the composition of extracellular matrix proteins [16, 17]. Disruption of RAS signalling prevents cardiomyocyte proliferation and ECM remodelling, thus, reducing the onset of heart failure [18]. ATR2 signalling also reduces the detrimental effects of ATR1 through the upregulation of cyclic guanosine mononucleotide phosphate (cGMP], nitric oxide (NO) and the activation of protein kinase C (PKC) [19, 20]. Third major mechanism for cardioprotective effects is the stimulation of bradykinin receptors, B1 and B2 [21]. Kinins are known to increase endothelial NO synthase activity, prostaglandins like prostacyclin ($PGI_{2,}$), endothelial-derived hyperpolarizing factor (EDHF) and tissue-thromboplastin activator (t-PA) [22, 23]. These mechanisms together lead to vasodilation, apoptosis and prevent fibrosis, inflammation and cell proliferation. Clinical data reveals that ACE inhibitors have proven to be most effective in preventing death and hospitalization of a wide range of patients with cardiac diseases [24].

RAS in Renal Aging

The evidence for the role of RAS in renal aging was first reported in mice. It was observed that mice 129/Sv strain displayed an early onset aging-related phenotypes in kidney as compared to C57BL/6; 129/Sv mice showed Sclerosis, interstitial fibrosis, and glomerular mesangial expansion at 15 months while these phenotypes occurred in C57BL/6 mice at 18–20 months [25]. The 129/Sv strain has one active copy of the renin gene while the C57BL/6 mice have two functional copies, resulting in plasma renin levels that are almost ten times higher in C57BL/6 mice [26]. Ang-II promotes chronic kidney disease (CKD) by inducing vasoconstriction of the post-glomerular arterioles, raising its hydraulic pressure. [27]. This results in ultrafiltration of blood plasma proteins, thus, results in nephron hypertrophy and progression of CKD [28]. It has also been reported that RAS inhibitors effectively reduced the progression to chronic kidney disease in patients with acute kidney injury [29]. Evidence suggests that Ang-II induces cellular senescence in human glomerular mesangial due to telomere shortening and cell-cycle arrest. At the same time, the administration of losartan, an ATR1a inhibitor, rescues the phenotype [30]. Several studies have demonstrated that ATR1/ATR2 ratio in the mesenteric artery and intrarenal tissue was increased with aging [31] and led to the activation of the detrimental RAS pathway, followed by renal fibrosis [32].

The role of the protective axis of RAS on kidney senescence has not been elucidated well to date. However, some reports demonstrate that ATR2 receptor agonists preserve the structure of the kidney by preventing inflammation and collagen deposition in stroke-prone spontaneously hypertensive rats (SPSHRs) [33].

RAS in Brain Aging

Recently, it has been demonstrated that circulating levels of Ang-II, mediating central physiological action, are induced by the brain-specific localized RAS components. Besides acting in the classical pathway of vasopressin secretion, Na + and water balance, and blood pressure regulation, brain RAS is also engaged in the aging and pathophysiology of central nervous system (CNS) [34]. Labandeira-Garcia et al. showed that dysregulation of brain-specific RAS components led to aging-related changes and neurodegenerative diseases. And the associated mechanism proposed was an increase in free radical production and neuronal inflammation via switching from the pro-inflammatory phenotype (M1) to immune-regulatory phenotype (M2) [35]. Some reports suggest that the RAS activation in the brain of obese-diabetic mice KKAy, induced disruption of Blood–Brain Barrier (BBB), therefore, led to cognitive impairment, dementia, and accelerated brain aging [36]. This effect was reversed after the administration of the Ang-II receptor blocker. In a recent study by Wang et al., ARB treatment was shown to deal with neurodegenerative disorders.

It reduced the accumulation of β-amyloid proteins in CNS and improved cognitive power in mice model of Alzheimer's [37].

RAS in Skeletal Muscle Aging

Ang-II is responsible for muscle wasting due to increased expression of NADPH oxidase and mitochondrial ROS production. This was confirmed by scientists' experiment infused Ang-II in the wild-type C57BL/6 J mice or p47*phox* − / − mice. Results revealed that superoxide production increased by 2.5 folds in the wild-type mice compared to those in which NADPH oxidase subunit p47*phox* was deleted [38].

In contrast, deficiency or dysregulation of ACE2 leads to age-dependent sarcopenia and muscle weakness, suggesting the role of a protective axis of RAS in aging [39]. ACE2 knockout mice showed upregulation of senescence-associated genes like p16INK4a. This phenotype was rescued by the Ang-(1-7) application [40]. Intriguingly, Ang-(1-7)/Ang-II ratio was found to be significantly increased during vigorous muscle strength training and exercise [41]. Thus, protective ACE2 Ang-(1-7) and pathological Ang-II/ACE axis have antagonistic effects in muscle aging. This evidence suggests that RAS functions vary according to the type of receptor involved and, in the tissue-dependent manner.

RAS in Other Aging Induced Diseases

RAS components are also involved in the pathogenesis of other aging-related diseases like type-2 diabetes, osteoporosis, cancer, skin and muscle senescence.

RAS is implicated in promoting insulin resistance in patients who have diabetes mellitus. Ang-II has an inhibitory action on pancreatic islets leading to impaired beta-cell function. Recent reports suggest that RAS blockade improves the morphology of pancreatic islets and prevents its fibrosis, leading to improved insulin sensitivity [42]. Silva AC et al. reported that the cardio-protective RAS axis mediates RAS counter-regulatory effects, i.e., Ang-(1-7)-Mas Receptor pathway, which has potential as a therapeutic option against the detrimental effects of diabetes [43].

Due to its pleiotropic effects, RAS also exerts its role in osteoporosis. Research demonstrates that RAS inhibitors like ARB cause a decline in the activity of the osteoclasts and attenuates osteoporosis [44]. Moreover, this inhibition also improves bone density and lowers the risk of bone fractures [45].

Evidence of the role of RAS in tumour progression came from a study where RAS inhibitors were found to suppress tumour growth and proliferation. This is due to the high expression of ATR1 by cancer cell lines like pancreatic cancer [46]. ATR1 knockout was shown to reduce the growth and angiogenesis of ectopically given tumour populations in knockout mice, supporting a role for RAS in carcinogenesis [47]. Thus, RAS inhibitors and antagonists can also be used in tumour therapy.

Furthermore, some studies show that UVB radiation upregulates the expression of RAS components in the skin and induces wrinkles in the skin. UVB-induced wrinkles and Ang-II-induced photo aging is prevented by RAS inhibitors like enalapril maleate [48]. RAS is involved in the inflammatory pathways, collagen deposition, and TGF-β signalling cascade involved in wound healing [49]. It has been established that ATR1 blockers impair the wound healing process and interfere with fibroblast migration [50]. However, reports suggest that ATR2 deficiency accelerates the wound healing process but yields fragile and poor-quality healing [51]. Thus, ATR1/ATR2 ratio increases during wound healing in diabetic models [49].

RAS Involvement in Physiological Aging

RAS blockade has been proven to enhance longevity and attenuate age-related oxidative stress. Sirt3 is a member of sirtuins and is a deacetylase enzyme, which protects against stress-mediated cell death, and it requires NAD^+ as a cofactor. Nicotinamide phosphoribosyl transferase (Nampt) generates this NAD^+. Nampt takes its role in the salvage pathway as the rate-limiting enzyme catalyses the phosphoribosyl group's transfer to the nicotinamide (NAD) to form NAD mononucleotide (NMN). This NMN is further catalysed by another enzyme, NMN acetyltransferases, to form nicotinamide adenine dinucleotide [52] (Fig. 17.2). Sirtuins have established their role as the primary regulator of aging and longevity [53]. From the seven known sirtuins, three (Sirt 3-5) are localized in mitochondria. Only Sirt3 is reported to be linked with longevity due to the occurrence of a VNTR polymorphism in intron 5 in its enhancer region that stimulates the upregulation of specific proteins found in long-lived individuals [54]. In oxidative stress, Sirt3 has multiple protective effects, leading to the upregulation of mitochondrial antioxidant proteins manganese superoxide dismutase [55]. Sirt3 also increases cellular respiration and decreases ROS production and mitochondrial membrane potential. It also helps in regulating adaptive thermogenesis [56]. Moreover, Sirt3 overexpression has been shown to confer protection against Bax-mediated apoptosis in cardiomyocytes [57].

Studies reveal that the genetic disruption of the AT1a receptor in mice led to the upregulation of pro-survival genes in the aging kidney. Expression levels of Nampt and Sirt3 have also been found to be increased dramatically. Thus, AT1a deficiency in mice resulted in decreased oxidative stress [58]. ARB or ACE inhibitor was administered to the CF1 mice. The results revealed that their lifespan increased, accompanied by a decrease in fibrosis, apoptosis, and prevention of age-related reduction in the mitochondria [59, 60].

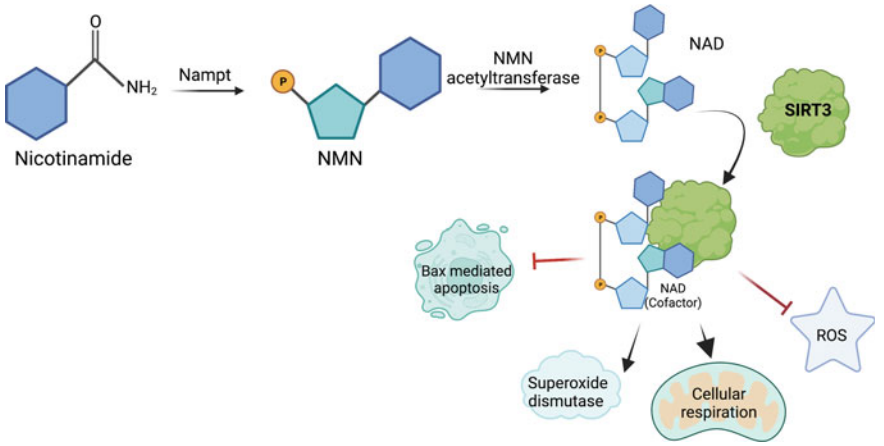

Fig. 17.2 Expression of SIRT3 prevents physiological aging. NAD is generated by the sequential action of Nampt and NMN on nicotinamide, NAD acts as a cofactor for SIRT3 mediated anti-aging effects

Mechanism of RAS Induced Aging

ROS is the dominant molecule involved in the aging process. ROS induces the chemical modification of accumulated damaged macromolecules like DNA, RNA, and proteins, accelerating the aging process. Mitochondria are majorly responsible for intracellular ROS production. Thus, oxygen-free radicals interfere with mitochondrial function, reduce ATP production, and lead to oxidative damage to mitochondria. Moreover, superoxide radicals combine with NO inside mitochondria to form peroxynitrite, a cytotoxic anion that hampers the electron transport chain in the mitochondrial inner membrane [61]. Prolonged activation of RAS leads to ATR1 receptor stimulation by Ang-II. This activates the NADPH oxidase to produce superoxide anions which delink the endothelial NO synthase. Therefore, inducing ROS production due to unavailability of NO. The catalytic subunit of NADPH oxidase, Nox1, is also activated by Ang-II, which leads to ROS production by downregulating peroxisome proliferator-activated receptor-gamma coactivator-1α activity. Ang-II mediated ROS production is also responsible for acute stress-induced premature senescence in telomere-independent manner and chronic telomere-dependent replicative senescence [62]. Together, these mechanisms contribute to mitochondrial oxidative stress and trigger the aging process in various organs (Fig. 17.3).

RAS in Regeneration

RAS inhibitors are widely used as a gold standard treatment option to treat hypertension, heart failure, and other such cardiovascular disease due to prolonged activation

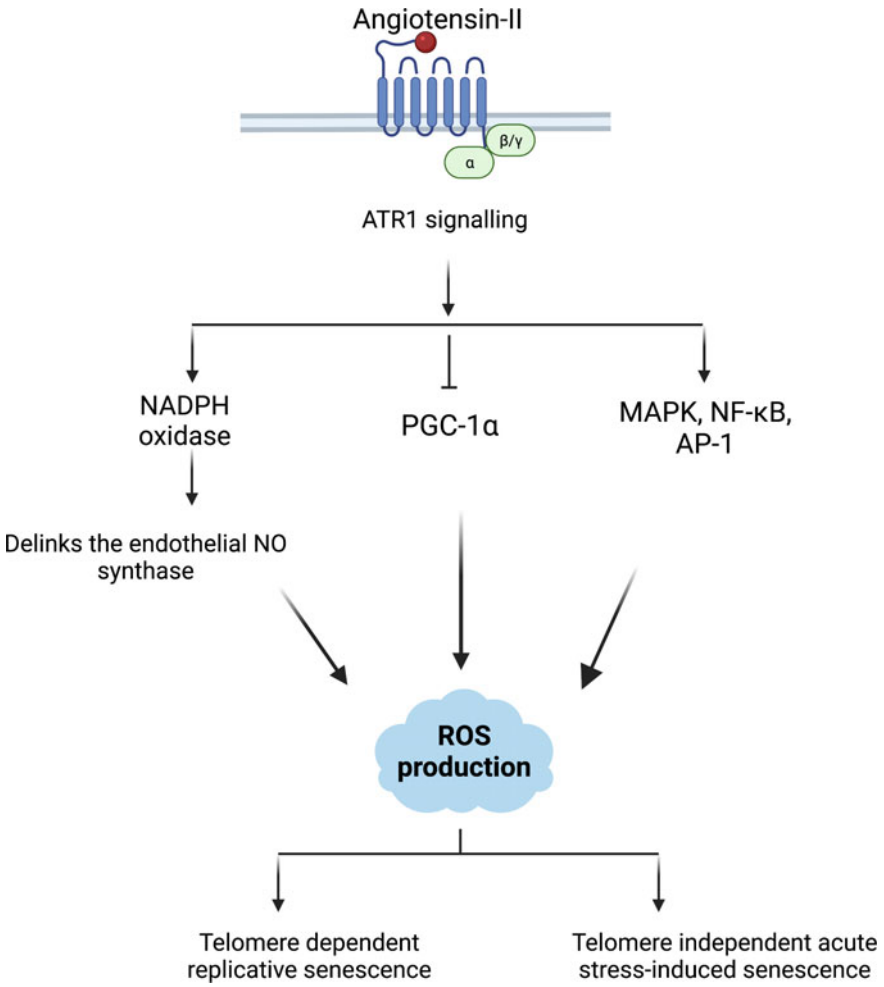

Fig. 17.3 Mechanism of induction of chronic replicative senescence and acute stress-induced senescence. ATR1 signalling upregulates the expression of NADPH oxidase which leads to ROS production due to unavailability of NO. ROS production also results from the inhibition of peroxisome proliferator-activated receptor-gamma coactivator-1alpha (PGC-1α) and activation of MAPK, NF-κB and AP1, which ultimately leads to senescence

of local RAS components in these diseases. But these treatments have side effects as high as 38%, most of which are in cutaneous tissue. This leads to psoriasis, urticaria, angioedema, photosensitivity, common skin rashes, and hair loss [63] Due to the cell-proliferative action of RAS, it has been implicated in maintaining the self-renewal and regenerative capacity of epidermal stem cells (ESCs). Experiments show that the functional RAS components were being expressed by ESCs in the wound edges and were promoting the wound healing process. Researchers have reported that

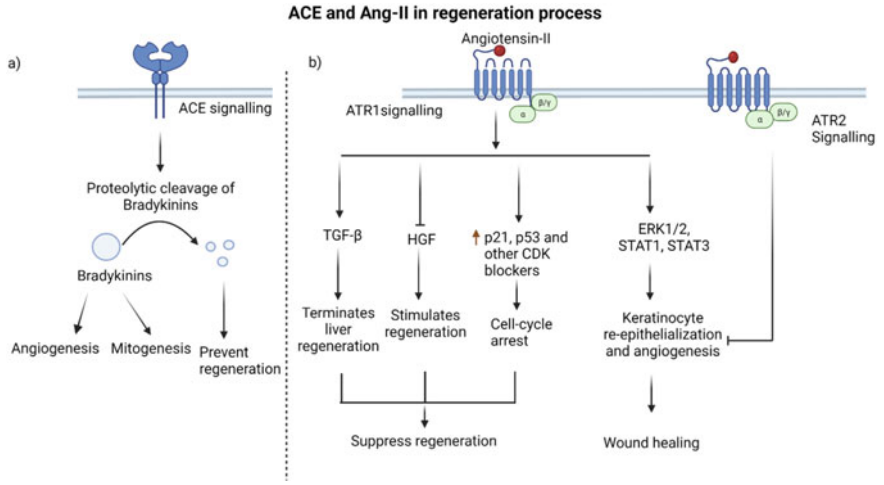

Fig. 17.4 Involvement of RAS components in regeneration process. a Stimulation of ACE leads to the cleavage of bradykinins which fail to impart angiogenic and mitogenic effects to promote regeneration. **b** ATR1 signalling inhibit regeneration due to activation of TGF-β cascade, suppression of HGF production and high expression of CDK blockers. In contrast, ATR1 pathway promotes wound healing through the activation of ERK1/2, STAT1 and STAT3, which is antagonized by ATR2 signalling

stimulation of ATR1 by Ang-II has a positive effect on ERK, STAT1, and STAT3 phosphorylation, whereas these phosphorylation reactions are inhibited by Ang-II through ATR2 [64]. Studies reveal that ATR1 activation accelerates keratinocyte re-epithelialization and angiogenesis, whereas ATR2 signalling has antagonistic effects [65] (Fig. 17.4).

Similarly, ACE, angiotensinogen, and ATR1 were upregulated following liver injury in the early stages of liver regeneration. However, other Ang-(1-7)-MasR axis mediators were upregulated later [66]. Ang-II critically works to inhibit the production of HGF, which has a potent proliferative effect and stimulates TGF-ß production, which essentially terminates liver regeneration. There is also supporting evidence that shows that blocking the Ang-II with inhibitors or using ATR1 antagonists helps in liver regeneration and significantly reduces liver fibrosis by increasing HGF levels in blood plasma and reducing TGF-ß expression [67, 68]. ACE inhibitors were also shown to increase the plasma levels of Bradykinins. ACE conducts the proteolytic cleavage of bradykinins and reduces the stimulation of Bradykinin receptor 2, which has angiogenic and mitogenic effects [69].

However, there is also evidence showing that the ARB treatment improved the regenerating capacity of skeletal muscle by inhibiting TGF-ß signalling [70]. Satellite cells constitute a pool of muscle stem cells involved in growth and regeneration. Ang-II induces mitochondrial oxidative stress, decreases the size and number of

regenerating myofibrils, and inhibits the ability of satellite cells for muscle regeneration. Studies have found that ARB suppressed the anti-proliferative reactions of Ang-II on the satellite cells, therefore increasing the number of regenerating myofibrils [71].

Conclusion

In this chapter, we have discussed the role of RAS in various types of physiological aging of the organs like liver, kidney, brain, heart and vascular system. RAS also plays a critical role in pathological conditions like type-2 diabetes, osteoporosis, cancer, skin and muscle senescence. Looking into regeneration, findings suggest contradictory role of RAS both as inducer as well as inhibitor of regeneration in spatio-temporal manner. RAS blockade has been proven to enhance longevity, regeneration and attenuate age-related oxidative stress thus retard the physiological as well as pathological aging. There is one agreeable point that blockade of RAS signalling is beneficial and future drug strategies should be based on developing specific small molecule inhibitors for RAS to reduce the bystander effects. There is more research required to streamline specific molecular signalling mediated by RAS in process of regeneration.

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Chapter 18
Renin Angiotensin System in the Maturation and Failure of Arterio-Venous Fistula

Vikrant Rai and Devendra K. Agrawal

Abstract An arteriovenous fistula (AVF) is the most used vascular access for hemodialysis but is associated with failure occurring within 3 months (early, primary) or after using it for dialysis (late or secondary). AVF maturation failure poses complications both technically and therapeutically as well as an increased socioeconomic burden. AVF maturation failure might be due to excessive neointimal hyperplasia, inward remodeling, endothelial dysfunction, excessive extracellular matrix deposition, and vascular stenosis due to thrombosis and stenosis. Neoangiogenesis, arteriogenesis, endothelial cells, and proliferation and migration of vascular smooth muscle cells play a crucial role in AVF maturation and maturation failure through vessel remodeling. Regulation of these mechanisms by the components of the renin-angiotensin system (RAS) suggests a role of RAS in regulating AVF maturation and maturation failure. Previous studies have documented the role of RAS in vessel remodeling in pulmonary hypertension and other organ system but not in AVFs. This chapter discusses the potential role of RAS components and other genes regulated by RAS components in vessel remodeling in AVF.

Keywords Arteriovenous fistula · Maturation failure · Neointimal hyperplasia · Renin angiotensin system · Vessel remodeling

Introduction

An autologous arteriovenous fistula (AVF) is the most common and suitable vascular access in patients undergoing hemodialysis because of the lowest mortality and re-intervention rate compared to arteriovenous graft and central venous catheter [1]. The obstruction of the vessels involved in AVF and the AVF immaturity is the most common impediments for AVF maturation causing AVF failure and increased morbidity and mortality in patients undergoing hemodialysis. AVF maturation failure

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may be early (fistula that never matured to the extent that it could be used) or primary (thrombosis of the fistula, never usable, or inability for cannulation for hemodialysis within 3 months of AVF creation) failure accounting for 20–54% of cases [2, 3] and late or secondary failure defined as AVF failure occurring after meeting dialysis suitability criteria and after 3 months of creation [4, 5]. In humans, AVF maturation is considered mature if, after 6 weeks of surgery, the fistula is located at a maximum of 6 mm from the skin surface with vessel diameter of 6 mm and blood flow of at least 600 ml/min. The successful and reliable use of AVF using needles during dialysis with minimal bleeding and successful removal of the tunneled catheter placed for initiation of dialysis also characterize matured fistula [6, 7]. Experience of the surgeon creating AVF, inflow artery diameter, outward vein remodeling, patient-related factors (inflammation at AVF site, hypercoagulability, hyperlipidemia, and comorbid conditions), monitoring and follow up after AVF creation, and the use of AVF creation during hemodialysis are determinants of AVF maturation [7–10] (Fig. 18.1).

Inflow blood volume and heart rate determine the flow volume rate through AVF, and this may increase 10–80-folds due to increased resistance. Increased blood flow rate and arterial pressure in the artery and vein involved in AVF causes outward arterial and venous remodeling, and shear stress plays a crucial role in remodeling [7, 11]. Medial fibrosis, intimal hyperplasia, and collagen fiber organization also play a crucial role in AVF maturation and maturation failure [12]. Outward remodeling, an important requisite for AVF maturation, is highly dependent on the composition and organization of extracellular matrix (ECM) and excessive collagen deposition in the adventitia leads to stenosed and failed AVF. Outward remodeling of the vein involved in AVF is a must to withstand the hemodynamic change of increased flow and pressure due to inflow blood directly from the artery. Excessive ECM deposition

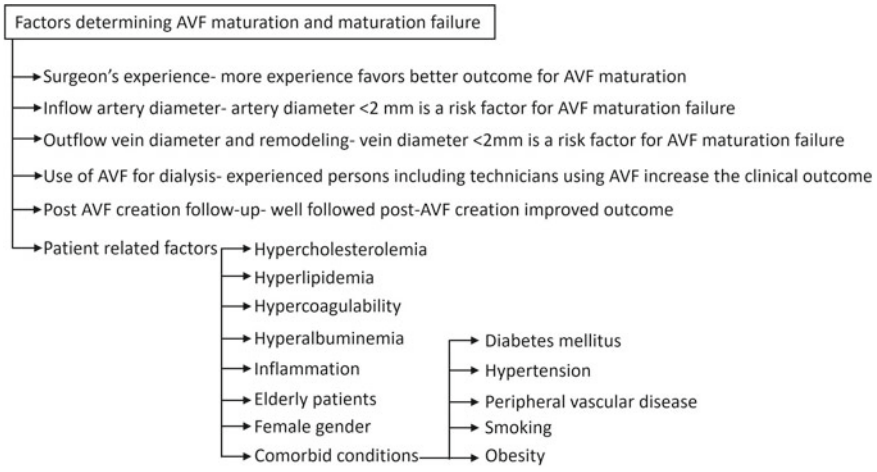

Fig. 18.1 Factors affecting arteriovenous fistula (AVF) maturation and maturation failure

in the vascular intimal layer, proliferation of vascular smooth muscle cells (VSMCs), and luminal stenosis due to neointimal hyperplasia (NIH) are the most common causes of AVF failure [12–14].

The hemodynamic changes in the vessels involved in AVF are sensed by endothelial cells (ECs) and these ECs respond to shear wall stress and hemodynamic changes via vasodilatation and structural changes [15]. Vascular wall (intima) injury during the vascular intervention and AVF creation also cause EC dysfunction and intimal hyperplasia (IH) [16, 17]. Endothelial injury is followed by the death of medial VSMCs and the proliferation and migration of remaining medial VSMCs to intimal area and deposition of ECM [18]. Intimal injury following vascular intervention is associated with increased expression of angiotensin-converting enzyme (ACE), angiotensin II (Ang II), and angiotensin II receptor type 1 (AT_1R) in intima and VSMCs causing proliferation of VSMCs [18]. Furthermore, a decreased neointima formation with ACE inhibitors and AT_1R receptor antagonists after balloon injury suggests the potential of inhibiting the renin angiotensin system (RAS) in attenuating neointima formation and thereby vessel stenosis [19]. Since increased neointima formation, VSMCs proliferation, and excessive ECM deposition on intima are the causative mechanisms for AVF maturation failure, it is imperative to associate the role of RAS in AVF maturation and maturation failure.

Renin Angiotensin System

The renin–angiotensin–aldosterone system (RAAS) regulates blood pressure, blood volume, and systemic vascular resistance. During decreased renal perfusion and dysregulated electrolytes balance in the distal convoluted tubule, RAAS responds by increased secretion of renin, angiotensin I (Ang-I), and angiotensin II (Ang-II) mediating vasoconstriction and increased sodium absorption, thereby increasing blood volume and blood pressure. RAAS involves the lung, kidney, systemic vasculature, and brain. During decreased blood flow to the kidney (renal perfusion) and imbalanced electrolytes, RAAS is activated resulting in increased secretion of renin from juxtamedullary cells. Subsequently, angiotensinogen (AGT) secreted from the liver is converted to angiotensin I (Ang I) via renin followed by conversion to angiotensin II via the angiotensin-converting enzyme (ACE) found in the vascular endothelial cells surface. Angiotensin II binds to the angiotensin receptor (ATR) and causes vasoconstriction. Additionally, angiotensin II also enhances the secretion of aldosterone from the adrenal cortex which increases the reabsorption of sodium in the proximal tubule and enhances the secretion of potassium. This causes increased water reabsorption and increases extracellular body fluids finally leading to increased blood volume and blood pressure [20–22] (Fig. 18.2). RAS components play a pivotal role in vascular remodeling after vascular intervention and intimal injury [18], in the pathophysiology of atherosclerosis [22, 23], increased RAS components after vascular injury [24], and attenuated neointima formation, inflammation, and atherosclerotic lesion after vascular injury with RAS inhibition [25–27]. These effects suggest the

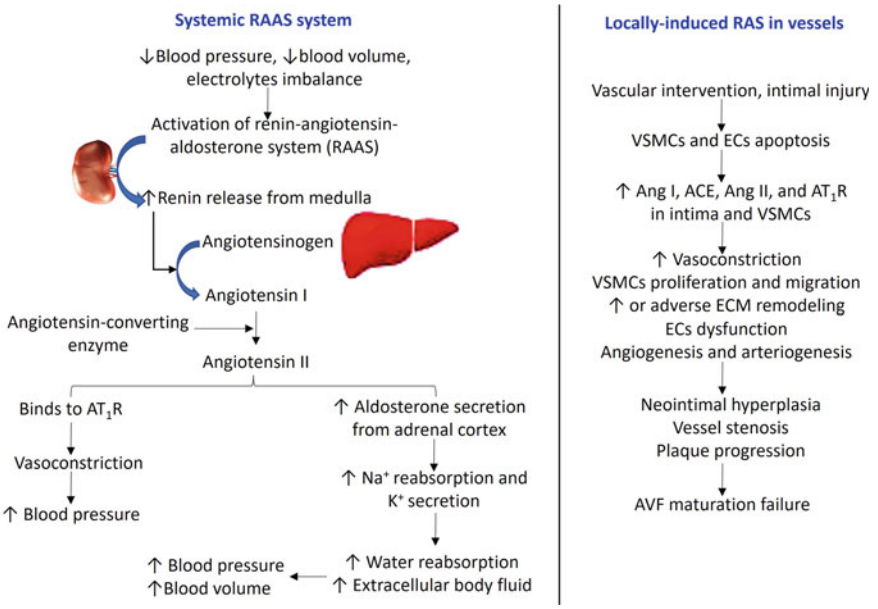

Fig. 18.2 Systemic and locally induced renin angiotensin system (RAS). Hemodynamic changes and electrolyte imbalance induce systemic RAS while vessel injury due to vascular intervention induce local RAS and led to increased expression of RAS components and RAS regulated genes in vascular intima and vascular smooth muscle cells (VSMCs) and regulate endothelial cells (ECs) function, vessel remodeling, intimal hyperplasia, neoangiogenesis, and thrombosis. AT1R—Angiotensin II receptor type 1, Ang I-angiotensin I, Ang II-angiotensin II, ACE-angiotensin converting enzyme

role of locally activated RAS in vascular pathologies and remodeling [28]. Since vascular inflammation, neointimal hyperplasia, VSMCs proliferation and migration, and vascular remodeling play a critical role in AVF maturation and maturation failure, understanding the role of RAS components in vascular remodeling is warranted.

Renin Angiotensin System and AVF

Vascular remodeling, the underlying mechanism for AVF maturation, also plays a role in atherosclerosis. ECs in the intima are exposed to all hemodynamic changes as well as vascular intervention and apoptosis of ECs has been implicated in local activation of RAS in vasculature. AGT released from apoptotic ECs and VSMCs increases the expression of Ang II, the factors playing a critical role in vascular promoting vascular remodeling [29]. Similarly, aldosterone also plays a role in vascular remodeling via activated angiotensin type 1a receptors (AT1aR) and mineralocorticoid receptors

after vascular injury [30]. This suggests a possible regulatory role of RAAS in AVF maturation and maturation failure.

AVF creation exposed the inflow artery and outflow vein to hemodynamic changes with increased luminal pressure and shear stress [31]. These changes mediate functional and structural changes in the vascular wall modulating vessel diameter and vascular wall thickness and outflow vein undergoes outward remodeling with increased vein diameter and stiffer vascular wall to withstand increased luminal pressure and lower shear stress. Vascular intervention or intimal injury is associated with local activation of RAS and increased production of ACE and Ang II [29]. ACE mediates vasoconstriction by increasing Ang II and attenuates vasodilatation by degrading bradykinin, thus regulating vascular tone. The increased ACE expression is also associated with neointima formation and attenuated neointima with ACE inhibitors suggests the potential role of ACE in adverse remodeling and protective effect of ACE inhibition [32]. Angiotensin-converting enzyme (ACE) converts Ang I to Ang II and mediate vascular remodeling whereas ACE2, a homolog of ACE metabolizes Ang II into Ang 1–7 and is a key negative regulator of RAS, regulates Ang II-mediated vascular remodeling. ACE2 expression is upregulated in vascular disease as a compensatory response to decrease vascular injury and its attenuated expression is associated with increased Ang II-mediated vascular remodeling. Attenuated ACE2 expression is associated with vascular stiffness, decreased media-to-lumen ratio, and loss of vascular smooth muscle cells. ACE2 by regulating Ang II regulates oxidative stress and apoptosis of VSMCs and plays a critical role in the prevention of adverse vascular remodeling in arteries [33] (Fig. 18.3).

Increased luminal pressure and shear stress mediate NIH and initial neointima formation is necessary for AVF maturation, however, excessive NIH leads to vascular stenosis and AVF failure. Adequate ECs function and ECM remodeling are a must for AVF maturation, and this depends on the production of endothelial progenitor cells (EPCs) and their migration to the site of injury. CD34 and CD133 expressed on EPCs mediate angiogenesis and endothelialization [34]. Activation of another

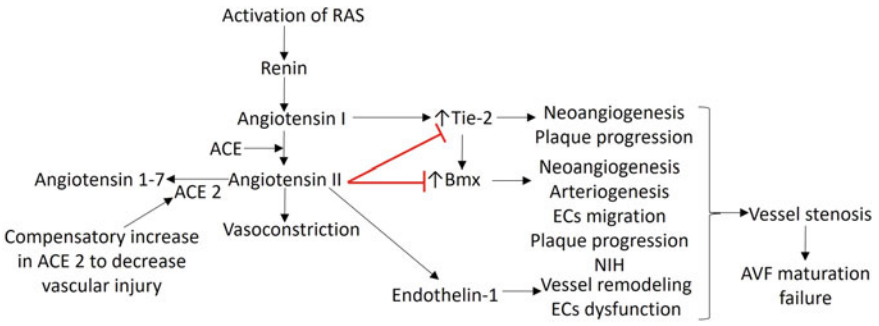

Fig. 18.3 The probable role of the renin angiotensin system (RAS) in vessel remodeling and arteriovenous fistula (AVF) maturation. Ang I-angiotensin I, Ang II-angiotensin II, ACE-angiotensin converting enzyme, ECs-endothelial cells, Tie-2 (TEK)—gene encoding Angiopoietin-1 receptor also known as CD202B, Bmx—BMX Non-Receptor Tyrosine Kinase

angiogenic cytokine vascular endothelial growth factor (VEGF) and its receptors VEGFR-1, and VEGFR-2 were found increased in experimental AVF models and associated with venous NIH [35]. This suggests a possible role of neoangiogenesis in vascular plaque and AVF maturation failure and is supported by the fact that non elevated levels of VEGF and disrupted angiogenesis are associated with venous stenosis and dysfunction of hemodialysis AV fistula [36]. Neoangiogenesis plays a crucial role in plaque formation and angiogenic factor Tie-2 (TEK) plays a critical role in angiogenesis and new vessel stabilization. The role of Tie-2 in vessel stabilization and destabilization has been suggested in association with Ang-1 mediated Tie-2 activation and Ang-II mediated inhibition of Tie-2 activation, respectively [37]. Since Tie-2 activation is regulated by Ang-I and Ang-II (components of RAAS), it is imperative to think of a role of RAAS-Tie-2 regulation in neoangiogenesis involved in plaque formation, the factors underlying vessel stenosis and AVF failure. Thus, regulating RAS (Ang-I and Ang-II) and Tie-2 expression might play a role in AVF maturation and maturation failure (Fig. 18.3).

Tie-2 regulates the expression of Bmx protein by inducing tyrosyl phosphorylation and increased expression of Bmx protein was found in endothelial cells of large arteries while venular endothelium revealed weak Bmx expression. Tie-2 and VEGFR-1 stimulate Bmx tyrosine kinase activity. This suggests that Bmx tyrosine kinase functions downstream to Tie-2 and VEGFR in arterial endothelium or tie-2 and VEGFR are upstream regulators of Bmx [38]. As discussed above, Tie-2 might have a critical role in AVF pathogenesis, Bmx may also play a role in vessel remodeling and ECs function. This notion is supported by the reports of Bmx playing a role in cardiac remodeling and might be a therapeutic target as Bmx deficiency results in a significant reduction in Ang-II-induced cardiac hypertrophy by attenuating STAT3 signaling [39]. The role of Bmx in AVF maturation failure may also be implicated by the role of Bmx in ECs migration, arteriogenesis, and angiogenesis in ischemic conditions [40]. Increased Bmx expression was found in ischemic vessels of peripheral arterial disease. This suggests that ischemic and vascular stenosis induce Tie-2 which increases its downstream Bmx and these might be therapeutic targets to attenuate stenosis [40]. Since Ang-I regulates Tie-2 expression and Bmx is downstream to Tie-2, it is imperative to hypothesize that locally active RAS after vascular intervention/injury may regulate Bmx expression and Bmx might play a role in AVF maturation failure by ECs dysfunction and vascular stenosis. However, this relationship warrants investigations. Further, the involvement of Bmx in endothelial cell migration and angiogenesis through tumor necrosis factor receptor type 2 (TNFR2) [41] and activation of VEGF2 and PIK-3 to induce angiogenesis via TNF-α [42] further suggest a possible role of Bmx in vascular pathology (Fig. 18.3).

AVFs are associated with increased expression of endothelin-1 (ET-1) in ECs of the outflow vein [43] and the intima of the stenosed AVF vessels [44]. Increased plasma ET-1 expression is also associated with recombinant human erythropoietin therapy during hemodialysis [45]. Increased expression of ET-1 in endothelium through the activation of vascular NAD(P)H oxidase mediates vascular remodeling and ECs dysfunction [46], two factors related to AVF maturation failure, and thus, increased ET-1 expression may precipitate AVF maturation failure. ET-1 also

increases the expression of vessel remodeling genes in VSMCs through G-protein coupled receptor-linked mediators [47]. These results suggest that increased ET-1 expression plays a critical role in vascular remodeling and thus might play a probable role in AVF maturation failure through vascular stenosis, however, no study has investigated this direct link. Increased expression of Ang-II is associated with increased expression of the preproendothelin-1 (ppET-1) gene and ET-1 synthesis in VSMCs and ECs [48]. This suggests a link between RAS and ET-1 and targeting both might be useful in improving the outcome of AVF creation. This notion is supported by the fact that dual inhibition of RAS and ACE improves renal function by decreasing proteinuria, renal structural change, renal fibrosis, and inflammation [49]. Targeting RAS in AVF also seems promising because inflammation plays a crucial role in AVF maturation failure [50] and RAS inhibition attenuates inflammation [49] (Fig. 18.3).

In addition to AGT, ACE, Tie-2, and Bmx, CMA1 (chymase 1) produced by vascular endothelial cells and Ang-II forming enzyme may be involved in vascular remodeling because CMA1 is involved in acute and chronic tissue injury and vascular remodeling [51, 52]. ECM remodeling is an important mechanism in the pathogenesis of AVF maturation and maturation failure. Excessive ECM deposition in the intima causes inward remodeling and vessel stenosis whereas ECM remodeling in adventitia mediating outward remodeling helps in AVF maturation. KLK4 (Kallikrein Related Peptidase 4) involves in the ECM remodeling [53] and the interaction of RAS and kallikrein-kinin systems contributes to vascular hypertrophy in angiotensin II-induced hypertension [54] and diabetic nephropathy [55]. ELANE (Elastase, Neutrophil Expressed) is another gene that may have implications in AVF vessel remodeling. Neutrophil elastase is produced by neutrophils, macrophages, and VSMCs. Neutrophil elastase is involved in adverse ECM remodeling by regulating the degradation of elastin, collagen, fibronectin, and laminin and increased activity of matrix metalloproteinases (MMPs). ECM remodeling is involved in AVF vessel remodeling and hence, ELANE might play a role in AVF maturation and maturation failure. This is supported by an increased expression of neutrophil elastase in fibrous and atheromatous plaques and its involvement in neointima formation [56–58]. The possible role of these genes in AVF vascular remodeling in based on their role in vessel remodeling in other organ system and their association with RAS (predicted based on Signor and STRING network analysis for AGT and Renin—Fig. 18.4).

RAS activation is associated with change in the hemodynamics in the vascular system with large AVF causing increased cardiac output and activation of vasoconstrictors, however, this initiates a compensatory mechanism for vasodilatation by increasing Atrial natriuretic peptide (ANP) and Nitric oxide (NO). The management of electrolyte imbalance, blood pressure, and blood volume using angiotensin converting enzyme inhibitors (ACEi) and angiotensin II (ATII) blockers (ARBs) suggest the possible therapeutic role of RAS in AVF maturation [59]. This notion is supported by the increased AVF patency with ACEi, ARBs, and calcium channel blockers [60]. However, it has also been reported that combined use of ACEi and ARBs increase risk of cardiovascular death and thus, no-ACEi therapy in combination with ARBs may have beneficial outcome [61].

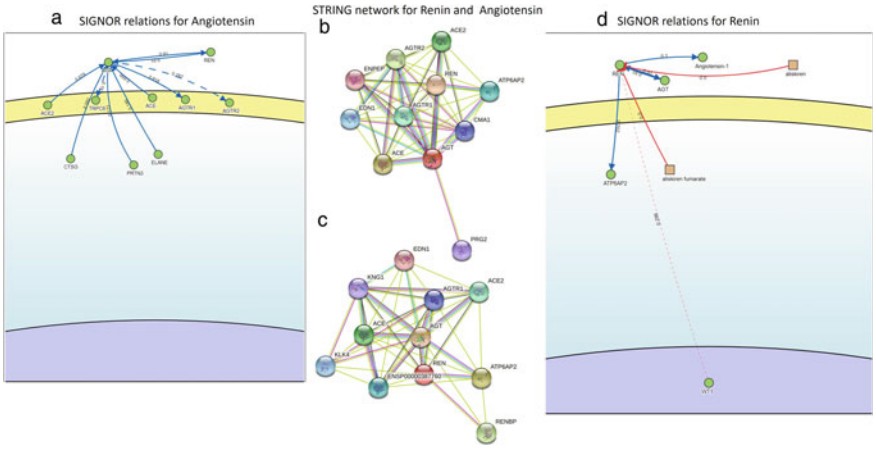

Fig. 18.4 Regulatory network and signaling pathways for renin (REN) and angiotensin (AGT) using https://signor.uniroma2.it/ and https://string-db.org/. Renin—panels (**a**) and (**b**); and angiotensin—panels (**c**) and (**d**). The network analysis shows the regulation of ELANE (neutrophil elastase), CMA1 (chymase 1), and kallikrein 4 (KLK4) involving renin and angiotensin

Conclusion

The component of RAS regulates neointimal hyperplasia, ECs dysfunction, VSMCs proliferation and migration, neoangiogenesis, arteriogenesis, and vasoconstriction. Increased expression of Tie-2, Bmx, ET-1, Ang II, CMA1, ELANE, and KLK4 is associated with adverse vessel remodeling. Thus, increased expression of these genes which are regulated by components of RAS might contribute to AVF maturation failure due to thrombosis or stenosis. This also indicates that targeting these genes might have therapeutic potential and may have a better clinical outcome. This is supported by the beneficial outcome of ACE inhibitors and ARBs. However, a direct relation between RAS and AVF maturation failure warrants investigation.

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Part III
Pharmacotherapeutic Aspects of Renin Angiotensin System

Chapter 19
Renin–Angiotensin–Aldosterone System as an Old New Target in Heart Failure Therapy

RAAS Inhibition in Heart Failure

Árpád Kovács and Judit Barta

Abstract The prevalence of heart failure (HF) increases worldwide. Long-term maladaptive activation of the renin–angiotensin–aldosterone system (RAAS) contributes to pathological left ventricular (LV) remodeling in the failing heart. Accordingly, RAAS blockade induces reverse remodeling in HF patients. To date, number of large, randomized clinical trials have confirmed the efficacy of different RAAS inhibitors in the management of HF with reduced LV ejection fraction (HFrEF). Therefore, actual HF guidelines recommend broad spectrum of RAAS inhibitors including angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers, and aldosterone antagonists to reduce morbidity and mortality of HFrEF patients. In addition, novel therapeutic approaches targeting the RAAS such as dual angiotensin receptor and neprilysin inhibition (ARNi) with sacubitril/valsartan still open new avenues for HF patients. In contrast to HFrEF, RAAS inhibitors have not been proven in HF with preserved LV ejection fraction (HFpEF). This review aimed to summarize the rationale for and our current knowledge of RAAS inhibition in the clinical management of human HF.

Keyword RAAS · Heart failure · Left ventricle · HFrEF · HFpEF · ACE inhibitor · ARB · MRA · Angiotensin receptor and neprilysin inhibition (ARNi) · Sacubitril/valsartan

Rationale for Renin–Angiotensin–Aldosterone System (RAAS) Inhibition in Heart Failure (HF)

According to the universal definition, HF is a complex clinical syndrome that comprises 3 elements: (1) structural or functional heart disease; (2) HF symptoms including dyspnea, fluid retention/edema, fatigue and exercise intolerance; (3) and

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objective signs commonly seen in HF [1]. The prevalence of HF increases with age up to 1–2% among adults [2, 3], however, taking into account unrecognized HF cases this number is presumably higher [4]. In addition, growing number of patients progress into a phase of advanced HF with persistent symptoms at rest and recurrent hospitalizations despite guideline-directed management and therapy (GDMT) [5–7]. HF is therefore a global challenge as prognosis remains poor with a 1-year mortality of 25–75% [8–10]. HF is classified based on left ventricular (LV) ejection fraction (EF) as follows: HF with reduced EF (HFrEF) with LV EF of $\leq 40\%$; HF with mildly reduced EF (HFmrEF) with LV EF of 41–49%; and HF with preserved EF (HFpEF) with LV EF of $\geq 50\%$ [11, 12]. Moreover, those patients with HFrEF at baseline who show an increase at second measurement of LV EF of $> 40\%$ are referred to have HF with improved EF (HFimpEF) [1].

Distinct HF phenotypes reflect to different etiologies and complex pathophysiology of HF. Nonetheless, neurohormonal activation seems to be a common trigger in the development and progression of chronic HF. The adrenergic nervous system, the RAAS and the cytokine systems are initially activated as short-term compensatory mechanisms to maintain hemodynamic stability in a clinically asymptomatic patient. However, long-term maladaptive neurohormonal activation contributes to pathological LV remodeling and secondary end-organ damage with subsequent cardiac decompensation, collectively leading to symptomatic HF [13]. As a proof of concept, during the past decades, number of large, randomized clinical trials confirmed the efficacy of different RAAS inhibitors in the management of HF. Nonetheless, novel medications targeting the RAAS have been still proven in recent clinical HF studies. Accordingly, HF guidelines have been updated lately by the American College of Cardiology/American Heart Association (ACC/AHA), the Heart Failure Association of the European Society of Cardiology (HFA/ESC) and the Canadian Cardiovascular Society/Canadian Heart Failure Society (CCS/CHFS)—in part—to introduce pharmacological innovations of RAAS inhibition into GDMT of HF [11, 14, 15].

RAAS Inhibition is the Cornerstone of GDMT in HFrEF

Angiotensin-Converting Enzyme (ACE) Inhibitors

Unless contraindicated or not tolerated, ACE inhibitors are recommended in all—symptomatic and asymptomatic—patients with HFrEF to increase survival and improve symptoms [16–19]. ACE inhibitors should be initiated in low doses and thereafter uptitrated to the maximum tolerated recommended doses. Higher doses of ACE inhibitors are more effective than lower doses in preventing hospitalization. However, adjustment of the dose of diuretics may be necessary, because fluid retention can interfere with ACE inhibitory therapy and symptomatic hypotension can occur during the initiation of ACE inhibitors. ACE inhibitors act on the RAAS by inhibiting the enzyme that is responsible for the conversion of angiotensin I to the

biologically active angiotensin II [20]. In addition, ACE inhibitors also inhibit kininase II and thereby may induce the upregulation of bradykinin, which may potentiate the effects of angiotensin suppression. All together, ACE inhibitors promote reverse LV remodeling and improve HF outcome.

The effectiveness of ACE inhibitors has been consistently reported in clinical trials including broad varieties of symptomatic and asymptomatic patients as well as causes and severity of LV dysfunction. Studies of Left Ventricular Dysfunction (SOLVD prevention) [21], Survival and Ventricular Enlargement (SAVE) [22] and Trandolapril Cardiac Evaluation (TRACE) [23] clinical studies demonstrated that ACE inhibitors limit the progression to symptomatic HF and the need of hospitalization in asymptomatic patients with LV dysfunction. Likewise, ACE inhibitors showed similar benefit for patients with symptomatic LV dysfunction. Moreover, all the three placebo-controlled chronic HF trials demonstrated a reduction in mortality [16, 19, 21]. Although placebo-controlled mortality trials have been conducted only with enalapril in patients with chronic HF, ACE inhibitors reduce mortality in direct relation to the degree of severity of chronic HF. Indeed, among HF patients with NYHA class IV, the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS I) [16] had a much larger effect size than the SOLVD treatment trial on mild congestive HF [19], which in turn had a larger effect size than the SOLVD prevention trial on asymptomatic LV dysfunction [21]. In line, enalapril improves HF with a mechanism independent of vasodilation, and this positive effect is related to the extent of neurohormonal activation as a prognostic factor for patients with HF. Accordingly, in the Vasodilator Therapy of Heart Failure II (V-HeFT-II) trial enalapril had significantly lower mortality as compared with the vasodilatory combination of hydralazine plus isosorbide dinitrate—with no direct action on the neurohormonal systems [24]. Beyond enalapril, multiple ACE inhibitors have been proven in myocardial infarction (MI) trials. ACE inhibitors including captopril, trandolapril and ramipril similarly improve both survival and functional NYHA class in HFrEF patients following acute MI [22, 23, 25]. Use of ACE inhibitors—or ARBs—are therefore recommended as soon as safely possible after MI in hemodynamically stable patients with HF or an LV EF < 40% [11, 14, 15]. In conclusion, the effects of ACE inhibitors on the natural history, development and progression of HF and post-MI LV systolic dysfunction represent "class effects" of these agents. Nonetheless, the effectiveness of ACE inhibitors is less well established in HFrEF patients with hypotension and impaired renal function.

Over the past decades, number of experimental and clinical studies have provided evidence that ACE inhibitors promote reverse cardiac remodeling as shown by reduced LV volumes and increased LV EF [26]. As a matter of fact, one of the first treatments among RAAS inhibitors shown to reverse cardiac remodeling was ACE inhibition [27]. Considering that both plasma and cardiac RAAS are activated in infarcted animals to promote angiotensin II formation, in pioneer works by Pfeffer et al. treatment with captopril could reduce chamber dilatation and infarction size, as well as improve survival in post-MI rats [28, 29]. Following experimental studies with ACE inhibitors and ARBs on post-MI rats have shown enhancement in intracellular Ca^{2+} handling, cellular and membrane protein expression and gene expression

levels [30]. ACE inhibition can also enhance cardiac NO production and attenuate beta adrenergic signaling [31]. Afterwards, reverse remodeling due to ACE inhibitory therapy was demonstrated in the human SAVE trial [22]. Likewise, in the SOLVD trial enalapril reduced LV volumes regardless of symptomatic status and improved EF as these effects were related to reverse LV remodeling [32].

ACE inhibitors are contraindicated in cardiogenic pre-shock/shock and with history of angioedema, bilateral renal artery stenosis, pregnancy and known adverse (e.g. allergic) reaction. Majority of the adverse effects of ACE inhibitors are related to suppression of the RAAS. Significant hyperkalemia (>5.0 mM/L), significant renal dysfunction [creatinine > 221 μM/L (>2.5 mg/dL) or eGFR < 30 mL/min/1.73 m^2], symptomatic or severe asymptomatic hypotension (systolic blood pressure < 90 mmHg) need caution. Light hypotension and mild azotemia are often seen during the initiation of therapy and well tolerated. Blood chemistry (serum potassium level, creatinine, urea/BUN) needs to be monitored in 1–2 weeks after initiation, then in 1–2 weeks after final dose titration and 4-monthly thereafter. Side effects of ACE inhibitors including bothersome nonproductive cough (10%), skin rash or angioedema (<1%) are related to kinin potentiation. In such patients, angiotensin receptor blockers (ARBs) are the recommended alternative line of therapy. Except serious complications (e.g. angioedema, hyperkalemia) occur, abrupt withdrawal of an ACE inhibitor should be avoided as it may cause clinical deterioration [11, 14, 15].

Angiotensin Receptor Blockade

ARBs are recommended and well tolerated in symptomatic and asymptomatic patients with reduced EF who are intolerant of ACE inhibitors for reasons other than hyperkalemia or renal insufficiency such as cough, skin rash, and angioedema. Like ACE inhibitors, ARBs have similar rates of hypotension, hyperkalemia or renal dysfunction. ARBs inhibit RAAS through the angiotensin type 1 (AT_1) receptor that mediates majority of relevant adverse effects of angiotensin II on cardiac remodeling. Similar to ACE inhibitors, ARBs exert their beneficial effects on reverse LV remodeling by reducing LV dimensions and improving LV EF [33] as this process is predictive of a long-term prognosis [34]. ARBs including candesartan, valsartan and losartan have been evaluated in placebo-controlled HF trials [33, 35–37]. Candesartan significantly reduced all-cause mortality in the Candesartan Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM-Alternative) trial [35], irrespective of background ACE inhibitor or beta blocker therapy. Valsartan had a similar favorable effect on survival in patients not receiving an ACE inhibitor in the Valsartan Heart Failure Trial (Val-HeFT) [33]. The Losartan Heart Failure Survival Study (ELITE II) was designed to directly compare the ARB losartan with the ACE inhibitor captopril on survival and tolerability of NYHA II-IV class HFrEF patients. There were no significant differences in all-cause mortality or sudden death between the two treatment groups, but losartan was significantly better tolerated [38]. Afterwards, two captopril-controlled trials aimed to compare an ARB and an ACE inhibitor

in post-ST elevation MI patients with subsequent LV systolic dysfunction or HF. In the Optimal Therapy in Myocardial Infarction with the Angiotensin II Antagonist Losartan (OPTIMAAL) study losartan (18%) was not as effective as captopril (16%) on all-cause mortality [39]. Conversely, in the Valsartan in Acute Myocardial Infarction Trial (VALIANT) on 14703 patients with acute MI valsartan was shown to be noninferior to captopril on all-cause mortality (valsartan 19.9%, captopril 19.5%) [40]. However, the combination of captopril and valsartan increased the number of adverse events with no further reduction in mortality (19.3%) in VALIANT. Valsartan (80 mg twice daily) therefore represents an alternative to ACE inhibitors. As a matter of fact, when complement ARBs were tested on top of ACE inhibitory therapies, neither candesartan in the CHARM-Added trial [36], nor valsartan in the Val-HeFT trial [33] added significant positive effect on mortality. Based on the results of the Heart Failure Endpoint Evaluation of Angiotensin II Antagonist Losartan (HEAAL) trial comparing high-dose versus low-dose ARB effect on all-cause death and HF admissions [37], ARBs should be initiated with the starting doses and thereafter uptitrated to the recommended doses [11, 14, 15]. Nevertheless, ACE inhibitors remain first-line options in the GDMT of HF, while ARBs are recommended for ACE-intolerant patients [11, 14, 15]. As with ACE inhibitors, blood pressure, renal function and potassium should be reassessed within 1–2 weeks after initiation of ARBs and monitored closely after changes in doses. Side effect profile of ARBs is similar to those of ACE inhibitors in terms of hyperkalemia or renal insufficiency.

There is lack of strong evidence regarding ACE inhibitors and ARBs in the early management of acute or worsening HF [15]. Observational data are available from the Get With The Guidelines-HF Registry ($n = 16052$) showing lower mortality and first year readmission rates in patients treated with ACE inhibitor/ARB treatment before discharge [41]. Indeed, a matched-cohort analysis showed that all-cause mortality was lower in patients who initiated in-hospital ACE inhibitor/ARB treatment compared with those for whom ACE inhibitor/ARB treatment was discontinued [42]. At the same time, in such patients hospitalized for HFrEF hemodynamic instability and/or worsening renal function much interfere with the otherwise optimal GDMT [43–45]. Important to note, a preventive reduction or withdrawal of ACE inhibitor/ARB therapy is carried out in a significant portion of patients with no documented impairment of renal function [46].

Dual Angiotensin Receptor and Neprilysin Inhibition

The Prospective Comparison of ARNi With ACEi to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF) trial [47] was the first multicentre, randomized, double-blind trial to evaluate the efficacy and safety of LCZ696 (200 mg twice daily) versus enalapril (10 mg twice daily)—in addition to recommended therapy—on morbidity and mortality in HFrEF. It is noteworthy that the trial was stopped early, because of the overwhelming benefit on the LCZ696 arm. Among 8442 HFrEF patients with NYHA class II-IV LCZ696 was superior to

enalapril in the primary composite endpoint (death from cardiovascular causes or first hospitalizations for HF) forasmuch that occurred in 21.8% in the LCZ696 group and 26.5% in the enalapril group ($P < 0.001$). Similar efficacy was observed with LCZ696 as compared with enalapril in death from any cause, death from cardiovascular causes and risk of hospitalization (all $P < 0.001$). In addition, LCZ696 significantly decreased the symptoms and physical limitations of HF ($P = 0.001$). Although the LCZ696 group had higher proportions of patients with hypotension and non-serious angioedema than the enalapril group, but had a simultaneous reduction in the decline in eGFR [48], in hyperkalemia [49], in the incidence of diabetes requiring insulin treatment [50], cough and even loop diuretic requirement [51]. Patients developing symptomatic hypotension also gained clinical benefits from LCZ696 therapy [52].

LCZ696 is the first-in-class drug with dual angiotensin receptor and neprilysin inhibition (ARNi) as a novel therapeutic strategy in HF [53]. LCZ696 is a salt complex that comprises two active components, i.e. the pro-drug sacubitril and the ARB valsartan [54], and thereby delivers simultaneous neprilysin inhibition and AT_1 receptor blockade [55]. Sacubitril is further metabolized to the neprilysin inhibitor LBQ657. In the failing heart natriuretic peptide release from the heart (ANP and BNP) and the vasculature (CNP) have potential beneficial actions on the regulation of blood pressure and volume [56–58]. Furthermore, preclinical studies indicate that natriuretic peptides also exert potent cardiac antihypertrophic and antifibrotic effects [59, 60]. Since the endopeptidase neprilysin is responsible for natriuretic peptide degradation, targeting neprilysin appears a reasonable therapeutic approach in HF. Nonetheless, neprilysin metabolizes angiotensin I and II via several pathways, inhibition of neprilysin alone is therefore insufficient as it is associated with an increase in angiotesin II levels, counteracting the potential benefits of neprilysin inhibition [61]. For this reason, neprilysin inhibition must be accompanied by simultaneous RAAS blockade (e.g. AT_1 receptor blockade). ARNi prevents both the counterproductive RAAS activation seen with neprilysin inhibition alone and the increase in bradykinin seen with neprilysin inhibition plus ACE inhibitor [61, 62]. Complementary effects of simultaneous inhibition of neprilysin and suppression of the RAAS with ARNi lead to enhancing cGMP-mediated benefical effects of natriuretic peptides and suppressing RAAS-mediated detrimental effects [63]. Natriuretic peptide levels are diagnostic and prognostic biomarkers in HF. Unlike BNP, NT-proBNP is not a substrate for neprilysin, consequently, NT-proBNP remains a useful biomarker of therapeutic effect and prognosis during neprilysin inhibition [47, 53]. Bradykinin is a substrate of neprilysin and other vasopeptidases and its elevation has been associated with cough and angioedema. However, LCZ696 opens alternative degradation pathways for bradykinin in accordance with a lower incidence of cough and a higher proportion of non-serious angioedema in patients treated with LCZ696 versus enalapril in PARADIGM-HF.

Growing evidence suggests that ARNi can also reverse LV remodeling in the failing heart. In rodent models of MI or ischemia–reperfusion injury ARNi could attenuate LV remodeling by reducing cardiac fibrosis and hypertrophy [64, 65]. Likewise in HFrEF patients, ARNi leads to a dose-dependent reverse remodeling

involving both systolic and diastolic LV function [66, 67]. In addition, two trials on patients with HFrEF have been presented lately that support the role of ARNi in cardiac reverse remodeling. The Study of Effects of Sacubitril/Valsartan versus Enalapril on Aortic Stiffness in Patients With Mild to Moderate HF With Reduced Ejection Fraction (EVALUATE-HF) trial demonstrated reduction in both atrial and ventricular volumes, improvement in diastolic function and reduction in NT-proBNP after 3 months of treatment with ARNi as compared to enalapril [68]. The Prospective Study of Biomarkers, Symptom Improvement, and Ventricular Remodeling During Sacubitril/Valsartan Therapy for Heart Failure (PROVE-HF trial) demonstrated similar improvements in LV EF, as well as reduction in left atrial and LV volume indicies [69]. Finally, a sub-study of PROVE-HF established a strong predictive value of the rise in ANP concentration on later improvements in LV EF and reductions in left atrial volume index [70]. This observation was later proven single-centre retrospective study of patients with HFrEF showing ARNi had greater influence on left atrial reverse remodeling and was associated with a better prognosis compared with ACE inhibitor/ARB use [71]. However, the mode of action of ARNi on reverse remodeling warrants further mechanistic studies.

Accordingly, ARNi (sacubitril/valsartan) is recommended as a replacement for ACE inhibitor/ARB therapy in symptomatic HFrEF patients despite optimal GDMT (strong recommendation). A candidate for sacubitril/valsartan therapy should be hemodynamically stable and have an adequate blood pressure and an eGFR $\geq$ 30 mL/min/1.73 m^2. A washout period of at least 36 h after ACE inhibitor therapy is required in order to minimize the risk of angioedema. Nevertheless, 36 h washout period is not necessary for those receiving ARB therapy at the time of hospitalization [11, 14, 15]. Recently, two studies have examined the safety and efficacy of ARNi in patients hospitalized with acute HF, including de novo HF, with or without previous exposure to RAAS inhibition. The Comparison of Pre-discharge and Post-Discharge Treatment Initiation With LCZ696 in Heart Failure Patients With Reduced Ejection-Fraction Hospitalized for an Acute Decompensation Event (TRANSITION) study [72] showed the safety of initiating ARNi in HFrEF patients with decompensated HF compared with initiation of ARNi after discharge. In addition, patients with newly diagnosed HF were shown to be more likely to achieve target dose of sacubitril/valsartan at 10 weeks with fewer serious adverse reactions in TRANSITION [73]. Patients with de novo HFrEF who started ARNi therapy had a greater decrease in NT-proBNP and lower rates of rehospitalization without compromising up-titration of other GDMT. Likewise, in-hospital initiation of sacubitril/valsartan was compared with enalapril for 8 weeks in hemodinamically stable HFrEF patients ($n = 881$) hospitalized with acute decompensated HF and resulted in a significantly greater proportional reduction in NT-proBNP in the Comparison of Sacubitril/Valsartan Versus Enalapril on Effect on Nt-Pro-Bnp in Patients Stabilized From an Acute Heart Failure Episode (PIONEER-HF) trial [74]. This change was consistent across all subgroups, irrespective of previous HF or RAAS inhibition. When in-hospital initiation of sacubitril/valsartan and switch from enalapril to sacubitril/valsartan were followed-up for an additional 4 weeks, a further 17.2% and 37.4% reduction in NT-proBNP was observed, respectively [75]. ARNi as an in-hospital first choice therapy resulted in a

lower incidence of HF rehospitalization or cardiovascular mortality over the entire 12-week trial period compared with the conversion of enalapril to ARNi after the first 8 weeks (13.0% versus 18.1%; $P = 0.03$) [75]. A recent additional analysis has shown that in hemodynamically stabilized patients with acute decompensated HF the efficacy and safety of sacubitril/valsartan are generally consistent across dose levels, even in patients who might not tolerate early up-titration to target dose [76]. In summary, first-line ARNi therapy as an alternative to either an ACE inhibitor or ARB may be considered with a new diagnosis of HFrEF (weak recommendation) [11, 14, 15]. ARNi might reduce diuretic requirements and diuretic dosing should be carefully evaluated when starting ARNi therapy. Initial dosing and titration schedule should be individualized. Drug tolerability, side effects, and laboratory follow-up (renal function and serum potassium level) of ARNi is similar to that of ACE inhibitors or ARBs, and essential after discharge to monitor for adverse events [11, 14, 15]. Last but not least, important lacking clinical information has been reported lately on the utility of ARNi in patients with acute MI complicated by HF. The Prospective ARNI versus ACE Inhibitor Trial to Determine Superiority in Reducing Heart Failure Events After MI (PARADISE-MI) trial aimed to compare sacubitril/valsartan (200 mg twice daily) with ramipril (5 mg twice daily) treatment early after high-risk MI (12 h to 7 days) on the composite endpoint of cardiovascular death or incident HF [77]. A total of 5661 patients were involved for a median of 22 months. Of note, a primary outcome event occurred with a similar rate in the sacubitril/valsartan group (11.9%) and in the ramipril group (13.2%). Accordingly**,** ARNi and ACE inhibiton show comparable outcome on the incidence of death from cardiovascular causes or incident HF among patients with acute MI [78].

Aldosterone Antagonists

Mineralocorticoid receptor antagonists (MRAs: spironolactone or eplerenone) are recommended, in addition to an ACE inhibitor and a beta blocker, in all patients with HFrEF to reduce mortality and the risk of HF hospitalization, as well as to improve symptoms [79, 80]. As a matter of fact, aldosterone levels are not reduced by long-term treatment with ACE inhibitors [81]. MRAs are classified as potassium-sparing diuretics with additional benefits independently of the effects of these agents on sodium balance. MRAs block receptors that bind aldosterone and, with different degrees of affinity, other steroid hormone receptors (e.g. corticosteroid and androgen). In contrast to spironolactone as a competitive aldosterone antagonist, eplerenone is a selective aldosterone inhibitor, and thereby causes less gynaecomastia [82, 83].

More than two decades ago spironolactone produced a positive outcome on survival in the Randomized Aldactone Evaluation Study (RALES) trial, which evaluated the spironolactone versus placebo in HFrEF patients with NYHA class III-IV and ongoing ACE inhibitor, loop diuretic and typically digoxin therapy [79]. The trial was stopped prematurely, because spironolactone resulted in 30% reduction in

total mortality and 35% reduction in hospitalization for worsening HF as compared to placebo (both $P < 0.001$). In addition, spironolactone also improved symptoms of HF ($P < 0.001$), although gynecomastia was significantly higher in men who were treated with spironolactone (10%) versus placebo (1%; $P < 0.001$). Actions of aldosterone are mediated through the mineralocorticoid receptor, and lead to myocardial fibrosis [84] and ventricular arrhythmias [85]. Thus, the beneficial effect of spironolactone seen in RALES might be attributable to the prevention of extracellular matrix remodeling and increasing potassium levels. Thereinafter, the Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF) trial demonstrated an unambiguous positive effect of eplerenone (titrated to 50 mg daily) over placebo on composite of death from cardiovascular causes or hospitalization for HF in HFrEF patients (eplerenone: 18.3% versus placebo: 25.9%; hazard ratio, 0.63; 95% confidence interval, 0.54 to 0.74; $P < 0.001$) [80]. Similarly, eplerenone also resulted in significant decreases in all-cause death (24%), cardiovascular death (24%), all-cause hospitalization (23%) and HF hospitalizations (43%). Of importance, in contrast with the RALES trial, which was conducted before the widespread adoption of beta blockers, the background therapy for EMPHASIS-HF included ACE inhibitors/ARBs and beta blockers.

The findings in RALES and EMPHASIS-HF are consistent with those in randomized clinical trials in patients with acute MI and LV dysfunction. The Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival (EPHESUS) study evaluated the effect of eplerenone (titrated to 50 mg daily) on morbidity and mortality among patients with acute MI complicated by LV dysfunction and HF [86]. Treatment with eplerenone led to a 15% decrease in all-cause death in EPHESUS.

Although the aldosterone receptor is upregulated in failing human hearts, reverse remodeling was also shown with aldosterone antagonists [87]. Under experimental settings MRAs (spironolactone and eplerenone) were shown to restore endothelium-dependent vasodilation [88], reduce post-MI fibrosis [89–91] and normalize echocardiographic and hemodynamic parameters of diastolic function [92]. Moreover, the combination of eplerenone with the ACE inhibitor trandolapril in post-MI rats further improved LV remodeling and neurohormonal activation [93]. As a matter of fact, electrical remodeling of the myocardium precedes myocyte hypertrophy following experimental MI, but appears to be attenuated by mineralocorticoid receptor antagonism [94]. Aldosterone blockade is therefore likely to attribute to the prevention of sudden cardiac death after MI. As a proof of principle, cardiac remodeling (e.g. fibrosis) was prevented with spironolactone after MI in human patients [95].

On the basis of the results of the RALES and EMPHASIS-HF trials [96], MRAs currently are recommended for all patients with HFrEF in addition to a RAAS inhibitor (ACE inhibitor/ARB/ARNi) and a beta blocker to increase survival, reduce the risk of HF hospitalization and improve symptoms [11, 14, 15]. Spironolactone should be initiated at a dose of 12.5–25 mg/day and uptitrated to 25–50 mg/day, whereas eplerenone should be initiated at a dose of 25 mg/day and increased to 50 mg/day. Important to note, MRAs are not recommended in significant hyperkaleamia and kidney failure, therefore caution is needed with high serum potassium levels (>5.0 mM/L) or renal dysfunction [creatinine > 221 μM/L (> 2.5 mg/dL) or

eGFR < 30 mL/min/1.73 m^2] to avoid life-threatening hyperkalemia as the main concern. Worsening renal function might lead to dose reduction or discontinuation of MRA therapy, as well as adjustment of potassium supplementation if any. Monitoring of serum creatinine and potassium should be repeated within 1 week of initiation or dose change. Switching to eplerenone should be considered because of breast discomfort or gynaecomastia in ca. 10–15% of male patients who use spironolactone. MRAs, when used for HF, have very little effect on BP.

However, despite established guideline recommendations to initiate MRAs as part of standard therapy, a report of the US CHAMP-HF registry [97] showed that MRA was used in only 33.4% of patients with HFrEF without documented contraindication. Likewise in the more recent PARADISE-MI trial (with ARNi in post-MI HF), in which only 42% of patients were treated with MRAs almost 20 years after the randomized control trial evidence (EPHESUS) [98]. Randomized controlled trial data regarding in-hospital initiation of MRA therapy among patients with HFrEF is limited to the EPHESUS trial. In the PIONEER-HF study (with ARNi in acute decompensated HF) it was noted that in patients admitted with acute decompensated HFrEF, 65% had a history of HF but only 10% were receiving an MRA at the time of admission [74]. It appears that GDMT—at least regarding MRAs—is sub-optimal in the clinical practice and should be revised and improved to achieve better overall outcome for HF patients. Taken together, CCS/CHFS now recommend MRA treatment for patients with acute MI and LV EF $\leq$ 40%, and HF symptoms or diabetes, to reduce mortality and hospitalization for cardiovascular events (strong recommendation) [15].

Concerns and Limitations of RAAS Inhibition in HF

Renin Inhibition

Aliskiren, an orally active direct renin inhibitor appeared to suppress the RAAS as effectively as the ACE inhibitor ramipril in the short term [99]. The concept of renin inhibition has been encouraged by the phenomenon of "RAAS escape" that means a counterproductive increase in renin and downstream intermediaries of the RAAS upon ACE inhibitor and ARB therapy [81]. In the Aliskiren Observation of Heart Failure Treatment (ALOFT) trial HFrEF patients with NYHA class II-IV ($n = 302$) were randomized to test the addition of aliskiren to an ACE inhibitor/ARB and beta blocker [100]. After 3 months of treatment plasma NT-proBNP and urinary aldosterone were reduced by aliskiren in ALOFT. Despite these promising early results, however, the Aliskiren Trial on Acute Heart Failure Outcomes (ASTRONAUT) study did not reach the primary endpoint in HFrEF patients following an episode of acute decompensation [101]. No significant difference in cardiovascular death or HF rehospitalization at 6 months was observed in patients treated with aliskiren versus standard medical therapy for HF. However, hyperkalemia, hypotension and

renal impairment/failure were more frequent in the aliskiren group than in the placebo group. Subsequently, the Efficacy and Safety of Aliskiren and Aliskiren/Enalapril Combination on Morbi-mortality in Patients with Chronic Heart Failure (ATMOSPHERE) study was designed to compare enalapril ($n = 2336$) with aliskiren ($n = 2340$) and with the combination of the two treatments ($n = 2340$) in NYHA class II-IV HFrEF patients [102]. Nonetheless, the addition of aliskiren to enalapril led to more adverse events, while neither an increase in benefit, nor noninferiority was shown for aliskiren as compared with enalapril. Therefore, aliskiren has not been introduced to GDMT of HF.

HFmrEF

There are no specific trials of RAAS inhibitors in patients with HFmrEF. However, limited data are available from retrospective and group analyses of large clinical studies on HFpEF with LV EF > 40%. Accordingly, treatment with ACE inhibitors, ARBs, ARNi and MRAs may be considered in patients with HFmrEF. As a matter of fact, many patients with HFmrEF receive an ongoing RAAS inhibitor therapy because of ischemic heart disease and co-morbidities. Moreover, in contrast to HFpEF, it seems that guidelines recommend to treat and manage HFmrEF more similar to HFrEF [11, 14, 15].

HFpEF

Unlike in HFrEF, clinical studies on RAAS inhibition have failed to achieve their primary endpoints in HFpEF. These large, randomized, controlled trials include The perindopril in elderly people with chronic heart failure (PEP-CHF) [103], the CHARM-Preserved study [104], the Irbesartan in Heart Failure with Preserved Ejection Fraction Study (I-PRESERVE) [105], the Aldosterone Antagonist Therapy for Adults With Heart Failure and Preserved Systolic Function (TOPCAT) [106] and the Prospective Comparison of ARNI with ARB Global Outcomes in Heart Failure With Preserved Ejection Fraction (PARAGON-HF) [107], all with neutral outcomes on survival. Of note, hospitalizations for HF were reduced by candesartan and spironolactone, and there was a trend towards reduction with sacubitril/valsartan. In addition, subgroups of patients such as women and individuals with a lower LV EF may gain benefit with ARNi. As a matter of fact, because of similar reasons seen in HFmrEF, many HFpEF patients are already treated with RAAS inhibitors. In the recent Effect of Sacubitril/Valsartan versus Standard Medical Therapies on Plasma NT-proBNP Concentration and Submaximal Exercise Capacity in Patients With Heart Failure and Preserved Ejection Fraction (PARALLAX) study the effect of sacubitril/valsartan was compared with that of a RAAS inhibitor (ACE inhibitor/ARB) or placebo in HF patients ($n = 2572$) with LV EF of > 40% including both HFmrEF and HFpEF [108].

Sacubitril/valsartan treatment versus standard RAAS inhibitor or placebo resulted in a greater decrease in plasma NT-proBNP levels at 12 weeks but failed to improve 6-min walk distance at 24 weeks. Taken together, none of the RAAS inhibitory compounds met clinical significance, and thereby evidence-based recommendation in HFpEF [11, 14, 15, 109]. To date, only the sodium-glucose co-transporter 2 (SGLT2) inhibitor empagliflozin has been proven in HFpEF [110], and supposed to be incorporated into future HFpEF guidelines.

Coronavirus Disease 2019 (COVID-19)

Lately, COVID-19 has spread and been responsible for vast majority of morbidity and mortality as well as of HF decompensation worldwide. HF is an important risk factor for death in COVID-19 [111]. COVID-19 is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through a RAAS-mediated mechanism. Although angiotensin II is generated by ACE, it is eliminated by the so-called ACE2 isoform [112]. Important to note, ACE2 is not only responsible for the breakdown of angiotensin II but the cellular binding site for the SARS-CoV-2 [113, 114]. For this reason, uncertainties have arisen regarding the use of RAAS inhibitory medications in the management of COVID-19 infection.

An early prospective cohort study on 8.3 million patients aged 20–99 years reported that neither ACE inhibitors, nor ARBs were associated with increased risk of COVID-19 disease or receiving intensive care unit [115]. It appears that discontinuation versus continuation of RAAS inhibition in COVID-19 has no significant influence on the seriousness of COVID-19 [116]. In patients hospitalized for COVID-19, however, ACE inhibitor or ARB use is associated with lower levels of inflammation and lower risk of in-hospital outcomes [117]. On the basis of the data of two randomized clinical trials, namely the Randomized Elimination or ProLongation of Angiotensin Converting Enzyme inhibitors and angiotensin receptor blockers in Coronavirus Disease 2019 (REPLACE COVID trial) [118] and Angiotensin Receptor Blockers and Angiotensin-converting Enzyme Inhibitors and Adverse Outcomes in Patients With COVID19 (BRACE-CORONA) [119], RAAS inhibitors can be safely continued in patients admitted to hospital with COVID-19. Nonetheless, both studies predominantly evaluated patients with previously receiving ACE inhibitors or ARBs, whilst HF was an important exclusion criterion. Unlike the 1.4 million nationwide cohort from the Swedish National Patient Registry, in which patients with hypertension, HF, diabetes, kidney disease or ischemic heart disease were included, and was found that use of RAAS inhibition was not associated with increased risk of hospitalization for or death from COVID-19 [120]. On the contrary, the withdrawal of GDMT (e.g. ACE inhibitor/ARBs and MRAs) is associated with a significant increase of in-hospital mortality [111]. Moreover, it turned out that discontinuation of RAAS inhibitory medication worsens cardiovascular status without affecting ACE2 levels [112] (Tables 19.1, 19.2 and 19.3)..

Table 19.1 Clinical trials with RAAS inhibitors in HFrEF

HFrEF	Compound	Compared with	*n* (randomized)	NYHA class	EF	Follow-up (average)	Outcome on primary endpoint	Reference
ACE inhibitor								
CONSENSUS I	Enalapril	Placebo	253	IV	No data	188 days	Positive ($P = 0.002$)	[16]
SOLVD treatment	Enalapril	Placebo	2569	I–IV	$\leq 35\%$	41.4 months	Positive ($P = 0.0036$)	[19]
SOLVD prevention	Enalapril	Placebo	4228	I–II	$\leq 35\%$	37.4 months	Neutral ($P = 0.30$)	[21]
SAVE	Captopril	Placebo	2231	No data	$\leq 40\%$	42 months	Positive ($P = 0.019$)	[22]
AIRE	Ramipril	Placebo	2006	No data	No data	15 months	Positive ($P = 0.002$)	[25]
ATLAS	Lisinopril (high dose)	Lisinopril (low dose)	3164	II–IV	$\leq 30\%$	45.7 months	Positive ($P = 0.002$)	[18]
TRACE	Trandolapril	Placebo	1749	No data (post-MI)	$\leq 35\%$	24–50 months	Positive ($P = 0.001$)	[23]
ARB								
ELITE-II	Losartan	Captopril	3152	II–IV	$\leq 40\%$	555 days	Neutral ($P = 0.16$)	[38]
VAL-HeFT	Valsartan	Placebo	5010	II–IV	$< 40\%$	23 months	Neutral ($P = 0.80$)	[33]
OPTIMAAL	Losartan	Captopril	5477	No data	$< 35\%$	2.7 years	Neutral ($P = 0.07$)	[39]

(continued)

Table 19.1 (continued)

HFrEF	Compound	Compared with	*n* (randomized)	NYHA class	EF	Follow-up (average)	Outcome on primary endpoint	Reference
CHARM-Alternative	Candesartan	Placebo	2028	II–IV	≤ 40%	33.7 months	Positive ($P < 0.001$)	[35]
CHARM-Added	Candesartan	Placebo	2548	II–IV	≤ 40%	41 months	Positive ($P = 0.01$)	[36]
VALIANT	Either valsartan or valsartan + captopril	Captopril	14,703	No data (post-MI)	≤ 35%	24.7 months	Neutral ($P = 0.98$ valsartan versus captopril; $P = 0.73$ valsartan + captopril versus captopril)	[40]
HEAAL	Losartan (high dose)	Losartan (low dose)	3846	II–IV	≤ 40%	4.7 years	Positive ($P = 0.027$)	[37]
ARNi								
PARADIGM-HF	Sacubitril/valsartan	Enalapril	8442	II–IV	≤ 40%	27 months	Positive ($P < 0.001$)	[47]
TRANSITION	Sacubitril/valsartan (pre-discharge)	Sacubitril/valsartan (post-discharge)	1002	I–IV	≤ 40%	10 weeks	Neutral ($P = 0.099$)	[72]
PIONEER-HF	Sacubitril/valsartan	Enalapril	881	I–IV	≤ 40%	8 weeks	Positive ($P < 0.001$)	[74]
PARADISE-MI	Sacubitril/valsartan	Ramipril	5661	No data	No data	22 months	Neutral ($P = 0.17$)	[78]

(continued)

Table 19.1 (continued)

HFrEF	Compound	Compared with	*n* (randomized)	NYHA class	EF	Follow-up (average)	Outcome on primary endpoint	Reference
MRA								
RALES	Spironolactone	Placebo	1663	II–IV	≤ 35%	24 months	Positive ($P <$ 0.001)	[79]
EPHESUS	Eplerenone	Placebo	6642	No data (post-MI)	≤ 40%	16 months	Positive ($P =$ 0.008)	[86]
EMPHASIS-HF	Eplerenone	Placebo	2737	II	≤ 35%	21 months	Positive ($P <$ 0.001)	[80]
Renin inhibitor								
ALOFT	Aliskiren	Placebo	302	II–IV	31 ± 5.5% (mean ± SD)	3 months	Positive ($P =$ 0.0106)	[100]
ASTRONAUT	Aliskiren	Placebo	1639	I–IV	≤ 40%	11.3 months	Neutral ($P =$ 0.41 6 months; $P = 0.36$ 12 months)	[101]
ATMOSPHERE	Either aliskiren or aliskiren + enalapril	Enalapril	7016	II–IV	≤ 35%	36.6 months	Negative ($P =$ 0.91 aliskiren versus enalapril; $P =$ 0.17 aliskiren + enalapril versus enalapril)	[102]

Note That V-HeFT-II on enalapril versus hydralazine-isosorbide dinitrate is not included as corresponding data are missing [24]

Table 19.2 Clinical trials with RAAS inhibitors in HFpEF

HFpEF	Compound	Compared with	*n* (randomized)	NYHA class	EF	Follow-up (average)	Outcome on primary endpoint	Reference
ACE inhibitor								
PEP-CHF	Perindopril	Placebo	850	I–IV	56–66%	2.1 years	Neutral ($P = 0.545$)	[103]
ARB								
CHARM-Preserved	Candesartan	Placebo	3023	II–IV	> 40%	36.6 months	Neutral ($P = 0.118$)	[104]
I-PRESERVE	Irbesartan	Placebo	4128	II–IV	≥ 45%	49.5 months	Neutral ($P = 0.35$)	[105]
ARNi								
PARAGON-HF	Sacubitril/valsartan	Valsartan	4822	II–IV	≥ 45%	35 months	Neutral ($P = 0.06$)	[107]
PARALLAX	Sacubitril/valsartan	Enalapril or valsartan or placebo	2572	No data	> 40%	12/24 weeks	Positive ($P < 0.001$ 12 weeks)/neutral ($P =$ 0.42 24 weeks)	[108]
MRA								
TOPCAT	Spironolactone	Placebo	3445	I–IV	≥ 45%	3.3 years	Neutral ($P = 0.14$)	[106]

Table 19.3 Actions of RAAS inhibition on reverse remodeling in HF

	Species	References
ACE inhibitor/ARB		
Reduced chamber dilatation and infarction size and improved survival	Rat	[28, 29]
Enhanced intracellular Ca^{2+} handling, cellular and membrane protein expression and gene expression levels	Rat	[30]
Prevention of LV dilatation and increased EF	Human	[27]
Reduced LV volumes and increased EF	Human	[32, 33]
Enhanced cardiac NO production and attenuated beta adrenergic signaling	Human	[31]
Improved LV end-diastolic dimension and fractional shortening	Human	[34]
ARNi		
Reduced cardiac fibrosis and hypertrophy	Rat	[64]
Reduces fibrosis and increased EF	Mouse	[65]
Improved systolic and diastolic LV function	Human	[66, 67]
Reduced left atrial and LV volumes, improved diastolic function and reduced NT-probnp	Human	[68]
Increased EF and reduced left atrial and LV volume indices	Human	[69]
Reversed left atrial remodeling	Human	[71]
MRA		
Restored endothelium-dependent vasodilation	Rat	[88]
Reduced post-MI fibrosis	Rat	[89, 90]
Preserved LV volumes and EF, reduced LV end-diastolic wall stress, cardiomyocyte cross-sectional area and fibrosis	Dog	[91]
Improved diastolic function	Rat	[92]
Improved LV remodeling and neurohormonal activation	Rat	[93]
Attenuated electrical remodeling	Rat	[94]
Reduced LV end-diastolic volume index and fibrosis and increased EF	Human	[95]

Summary

In summary, RAAS inhibition remains the cornerstone of GDMT in HF. Although there is clear evidence of targeting RAAS in the failing heart, mechanistic gaps and practical insufficiencies still require attention. On the one hand, research should be encouraged on novel RAAS inhibitory therapies (e.g. ARNi) regarding their mechanism of action and contribution to reverse cardiac remodeling. On the other hand, clinical practice should be improved to achieve maximal optimal therapy for HF patients. Finally, ongoing experimental and clinical studies presumably provide further evidence on broader indications of ARNi.

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Chapter 20
Renin Angiotensin System Inhibitors in Heart Failure with Reduced Ejection Fraction: Clinical Evidence and Considerations for Use

Sigurd Hartnett and Buddhadeb Dawn

Abstract The renin angiotensin system (RAS) plays an important role in the regulation of body fluids and blood pressure. Therapy with various agents that modulate the components of RAS has been associated with improvement in outcomes in patients with heart failure. Although angiotensin-converting enzyme inhibitors (ACE-Is), angiotensin II receptor blockers (ARBs) and the angiotensin receptor neprilysin inhibitors (ARNI) are all associated with clinical benefits, some controversies persist with regard to the superiority of one class over the other. A careful review of data generated in numerous clinical trials testing the safety and efficacy of these classes of agents conducted over nearly four decades may provide insights necessary to make informed clinical decisions when treating patients with heart failure with reduced ejection fraction. This review is focused on the scientific evidence supporting the efficacy of these widely used classes of medicines and comparative data when available.

Keywords Renin angiotensin system · Heart failure with reduced ejection fraction · Angiotensin-converting enzyme inhibitors · Angiotensin II receptor blockers · Angiotensin receptor neprilysin inhibitors

Introduction

Cardiovascular disease (CVD) continues to be the leading cause of death in the USA [1, 2]. Globally, in 2017, approximately 17.8 million deaths were attributed to CVD, which was an increase of 21.1% from 2007 [2]. A major component of the overall CVD burden is heart failure (HF), which contributes significantly toward CV deaths. Furthermore, the prevalence of HF is increasing with an aging population worldwide. Between 2013 and 2016, approximately 6.2 million American adults ≥ 20 years of age had HF as compared with 5.7 million between 2009 and 2012

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[2]. At this trajectory, it is predicted that > 8 million people in the US, about 1 in 33 adults, will have HF by 2030 [3]. The financial cost of the total direct medical expenses for HF between 2012 and 2030 are projected to increase from $21 billion to $53 billion [3]. In order to slow the progression of HF with reduced ejection fraction (HFrEF) and associated expenses, the importance of clear understanding of the guideline-directed HFrEF medications as well as the underlying clinical trials is paramount. This chapter will focus on one such class of HFrEF medications that includes Angiotensin Converting Enzyme Inhibitors (ACE-I), Angiotensin II Receptor Blockers (ARB), and Angiotensin Receptor-Neprilysin Inhibitor (ARNI), all of which modulate the renin angiotensin system (RAS).

Renin Angiotensin System

The RAS plays an important role in the systemic regulation of water and electrolytes in various fluid compartments throughout the body and also impacts blood pressure through vasoconstriction. Alterations in the functionality of this system leads to dysregulation of cardiovascular homeostasis and results in acute as well as chronic pathological states. Given the complexity of interactions among the diverse components of this hormone system, understanding the roles of individual molecules that comprise RAS is important before discussing specific pharmacologic inhibitors. Renin is produced from the precursor prorenin synthesized in the juxtaglomerular cells in the kidney and secreted in response to decreased arterial pressure, low sodium in the distal tubule, and sympathetic nervous system activation (beta-1 adrenoceptors) [4]. Renin cleaves the liver-secreted peptide angiotensinogen to angiotensin I (Ang I), which is subsequently converted to angiotensin II (Ang II) by the angiotensin-converting enzyme (ACE) found in the lungs, kidneys, and endothelial cells. Ang II is a potent vasoconstrictor acting through smooth muscle in arterial walls leading to elevated blood pressure. In addition, Ang II stimulates aldosterone secretion from the adrenal glands causing water and sodium retention, and influences prostaglandins that regulate renal vasoconstriction [5]. Most of effects of Ang II are mediated through the Ang II type 1 receptor (AT1R) [6]. Importantly, ACE is also a component of the kinin-kallikrein system (KKS) and participates in the cleavage of bradykinin, a vasodilator peptide, which has been implicated in the mediation of vascular beneficial effects of ACE-Is [7]. Although several different classes of agents have been used to inhibit the effects of RAS in clinical practice, three major types of RAS inhibitors, ACE-I, ARB, and ARNI, are commonly used for the treatment of HFrEF [8]. The current review will focus on these agents.

Clinical Trials of Angiotensin-Converting Enzyme Inhibitors in HFrEF

ACE-Is block the conversion of Ang I to Ang II, a vasoactive peptide with multifaceted effects leading to retention of sodium and water and increased blood pressure [5]. Several ACE-Is have been studied in patients with HFrEF, including captopril, lisinopril, and enalapril. In a placebo-controlled randomized clinical trial (RCT) involving 92 patients with refractory HF, treatment with captopril was associated with clinical improvement in 80% of patients compared with 27% in the placebo group over a 10-week period [9]. The New York Heart Association (NYHA) functional class improved by 0.52 and exercise tolerance increased by 24% in captopril-treated patients [9]. In a subsequent RCT involving 130 patients with HFrEF, treatment with lisinopril resulted in greater improvement in EF (8% versus 2% in placebo-treated patients), improvement in NYHA class, and exercise duration on the background of digoxin and diuretics [10]. Finally, in three trials reported between 1987 and 1991, treatment with enalapril was able to reduce mortality by 16–40% in patients with HFrEF. In the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) in 253 patients with NYHA class IV severe congestive HF on background medications including vasodilators, the crude mortality was reduced by 40% in the enalapril arm compared with the placebo arm at 6 months [11]. Reported in 1991, the results of the Studies of Left Ventricular Dysfunction (SOLVD, N = 2,569 patients) trial showed a 16% reduction in mortality with the addition of enalapril to heart failure medications [12]. Furthermore, the results from the Veterans Administration Cooperative Vasodilator-Heart Failure trial II (V-HeFT II, N = 804 patients) showed that the addition of enalapril to a combination of vasodilators (hydralazine-isosorbide dinitrate) as well as digoxin and diuretics could reduce mortality by an additional 28% at 2 years [13]. The placebo-controlled RCTs comparing the effects of ACE-Is in the background of various contemporary therapies of HFrEF are summarized in Table 20.1.

Clinical Trials of Angiotensin II Receptor Blockers in HFrEF

ARBs inhibit the effects of Ang II by selectively blocking the AT1R [14]. Several ARBs, including candesartan and valsartan, have been studied in patients with HFrEF. In the Candesartan in Heart Failure: Assessment of Reduction in Mortality and Morbidity (CHARM)-Alternative trial (N = 2,028 patients) in patients with ACE-I intolerance, candesartan reduced the composite endpoint of cardiovascular death or HF hospitalization by 7% (relative risk reduction of 30%) with no difference in discontinuation rates between candesartan and placebo over 2.75 years (33 months) with background HF medications including beta-blockers (54%), spironolactone (23%), vasodilators (42%), and diuretics (85%) [15]. However, the absolute reduction of 3.2% (relative reduction of 12.1%) in cardiovascular death in the candesartan group

Table 20.1 Clinical trials of ACE-Is in HFrEF

Trial	N	Agent	Entry requirements	Primary endpoints		Secondary endpoints			
				Endpoint	Findings	Mortality (all-cause)	Mortality (CV)	MI	CVA
Captopril versus placebo, refractory HF [9]	92	Captopril	Chronic refractory HF, exercise 3–12 min	Clinical improvement (NYHA class)	−0.52	–	–	–	–
Lisinopril versus placebo, HF [10]	130	Lisinopril	EF < 45, NYHA class II-IV HF	Clinical improvement (EF)	+ 6%	–	–	–	–
CONSENSUS [11]	253	Enalapril	NYHA class IV HF	Mortality (6-month) and cause of death	−40%	−27 (6–12 months)	–	–	–
SOLVD [12]	2,569	Enalapril	EF ≤ 35	i) Mortality ii) Composite (mortality or HF hospitalization)	i) −16% ii) −26%	i) Primary	−18%	–	–
V-HeFT II [13]	804	Enalapril	EF < 45, decreased exercise tolerance	Morality (2-yr)	−28%	Primary	–	–	–

was not statistically significant. Additionally, results from the Valsartan Heart Failure Trial (Val-HeFT, N = 5,010 patients) demonstrated that treatment with valsartan reduced the composite endpoint of mortality and HF hospitalization in patients with NYHA class II, III, IV at 27 months [16]. However, this difference was again driven primarily by reduced hospitalizations, while there was no difference in mortality between groups [16]. Importantly, more than 92% of patients in each arm were receiving ACE-Is at baseline. Interestingly, the results of sub-group analysis showed that valsartan reduced mortality in patients who were not on ACE-1 or beta-blocker, however, significantly increased the risk of mortality in patients receiving both ACE-1 and beta-blocker [16]. This trend toward harm was consistent in three additional trials comparing ramipril and telmisartan as well as valsartan and captopril [17–19]. Thus, in general, the combination of ACE-I and ARB is less likely to be beneficial, making the selection of ACE-I or ARB an important decision. The findings from RCTs examining the efficacy of ARBs in HFrEF are summarized in Table 20.2.

Clinical Trials Comparing the Efficacy of ACE-Is and ARBs in HFrEF

Since both ACE-Is and ARBs were shown to be beneficial for the treatment of HFrEF, the effects of ACE-Is and ARBs have been directly compared in several RCTs. The first such RCT (Evaluation of Losartan in the Elderly Study, ELITE, N = 722 patients) compared the efficacies of losartan versus captopril in patients over 65 with NYHA class II-IV HFrEF. The results showed that fewer patients discontinued losartan (12.2% versus 20.8% in captopril arm) due to adverse effects, while losartan also reduced all-cause mortality by 3.9% (46% risk reduction) as compared with captopril [20]. Based on these encouraging data, the investigators embarked upon a subsequent larger RCT, ELITE II (N = 3,152 patients), which was designed to assess the impact of losartan on survival. The results of ELITE II failed to identify any significant differences in mortality, sudden death, or resuscitated cardiac arrest between losartan 50 mg daily and captopril 50 mg three times daily in patients with EF $\leq$ 40% and NYHA class II-IV HFrEF [21]. However, significantly fewer patients discontinued ARB as compared with ACE-I (9.7% versus 14.7%) due to adverse effects, which included cough. In the Optimal Trial in Myocardial Infarction with the Angiotensin II Antagonist Losartan (OPTIMAAL, N = 5,477 patients), the effects of valsartan and captopril on all-cause mortality was directly compared in patients (age $\geq$ 50 years) with confirmed myocardial infarction (MI) and HFrEF [22]. After a mean follow-up of 2.7 years, the results identified a non-significant reduction (P = 0.07) in total mortality in favor of captopril. Finally, in the Valsartan in Acute Myocardial Infarction Trial (VALIANT, N = 9,818 patients) in patients with acute MI and EF $\leq$ 35% and/or signs of clinical HF, the results showed no difference in all-cause mortality in the valsartan group compared with captopril at 24.7 months [19].

Table 20.2 Clinical trials of ARBs in HFrEF

Study	N	Agent	Entry requirements	Primary endpoints		Secondary endpoints			
				Endpoint	Findings	Mortality (all cause)	Mortality (CV)	MI	CVA
Val-HeFT [16]	5,010	Valsartan	EF < 40, NYHA class II–IV HF	i) Mortality (27 months) ii) Composite (mortality, cardiac arrest, HF hospitalization, IV inotrope or vasodilator Tx × 4 h)	i) NS ii) −13%	i) Primary	–	–	–
CHARM-Alternative [15]	2,028	Candesartan	EF ≤ 40, NYHA class II–IV HF	Composite (CV death or HF hospitalization, 33 months)	−30%	–	−20%	–	–

In addition to the above RCTs that provide the highest quality of clinical evidence, several meta-analyses have been performed to address the question whether one group of RAS inhibitor was superior to another in patients with HFrEF. One such meta-analysis of ACE-Is and ARBs in patients with HFrEF analyzed data from 38 RCTs with 47,662 patients [23]. The results showed that ACE-Is reduced all-cause mortality by 11% and CV death by 14%, while ARB use was not associated with reduced mortality [23]. Consistently, in a larger meta-analysis (106 RCTs enrolling 254,301 patients) that also included patients without HF, ACE-Is, but not ARBs, reduced all-cause mortality, CV death and MI [24]. However, in sensitivity analysis, the differences were not significant when the analysis was restricted to data from RCTs published after 2000, suggesting that the survival benefits of ACE-I over ARBs in these meta-analyses might possibly be attributed to higher rates of mortality in the placebo arms in the earlier ACE-I inhibitor trials [24]. Regardless, it is important to remember that results from meta-analyses are generated using data from prior trials, and therefore suffer from inherent limitations, including those resulting from heterogeneity [25]. For most purposes of clinical decision making, meta-analyses should not be used to arrive at conclusions, but these may serve as a consensus builder when appropriate [26]. Meta-analysis may also be used for the generation of hypothesis for future trials. The results from RCTs comparing ARBs versus ACE-Is in patients with HFrEF are summarized in Table 20.3.

Clinical Trials with Angiotensin Receptor-Neprilysin Inhibitor (ARNI)

Angiotensin receptor-neprilysin inhibitor (ARNI) is a combination of a neprilysin inhibitor (secubitril) and an ARB (valsartan) that was recently approved for the treatment of HFrEF [8]. Neprilysin is a neutral endopeptidase or membrane metalloendopeptidase that is expressed primarily in the kidney. It catalyzes the degradation of natriuretic peptides (NP) such as the atrial NP (ANP), brain NP (BNP), C-type NP (CNP), bradykinin, and even contributes to the breakdown of angiotensin II [27]. The roles of ANP and BNP in vasodilation, natriuresis, and diuresis have been well established. Although HFrEF is typically associated with an increase in levels of various NPs, even the elevated levels prove inadequate toward alleviating the fluid overload in HF. Thus, a strategy to manage HFrEF would be to increase circulating NPs, which can be accomplished by either increasing the production of NPs or decreasing their degradation. In the past, intravenous administration of nesiritide, a recombinant BNP analogue, resulted in a reduction in pulmonary-capillary wedge pressure and improvement in global clinical status, including reduced dyspnea and fatigue in hospitalized patients with decompensated HF [28]. However, the Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure (ASCEND-HF) trial failed to show any reduction in mortality or HF rehospitalization at 30 days when treated with nesiritide versus placebo in patients with decompensated HF [29].

Table 20.3 Clinical trials comparing ACE-Is and ARBs in HFrEF

Study	N	Agent	Entry req	Primary endpoints		Secondary endpoints			
				Endpoint	Findings	Mortality (all-cause)	Mortality (CV)	MI	CVA
ELITE [20]	722	Losartan versus captopril (CTRL)	EF ≤ 40, NYHA II–IV HF	i) Safety-increased creatinine > 0.3 mg/dL (48 weeks) ii) Composite (all−cause mortality and/or HF hospitalization)	i) NS ii) −32%	−46%	–	–	–
ELITE II [21]	3,152	Losartan versus captopril	EF ≤ 40, NYHA II–IV HF	All-cause mortality (1.5 yrs)	NS	Primary	–	NS	NS
OPTIMAAL [22]	5,477	Losartan versus captopril	AMI, EF < 35, HF (signs or symptoms)	All-cause mortality (2.7 yrs)	NS	Primary	+ 17%	NS	NS
VALIANT [19]	14,703	Valsartan versus captopril versus both	AMI, EF ≤ 35, ± HF (signs)	All-cause mortality (24.7 months)	NS	Primary	NS	–	–
VALIANT [18]	As above	As above	As above	As above	As above	NS	–	NS	NS

Thus, research efforts were directed to investigations into inhibiting the breakdown of NPs in the setting of HF. While sacubitril-valsartan is the only combination ARNI in this group at this time, several compounds such as ecadotril, candoxatril, and omapatrilat have been tested with neprilysin inhibitors but were discontinued due to underwhelming efficacy or side-effect profiles [27].

The Prospective Comparison of ARNI with ACEI to Determine Impact on Global Mortality and Morbidity in Heart Failure (PARADIGM-HF, N = 8,442 patients) was the first pivotal ARNI trial. Patients with NYHA class II-IV HF with EF ≤ 40% were randomized to receive sacubitril-valsartan (LCZ696) or enalapril with the primary outcome of a composite of CV death and HF hospitalization [30]. The trial was stopped early with a median follow-up of 27 months after safety committee found overwhelming benefit of sacubitril-valsartan versus enalapril group. There was a 4.7% absolute reduction (hazard ratio [HR] 0.80) in the primary outcome; a 2.8% reduction (HR 0.84) in all-cause mortality; and a 3.2% reduction (HR 0.80) in CV death in the LCZ696 group versus enalapril group. HF hospitalization and patient symptoms improved as well. As for the safety profile, sacubitril-valsartan had increased proportions of patients with hypotension and non-serious angioedema but decreased renal impairment, hyperkalemia, and cough compared with enalapril [30].

Next, the PIONEER-HF (Comparison of Sacubitril-Valsartan versus Enalapril on Effect on NT-proBNP in Patients Stabilized from an Acute Heart Failure Episode) trial randomized 881 patients hospitalized with acute decompensated HFrEF with EF ≤ 40% as well as NT-proBNP ≥ 1600 pg/mL or BNP ≥ 400 pg/mL to sacubitril-valsartan or enalapril after hemodynamic stabilization and evaluated changes in biomarkers and safety parameters at 8 weeks [31]. Hemodynamic stabilization was defined as having a systolic blood pressure ≥ 100 mmHg, no use of intravenous vasodilators for 6 h, no use of intravenous inotropes for 24 h, and no increase in intravenous diuretics. A minimum of 36 h of washout period was given prior to the initiation of sacubitril-valsartan to reduce the risk of angioedema with recent ACE-I usage. NT-proBNP levels decreased in both treatment groups with a significantly greater reduction in the sacubitril-valsartan group (-46.7%) compared with the enalapril group (−25.3%). Importantly, for safety outcomes, there were no significant differences with worsening renal function, hyperkalemia, symptomatic hypotension and angioedema. These data suggested that sacubitril-valsartan could be safely started as inpatient prior to discharge (hemodynamically stable) after 36 h washout of ACE-I.

However, emerging evidence with sacubitril-valsartan indicates that the selection of a class of RAS inhibitors in patients with HFrEF may not be straightforward. The data from two recent RCTs, PARADISE-MI (Angiotensin Receptor-Neprilysin Inhibtion in Acute Myocardial Infarction [32]) and HFN-LIFE (Entresto [LCZ696] in Advanced Heart Failure [33]) have challenged the philosophy that ARNI is superior to ACE-I and ARB, respectively, in all situations of heart failure. In PARADISE-MI, a total of 5,661 patients with acute MI complicated by a reduced EF, pulmonary congestion or both were randomized (1:1) to receive either sacubitril-valsartan or ramipril and followed for a median of 22 months with primary outcomes of death

from cardiovascular causes or incident HF [32]. While approximately 1,000 patients had EF > 40%, the percentage of remaining patients were similarly divided between having EF < 30%, between 30–35, and 35–40%. While the events were numerically lower compared with the ramipril group, sacubitril-valsartan was not associated with a significant reduction in the primary endpoints of CV death or incident HF (11.9% versus 13.2% in ramipril group, $P = 0.17$), or in sub-group analysis (CV death 5.9% versus 6.7% in ramipril group) as well as mortality from any cause (7.5% versus 8.5% in ramipril group) [32].

Importantly, less than 1% of patients enrolled in the above PARADIGM-HF had NYHA class IV symptoms. To examine any potential benefit of ARNI (sacubitril-valsartan) in patients with advanced HFrEF, the HFN-LIFE trial randomized (1:1) 335 patients with advanced HFrEF and recent NYHA class IV symptoms to ARNI versus valsartan with a primary endpoint of change in area under the curve (AUC) for the ratio of NT-proBNP levels compared with baseline during 24 weeks of therapy [33]. Although 49 patients (29%) discontinued ARNI during the trial, there was no significant change found in the median NT-proBNP AUC in patients assigned to sacubitril-valsartan as compared with valsartan. In addition, sacubitril-valsartan also failed to improve the clinical composite outcomes of the number of days alive, days out of hospital, and days free from heart failure events. Thus, these two trials suggest that the etiology (such as acute MI) and/or severity of disease (end-stage HFrEF with NYHA class IV symptoms) may impact the efficacy of therapy with ARNI.

A recent article reported a retrospective analysis of the effectiveness of sacubitril-valsartan versus ACE-I or ARB with a total of 7,893 propensity score-matched pairs of patients in routine clinical practice with a mean follow-up of 6.3 months [34]. Sacubitril-valsartan reduced the HR of composite all-cause mortality or all-cause hospitalization with each individual component also significantly reduced. Interestingly, it did not reduce the hospitalization due to HF. Furthermore, in sub-group analysis, the reduced risk of composite endpoint was seen only in white patients, but not in black patients (21% of population). In the setting of black/African American representation in previous trials (5% of 8,442 patients in PARADIGM-HF and 35% of 881 patients in PIONEER-HF), this discrepancy in outcomes will require further studies. Together, the emerging evidence from recent clinical trials question the broad applicability of the American College of Cardiology (ACC) Expert Consensus Decision Pathway for Optimization of Heart Failure Treatment in recommending that ARNI are the preferred agent among angiotensin inhibitors [8]. Trials comparing ARNI versus ACE-I or ARB in heart failure are summarized in Table 20.4.

Selection of ACE-I versus ARB in HFrEF

The systemic RAS is a complex network of molecules that influences the regulation of blood pressure and body fluids, impacting the physiology and function of the cardiovascular and renal systems, which are affected in HFrEF. Given the attendant signaling complexities through various receptors, some of the agents that modulate

Table 20.4 Clinical trials comparing ARNI versus ACE–I or ARB in HFrEF

Study	N	Agent	Entry req	Primary endpoints		Secondary endpoints			
				Endpoint	Findings	Mortality (all–cause)	Mortality (CV)	MI	CVA
PARADIGM–HF [30]	8,442	Sacubitril-valsartan versus enalapril	EF $\leq$ 40, NYHA class II–IV HF	Composite (CV mortality and/or HF hospitalization, 27 months)	−20%	−16%	−20%	–	–
PARADISE-MI [32]	5,661	Sacubitril-valsartan versus ramipril	AMI and EF $\leq$ 40 $\pm$ signs HF	Composite (CV mortality and/or symptomatic HF (outpatient or hospitalized, [22 months])	NS	NS	NS	–	–
HFN-LIFE [33]	335	Sacubitril-valsartan versus valsartan	EF < 35, NYHA class IV HF	NT-proBNP level proportional change (area under the curve), (24 weeks)	NS	–	–	–	–

RAS may also lead to unwanted adverse effects despite improvements in clinical outcomes. Since all patients with HFrEF are not the same, treatment with RAS inhibitors should be individualized and guided by evidence from RCTs whenever available.

As discussed above, data from clinical trials over the past several decades have established that both ACE-Is and ARBs improve certain clinical outcomes in patients with HFrEF. For the treatment of hypertension, it has been suggested that perhaps ARBs should be preferred over ACE-Is as first-line agents [35]. While select results stemming from the analysis of heterogeneous large datasets may indicate that ACE-Is and ARBs are similar in efficacy in treating hypertension [36], the superiority of ARBs over ACE-Is has not been definitively proven in RCTs in patients with hypertension. Furthermore, it will be prudent to remember that clinical evidence from trials of hypertension may not be entirely applicable to patients with HFrEF. The following additional considerations should be taken into account while comparing the suitability of ACE-Is versus ARBs in HFrEF.

Adverse effects and patient intolerance. The use of both ACE-Is and ARBs are associated with side-effects such as hypotension, hyperkalemia, renal dysfunction, dizziness and/or syncope, dry cough, and angioedema. The reported percentages of these side-effects have varied among trials. In particular, the incidence of cough has varied considerably not only from trial to trial, but also with the use of specific ACE-Is [37]. Aside from converting Ang I to Ang II, ACE also breaks down bradykinin, a potent vasodilator, which can sensitize somatosensory nerve fibers in the airways. In guinea pigs, administration of a bradykinin receptor antagonist was able to suppress cough triggered by inhaled citric acid after treatment with captopril for 2 weeks [38]. Although the etiology of cough may potentially involve additional mechanisms [37], ARBs offer suitable alternatives for patients who are unable to tolerate ACE-Is due to cough and other side-effects. In this regard, the rates of discontinuation due to side-effects have been lower with ARBs in trials that have compared ACE-I and ARB therapies [21, 22].

A potentially life-threatening side-effect with the use of RAS inhibitors is angioedema. With ACE-I use, angioedema likely results from increased bradykinin levels secondary to ACE inhibition [39]. Although ARBs do not impair the degradation of bradykinin, these are also known to cause angioedema through unclear mechanisms. The incidence of angioedema with ACE-Is and ARBs has been evaluated in analysis of pooled data from clinical trials. In a meta-analysis by Makani et al., the weighted incidence of angioedema with ACE-Is was 0.30% compared with 0.11% with ARBs [40]. In another analysis, the incidence rates of angioedema per 1000 person-years were 4.28 (95% CI: 4.24–4.54) and 1.66 (95% CI: 1.47–1.86) for ACE-Is and ARBs, respectively [41]. However, in another large dataset, the incidence of angioedema with ACE-I was noted to be 1.97 (95% CI: 1.77–2.18) cases per 1000 person years [42]. Despite the rather low incidence, the rates of angioedema were nearly 4 times higher in blacks and 50% higher in women [42].

Impact on mortality. The beneficial effects of ACE-Is on all-cause as well as cardiovascular mortality in patients with HFrEF have been documented in multiple trials. In CONSENSUS, the relative risk for all-cause mortality in the enalapril group

was reduced by 40% at 6 months, and by 27% at the end of study [11]. In SOLVD, the risk for all-cause mortality was reduced by 16% in the enalapril arm, with the largest reduction in deaths due to progressive HF [12]. In V-HeFT II, after 2 years of follow-up, enalapril reduced mortality by 28% compared with hydralazine-isosorbide dinitrate therapy [13], which was already shown to improve mortality in V-HeFT I [43].

In contrast, the impact of ARB treatment on mortality in patients with HFrEF has been tenuous. In Val-HeFT, all-cause mortality was nearly identical in valsartan and placebo arms (19.7% versus 19.4%, respectively) [16]. In CHARM Alternative, the HR for both all-cause mortality (unadjusted HR 0.87 [0.74–1.03]) and cardiovascular mortality (unadjusted HR 0.85 [0.71–1.02]) achieved statistical significance in favor of candesartan only after adjustment for co-variates [15]. Among trials that compared ACE-I and ARB treatments directly, results have been discordant. In ELITE, the risk of all-cause death was reduced significantly in the losartan arm compared with captopril [20]. However, and intriguingly, this survival benefit with losartan was not recapitulated in the larger ELITE II trial [21]. In OPTIMAAL, the total mortality was non-significantly ($P = 0.07$) reduced in favor of captopril [22]; and in VALIANT, there was no difference [19]. Although these data may suggest that the effects of ARB therapy on survival may not be inferior compared with ACE-I therapy in HFrEF, the cumulative evidence from a number of RCTs certainly does not prove superiority of ARBs over ACE-Is. Indeed, in meta-analysis, ACE-I treatment was associated with protection against all-cause and cardiovascular mortality in patients with heart failure, while ARB treatment was not [23]. Similar observations regarding the efficacy of ACE-I toward reducing mortality and the lack of such benefits with ARB treatment have been made in meta-analysis of data from patients with hypertension [44] and diabetes mellitus [45].

Impact on myocardial infarction. Although both ACE-Is and ARBs reduce blood pressure, the outcomes of treatment with these two classes of agents on incident cardiovascular events, especially myocardial infarction (MI), have varied in clinical trials [46, 47]. In CHARM-Alternative, which included 2028 ACE-I-intolerant patients with reduced EF, the incidence of MI was significantly more in the candesartan group compared with the placebo group [15]. Analogous observations were made in patients with hypertension in the Valsartan Antihypertensive Long-term Use Evaluation trial (VALUE, N = 15,245 patients), wherein patients treated with valsartan experienced increased incidence of MI and angina pectoris compared with amlodipine-treated patients [48]. Although the specific molecular basis for such observations remain unclear, increased activation of AT2Rs by the elevated levels of Ang II subsequent to ARB therapy may potentially play a role [46, 47, 49]. However, additional data from clinical trials will be necessary to arrive at definitive conclusions.

Conclusions

ACE-I, ARB, and ARNI represent key components of optimal medical therapy for patients with HFrEF. Given the widespread use of these agents, it is important to understand that all heart failure patients are not the same, and treatment should be individualized and guided by high-quality data. Cumulative evidence from various RCTs and meta-analyses suggests that clinical cardiovascular outcomes of therapy with ACE-I and ARB are largely similar in HFrEF. However, notable differences include a proven survival benefit with ACE-Is, increased discontinuation rates with ACE-Is, and a greater potential for coronary events with ARBs. Although ARNIs are currently recommended as the preferred agents for RAS inhibition in HFrEF, newer data do not support their superior efficacy in the settings of acute MI with new-onset HFrEF or advanced HFrEF. Thus, a clinician's decision to use ACE-I versus ARB versus ARNI in HFrEF should be based not only on guidelines, but also on emerging evidence in conjunction with patient characteristics and wishes.

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Chapter 21
Protective Role of the AT_1 Receptor in the Heart: A Biosensor of Stress

Fouad A. Zouein, Babbette B. LaMarca, and George W. Booz

Abstract The renin angiotensin system plays an important role in blood pressure regulation and its primary component angiotensin II has been implicated in adverse cardiac remodeling in response to hypertension. However, questions remain as to whether these actions of angiotensin II are mediated directly at the level of the heart via its principal receptor, AT_1R. A more nuanced reading of the literature suggests that AT_1R acts in concert with other signaling events, such as inflammation and tyrosine kinase receptor transactivation, to induce cardiac hypertrophy under chronic conditions. Here we highlight evidence for an underappreciated, but equally relevant role for AT_1R in coupling to a protective program in cardiomyocytes with hypertension. Recent advances in identifying receptor conformations specific to membrane stretch using nanobodies will allow for further study on AT_1R coupling to protective signaling. These studies will expand our understanding of AT_1R regulation and possibly lay the groundwork for pharmaceutical exploitation. In any event, AT_1R is likely to reveal additional surprises with relevance to cardiac function and remodeling under stress conditions.

Keywords Cardiac hypertrophy · β-arrestin biased signaling · G protein-coupled receptor · Hypertension · Heart failure

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Introduction

The angiotensin II type 1 receptor (AT_1R) is a 7 transmembrane spanning G protein-coupled receptor that predominately couples to the activation of G_q, and to a somewhat lesser extent $G_{12/13}$, to affect a variety of physiological processes, including vasoconstriction, positive inotropy and chronotropy of the heart with enhanced cardiac output, and enhanced aldosterone release with increased salt and water intake [1]. AT_1R stimulates the sympathetic nervous system both centrally and peripherally and can increase the metabolic rate. AT_1R may couple as well to G_i activation, the extent to which may be cell type-dependent [2] and influenced by posttranscriptional modification [3] or extraneous forces such as mechanical stress [4]. At least under experimental conditions, AT_1R may atypically couple to G_s [5]. Hyperactivation of AT_1R resulting from a sustained increase in angiotensin II (Ang II) levels has been implicated in various pathologies, such as cardiac hypertrophy and fibrosis, hypertension, and inflammation [1].

Cardiac hypertrophy, reflecting an increase in the size of individual cardiomyocytes, has been a well-studied aspect of Ang II signaling with Ang II implicated in left ventricular hypertrophy associated with hypertension [6, 7], diabetic cardiomyopathy [8–10], and the remote myocardium after myocardial infraction (MI) [11]. However, although supported by numerous in vitro experiments, the role of AT_1R via AngII in inducing cardiac hypertrophy in response to pressure or volume overload in vivo remains unsettled, with evidence that AT_1R in the heart may be activated independently of its ligand under conditions of increased membrane stretch. Moreover, evidence against any direct role for cardiac AT_1R in stimulating cardiac hypertrophy has also been reported. In this review, we discuss the ongoing controversy surrounding a direct role for the AT_1R of cardiomyocytes in contributing to a pathological phenotype with an emphasis on unresolved issues and pharmacological opportunities.

Role of AT_1 Receptor in Cardiac Hypertrophy

Cardiac hypertrophy, specifically increased left ventricular mass, is an independent predictor of morbidity and mortality [12]. Ang II-induced AT_1R activation has been implicated in cardiac hypertrophy via $G\alpha_{13}$ and $G\alpha_{q/11}$-mediated signaling involving increases in intracellular Ca^{2+} and/or conventional PKC activation along with recruitment of one or more mitogen-activated protein kinase (MAPK) cascades [1]. These canonical signaling events are postulated to be sustained or amplified by Ang II/AT_1R-mediated upregulation of an immune response together with inflammation and oxidative stress [1, 13–15]. Ang II-induced transactivation of the epidermal growth factor receptors (EGFRs) may also contribute to cardiac hypertrophy [16, 17]. This process is dependent on Src-mediated activation of ADAM17, a membrane protein with extracellular sheddase activity that cleaves the ectodomain of several

precursor molecules including that of heparin-binding EGF-like growth factor, an agonist of EGFR. Knockdown of ADAM17 attenuated cardiac hypertrophy in the spontaneously hypertensive rat without reducing blood pressure [18]. Similarly, ADAM17 knockdown prevented cardiac hypertrophy and fibrosis in the mouse infused with Ang II, although increased blood pressure was moderately attenuated [18].

Many of the studies implicating AT_1R in cardiac hypertrophy were performed in vitro using cultured cells, most often neonatal rat ventricular myocytes. These cells exhibit notable differences in morphology and function compared to adult cardiomyocytes [19–22]. For the studies performed in vivo, teasing apart the effects of increased blood pressure, itself a potent inducer of cardiac hypertrophy, from any direct growth-promoting effects of Ang II on cardiomyocytes has proven challenging. This question has relevance for understanding the purported greater efficacy of ACE inhibitors or AT_1R blockers (ARBs) in reducing the left ventricular hypertrophy and cardiovascular mortality associated with hypertension, compared to some other antihypertensive therapies [6, 23]. Preclinical studies that attempted to do so using a subpressor dose of Ang II did not deal with diurnal variations in blood pressure nor the fact that chronic infusion of subpressor levels of AngII invariably leads to hypertension [24–26].

Receptor Knockout Studies

Genetic deletion of the dominate AT_{1A} receptor gene in the mouse did not affect pressure overload–induced cardiac hypertrophy [27, 28]. Besides the fact that pressure overload is a potent stimulus that would likely mask the actions of Ang II, these germline knockouts may be confounded by potential genetic compensation in the heart. A study that involved kidney transplants in mice reported that extra-renal AT_1A receptors are not needed for hypertension or cardiac hypertrophy with Ang II infusion [29]. However, confounding factors such as experimental immunosuppression may complicate interpretation of these results [1].

A recent study compared two strains of mice in which AT_1R was deleted in the heart and conduit vessels of both, but in one strain was also deleted in resistance vessels [30]. Infusion of Ang II caused hypertrophy and hypertension in the mice without AT_1R in the heart and conduit vessels, but not in mice lacking receptors in resistance vessels. These results would seem to support the conclusion that blood pressure is the dominant contributor to cardiac hypertrophy over Ang II, at least when the two are rapidly introduced concurrently. But the heart also has "resistance vessels" and Ang II has been implicated in vascular smooth muscle cell (VSMC) hypertrophy. This occurs via its upregulation of ADAM17 and enhanced coupling of AT_1R to EGF receptor (EGFR) signaling and endoplasmic reticulum (ER) stress [31]. In Ang II-infused mice, inhibition of EGFR or ER stress attenuated not only vascular remodeling but cardiac hypertrophy as well, without any effect on the induced hypertension. Another study also implicated ADAM17, together with matrix metalloproteinase 7

(MMP-7), in Ang II-induced cardiac hypertrophy and remodeling [32]. Of note, hypertension was found to occur downstream of MMP-7 and ADAM17 activation (and cardiac hypertrophy) at the level of MMP-2. A recent study involving vascular ADAM17-deficient mice further implicated VSMC ADAM17 in Ang II-induced cardiac hypertrophy, independent of increased blood pressure [33]. Altogether, these studies support the idea that smooth muscle AT_1R may couple to cardiac hypertrophy by intercellular signaling and paracrine factors involving the vasculature [34].

Alternative Interpretations

Surprisingly, mice that lacked AT_1R in the heart, as well as both conduit and resistance vessels developed ventricular dilation and eccentric hypertrophy in response to acute pressure overload, rather than the anticipated concentric hypertrophy [30]. These hearts also exhibited reduced cardiac function with pressure overload in contrast to the hearts of wild type mice. These finding indicate that AT_1R may provide protection against adverse remodeling with acute pressure overload. Similarly, genetic deletion of the Mas-related G protein–coupled receptor member D (MrgD) was found to be associated with pronounced dilated cardiomyopathy [35]. MrgD, which is activated by the natural analog of Ang-(1–7), alamandine, is expressed by cardiomyocytes and is linked to antihypertrophic and antifibrotic effects independent of blood pressure lowering actions. It is possible that membrane stretch due to pressure overload stabilizes a conformation of AT_1R that is more "MrgD like" in its signaling.

AT_1R as a Mechanosensor

The evidence from receptor knockout studies suggests that AT_1R may function as a biosensor of increased blood pressure in small vessels (perhaps in the heart) and in the myocardium itself (Fig. 21.1). The signaling pathways elicited and their functional consequences would vary depending upon its cellular localization. In vessels, AT_1R would couple to cardiac hypertrophy downstream of EGFR transactivation in a paracrine manner [33, 34]. In cardiomyocytes, AT_1R would couple to concentric cardiac hypertrophy and anti-apoptotic or cardioprotective signaling [30].

Multiple studies support the idea that AT_1R couples to the hypertrophy of cardiomyocytes in response to mechanical stretch [36]. Early studies on neonatal rat ventricular myocytes provided evidence for a contribution of autocrine activation of AT_1R in this process by the upregulation of the angiotensinogen gene and release of stored Ang II [37, 38]. Subsequent studies, however, established that stretch itself is a potent activator of AT_1R. One of the first such studies showed that mechanical stretch activated AT_1R to potently induce activation of extracellular signal regulated kinases (ERKs), possibly downstream of JAK2 recruitment to the receptor, as well as phosphoinositide production, independently of an increase in extracellular

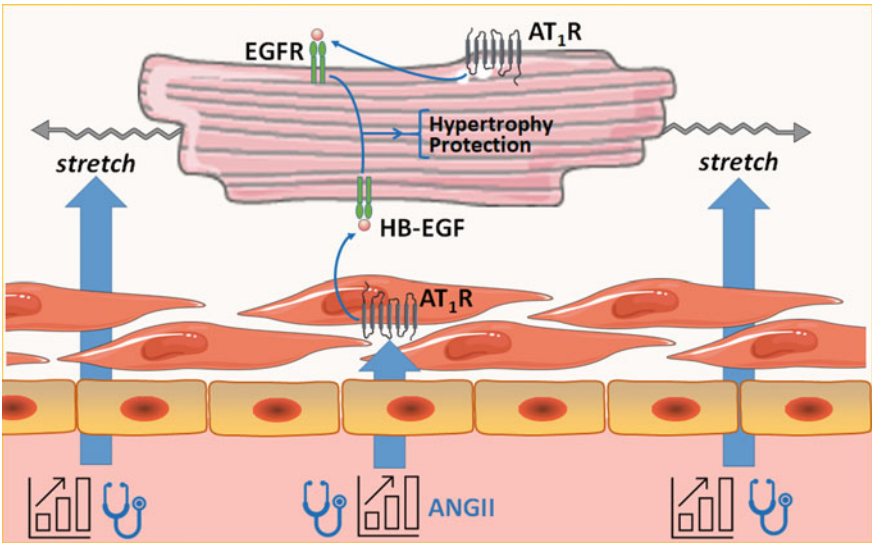

Fig. 21.1 AT_1R as biosensor of stress in the heart. In response to either increased Ang II or blood pressure, AT_1R in the smooth muscle of small vessels of the heart, transactivates the epidermal growth factor receptor (EGFR). This occurs through Src-mediated activation of ADAM17, a membrane protein that cleaves the ectodomain of heparin-binding EGF-like growth factor (HB-EGF), an agonist of EGFR. Additionally, mechanical stretch may act directly on the AT_1R of cardiomyocytes. EGFR has been shown to couple to both protective signaling, as well as cardiac hypertrophy. Whether this hypertrophy is physiological or pathological is uncertain, as is the relative extent of hypertrophy versus protection. Some of the content is adapted from Servier Medical Art (https://smart.servier.com/)

Ang II [39]. Activation could be blocked by the ARB candesartan, but not with a competitive inhibitor peptide or by mutating the Ang II binding site of AT_1R to prevent its binding. Moreover, candesartan was found to attenuate pressure overload-induced cardiac hypertrophy in mice lacking the angiotensinogen gene [39], a finding replicated by others [40].

Mechanical responsiveness is not a feature shared by all GPCRs. Neither the endothelin (ET-1) type A (ET1A) receptor nor the β_2-adrenoceptor (β_2-AR) are activated by membrane mechanical stretch [39]. Although the possibility was considered that AT_1R is activated indirectly via an interaction with a conventional mechanical sensors, such as integrins, muscle LIM protein, or stretch-sensitive ion channels, subsequent research provided compelling evidence that mechanical forces directly induce a conformational change in AT_1R [41]. Through substituted cysteine accessibility mapping (SCAM) and molecular modeling, cell stretch was shown to induce a conformational anticlockwise rotation and shift of transmembrane (TM) 7 into the ligand binding pocket (Fig. 21.2). This conformational "switch" is distinct and different from two other stabilized conformational states, the activation state stabilized by Ang II and an inactivation state stabilized by the inverse agonist candesartan. Similar models of multiple AT_1R functional states are reported by others (for example

[42]). Notably, the activation state of AT_1R attenuates the inverse agonism of most ARBs that are based on a biphenyl-tetrazole scaffold (such as losartan, valsartan, and irbesartan), shifting their binding interactions and functional response towards agonism [43]. The structurally distinct non-biphenyl, non-tetrazole ARB, eprosartan is described as a robust inverse agonist of the active state of AT_1R [44], at least the receptor activated by molecular mutation. Interestingly, multiple lines of evidence indicate that the second extracellular loop of AT_1R is a key regulator of its functional state (including those for both inverse agonism and agonism) [43]. In preeclampsia, posttranslational modification of glutamine residue in the second extracellular loop creates a neoepitope that fosters production of an autoantibody by the immune system that activates AT_1R [45]. As a final point, mutational studies have identified amino acids of AT_1R that are important for sensing mechanical stretch and inducing cardiomyocyte hypertrophy, distinguishable from Ang II responsiveness [46].

β-Arrestin Biased Signaling

Mechanical forces in the heart can couple to cardiac hypertrophy by a variety of mechanisms, including the activation of G_q-coupled signaling that may also involve the recruitment of AT_1R [47, 48]. Common downstream targets are the TRPC3/6 channels, resulting in an increase in intracellular Ca^{2+} with activation of the calcineurin-NFAT pathway and pathological hypertrophy [36]. Besides that, evidence indicates that mechanical forces in the heart signal through AT_1R, independently of G proteins, through recruitment of β-arrestins, which serve as signal transduction scaffolds that connect to beneficial actions and (although not definitively shown) physiological hypertrophy [49]. During this process, the β-arrestins associate with the AT_1R receptor and undergo phosphorylation and conformational changes [49]. One such beneficial action in the heart for AT_1R β-arrestin biased signaling would seem to be the Frank–Starling mechanism, which is the enhanced contractility that is observed with increased filling of the heart with blood [50].

GPCRs are thought to adapt different high-affinity conformations that signal through G protein activation or β-arrestins. Different ligands stabilize different conformations and Ang II is a balanced agonist at AT_1R, meaning that it activates both G protein and β-arrestin signaling pathways. Mechanical stretch skews AT_1R towards β-arrestin biased signaling that is distinguishable from that of Ang II by the recruitment of the inhibitory G protein ($G_{\alpha i}$) upstream of β-arrestin [4], although the details as to how G_i recruitment and activation is involved in β-arrestin recruitment are not clear (Fig. 21.3). Moreover, at least in the heart, mechanical stretch favors the recruitment of β-arrestin 2 rather than β-arrestin 1 [49]. Other evidence indicates that mechanical stretch activates AT_1R in cardiac myocytes, leading to the prominent stimulation of ERK and AKT, downstream of Src-mediated transactivation of EGFR [49]. EGFR transactivation in the heart is thought to promote cell survival signaling [51] and AT_1R was linked to anti-apoptosis effects in ex vivo hearts by the blocking actions of an ARB, losartan [49]. The role of ERK1/2 in the associated

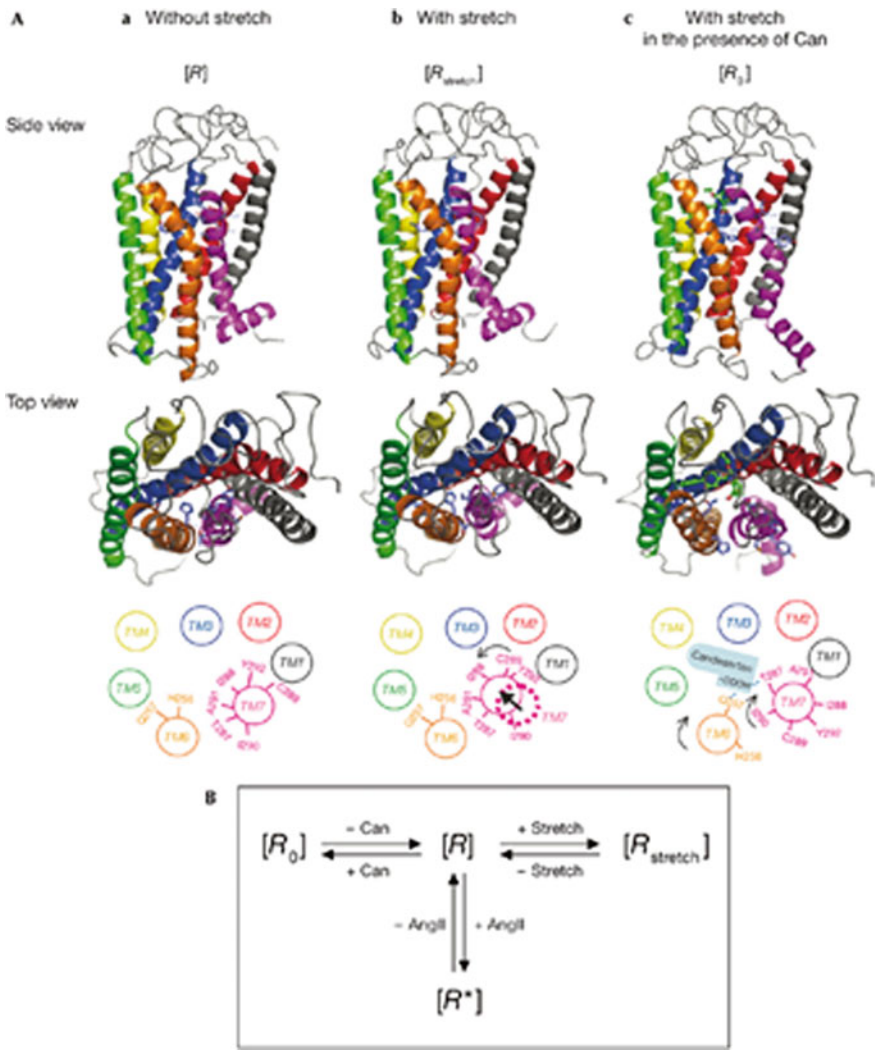

Fig. 21.2 Conformational switch of AT_1R underlying mechanical stress-induced activation. (A) A three state molecular model of AT_1R without stretch, with stretch, and with stretch in the presence of candesartan (Can). (B) Predicted distinct AT_1R conformations: [R], unaligned inactive state: [R_0], inactive state stabilized by inverse agonist candesartan; [R*], active state stabilized by agonist Ang II; and [$R_{stretch}$], distinct active state stabilized by mechanical stretch. Reproduced with permission [41]

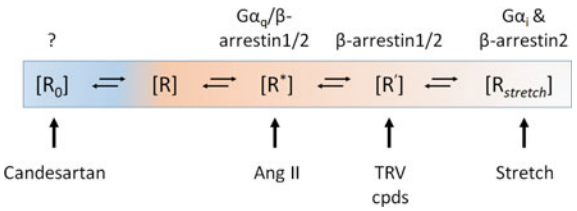

Fig. 21.3 Various agents stabilize distinct activation states of AT_1R that involve the recruitment of various signaling components that couple to different outcomes. Although the relationship among the functional AT_1R states is depicted as a continuum, the transition among the various states is likely more scattered or random

cardiac hypertrophy is not clear, although ERK1/2 signaling has been implicated in coordinating concentric versus eccentric growth of the heart [52].

Several "TRV" compounds that function as β-arrestin-biased AR1R agonists have been designed [4, 53]. TRV120027 activates ERK1/2 and Src and induces eNOS activation. This compound enhances cardiac contractility due to phosphorylation of contractile machinery [1, 54]. It preserves cardiac stroke volume without increasing myocardial oxygen demand, since it also reduces blood pressure and afterload. However, in the BLAST-HF trial on patients with acute heart failure, TRV120027 did not exhibit any beneficial actions [55]. A number of reasons might explain the failure to translate, including duration of treatment. Additionally, a further distinction between β-arrestin-biased AT_1R signaling of the "TRV" compounds and mechanical stress is that the later also involves G_i [56], which may be important for the full protective profile in the heart. $G_{\alpha i}$ was also shown to be required for carvedilol-induced β_1 adrenergic receptor β-arrestin biased signaling involving EGFR transactivation-induced ERK activation [57]. Studies have shown that carvedilol exerts beneficial effects on cardiomyocyte cell survival by regulating microRNA processing [58–60]. Of note, a sizable subset of genes induced by mechanical stretch of neonatal rat ventricular myocytes was related to AT_1R activation [61], although a resolution of the signaling networks involved in hypertrophy versus cellular protection is needed.

Nanobodies as Potential Conformation-Specific Drugs

There is evidence that AT_1R independently of Ang II couples mechanical forces to concentric cardiac hypertrophy as seen with pressure-overload or hypertension [39, 42, 62]. However, there is evidence as well that under these conditions AT_1R has beneficial actions by increasing cardiac myocyte isotropy, attenuating apoptosis and fibrosis, limiting cardiomyocyte hypertrophy, and activating autophagy [30, 49, 63–66]. Moreover, AT_1R seems to be an important determinant for preventing eccentric cardiac hypertrophy and ventricular dilation, as seen with volume overload [30].

Additional studies are needed to distinguish the agonist-independent activation states of AT_1R responsible for these harmful and beneficial actions.

Nanobodies, which are based on the variable regions of camelid-derived heavy chain–only antibodies, might be used to better define the specific conformation of AT_1R that responds to mechanical forces, as opposed to the β-arrestin biased TRV agonists [67]. A synthetic yeast-displayed nanobody library was recently used for crystallization experiments to define the molecular activation mechanisms of AT_1R [68]. In this study, an intracellular binding nanobody was identified that stabilized binding of an Ang II analog with partial agonist activity. Nanobodies may bind extracellularly as well to either orthosteric or allosteric binding sites. Recently, a library screen identified a nanobody that binds to the extracellular side of the AT_1R and antagonizes signaling through both the G protein and β-arrestin pathways [69]. This nanobody was effective in mice in reducing Ang II-induced hypertension. Finally, nanobodies are ideal potential drug candidates for GPCRs, given their solubility, small size and unique structural features that include long protruding CDR3 loops, small convex paratope, and ability to bind conformational epitopes. The European Medicines Agency and the US Food and Drug Administration recently approved the bivalent nanobody caplacizumab, as a first-in-class medication for treating thrombotic thrombocytopenic purpura [70]. Conceivably, a similar approach could be used to produce a drug that stabilizes the stretch-induced AT_1R conformation that is cardioprotective.

Conclusions and Future Directions

There is a strong body of evidence that AT_1R serves as a biosensor in the heart to mechanical forces for instance that are relevant to hypertension and heart failure. The AT_1R of both small vessels and cardiomyocytes may be engaged in this role with transactivation of EGFR serving as a major signaling platform that elicits protective actions. Although studies have documented molecular changes in AT_1R in response to mechanical force, a clear understanding of the downstream signaling events from the perspective of which couple to hypertrophy and which to protection is still not known. Pathway analysis is needed to address these questions and whether the hypertrophy is pathological or physiological and whether the hypertrophic events can be separated from those that convey protection. Another issue is why given the relatively low number of AT_1R in the heart, this receptor plays such an important role. A better idea as to its localization would help in this regard. Finally, the emergence of nanobodies as drugs presents the possibility of designing drugs that selectively activate the mechanosensor conformation of AT_1R.

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Chapter 22
Cardiovascular Protective Arm of Renin Angiotensin System

Yagna P. R. Jarajapu

Abstract Cardiovascular protective axis of Renin Angiotensin System is relatively newer concept and largely originated from the discoveries of an alternative angiotensin-converting enzyme (ACE), ACE2 and a heptapeptide metabolite of RAS angiotensin-(1–7) (Ang-(1–7)). The property of ACE2 to cleave angiotensin II (Ang II), which produces pathological effects in cardiovascular system, to a cardiovascular protective peptide Ang-(1–7) makes this enzyme an attractive molecular target for the treatment of cardiovascular disorders. The discovery of Mas receptor, as the cognizant receptor for the biological actions of Ang-(1–7) provided novel therapeutic opportunities. Protective functions of ACE2 or activators of MasR are now being explored in cardiac, pulmonary, renal, central nervous system, and many non-cardiovascular disorders. In addition to Ang-(1–7) few other peptide fragments of Ang I were identified to have protective functions in cardiovascular system. Alamandine is the newest member of RAS and is gaining a lot of interest among research community. This chapter provides an overview of recent literature on the cardiovascular protective functions of angiotensin peptides. The overview will include a brief mention on the role of ACE2 and Ang-(1–7) in the vascular regenerative functions of stem/progenitor cells.

Keywords Angiotensin converting enzyme-2 · Angiotensin-(1–7) · Angiotensin-(1–9) · Alamandine · Mas receptor and MrgD

Introduction

Renin angiotensin system (RAS) has been studied for more than a hundred years and yet, the physiology of this system is expanding with novel functions in cardiovascular and non-cardiovascular organ systems. Importantly, several small molecules that antagonize the classical axis of RAS were developed for pharmacotherapeutics of

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cardiovascular disorders such as hypertension, heart failure, pulmonary hypertension, etc. Classical axis consists of angiotensin-converting enzyme (ACE), which cleaves Angiotensin I (Ang I) to Ang II. By activating AT1 receptor (AT1R), Ang II produces cardiovascular detrimental effects such as vasoconstriction, increased blood pressure, heart rate, myocardial contractility, fibrosis and inflammation. ACE inhibitors and angiotensin receptor blockers (ARBs) are clinically successful in the treatment of cardiovascular disorders.

With the availability of state-of-the-art analytical and proteomic approaches in the past two decades RAS has been expanded with the addition of new members including novel enzymes and peptide fragments of Ang I. Incidentally, some of these new members were identified for their counter-regulatory effects on the classical axis of RAS. While some of these require further investigation, a few have attracted massive interest from academic and industrial research community. Namely, the new members of RAS include the enzyme angiotensin-converting enzyme-2 (ACE2), peptides Ang-(1–9), Ang-(1–7), alamandine, Ang-(2–8) (Ang III) and Ang-(3–8) (Ang IV) (Fig. 22.1) and receptors AT2R, Mas receptor (MasR) and Mas-related G-protein-coupled receptor, D (MrgD) . Evidence in support of cardiovascular physiology and pharmacology of the new members of RAS is now accumulating, which offers novel therapeutic targets for the treatment of cardiovascular disorders (CVDs). This review provides an overview of physiology and pharmacology of the new members in cardiovascular system and where applicable, in the non-cardiovascular organ systems.

Angiotensin-Converting Enzyme-2 (ACE2)

The alternative ACE, angiotensin-converting enzyme-2 (ACE2), was first discovered for its ability to convert A Ang I to Ang-(1–9), which is a substrate for ACE resulting in Ang-(1–7) formation [3, 4]. Furthermore, Ang II cleavage by ACE2 was shown to produce Ang-(1–7) with high catalytic efficiency [5, 6]. Both Ang-(1–7) and Ang-(1–9) have cardiovascular protective properties via acting on MasR or MrgD (see below). The recombinant ACE2 effectively metabolized Ang I and Ang II to Ang-(1–9) and Ang-(1–7) in patients with heart failure suggesting therapeutic feasibility of this concept [7]. Therefore, ACE2 peptide or small molecule activators of ACE2 by increasing local and systemic Ang-(1–7) and Ang-(1–9) are likely to produce therapeutic effects in cardiovascular diseases. Another study reported a modified form of ACE2, ACE2-Fc chimeric fusion protein, free from immunogenicity, produced long-lasting decrease in blood pressure in mouse models of Ang II-induced hypertension [8].

Crackower et al. [9] first showed in multiple rat models of hypertension, ACE2 expression is downregulated in kidneys, and deletion of ACE2 impaired cardiac contractility that was attributed to increased Ang II levels and could be overcome by concurrent ACE-deletion. These findings were then corroborated by Yamamoto et al. [10]showing that ACE2-eficiency accelerates pressure overload-induced cardiac

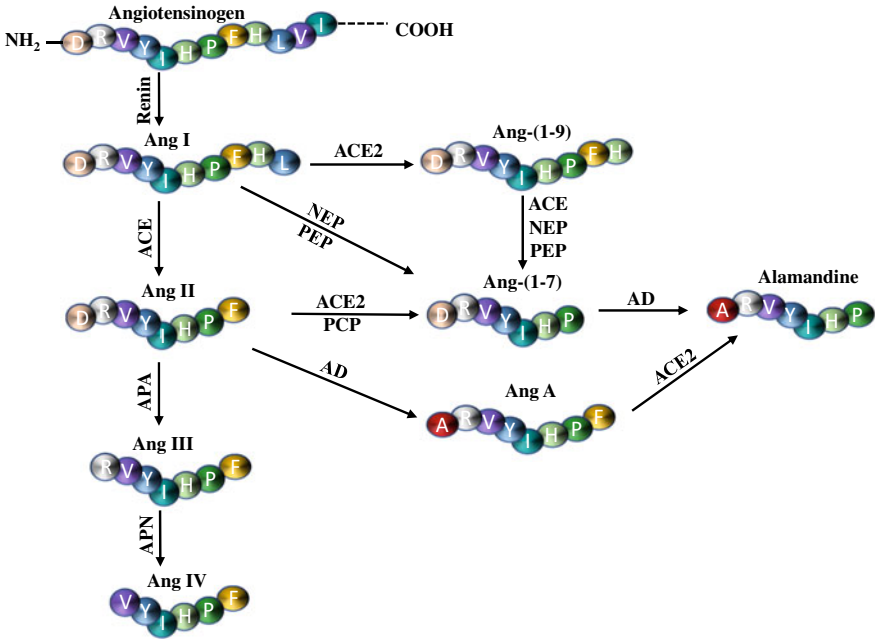

Fig. 22.1 Synthesis of cardiovascular protective angiotensin peptides. Angiotensinogen derived from liver is cleaved by renin, which is secreted from juxtaglomerual cells in the kidney, to angiotensin-(1–10) (Ang I). Further metabolism of Ang I generates biologically active peptides. Ang-(1–8) (Ang II) is generated by angiotensin-converting enzyme (ACE), Ang-(1–9) by ACE2, and Ang-(1–7) by endopeptidases such as neprilysin (NEP) and prolylendopeptidase (PEP) from Ang I. Ang-(1–7) is also generated from Ang II by ACE2 or prolylcarboxy peptidase (PCP). Ala^1-Ang-(1–8) or Ang A is generated from Ang II by the enzyme aspartate decarboxylase (AD). Ang A is converted to Ala1-Ang-(1–7) or alamandine by ACE2. Alternatively, alamandine can also be generated from Ang-(1–7) by the enzyme AD. Further cleavage of Ang II by aminopeptidase A (APA) generates Ang-(2–8) aka Ang III [1]. Ang III can be cleaved by Alanyl aminopeptidase N (APN) cleaves Ang III to Ang-(3–8) aka Ang IV [2]

dysfunction which was attributed to increased levels of Ang II. In agreement with this, ACE2 gene transfer attenuated hypertension-induced pathophysiological changes in SHR [11]. Cardiac overexpression of ACE2 in the myocardium offered ischemic protection in a rat model of myocardial infarction [12].

A meta-analysis of pharmacological interventions in animal models of pulmonary arterial hypertension (PAH) revealed that ACE2 activators are most potent class of agents for the treatment of PAH) [13]. First evidence for the therapeutic benefit of ACE2 in PAH was shown by Yamazoto et al. [14] using lentiviral approach for ACE2 gene transfer, which prevented right ventricle hypertrophy and functional deficits in a rat model of monocrotaline-induced PAH (Fig. 22.2). These findings were later corroborated by using similar approach for increased ACE2 expression in a rat model of bleomycin-induced PAH and lung fibrosis [15]. Similar protection was also observed by infusion of rhACE2 in bleomycin-induced PAH in mice [16]. In a

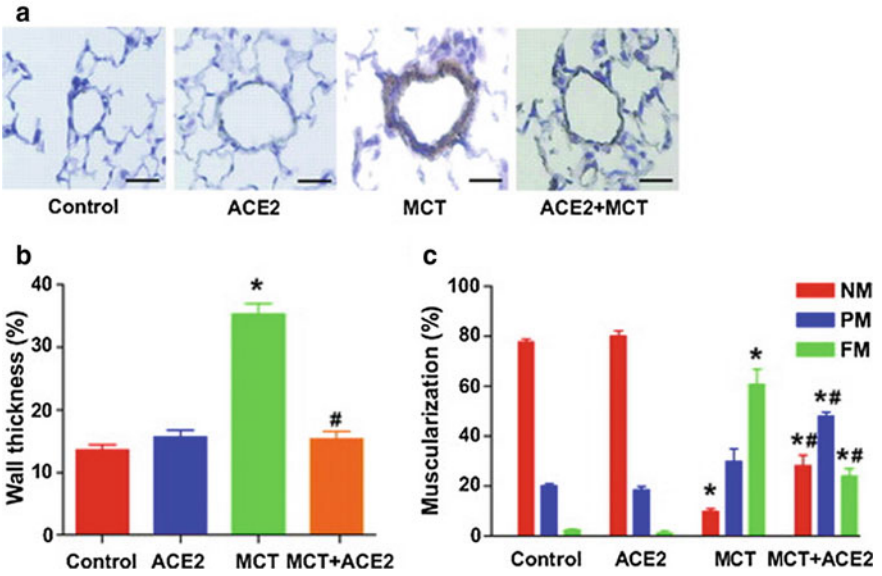

Fig. 22.2 Prevention of pathology in pulmonary hypertension induced by monocrotaline (MCT) by ACE2 gene transfer. **a** Representative microphotographs of pulmonary vessels (scale bar: 50 μm). **b** Quantification of wall thickness as described in the Methods section. Data are expressed as mean ± SEM. $*P < 0.05$ versus control group; $\#P < 0.05$ versus MCT group (n = 4 to 5). **c** Quantification of vessel muscularization: degree of muscularization of vessels was carried out as described in the Methods section. Data are expressed as mean ± SEM. $*P < 0.05$ versus control group; $\#P < 0.05$ versus MCT group (n = 4 to 5). NM indicates nonmuscularized vessels; PM, partially muscularized vessels; FM, fully muscularized vessels. (*Taken from* Yamazoto et al. [14])

small cohort of patients with PAH, rhACE2 improved pulmonary vascular resistance and cardiac output [17]. These benefits were associated with decreased markers of inflammation and increased levels of superoxide dismutase in the circulation.

Elegant studies showing pharmacology of small molecule activators of ACE2 were reported in experimental models of cardiopulmonary disorders. Xanthenone and resorcinol-naphthalein were first identified by virtual screening approach as potent ACE2 activators [18]. Infusion of XNT decreased blood pressure in a spontaneously hypertensive rat with no effect in a normotensive rat [18]. Pulmonary arterial endothelial function was improved by xanthenone (XNT) derivative and resorcinol-naphthalein that was associated with endothelial nitric oxide synthase (eNOS) activation [19, 20]. Diminazene aceturate, another small molecule, was shown to produce protective effects in experimental models of cardiopulmonary disorders that were shown to be ACE2-dependent. Diminazene restored cardiac functions in a rat model of myocardial ischemia, which was accompanied by increased expression and activity of ACE2 in the myocardium [21]. However, direct ACE2 activation by XNT and diminazene was questioned by another study and suggested

involvement of ACE2-independent mechanisms [22]. Upregulation of ACE2 expression is a possible underlying mechanism in studies involving chronic administration of activators that showed promising actions in cardiopulmonary disorders.

Our group has reported the expression of ACE2 in bone marrow-derived stem/progenitor cells of human and murine origin. This cell population with hematopoietic markers have the propensity of stimulating vascular regeneration following ischemic vascular injury. Our studies showed imbalance in ACE versus ACE2 expression/activity is associated with dysfunctional regenerative capacity of vasculogenic progenitor cells derived from diabetic or aging individuals as well as in cells derived from mouse models of diabetes or aging. Of particular interest, in human $CD34^+$ hematopoietic stem/progenitor cells ACE2/ACE ratio was found to be declined with HbA1C, which was correlated with prevalence of cardiovascular complications (Fig. 22.3) [23]. ACE2 gene-transfer reversed reparative dysfunction in $CD34^+$cells derived from diabetic older adults and restored blood flow in vivo following ischemic injury. Interestingly, expression of ACE2 was higher in a unique group of diabetic individuals who are resistant to the development of microvascular disease despite long-term diabetes with HbA1C of 11, compared to that in cells from diabetics with microvascular complications [24]. Therefore, therapeutic potential of ACE2 gene therapy is rather strongly supportive based on a variety of preclinical models and translational studies.

Angiotesnin-(1–7)

Ang-(1–7) was thought to be acting as a physiological counter-regulatory peptide for the actions of Ang II. Importantly, Ang-(1–7) has been shown to inhibit ACE activity [25]. Santos et al. [26, 27] discovered that the Mas receptor as the specific receptor for Ang-(1–7) [28, 29]. Mas was originally identified as a protooncogene with a potential to induce tumorignecity and it has sequence homology with G protein-coupled receptor subfamily of hormone-receptor proteins [30, 31]. Subsequently, several studies identified Mas expression in several tissues including cardiac myocytes, neurons and vascular endothelium. Ang-(1–7) activates eNOS by Mas receptor/Akt-dependent mechanism and attenuates NADPH oxidase via MasR activation [32, 33]. Ang-(1–7) failed to stimulate nitric oxide (NO) levels in cells or tissues derived from MasR -deficient mice [34]. Mas deficient mice show mild hypertension and sustained activation of ACE/Ang-II/AT1R pathway that promotes cardiac ventricular dysfunction [29]. Cumulatively, these studies support the concept that ACE2/Ang-(1–7)/MasR axis promotes protection against CVDs. By acting through MasR, Ang-(1–7) promoted vasodilation, antihypertensive, antifibrotic, antithrombotic and antihypertrophic effects in several in vitro and in vivo experimental studies thus making the protective pathway of RAS a potential therapeutic target for CVDs [35].

Pharmacological activation of MasR was proven to restore wound healing in experimental models of diabetes, and the reparative end-points were associated

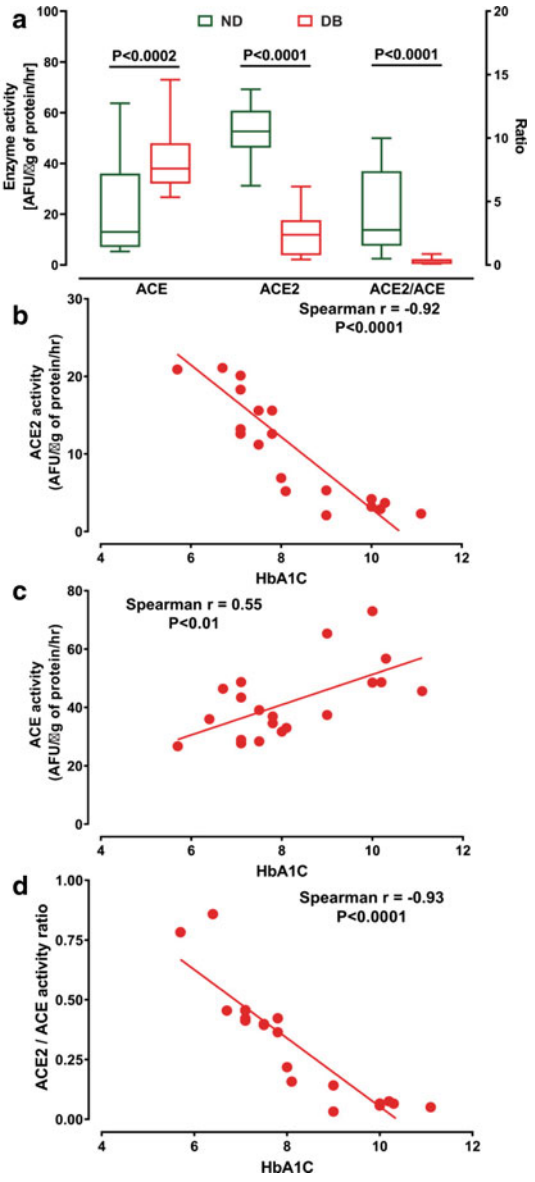

Fig. 22.3 ACE2/ACE imbalance in human vasculogenic progenitor cells in diabetes. **a** ACE activity is increased in diabetic (DB) $CD34^+$ cells (n = 20) compared to nondiabetic (ND) cells (n = 23). ACE2 activity is decreased in DB cells compared to ND (P < 0.0001). These changes resulted in decreased ACE2/ACE ratio in DB cells. **b** ACE2 activity is inversely correlated with HbA1C in DB cells (spearman correlation 'r' −0.92). **c** ACE activity is directly correlated with HbA1C in DB cells (spearman correlation 'r' −0.55) **d**. ACE2/ACE ratio is inversely proportional to the HbA1C in DB cells with a spearman correlation 'r' −0.93. [23] (*Taken from* Joshi et al.)

with increased vascular regenerative capacity [36, 37, 38]. Our group has extensively studied pharmacology of Ang-(1–7) in vasculogenic progenitor cells in the context of ischemic vascular injury. Ang-(1–7) stimulated mobilization of bone marrow-resident cells into the blood stream which was dysfunctional in diabetic mice [39]. Importantly, mobilization in response to ischemic injury, which is impaired in diabetes was restored and vascular regeneration following ischemic injury was

accomplished (Fig. 22.4) [39]. Lentiviral approach for Ang-(1–7) transgene restored vascular repair-relevant functions in dysfunctional $CD34^+$ cells of diabetic origin—migration of cells to hypoxia-regulated factors in vitro and to the areas of ischemic injury in vivo in a model of retinal ischemic-reperfusion injury [24]. The vasculogenic effects of Ang-(1–7) were mediated by MasR in stem/progenitor cells including migration, proliferation and NO generation in basal or in response to hypoxia-regulated factors such as stromal-derived factor-1 or vascular endothelial growth factor [24, 40]. In mice with genetic deficiency of MasR, number of circulating progenitor cells were lower and ischemic injury failed to mobilize cells from bone marrow into the circulation resulting in partial recovery of blood flow [39].

NO has an important role in the maintenance and mobilization of progenitor cells from bone marrow [41]. NO at least in part mediates protective functions of Ang-(1–7) in the progenitor cells. In agreement with initial studies inferring MasR-dependent activation of eNOS in endothelial cells [42, 43], PI3K/Akt pathway has been shown to be involved in MasR-dependent NO release by Ang-(1–7) in human and murine HSPCs [24]. Oxidative environment induces uncoupling of eNOS resulting in superoxide generation which further exacerbates oxidative stress. Ang-(1–7) is able to restore NO/cGMP levels in the bone marrow at least in part by reducing oxidative stress in diabetes and obesity [36, 38]. $CD34^+$ cells derived from diabetic individuals have shown increased ROS levels that were normalized by Ang-(1–7), which was associated with increased NO levels [24].

Despite the shorter biological half-life, Ang-(1–7) administration has been shown to be effective even in nanomolar doses in a wide range of experimental models that are described above. More stable analogues of Ang-(1–7) such as $Norleu^3$-Ang-(1–7) or glycosylated Ang-(1–7) would be better alternatives for clinical use [44, 45].

Angiotesnin-(1–9)

Ang-(1–9) was first shown to be produced by the alternative ACE, ACE2, [3, 4] or cathepsin A from Ang I [46]. Interestingly, Ang-(1–9) levels in the circulation increased following myocardial infarction in ACE2-dependent manner [47]. Later studies showed evidence for cardioprotective effects in experimental studies and human individuals with cardiopulmonary disease.

Evidence of the cardioprotective effect of Ang-(1–9) was shown in a rat model of myocardial infarction [48]. Another study in stroke-prone SHR model, Ang-(1–9) administration protected from the development of cardiac fibrosis and increased basal NO release in the conduit blood vessel [49]. Subsequent studies, demonstrated that antihypertensive effect of Ang-(1–9) in Ang II-or renal-clipping-induced hypertension in rats, which accompanied reduced ventricular hypertrophy and fibrosis [50]. These effects were reversed by AT2R antagonist (PD123319) but not by MasR-selective antagonist, A779. Another study in mice [51] with coronary ligation reported reduction in systolic blood pressure, cardiac output and enhanced survival by adenoviral (AAV) gene transfer of Ang-(1–9) though no significant protection was

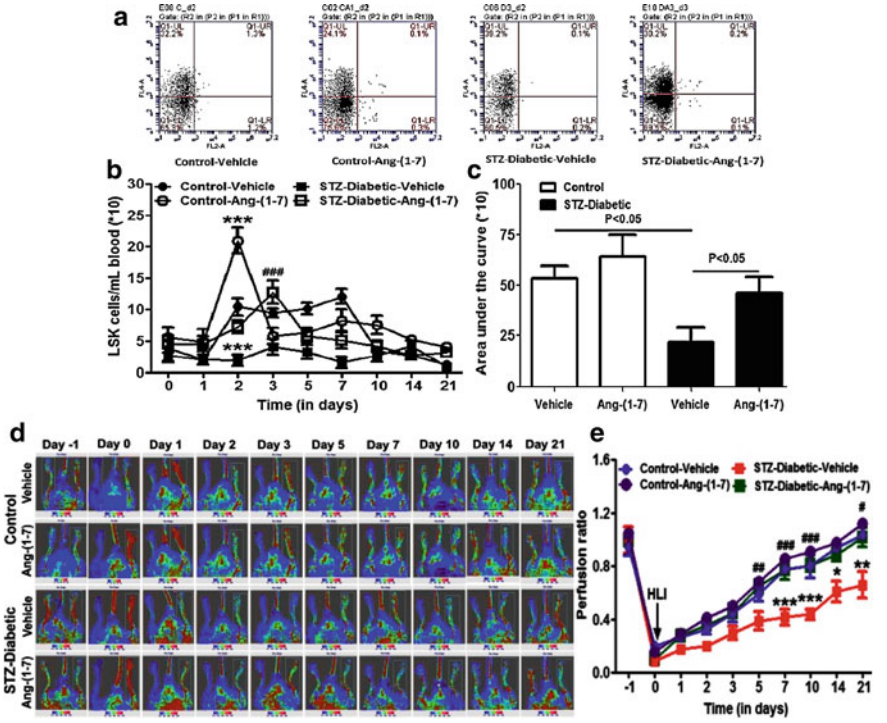

Fig. 22.4 Ang-(1–7) and ischemic vascular repair in diabetes. **a** Representative flow cytometric dot plots of progenitor cells at the time of peak mobilization following hind-limb ischemia (HLI) in different treatment groups involving control and streptozotocin (STZ)-Diabetic mice. **b** $Lin^{-}Sca\text{-}1^{+}cKit^{+}$ (LSK) cell mobilization kinetics in different treatment groups involving control and STZ-Diabetic mice over a period of 21 days after HLI (n = 6–8), where day 0 was prior to HLI. ***P < 0.001 versus Control-Vehicle; ###P < 0.001 versus STZ-Diabetic-Vehicle as analyzed by using two-way ANOVA. **a** Representative Laser Doppler images (LDI) of blood flow in different treatment groups before and after HLI. **b** Blood flow was quantified by red blood cell (RBC) flux (blood × $area^{-1}$ × $time^{-1}$) expressed as percent of the respective contralateral limb. Blood flow recovery was lower in STZ-Diabetic mice which was restored to normal by Ang-(1–7) (n = 6). *P < 0.05, **P < 0.01, and ***P < 0.001 versus Control-Vehicle; #P < 0.05, ##P < 0.01, and ###P < 0.001 versus STZ-Diabetic-Vehicle as analyzed by using two-way ANOVA (*Taken from* Vasam et al. [39]).

observed in cardiac hypertrophy and fibrosis. In vitro studies using neonatal cardiac myocytes or Langendorff preparation demonstrated positive inotropic response to Ang-(1–9). Similar protections was observed in a rat model of cardiac ischemia induced by left anterior descending (LAD) coronary artery ligation [52]. This study also showed AT2R-dependent Akt generation by using the antagonist, PD123319, in the cardiac protective effects of Ang-(1–9) in cardiac myocytes subjected to simulated ischemia/reperfusion. It is important to note that PD123319 was shown to antagonize MrgD receptor in later studies [53] therefore receptor(s) mediating responses to Ang-(1–9) require further systematic investigations.

Lastly, in a small cohort of individuals with acute respiratory distress syndrome (ARDS), survival was closely associated with increased Ang-(1–9) levels and mortality was closely associated with accumulated Ang-(1–10) levels in the circulation [54]. Increased ACE2 levels as indicated by Ang-(1–9)/Ang-(1–10) ratio was accompanied by increased ACE levels as indicated by Ang-(1–7)/Ang-(1–10).

Alamandine

Alamandine is a newly discovered member of RAS with cardiovascular functions that are protective and opposing to the classical axis. Alamandine is a product of ACE2-dependent catalytic hydrolysis of Angiotensin A (Ang A) and can also be generated by decarboxylation of Aspartic acid residue of Ang-(1–7). Ang A is Ala^{1}-Ang II (alanine in place of aspartic acid). In mononuclear leucocytes Ang II is converted to Ang A by decarboxylation of aspartic acid.ref Ang A is detected in human circulation and was shown to be higher in individuals with end-stage renal disease [55]. Alamandine produced endothelium-dependent vasorelaxation that was blocked by D-Pro^{7}-Ang-(1–7), an MrgD receptor antagonist but not by A779, a MasR antagonist [56]. In agreement with this, Tetzner et al. [53] showed that alamandine activated cAMP formation in endothelial and mesangial cells transfected with MrgD. Importantly, oral administration of 2-Hydroxy-prolyl β-cyclodextrin (HPβCD)-alamandine produced antihypertensive effect in spontaneously hypertensive rat (SHR) [56]. Alamandine showed cardioprotective effects in experimental models of pressure overload. In mice undergoing transverse aortic constriction, Alamandine prevented cardiac hypertrophy and fibrosis that was shown to be mediated partly via decreased ERK1/2 phosphorylation, TGFβ1 and MMP2, and increased AMPKα phosphorylation [57]. In the same model, vascular remodeling in the ascending aorta was also prevented that was associated with decreased pro-inflammatory (IL1β, TNF and CCL2) and pro-fibrotic factors (MMP2 and TGF β1), and increased pro-resolution markers (MRC1 and FIZZ1) [58]. Mice deficient of MrgD receptor showed myocardial pathology with dilated cardiomyopathy and a marked decrease in systolic function further supporting cardioprotective pharmacology of alamandine. A study by Marins et al. [59] showed evidence for central regulation of hemodynamics specifically by acting on MrgD receptors in rostral insular cortex. Microinjection of alamandine in this area elevated mean arterial blood pressure and renal sympathetic activity that were blocked by D-Pro^{7}-Ang-(1–7), an MrgD antagonist. Angiotensin-converting enzyme 2 is an essential regulator of heart function.

Conclusions

RAS has given many therapeutic opportunities for the treatment of cardiovascular diseases that are exclusively targeting ACE or AT1R. This review has highlighted

therapeutic promise of ligands that are activators of ACE2 and at least two novel receptors MasR, or analogs of three novel peptides Ang-(1–7), Ang-(1–9) or alamandine for the treatment of cardiovascular disorders. The author strongly believes that the future pharmacotherapeutics is poised for activators of the protective members of RAS.

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Chapter 23
Quercetin: A Promising Flavonoid for the Therapy of Cardiac Hypertrophy and Heart Failure Mediated by the Renin Angiotensin System

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Abstract The Renin angiotensin system (RAS) plays an essential role in regulating the angiotensin II levels in the body and maintaining cardiovascular homeostasis. However, overactivation of RAS leads to hypertension and cardiac overload, especially in the left ventricle, causing cardiac hypertrophy. As a compensatory mechanism, the heart muscles undergo myocardial hypertrophy to maintain the cardiovascular function. Progression of cardiac hypertrophy along with other factors leads to heart failure. RAS mediated high blood pressure is one of the major risk factors that initiates pathological changes in the coronary blood vessels, sometimes resulting in heart attack and stroke. The results of preclinical and limited number of clinical studies demonstrated that quercetin possesses cardioprotective effects to prevent hypertension, cardiac hypertrophy, and heart failure. The multiple benefits of quercetin are attributed to ACE inhibition, strong antioxidant and anti-inflammation properties and protection of endothelial function which collectively prevent coronary heart disease, hypertension, myocardial hypertrophy and heart failure. This review aims to provide an overview of the current knowledge about the impact of RAS in causing CVDs, to highlight cellular and molecular mechanisms of quercetin involved in ACE inhibition via RAS pathway and to slow down the progression of cardiac hypertrophy and heart failure. Despite having an enormous therapeutic potential, the usefulness of quercetin at present is limited due to its low aqueous solubility and oral

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bioavailability. Different formulations are being developed to enhance the bioavailability of quercetin by employing novel drug delivery systems which will pave the ways for discovering cost-effective, affordable, and safe drugs for treating CVDs.

Keywords Renin angiotensin system · Cardiac hypertrophy · Heart failure · Cardiovascular diseases · Quercetin · Flavonoids · Quercetin formulations

Abbreviations

ACE	Angiotensin-Converting Enzyme
ACE2	Angiotensin-Converting Enzyme 2
AGT	Angiotensinogen
Akt	Protein kinase B
ANF	Atrial natriuretic factor
Ang I	Angiotensin I
Ang II	Angiotensin II
ARB	Angiotensin II type 1 receptor blockers
AT1	Angiotensin II type 1 receptor
AT2	Angiotensin II type 2 receptor
BK	Bradykinin
BNP	Brain natriuretic peptide
CVD	Cardiovascular Diseases
eNOS	Endothelial nitric oxide synthase
H9c2	Clonal cell line from rat heart
HFrEF	Heart failure with reduced ejection fraction
HUVEC	Human umbilical vein endothelial cells
IDH2	Isocitrate dehydrogenase
IL1	Interleukin-1
IL2	Interleukin-2
IL-1β	Interleukin 1beta
IL-6	Interleukin 6
iNOS	Inducible nitric oxide synthase
JG	Juxtaglomerular cells
LDL	Low density lipoprotein
LVH	Left ventricular hypertrophy
NADPH	Nicotinamide adenine dinucleotide phosphate oxidase (NADPH-oxidase)
NF-κB	Nuclear Factor kappa B
NO	Nitric Oxide
PGE2	Prostaglandin E2
RAS	Renin Angiotensin System
RAAS	Renin–angiotensin–aldosterone system
ROS	Reactive oxygen species

SAR	Structure-Activity Relationship
SHR	Spontaneously hyperactive rats
SIRT5	Sirtuin (silent mating type information regulation 2 homolog) 5 (S. cerevisiae)
SNS	Sympathetic nervous system
TNF-α	Tumour Necrosis factor alpha

Introduction

The Renin Angiotensin System (RAS) is a complex cascade of biochemical processes that occur in the plasma membrane of the cells, which regulate the functions of the body such as cell growth, cardiac output, regulation of body fluid volume, strength of blood vessels, electrolyte balance etc. RAS is a major risk factor for patients combating cardiovascular diseases (CVD). It's control over the circulatory system and endocrine factors leads to an increase in the body fluid volume, which induces vasoconstriction, muscular hypertrophy and fibrosis, therefore potentiating an additional strain onto the heart [1–3].

CVD consists of an array of diseases that influence the heart and the vascular system, which includes cerebrovascular disease, peripheral artery disease, coronary heart disease, rheumatic heart disease, congenital heart disease and deep vein thrombosis or pulmonary embolism [4]. With over 17.9 million people being affected globally by CVD, and 32% of deaths being attributed to this disease, the prevalence of CVD has become a global phenomenon, majorly affecting the low to middle income society and developing countries. Hypertension is regarded as one of the major causes of cardiovascular diseases, which in turn increases the prevalence of ventricular arrhythmias and atrial fibrillations [5]. A patient's susceptibility to ischemia and myocardial infarction is also directly proportional to changes in normal blood pressure. Since, the increase in Angiotensin II (Ang II) levels have a direct effect on fluid haemodynamics, the study of RAS unfolds an area of pathogenesis and treatment strategies, that can be used to counteract CVD [6].

Ang II is a peptide hormone that is a common target for CVD drugs as it perpetuates diseases like hypertension, stroke and heart failure. Therefore, current treatment strategies include angiotensin-converting enzyme (ACE) inhibitors, angiotensin II type 1 receptor blockers (ARBs) or a combination of both for a more intensive approach [3]. Other current therapies used in the treatment of heart failure include beta-blockers and aldosterone antagonists, whereas cardiac hypertrophy uses verapamil as the first line of treatment in conjugation with beta-blockers. The use of cardiac rehabilitation, lifestyle modifications such as dietary changes and exercise in combination with pharmacological therapy, contributes to an enhanced recovery rate [7, 8]. The use of these synthetic drugs is limited because of its side effects. A study on ACE inhibitors by Agusti et al. was conducted on 18,234 patients and a 24.3% withdrawal rate was observed due to side effects such as cough, hypotension

and dizziness. Other not so common adverse effects described in the study included nausea, headache and cold extremities [9, 10]. Thus, in view of recent studies, there is an unmet need for natural and safe drugs devoid of such side effects which leads to an opportunity to explore the nutritional and medicinal properties of flavonoids.

Flavonoids are polyphenolic compounds derived from plants, and depending on the synthesizing enzymes and their structure, they are separated into two classes. Flavones, flavonols, flavanones, flavan-3-ols, and anthocyanidins belong to the 2-phenyl chromen class and isoflavonoids belong to the 3-phenyl chromen class [11]. Flavonoids exhibit a wide range of pharmacological activities, the most prominent ones being antioxidant, anti-inflammatory and anti-hypertensive action. Based on their class and indications, the common flavonoids used for CVD are enlisted in Table 23.1 [11]. A meta-analysis by Peterson et al. extensively investigated the results of 13 publications of preclinical studies on flavonoids and their types on its association with CVD, and a decrease in risk of coronary heart disease was reported [12].

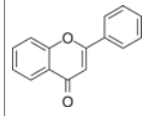
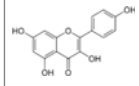
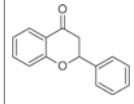
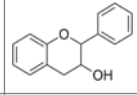
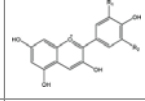
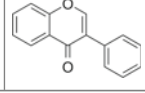
Quercetin is a safe flavanol that is found in abundance in nature and has shown potential in the treatment and prevention of CVDs. This polyphenolic flavonoid can inhibit ROS, protect the endothelial function, prevent LDL oxidation and offer a vasodilatory effect to the heart [24]. The anti-oxidant and anti-inflammatory properties of quercetin are instrumental in preventing diseases such as hypertension, atherosclerosis, ischemia and cardiotoxicity [25]. Various preclinical studies (Table 23.4) on antioxidant and anti-inflammatory activities and clinical studies (Table 23.5) on cardiovascular protection and hypertension have been conducted [24]. Therefore, in view of these studies conducted, there is a profound need to explore the mechanism of quercetin for its investigation and incorporation as an innocuous and effective drug to counteract RAS induced CVDs.

Relationship of RAS with the Pathogenesis of Cardiac Hypertrophy and Heart Failure

The RAS pathway follows the negative feedback inhibition cycle through a short loop system that begins with the production of prorenin in the afferent arteriole of the kidney by the juxtaglomerular (JG) cells. It splits to form activated renin which, in turn, catalytically cleaves a protein derived from the liver called Angiotensinogen (AGT) into Angiotensin I (Ang I) as seen in Fig. 23.1 [26]. This is the rate-limiting step [6, 27]. Ang I gets cleaved by angiotensin-converting enzyme (ACE) into Ang II. Ang II binds to Angiotensin II type 1 (AT1) and Angiotensin II type 2 (AT2) receptors in the plasma membrane to regulate various physiological functions. The AT1 receptor mediates most of the effects of Ang II, in particular, its vasoconstrictive effect that has been reported to contribute to hypertension, cardiac dysfunction such as arrhythmias, hypertrophy, atherosclerosis and aortic aneurysms [27, 28].

Cardiac hypertrophy is the enlarging of heart muscles which occurs via a compensatory mechanism to improve the pumping action of the heart. A slight increase in

Table 23.1 Different types of flavonoids, chemical structures and therapeutic uses

Class	Types	Dose	Structure	Sources	Uses	Refs.
Flavone	Luteolin	50 mg/kg		Chamomile and parsley	Decrease in tumor necrosis factor (TNF)-a (39.28 ± 3.17), interleukin (IL)-1b (12.07 ± 1.24), and IL-6 (24.72 ± 2.52) in MSU crystal-induced rats	[12, 13]
Flavonol	Quercetin, Kaempferol	50 μM/kg		Onions, broccoli, apple, green tea and fruits	Inhibits LDL oxidation; protective against oxidative stress; protects and prevents inflammatory damage	[14, 15]
Flavanone	Naringenin, Hesperetin	50 mg/kg		Grapefruit, oranges, tangerine, tomatoes, aromatic herbs like mint	Decrease levels of elevated anti-oxidative enzymes, decrease in lipid oxidation, anti-hypertensive and anti-inflammatory effect	[16, 17]
Flavan-3-ol	Catechin, Epicatechin	10 mg/kg		Cocoa, apples, pears, tea, and grape based products	Vasculo-protective effect, decrease in blood pressure, improved vascular function	[18, 19]
Anthocyanidin	Cyanidin, Malvidin	150 mg/kg		Grape seed, Acai, strawberry, elderberry, Black currents	Restoration of mitochondrial function, decrease in lysosomal enzymes, decrease in lipid peroxidation, suppression of ROS	[20, 21]
Isoflavone	Daidzein, Genistein	0.04 mM/kg		Soy, legume seeds (lentil, beans, peas), potatoes, milk, meat	Suppression of (iNOS), cyclooxygenase-2 (COX-2), and NF-κB proteins in HUVECs	[22, 23]

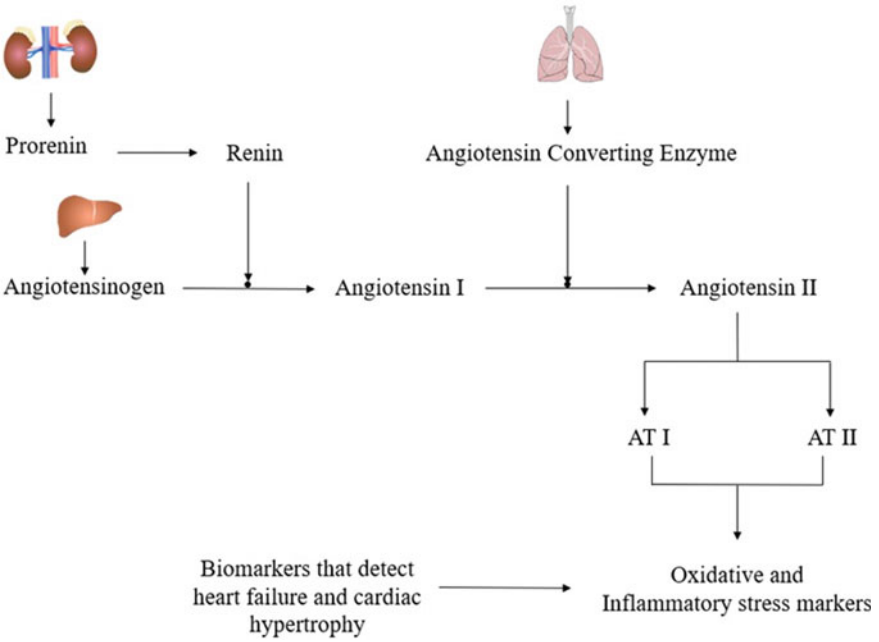


Fig. 23.1 Diagrammatic illustration of the components of the Renin Angiotensin System along with its correlation to biomarkers

cells occurs due to exercise or pregnancy, which is physiologically considered as a mild and reversible effect. However, a greater increase in the size of cells that is often irreversible, suggests that the heart is functioning under stress, which could be due to any pathological condition such as hypertension, cardiac disease or ischemia [29, 30]. Various internal and external factors are the cause of biomechanical stress which elicits a response leading to the thickening of the myocardium [31]. The various stress factors affecting the left ventricle include pressure–volume changes, genetic mutations of the sarcomeric proteins, and previous incidents of heart failure, which weaken the contractile tissue [29].

In hypertension, the pressure on the wall of the heart increases on account of the rise in afterload. As a result, the heart's compensatory mechanism triggers the production of collagen, fibroblasts and fibrous tissue as depicted in Fig. 23.2. An increase in interstitial myocardial fibrosis and myocardial mass, as well as significant changes to the coronary arteries, are some of the phenomena that contribute to a reduction in vascular coronary flow reserve [24]. Despite these changes, the ejection fraction of the heart is preserved. The consequence of these changes is a heightened cardiac performance and a diminution in the use of oxygen and wall tension of the ventricles as part of the compensatory mechanism. The visual result is the hypertrophic growth of the myocardium [25]. This asymmetric and necessitated growth, however, leads to the decrease in the volume of blood that can be accommodated

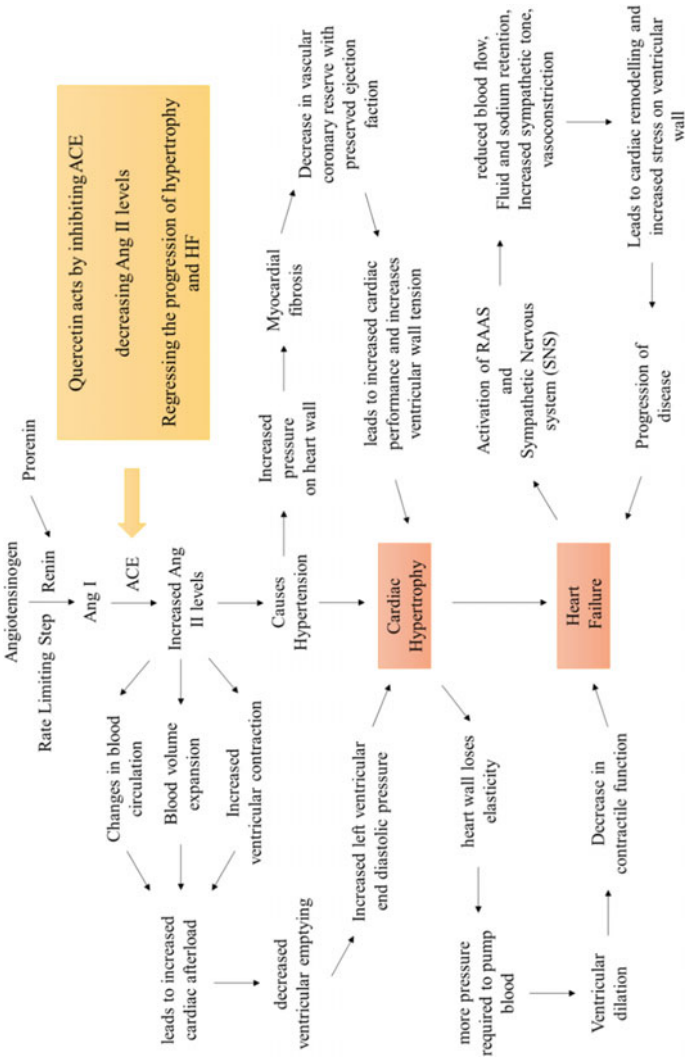


Fig. 23.2 Diagrammatic representation of the pathological basis of RAS-induced cardiac hypertrophy and heart failure and the mechanism involved in the protective action of quercetin

in the ventricle. The culmination of these effects lead to the potentiation of diseases such as ischemias and arrhythmias [28].

Cardiac Hypertrophy can also be induced by cardiac volume overload. Ang II present in the circulation has specific activities on the haemodynamics and fluid volume regulation to cause various effects such as changes in blood circulation, blood volume expansion, increase in ventricular contractions of the heart which brings about an increase in cardiac afterload and a decrease in left ventricular emptying. All these

effects cumulatively cause an increase in the left ventricular end-diastolic pressure leading to cardiac hypertrophy [32]. Ang II acts on the AT1R pathway and causes inflammation along with oxidative stress, thereby worsening the cardiac remodelling in hypertension [33]. Cardiac Hypertrophy is most common in the early stages of heart failure. There is a gradual progress towards the end stage of heart failure which includes reactivation of fetal gene programs as one of the many cellular changes it undergoes [34]. Ang II also plays an important role in the advacement of cardiac remodelling and consequent heart failure [35].

Heart Failure is a chronic pathophysiological condition wherein the heart cannot pump or eject adequate amounts of blood for the body's needs [5, 36]. Based on the location, heart failure can be classified into left ventricular, right ventricular and biventricular heart failure and based on the cardiac output, high output heart failure is potentiated by RAS and low output heart failure occurs due to ventricular dysfunctions [5]. Heart failure is usually anteceded by cardiac hypertrophy which is essentially the heart's response to greater workload or a cardiac arrest [37].

Due to low cardiac output produced during cardiac injury, the heart undergoes compensatory mechanisms such as the activation of renin–angiotensin–aldosterone system (RAAS) and sympathetic nervous system (SNS) [36]. The changes in the circulatory system which are caused by heart failure (low cardiac output) are detected by peripheral baroreceptors and chemoreceptors. The baroreceptors in the carotid sinus and aortic arch regulate the central nervous system's sympathetic and parasympathetic outflow. Changes in the cardiac output affect the effective arterial blood volume (EABV) that will result in a reduced parasympathetic response along with a rise in reflux of the sympathetic vascular tone. This in turn leads to vasoconstriction of the renal afferent arteriole and limited blood flow to the kidney [36, 38].

These compensatory mechanisms elevate the retention levels of sodium and water as well as increase vasoconstriction. RAS plays an important role in regulating blood pressure and electrolyte balance. Due to pathophysiological conditions, the RAS effects exhibited get amplified and cause inflammation which further leads to cardiac and endothelial vascular damage. Ang II by acting on AT1R and AT2R causes vasoconstriction and inflammation via the formation of reactive oxygen species (ROS) and multiplication of smooth muscle cells. This further advances into myocardial fibrosis which is responsible for left ventricular dysfunction [38]. The thickened heart wall in left ventricular hypertrophy loses elasticity and this prompts the heart to use more pressure in pumping out blood to the entire body. In due course, the heart will fall short of pumping blood with as much force as necessary. This chronic state results in ventricular dilation, fall in contractile function and eventually heart failure [39].

Biomarkers Involved in CVDs

Oxidative stress markers and inflammatory markers are essential markers used to detect heart failure and cardiac hypertrophy (Table 23.2). Oxidative stress and inflammation have been previously linked to increased blood pressure which in turn causes heart failure as seen in Figs. 23.1 and 23.2. Similarly, ROS from oxidative stress causes cardiac fibrosis, apoptosis, and cardiac hypertrophy [40].

Effects of Quercetin on the Cardiac Biomarkers

As shown in Table 23.2, fluctuation in the levels of cardiac biomarkers is used to assess the risk and identify the presence of CVD. Quercetin being a favourable candidate in reducing the risks involved in CVD and possibly attenuating its progression, was used in the study by Zahedi et al. [44]. In this study 72 women with type II diabetes were segregated into two groups, where one group was given the placebo and the other group was administered a daily dose of 500 mg quercetin capsule for 10 weeks. The inflammatory stress markers such as TNF-α and IL-6 presented a significant reduction in the serum concentration by demonstrating p values of $p = 0.01$ and $p < 0.001$ respectively. Yet, the overall mean serum level of IL-6, TNF-α and C-reactive protein between the test group and the placebo group showed no overall significant reduction as shown in Fig. 23.3.

The effect of quercetin on these inflammatory biomarkers can be enhanced by either adjusting the dose of quercetin, conducting more trials or using a larger patient pool, by dividing the dose of quercetin and increasing the dosing frequency. Thus, by conducting further investigation and employing a better study design, the effective role of quercetin on cardiac biomarkers can be established [44].

Quercetin as a Promising Cardioprotective Agent

Among all the naturally available flavonoids, quercetin has received much attention recently because of its beneficial effects in mitigating the CVDs. The versatile nature of this flavonoid has been explored in treating cancer and respiratory illnesses. Although well-designed clinical studies regarding the cardioprotective effects of quercetin are lacking, numerous clinical observations and nonclinical studies have shown its cardioprotective effects, which suggest that further studies are needed to evaluate the safety, efficacy, and optimal dose schedules of this promising flavonoid.

Table 23.2 Clinical biomarkers used in the detection of heart failure and cardiac hypertrophy

Biomarkers	Types	Inference	Refs.
1. Oxidative stress biomarkers	(a) Malondialdehyde (b) 8-Hydroxy-2-deoxyguanosine (c) Xanthine oxidase (d) Uric acid	(a) High levels show greater risk for heart failure (b) Plasma concentration determines the intensity and severity of heart failure (c) The higher levels of ROS indicate heart failure and cardiac hypertrophy (d) Hyperuricemia worsens oxidative metabolism, causing cardiac hypertrophy leading to heart failure	[40]
2. Inflammatory stress biomarkers	a) IL-1,6 & 8 b) C-reactive proteins c) TNF-α d) Pentraxin-3 and Galectin-3	a) In particular, IL-1 & 6 are the important markers, which rises with the onset of heart failure b) No major changes c) No major changes d) Increase in levels indicate heart failure	[40]
3. Other biomarkers of HF	a) Brain natriuretic peptide & N-terminal pro-BNP	a) BNP < 100 pg/ml may suggest less likely occurrence of HF b) BNP levels between 100 and 500 pg/ml may suggest the person has HF c) If levels are more than 500 pg/ml then the person should be treated immediately for HF	[41]
4. Other biomarkers of the cardiac hypertrophy	a) Micro-RNA b) NAD-dependent deacetylase sirtuin-3, growth/differentiation factor 15, glycoprotein 130, Endothelial growth factor c) Alpha and beta myosin heavy chains d) ANP, BNP and UBE3A	a) Controls the mRNA expression and any change in this expression indicates presence of cardiac hypertrophy b) No major changes c) No major changes d) Ubiquitin-protein ligase E3a (UBE3A) was increased on the induction of cardiac hypertrophy in rat heart cell line H9c2	[42, 43]

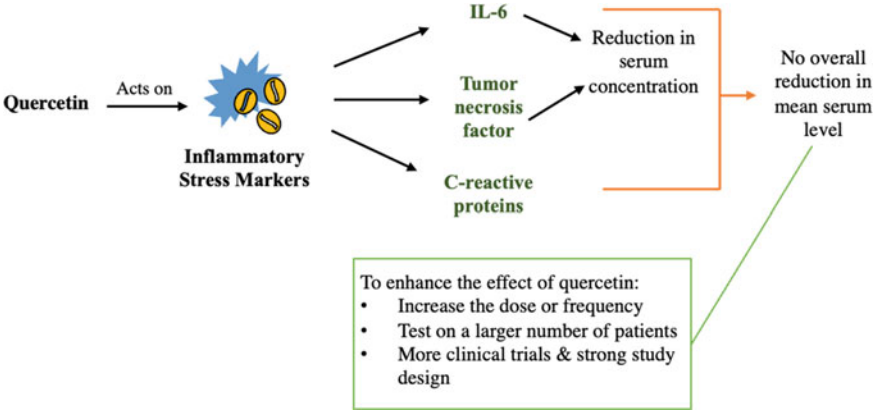


Fig. 23.3 Diagrammatic illustration of the mechanism of action of quercetin on the inflammatory stress biomarkers (IL-6, TNF-α and C-reactive proteins) produced during CVD

Chemistry and Structure-Activity Relationship (SAR) of Quercetin

From the historical perspective, quercetin is derived from the Latin word "Quercetum," which means an Oak Forest, and is named after Quercus which is a genus of hardwood [25]. Its IUPAC name is: 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one with a molecular formula of $C_{15}H_{10}O_7$, and the chemical nomenclature of quercetin is: 3,3',4',5,7-pentahydroxyflavone as shown in Fig. 23.4 [45, 46].

The major antioxidant potential of quercetin is attributed to the presence of hydroxyl groups attached at the ortho positions of ring B. The presence of hydroxyl

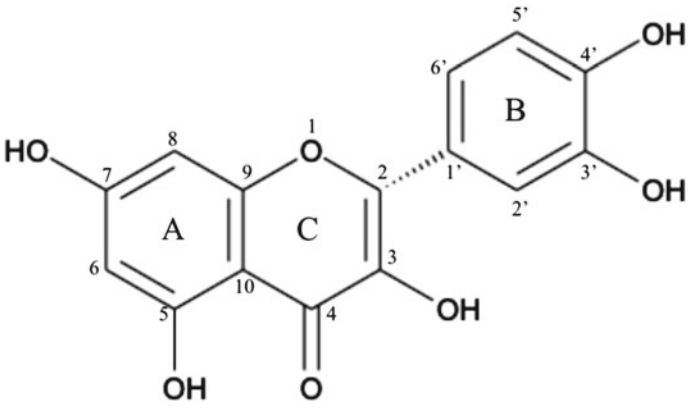


Fig. 23.4 Chemical structure of quercetin

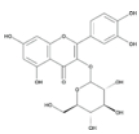
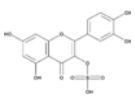
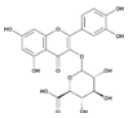
groups at position C3 and C5 as well as the carbonyl group on position C4 are also involved in the antioxidant activities. These groups react with metal ions such as Fe_3^{+++} and Cu_2^{++} via complexation mechanisms to prevent the progress of redox reactions [47].

Dietary Sources and Biological Properties of Quercetin

Quercetin as an aglycone is insoluble in water, moderately soluble in ethanol and acetic acid, and highly soluble in ether and methanol. These properties of quercetin give rise to the drawback of the low bioavailability of this molecule after oral administration. Efforts are being made to enhance the aqueous solubility of quercetin by nanotechnology and nanoemulsions. The different structural forms of quercetin which can be used in nanoformulations are shown in Table 23.3. Quercetin possesses a wide variety of pharmacological activities such as antioxidant, anti-inflammation, antibacterial, antihypertensive, anticholesterolemic, and anti-atherogenic [45]. It has been reported that the antioxidant activity of quercetin is greater than that of vitamin C and E [24].

This flavonoid is widely present in natural dietary sources such as citrus fruits, apples and berries, leafy vegetables such as broccoli and onions, and drinks such as tea and wine [45]. Quercetin is present in a high concentration in onion

Table 23.3 Various types of quercetin with their chemical structures and synonyms

Type	Structure	Synonym	Refs.
Quercetin 3-glucoside		Isoquercitrin Quercetin-3-glucoside Quercetin 3-O-beta-D-glucoside Quercetin-3-O-beta-D-glucoside	[51]
Quercetin sulphate		Quercetin 3-sulfate Quercetin 3-O-sulfate	[52]
Quercetin glucuronide		Miquelianin quercetin 3-O-glucuronide Querciturón Querciturone	[53]
Quercetin dihydrate		Quercetin dihydrate 6151–25-3 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one dihydrate MFCD00149487 Quercetine dihydrate	[54]

leaves (1497 mg/kg), bird chillies, tea, papaya, guava and semambu leaves [48]. Other sources include vegetables such as asparagus, tomatoes, lettuce and green pepper [49]. Medicinal plants such as Ginkgo biloba, and *Sambucus canadenis* and *Hypericum perforatum* are also significant sources of quercetin [50].

Cellular and Molecular Pharmacological Mechanisms of Quercetin

Among all the functions and activities of quercetin, some of the main molecular mechanisms are depicted in Fig. 23.5. The position of the hydroxyl groups in quercetin expresses a potent antioxidant effect to prevent the formation of superoxide radicals in the initiation stage, crypto-hydroxyl radicals in the chelation stage and lipid-peroxyl radicals in the peroxidation stage [47, 55].

A study was conducted in 2012 which enumerated the effect of quercetin in directly inhibiting ACE in the RAS pathway in a dose-dependent manner to decrease the mRNA production of ACE in the kidney [56]. This effect prevents hypertension which, in turn, inhibits smooth muscle proliferation and hypertrophy induced by Ang II [55]. Häckl et al. performed a study on rats which revealed that pre-treatment of 88.7 micromol/kg dose of quercetin for 45 min enhanced the hypotensive effect of Bradykinin (BK) (10 nmol/kg i.v.) when administered intravenously. Hence, this revealed a significant reduction in the hypertensive response to Ang I [28].

In humans, acute quercetin supplementation (200 mg) has also been shown to increase nitric oxide (NO) production, which could potentially prevent narrowing or twisting of blood vessels [57]. Quercetin alleviates ischemia reperfusion injury

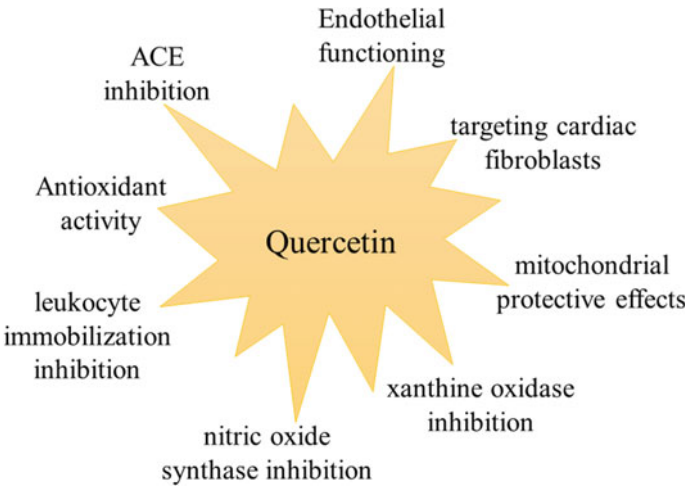


Fig. 23.5 Diagram showing cellular and molecular pharmacological actions of quercetin

by preventing endothelial dysfunction through nitric oxide synthase interference. It prevents high concentrations of NO from accumulation and causing oxidative damage. Additionally, quercetin inhibits xanthine oxidase activity and reduces free radical formation to prevent damage to the heart tissues [58].

Apart from these mechanisms, quercetin inhibits enzymes cyclooxygenase and lipoxygenase, which prevents the formation of inflammatory mediators. Quercetin also inhibits adhesion of leukocytes to the endothelial wall to prevent release of cytotoxic oxidants and inflammatory mediators [58]. Quercetin dihydrate was found to inhibit the secretion of inflammatory factors TNF-α, IL-1β and IL-6 of cardiac fibroblasts by decreasing the activation of protein expression of Nuclear Factor kappa B (NF-κB) p65 and Protein kinase B (Akt) in neonatal rats [59]. Quercetin also acts on the SIRT3/PARP-1 pathway by protecting the mitochondrial function in cells. These various pathways depict the regression of pathological state by maintaining the cellular functions and internal conditions of cardiac cells.

Promising Therapeutic Applications of Quercetin

Apart from its protective role in CVDs, quercetin may be useful for a variety of other pathophysiological conditions, such as to induce apoptosis and inhibit blood vessel proliferation in tumour cells. Its antitumor effects have been mainly studied in breast cancer cell lines and ovarian cancer [25, 60]. Quercetin can decrease toxicity from mycotoxins by protecting the cells from stress and apoptosis, and this is another pathway that supplements its antioxidant and anti-inflammatory properties [25].

Quercetin improves the responsiveness of insulin and regulates the levels of eNOS and iNOS which get imbalanced in hyperglycaemia. It prevents oxidation of cells in the liver, kidneys and the retina, preventing major diabetic complications. It also prevents human umbilical vein endothelial cells (HUVECs) from getting damaged by hyperglycaemia through autophagy.

Through its antioxidant, anti-inflammatory and apoptosis mechanism, quercetin has the capacity to protect the brain cells and inhibit the development of Alzheimer's disease. Quercetin can supress the formation of neurofibrillary tufts by inhibiting hyperphosphorylation of tau. Quercetin's anti-inflammatory effect is also used for the treatment of rheumatoid arthritis in conjugation with methotrexate. Studies on mice showed a decrease in hyperalgesia and oedema with no side effects to other organs [24].

Recent Advances in the Management of Cardiac Hypertrophy and Congestive Heart Failure

Congestive heart failure and cardiac hypertrophy have been on a rise for the last few decades. According to certain statistical data, around 80% of the world's population are inclined towards using naturally derived medicines, due to their fewer side effects and acceptability in the society. Hence, there is an ever-increasing demand and need for developing medicines that have a natural origin. The therapeutic effect of Quercetin on RAS in conditions like, heart failure and cardiac hypertrophy has been significant. Molecular docking and in-silico analysis has been proved, yet again, as an effective method to screen molecules and their interaction with biological targets like ACE [61]. The prolonged use of marketed antihypertensives and ACE inhibitors can cause many harmful side effects. This led to the exploration of new naturally derived alternatives to treat and prevent heart failure and cardiac hypertrophy.

Molecular Mechanisms of Quercetin in Improving CVDs Mediated by RAS

Quercetin can be used to target RAS-induced heart failure as depicted in Fig. 23.2. This bioactive phytochemical has been virtually screened for its use as an effective ACE inhibitor, which acts by preventing the conversion of Ang I to Ang II (See Fig. 23.1). After the docking tests, it was observed that quercetin showed a good binding affinity in comparison to certain standard marketed anti-hypertensive drugs to its biological target [61]. The Zutphen Elderly's Study suggested that there is a prominent relationship between quercetin like flavonoids and their cardioprotective action. Overactivation of RAS can gradually lead to hypertension which may cause heart failure (See Fig. 23.2). Quercetin has been proven to inhibit ACE in-vitro by using metal chelation as a molecular mechanism [62].

Endothelial cells are an important part of our human body and are responsible for controlling many mechanisms and processes such as angiogenesis, vasoconstriction, and vasodilation, etc. Endothelial malfunctioning and apoptosis are both associated with the pathogenesis of many cardiovascular diseases. HUVECs were used to describe the effect of quercetin on Ang II against endothelial cell apoptosis and cardiovascular diseases. Subsequently, it was found that quercetin posed as a good Ang II induced apoptosis inhibitor in HUVECs, by handling mitochondrial-dependent pathways. It prevents the Ang II based injury to endothelial cells thereby acting against a variety of cardiovascular diseases [63]. Figure 23.6, summarises the various ways by which quercetin acts on the RAS to prevent heart failure.

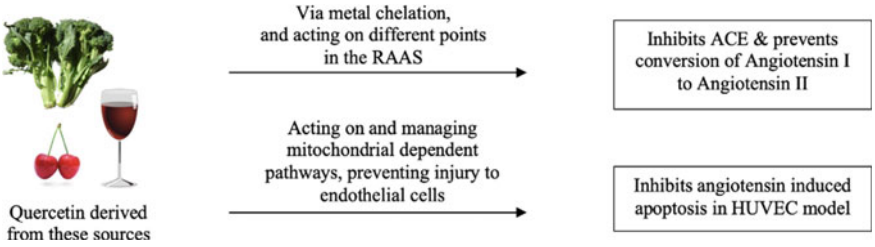


Fig. 23.6 Showing the multiple actions of quercetin derived from red grapes and broccoli on RAAS and prevention of heart failure

Targeting the Mechanism of Action Quercetin for Heart Failure

Myocardial fibrosis is a pathological condition that can lead to heart failure. This condition is often caused due to dysregulated extracellular matrix, various oxidative stress caused by ROS, improper collagen component ratio and inflammation. Oxidative stress and inflammation lead to various conditions, a major one being the damage to cardiomyocyte cells. Results of an in vivo study conducted by Chang et al. showed that quercetin can prevent heart failure caused by myocardial fibrosis by a number of actions: (a) regulating myocardial cell mitochondrial homeostasis, (b) inhibiting inflammation and oxidative stress, (c) increasing SIRT5 expression in cardiac cells d) promoting IDH2 desuccinylation (e) preventing cardiomyocyte death. Quercetin increases SIRT5 expression and the promotion of IDH2 desuccinylation that can extensively prevent heart failure by protecting the myocardial tissue [64].

Role of Quercetin in RAS Induced Cardiac Hypertrophy

Quercetin interrupts cardiac remodelling, thereby preventing and suppressing LVH (See Fig. 23.2). Ang II is known to play a major role in causing cardiac hypertrophy, by directly acting on the cardiac myocyte. The expression of both angiotensin receptors is modulated during cardiac hypertrophy [65].

Quercetin dihydrate in a recent study, has shown some inhibition in the induction and elevation of cardiac fibrosis caused by Ang II, both in vivo and in vitro, which is a key factor that causes cardiac hypertrophy. As cardiac fibrosis leads to cardiomyopathy, designing and creating effective anti-fibrosis agents is of significance. Targeting cardiac fibroblasts is perceived to be crucial as it is one of the main effector cells. Quercetin dihydrate could hinder the production of abnormal levels of the biomarker's collagen I and III in the mouse heart by inhibiting the mRNA. The flavonoid quercetin prevents oxidative stress and inflammation, cardiac fibrosis,

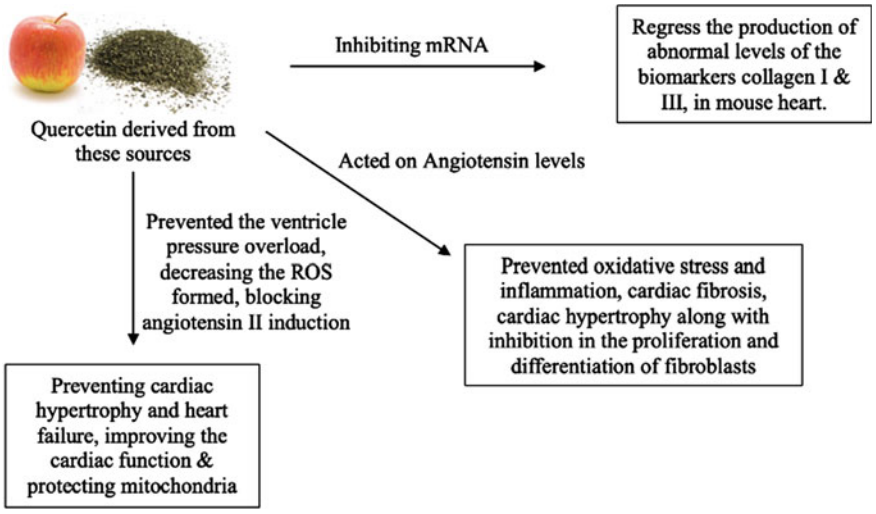


Fig. 23.7 Showing the multiple actions of quercetin derived from apple and green tea on RAS in preventing cardiac hypertrophy

cardiac hypertrophy and inhibits the proliferation and differentiation of fibroblasts caused by Ang II [66].

Various forms of quercetin (Table 23.3) elicit a cardioprotective effect in cardiac hypertrophy [67]. Quercetin was recently studied in rats to understand and discuss its effectiveness in preventing ventricular pressure overload (See Fig. 23.2), and thereby preventing cardiac hypertrophy and heart failure [68]. Ang II treated H9c2 cell line was evaluated, by testing the mitochondrial protective effect of quercetin. Similarly, spontaneously hyperactive rats (SHR) were treated with quercetin to check the effects on cardiac hypertrophy and blood pressure. In both the above treatments, quercetin proved to be an effective agent by improving cardiac function, decreasing the ROS formed, and blocking Ang II induction by protecting mitochondria [67].

Quercetin also acts by counteracting the elevated levels of ANF and β-myosin heavy chains which are often raised due to Ang II [67]. Figure 23.7 presents the different ways by which quercetin acts on RAS to curb the escalation of cardiac hypertrophy and all the conditions that lead to the same.

Preclinical and Clinical Studies Done with Quercetin

Alexandre et al. conducted a preclinical study on male swiss mice to demonstrate the effect of quercetin in blocking the H_2O_2 (hydrogen peroxide) along with some antioxidant activity. A dose of 10 mg/kg/day of quercetin was given orally to one set of mice that were pre-treated with 30 mg/kg/day of intraperitoneally injected isoproterenol. The results obtained from the trial suggested that on administration

of quercetin suppression of the ROS pathway, restoration of antioxidants, H_2O_2 blocking effect, antioxidant effect, as well as a decline in the advancement of cardiac hypertrophy was observed [69].

In yet another study performed by Hui et al., SD rats were used for understanding the possible anti-inflammatory and anti-oxidative stress effect of quercetin as well explained the mechanism of quercetin preconditioning on anti-myocardial ischemia-reperfusion [70]. About 250 mg/kg dose of quercetin was given subcutaneously to the SD rats that were anaesthetised with pentobarbital. After 10 days of conducting this study, the anti-apoptotic, anti-inflammatory and anti-oxidative stress effects of quercetin were observed (Table 23.4) [67].

HUVEC has also been used to study the effect of quercetin on Ang II levels to prevent heart failure. The HUVECs were specifically pre-treated with 100 μm of Ang II, with varying concentrations of 5,10 and 15 μm of quercetin, within a given period of 24 h. This study concluded that quercetin could successfully act as an Ang II induced apoptosis inhibitor (Table 23.4) [63].

In 2020, Wang et al. demonstrated the inhibitory action of quercetin on cardiac fibrosis caused by Ang II. Quercetin Dihydrate when injected in a male wild type

Table 23.4 Preclinical studies carried out in animal models to assess the cardioprotective effects of quercetin

Test organism	Dose	Duration	Study design	Result	Refs.
60 days old male Swiss mice (25–30 g)	10 mg/kg/day	8 days	Pre-treated with isoproterenol (30 mg/kg/day)	Quercetin exhibited H_2O_2 blocking effect, antioxidant effect and attenuation of cardiac hypertrophy	[69]
SD rats	250 mg/kg	10 days	Pre-treated with LY294002 (0.2 mg/kg, s.c.)	Anti-apoptotic effect along with the anti-inflammatory and anti-oxidative stress action	[70]
HUVECs-ex-vivo (human umbilical vein endothelial cell-line)	Varying concentrations of 5, 10 and 15 μm of quercetin	24 h	Pre-treated with 100 μm of Ang II	Quercetin acted as an angiotensin II induced apoptosis inhibitor	[63]
Male wild-type (WT) c57bl/6 Mice (8 weeks of age)	25 mM/kg body weight	Once every 2 days for 2 weeks	Treated with 1000 ng/kg/min Ang II 2 days after giving quercetin	Quercetin dihydrate decreased the production of collagen I and III by inhibiting the mRNA	[66]

C57BL/6 mouse, and when given in 25 mM/kg body weight dose, once every two days for a duration of 2 weeks, decreased the production of collagen I and III by inhibiting mRNA in the mouse heart. Quercetin Dihydrate also helped in improving cardiac functioning and prevented structural remodelling of the heart by acting on Ang II levels [66].

The results of clinical studies conducted with quercetin to evaluate its cardioprotective effects are summarised in Table 23.5. For example, Shatylo et al. did a clinical study in 110 elderly patients suffering from metabolic syndrome. A dose of 240 mg quercetin tablet when administered orally thrice a day for 3 months, showed significant decrease in systolic ($p < 0.001$) and diastolic blood pressure ($p = 0.001$), decrease in fasting plasma insulin ($p = 0.02$) and LDL cholesterol ($p = 0.007$). Most of the oxidative stress markers remained the same except for glutathione which showed a prominent decrease in levels ($p = 0.005$). This study confirmed the therapeutic benefits of quercetin in elderly patients for treating metabolic syndrome (MetS) [71].

The effect of quercetin on managing blood pressure was conducted via performing double blind, placebo-controlled crossover trials. 19 men and women with prehypertension and 22 men and women with stage 1 hypertension were subjected to a dose of 730 mg/day of quercetin for 28 days. The patients with stage 1 hypertension exhibited a drastic decrease in diastolic, systolic and mean arterial pressure, owing to a reduction in hypertension which is one of the leading causes of heart failure [75].

Zahedi et al. conducted a double-blind randomized controlled clinical trial, to verify and assess the result of quercetin on cardiovascular risk factors and inflammatory biomarkers in women with Type II Diabetes. A Quercetin capsule of 500 mg was administered once daily for 10 weeks in 72 women participants. The study concluded that even though quercetin exerted some decrease in levels of systolic blood pressure ($p = 0.04$), there were hardly any significant changes observed in alleviating cardiac risk factors [44].

Moonikh et al. performed a randomized double-blind clinical trial involving 24 men predisposed with hypertension and coronary heart disease after percutaneous coronary intervention. A dose of 250 mg/day of quercetin was given daily for about two months, and it showed a significant reduction in the systolic and diastolic blood pressure in subjects, along with a remarkable decrease in oxidative stress. However, no significant effect was seen in the case of ventricular hypertrophy (Table 23.5) [73].

To understand the cardioprotective effect of quercetin Chekalina et al. conducted an open randomized controlled trial in parallel groups, involving about 85 participants with pre-existing coronary heart disease. The patients were randomly segregated into two groups—a comparison group with 55 patients and a research group with 30 patients. They were given 120 mg/day of quercetin for over 2 months. The outcome of the study was that quercetin aided in the reduction of left ventricular ejection fraction and left ventricular systolic function showing a p value of ($p < 0.05$) along with some good cardioprotective action.

Table 23.5 Clinical studies conducted with quercetin to evaluate its cardioprotective effects

Test organism	Dose	Duration	Study design	Result	Refs.
110 Elderly patients (aged 60+) suffering from metabolic syndrome	240 mg	3 times a day for 3 months	Randomized, placebo-controlled, double-blinded	Decreased the systolic and diastolic blood pressure, decreased fasting plasma insulin and LDL cholesterol. Only glutathione amongst other biomarkers showed a significant reduction in levels	[71]
Men and women with pre-hypertension (n = 19) and stage 1 hypertension (n = 22)	730 mg/day	Daily for 28 days	Double blind, placebo-controlled crossover trial	stage 1 hypertensive population exhibited a drastic decrease in diastolic, systolic and mean arterial pressure, owing to a reduction in hypertension	[72]
Women (age 35–55 yrs) participants with type II diabetes (n = 72)	500 mg	Once daily for 10 weeks	Double blind randomized controlled trial	Exerted some decrease in levels of systolic blood pressure. No significant changes were observed in alleviating cardiac risk factors	[44]
Men with hypertension and CAD after PCI (aged 40–60 years) (n = 24)	250 mg/day	Every day for 2 months	Randomize, double-blind trial	Reduced the systolic and diastolic blood pressure in the subjects, along with a remarkable decrease in the oxidative stress. However, no reduction or regression of ventricular hypertrophy was noted	[73]

(continued)

Table 23.5 (continued)

Test organism	Dose	Duration	Study design	Result	Refs.
Patients with coronary heart disease (n = 85)	120 mg per os	Daily for 2 months	Open randomized controlled trial in parallel groups	Improved the left ventricular systolic function in terms of ejection fraction (EF) of LV. Overall the study proved the cardioprotective effect of quercetin	[74]

Quercetin Containing Dietary Supplements Marketed for Treating Different Ailments

There are over 40 branded marketed formulations of quercetin available in the market as dietary supplements and for cosmetic purposes. The few marketed capsules and tablet formulations of quercetin that are specifically used for anti-inflammatory, anti-oxidative benefits and other health concerns are listed down in Table 23.6.

Opportunities and Challenges of Quercetin Therapy

The use of quercetin for the purpose of treating CVD such as heart failure and cardiac hypertrophy has been researched thoroughly in the last few years. With the benefits of being a naturally derived phytochemical, quercetin shows natural ACE inhibition activity, vasodilatory action, along with anti-inflammatory action and also cardioprotective activity [77].

Despite manifesting its versatile nature in attenuating CVD's, cancer, etc., quercetin's use is limited due to its poor solubility, which in turn limits its bioavailability. Quercetin's instability in the physiological medium like that of the stomach, shorter half-life and the extensive metabolism in the liver are some of the drawbacks that restrict its application in translational therapy. The major issue of improving the bioavailability of poorly soluble flavonoids can be achieved by using nanotechnology. Thus, the study conducted by Salehi et al. showed that nanomedicines can improve the solubility and bioavailability of flavonoids, helping in achieving a better-targeted delivery and a good drug release profile [24].

Incorporating quercetin onto cellulose nanofiber surface can help with better drug loading, better drug release, solubility and targeting profile [78]. A study performed by Park et al. shows that the solubility of quercetin almost increased 35.1-fold when it was complexed with mono-6-deoxy-6-aminoethylamino-b-cyclodextrin [79]. Papan et al. took an interesting approach with respect to quercetin and its use by complexing it with iron [80]. An iron (III) quercetin complex was prepared and tested for

Table 23.6 Examples of the most popular quercetin formulations marketed as dietary supplements [76]

Product	Dose	Formulation	Indication
Quercetin 500 mg (Nature's Best)	500 mg	Tablets	Antioxidant benefits and cardiovascular health improvement
Sandhu's Zinc Quercetin	400 mg	Capsules	Antioxidant benefits, weight loss benefits due to zinc
Algonot plus (algonot)	150 mg	Capsules	May support the structure & function of cartilage and joint maintenance in the body while being a useful anti-inflammatory
Solaray	500 mg	Capsules	Respiratory and Immune support
HealthVit	100 and 500 mg	Capsules	Helps with seasonal discomfort and supports the immune system
Doctor's Best (Quercetin with bromelain)	500 mg quercetin 250 mg bromelain	Capsules	Builds up immunity and has an anti-inflammatory action
Natural Factors Bioactive Quercetin EMIQ	50 mg	Capsules	Reduces oxidative stress, acts as an anti-inflammatory
Bluebonnet Super Quercetin	500 mg	Very high bioavailability quercetin soft gel capsules	Antioxidant and anti-inflammatory
Thorne Research Quercenase	250 mg	Capsules	Immunity booster
MoxyVites Quercetin phytosome and bromelain	500 mg	Capsules	Phytosome is the most stable form of quercetin in the stomach helping with oxidative stress caused by free radicals and reduces inflammation

its potential application in labelling and tracking circulating proangiogenic cells (CACs) and stem cells for their possible use in tissue regeneration and cell therapy. Quercetin phytosome is a new type of delivery system developed from phospholipids, which greatly improved the bioavailability and stability of quercetin in a very recent study [81]. Several approaches have been taken to understand the mechanism of quercetin and its potential therapeutic effect in controlling the progression of cardiac hypertrophy and heart failure.

Pre-clinical studies on rats and mice along with clinical studies in human subjects have shown enough evidence regarding the benefits and possible therapeutic properties of the drug [69, 71]. However, the pre-clinical and clinical approaches still have not been able to successfully establish a credible bench to bed approach [82].

Discussion and Future Prospects

In today's world the constant change in lifestyle is taking an unhealthy toll on the health of humans. The shift in socio-economic status has caused a drastic increase in the prevalence of CVDs, which corresponds to 30% of all deaths globally [83].

Various life-threatening diseases such as arrhythmia, ischemia's, cardiac fibrosis, cerebrovascular disease, etc., are all interlinked with each other through hypertension. The RAS system plays an important role in regulating the Ang II levels, which if left unregulated can progress further into several cardiac disorders.

Quercetin is proven to be an efficient cardioprotective flavonoid, capable of regulating the levels of Ang II, by inhibiting ACE that can be used against a multitude of heart diseases through the inhibition of mRNA levels, decreasing ventricular pressure overload, decreasing ROS, preventing the injury to endothelial cells and exhibiting antioxidant and anti-inflammatory action.

Several research studies have been undertaken for the treatment of CVD using quercetin in the last decade. The adoption of naturally obtained drugs has demonstrated lesser side effects for the treatment of lifestyle diseases like CVD, obesity, etc. The existing preclinical and clinical data suggest that the advancement in establishing a better and more reliable connection between quercetin and its therapeutic mechanism is essential. The low solubility and bioavailability profile of quercetin acts as a barrier for introducing it into the market. The drawback of this naturally obtained flavonoid can be tackled by using a novel drug delivery system. Nanotechnology has emerged as the one true solution for all the poorly soluble, naturally derived phytochemicals. Incorporating quercetin in different types of nano formulation has resulted in its increased bioavailability and solubility. These nano formulations have also been successful in improving the drug loading capacity and delivery at the targeted site [84].

Future prospects of quercetin as an experimental drug for ameliorating cardiac hypertrophy and heart failure is subject to its improvement in bioavailability and better understanding in its specific target mechanism. Owing to a significant translational gap, further clinical studies can help in bringing quercetin into the market as a possible treatment option for patients suffering from CVDs.

Conclusions

Based on the current global scenario, the number of people suffering from hypertension, coronary heart disease, and stroke has escalated over the past decades. This is attributed to a wide array of risk factors such as lower consumption of fruits and vegetables, poor dietary habits, life-related stress, irregular sleep patterns, intake of high carbohydrate and cholesterol rich foods, excessive use of sugar loaded drinks, smoking and drinking, sedentary lifestyle, lack of physical exercise, and exposure to environmental toxicants. A direct correlation is observed between an increase in CVDs and such mentioned trends. Another underlying mechanism of CVDs can be attributed to the changes in the RAS. Current medications used for the treatment of CVD have certain side effects and limitations, which has led to the possibility of exploring flavonoids as affordable and safe treatment options.

Quercetin being a naturally derived flavonoid can potentially replace the allopathic ACE inhibitors used for hypertension. Even though quercetin is available as a daily intake dietary supplement for building immunity and showing some antioxidant properties, it has not been used as a preferential immediate treatment for CVD. In comparison to allopathic medication, quercetin shows lesser side effects, better bioavailability, and precise drug targeting when used as a nano formulation. Further investigation on the therapeutic effects of quercetin should be encouraged, to introduce it as a successful candidate to improve the quality of life of patients suffering from CVD.

Conflict of Interest The authors declare no conflict of interest.

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Chapter 24
H_2S Signals and Renin Angiotensin System in Cardiovascular Diseases

Jiechun Zhu and Guangdong Yang

Abstract Hydrogen sulfide (H_2S) is recently recognized as the third gasotransmitter alongside with nitric oxide and carbon monoxide. H_2S exerts multifaceted physiological functions, including antioxidant, anti-inflammation, anti-apoptosis, angiogenesis, vasodilatation, metabolic modulation, and mitochondria bioenergetics, etc. The beneficial importance of H_2S signals has been extensively demonstrated in various cardiovascular diseases (CVDs). The renin angiotensin system (RAS) plays a central role in the pathogenesis of CVDs. By inducing hypertrophy of cardiomyocytes, damaging endothelial cells, stimulating proliferation of smooth muscle cells and fibroblasts, and activating inflammatory immune cells, RAS impairs the structure and function of heart, blood vessels and kidney. Inhibition of RAS activation by angiotensin-converting enzyme inhibitors and/or angiotensin receptor blockers are clinically used for the treatment of CVDs. H_2S signals are capable of counteracting RAS activation in a variety of cardiovascular pathologies. This review aims to summarize the current knowledge on the interaction of H_2S signals and RAS in CVDs and provide future directions for the development of targeted drugs to reduce cardiac risks.

Keywords Hydrogen sulfide · Renin angiotensin system · Cardiovascular disease · Angiotensin-converting enzyme inhibitors

Abbreviations

ACE	Angiotensin-converting enzyme
Ang I	Angiotensin-I
Ang II	Angiotensin-II

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AT1R	Angiotensin receptor type I
AT2R	Angiotensin receptor type II
CBS	Cystathionine β-synthase
CSE	Cystathionine γ-lyase
CVDs	Cardiovascular diseases
HUVECs	Human umbilical vein endothelial cells
H_2S	Hydrogen sulfide
MST	3-Mercaptopyruvate sulfurtransferase
NO	Nitric oxide
RAS	Renin angiotensin system
ROS	Reactive oxygen species
SHR	Spontaneously hypertensive rat
SMCs	Smooth muscle cells
WKY	Wistar Kyoto

Introduction

Cardiovascular diseases (CVDs) are the leading cause of death worldwide. The pathogenesis of CVDs is very complicated and can be caused by aging, sedentary behavior, smoking, diet, and/or pre-existing medical conditions such as diabetes, kidney disorder, autoimmune diseases, and cancer, etc. [1, 2]. Inflammation and oxidative stress are believed to be the main driving forces for the initiation and development of CVDs [3, 4]. Accumulating evidence has demonstrated that the risk factors of cardiovascular outcomes are often associated with over-activation of the classical renin angiotensin system (RAS) and downregulation of H_2S signals [5–12]. The RAS is a complex hormonal system regulating the functions of multiple tissues, including cardiovascular organs [5, 8]. Traditionally known as an environmental pollutant with a distinctive rotten-egg smell, H_2S is recently recognized as a novel gasotransmitter with important physiological/pathophysiological functions [13–15]. Especially, H_2S acts as an unlikely hero in the cardiovascular system [16–19]. In a variety of cardiovascular pathologies, H_2S signals are capable of counteracting RAS activation. This current review briefly describes the physiological importance of RAS and H_2S signals and summarizes the recent research progress on the complexity and interaction of H_2S signals and RAS in CVDs. The therapeutic potential of H_2S as a new RAS inhibitor to treat CVDs is also highlighted.

Enzymatic Pathways of Endogenous H_2S Generation and Its Cardioprotective Roles

The endogenous production and metabolism of H_2S are mostly enzymatically regulated (Fig. 24.1). Three enzymes have been responsible for H_2S generation in different cells and tissues, including cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (MST) [14, 20–24]. As pyridoxal-5′-phosphate-dependent enzymes, both CSE and CBS can catalyze L-cysteine and/or homocysteine to release H_2S [22, 23]. MST produces H_2S through the cooperation with cysteine aminotransferase by using 3-mercaptopyruvate as substrate [21]. The distribution of these 3 enzymes is tissue specific. CSE is reported to be the major H_2S-generating enzyme in the cardiovascular system, while CBS is primarily detected in neurons and astrocytes of the central nervous system [14, 18, 23, 25]. MST also contributes to neuronal and endothelial production of H_2S [26, 27]. In some organs, all these 3 enzymes exist, i.e., liver and kidney [20, 28]. Under the physiological conditions, endogenously produced H_2S is quickly eliminated via ethylmalonic encephalopathy protein and sulfur:quinone oxidoreductase-mediated oxidation for forming sulfite and sulfate in mitochondria [29, 30]. H_2S also undergoes methylation by thiol S-methyltransferase to produce unharmful dimethylsulfide and methanethiol [31].

H_2S exerts multifaceted biological functions in the cardiovascular system, including antioxidant, anti-inflammation, anti-apoptosis, angiogenesis, vasodilatation, metabolic modulation, and mitochondria bioenergetics, etc. [9–18]. The underlying mechanism postulated to serve as an explanation of these effects is protein *S*-sulfhydration, also named persulfidation, which is a way of post-translational modification of proteins by yielding a hydropersulfide moiety (-SSH) in specific cysteine residue(s) [20, 32]. It has been widely demonstrated that H_2S signals contribute to cardio-protection and maintenance of vascular function and integrity [14, 15, 19, 33, 34]. Abnormal generation and metabolism of H_2S are linked to many CVDs [10, 12, 34]. Genetic knockout of CSE in mice results in significantly lower cardiac H_2S level, impaired endothelial function, marked hypertension, and accelerated atherosclerosis and heart disorders [14, 18, 35]. Cardiac-restricted overexpression of CSE in mice displays clear protection against heart disorders [34]. The mice with the deficiency of either CBS or MST also developed endothelial dysfunction and cardiac disorders [26, 36]. All the evidence indicates that H_2S acts as a novel and important gasotransmitter for the cardiovascular system.

The RAS in Cardiac Dysfunctions

The RAS is a well-known hormonal cascade regulating cardiac functions and plays a central role in the pathogenesis and development of various CVDs at all stages

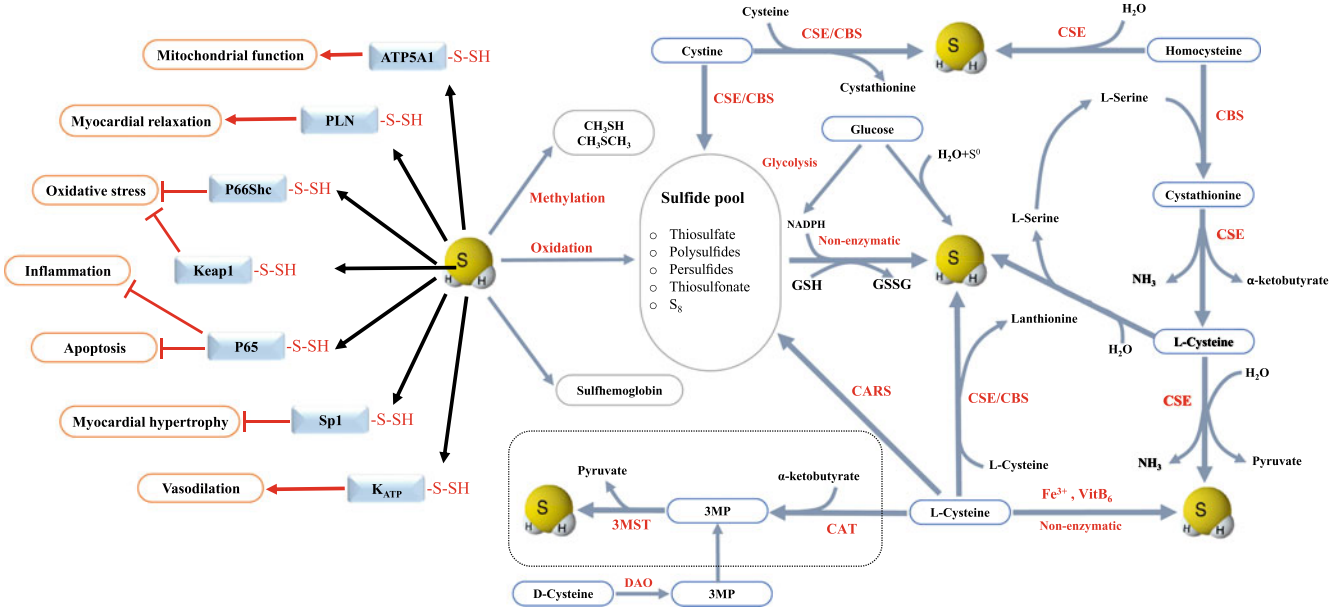

Fig. 24.1 The metabolism and cardioprotective role of endogenous H_2S. H_2S production occurs via both non-enzymatic and enzymatic pathways. Endogenously H_2S exerts cardioprotective functions via *S*-sulfhydration, participating in the regulation of oxidative stress, inflammatory processes, apoptosis, vasodilation, mitochondrial functions, myocardial relaxation and hypertrophy, etc. Abbreviation used: ATP5A1, ATP synthase subunit α; CARS, cysteinyl-tRNA synthetase; GSH, glutathione; GSSG, glutathione disulfide; K_{ATP}, ATP-sensitive potassium channel; Keap1, kelch-like ECH-associating protein 1; NADPH, nicotinamide adenine dinucleotide phosphate; PLN, phospholamban; SP-1, specific protein-1

[7, 8]. There are two major pathways for RAS activation, the classical and the non-classical pathway (Fig. 24.2). In the classical pathway of RAS activation, liver-synthesized precursor angiotensinogen is firstly cleaved by the enzyme renin to generate angiotensin-I (Ang I), which in turn is used as a substrate for the angiotensin-converting enzyme (ACE) giving rise to angiotensin-II (Ang II) [5]. As the main effector molecule of the RAS, Ang II is primarily binding to membrane protein angiotensin receptor type I (AT1R) for its intracellular activities [35]. AT1R activation often leads to the generation of aldosterone, an important player in the regulation of electrolyte balance [5, 35]. Under pathophysiological conditions, the activation of RAS and generation of Ang II lead to oxidative stress, inflammation, hypertrophy, fibrosis, and vasoconstriction, thus promoting compromised functions of several organs in the long run, including heart, blood vessels and kidney [37]. In contrast, by binding to AT2R, Ang II is able to antagonize the effect of AT1R by promoting vasodilation, anti-proliferation, anti-fibrosis, and anti-inflammation [38]. Application of ACE inhibitors and AT1R blockers are widely used as therapeutic agents in the clinic for the treatment of CVDs [39]. The non-classic RAS pathway is mostly mediated by the ACE2-Ang(1–7)-Mas receptor axis [40, 41]. A homolog of the ACE enzyme, named ACE2, can convert Ang I to Ang(1–9), which in turn can be converted to Ang(1–7) by other peptidases, thus ACE2 is able to antagonize ACE to reduce Ang II levels [40]. Ang(1–7) appears to improve CVDs via its anti-inflammatory and anti-oxidative stress properties [41]. The non-classical RAS components contribute to cardio-protection in competition with the classical system [40, 41].

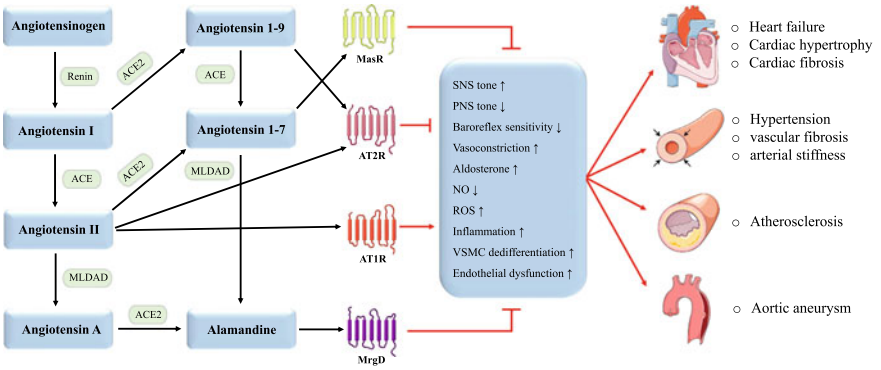

Fig. 24.2 The activation of RAS signaling and their pathophysiological effects on cardiovascular system. There are two major pathways for RAS activation, classical and non-classical pathway. Abbreviation used: ACE, angiotensin-converting enzyme; AT1R, Ang II type 1 receptor; AT2R, Ang II type 2 receptor; MasR, mas receptor; MLDAD, mononuclear leukocyte-derived aspartate decarboxylase; MrgD, member D of Mas1-related G-protein-coupled receptor; NO, nitric oxide; PSNS, parasympathetic nervous system; ROS, reactive oxygen species; SNS, sympathetic nervous system; VSMC, vascular smooth muscle cell

Interplay of H_2S Signals and RAS in CVDs

Animal models are often used to study pathophysiology and develop drugs for CVDs. Mounting evidence from animal studies has clearly revealed the interaction of H_2S signals and RAS activation in the development of various CVDs, including hypertension, cardiac hypertrophy and fibrosis, heart failure, atherosclerosis, aortic aneurysms, and endothelial dysfunctions, etc. (Table 24.1).

Hypertension

Hypertension is a chronic medical disorder with high prevalence in the elderly population and is also a high-risk factor for many other CVDs including stroke and heart failure. The negative coordination of H_2S signals and RAS have been widely demonstrated in different animal models of hypertension, including those with Ang II infusion, spontaneously hypertensive rats (SHR), DahI salt-sensitive rats, nitric oxide synthase inhibition, etc.

Ang II is a well-known inducer of hypertension. Chi et al. showed that chronic infusion of Ang II via an osmotic pump for up to 4 weeks induced higher blood pressure in mice. CSE expression and H_2S levels were much lower in aorta tissues, smooth muscle cells (SMCs) and endothelial cells (ECs) when incubated with Ang II [42]. It was further found that Ang II-induced proteasomal degradation of CSE via a deacetylase activity-dependent manner, the elevation in blood pressure mediated by Ang II was returned to normal level when CSE deacetylation was prevented by HDAC6 inhibitor tubastatin A, thus the vasoconstriction and hypertension caused by Ang II could be due to the dysregulation of CSE/H_2S system [42]. Similarly, Chi et al. further confirmed that Honokiol (a natural compound in the *Magnolia* plant) ameliorated Ang II-induced hypertension and endothelial dysfunction by inhibiting HDAC6-mediated CSE degradation and boosting H_2S generation in rat aortic tissues [43]. Snijder et al. observed that renal mRNA expression of CSE, CBS and MST was significantly decreased after 3 weeks of Ang II infusion in rats, and simultaneous injection of H_2S donors (NaHS or thiosulfate) decreased Ang II-induced blood pressure, proteinuria, and renal damage [44]. The protective effects of H_2S were possibly attributed to its strong inhibition of Ang II-induced reactive oxygen species (ROS) generation, MAPK activation, and AT1R binding affinity. In contrast, Oosterhuis et al. reported that blocking H_2S production with a CSE-specific inhibitor DL-propargylglycine reduced blood pressure, proteinuria, and renal injury in Ang II-infused rats [45]. The mechanism is not really clear, and it seems that the interplay of H_2S signals and RAS in the regulation of blood pressure is very complicated, since H_2S causes vasoconstriction at low concentrations but vasorelaxation at higher concentrations [75]. In addition, Cui et al. reported that conditional deletion of CSE in T cells exacerbated Ang II-induced hypertension in mice by reducing circulatory and renal T regulatory cell numbers [46].

Table 24.1 Animal models used for determining the interplay of H_2S signals and RAS in CVD

Animal model of CVD	Induction of RAS activation	Alteration of H_2S signals	Target signaling pathway	References
Hypertensive mouse model induced by Ang II	Ang II (1 mg/kg/day) infusion to C57BL/6 N mice for 4 weeks using osmotic pumps	Reduced CSE expression and lower H_2S level in the aorta	Upregulation of HDAC6 by Ang II leads to CSE ubiquitination and degradation	Chi et al. [42, 43]
Hypertensive rat model induced by Ang II	Ang II (435 ng/kg/min) infusion to Sprague Dawley (SD) rats for 3 weeks using osmotic pumps	IP injections with NaHS (5.6 mg/kg/day) or sodium thiosulfate (1 g/kg/day) twice a day for 3 weeks	H_2S attenuates Ang II-induced hypertension, proteinuria, oxidative stress and renal functional and structural deterioration	Snijder et al. [44]
Hypertensive rat model induced by Ang II	Ang II (435 ng/kg/min) infusion to SD rats for 3 weeks using osmotic pumps	IP injections with PPG (18.75 mg/kg) twice daily	Inhibition of H_2S induces renal HO-1 expression and reduces Ang II-induced hypertension and renal injury	Oosterhuis et al. [45]
Hypertensive mouse model induced by CSE knockout in T cells and Ang II	Ang II (1 mg/kg/day) infusion to C57BL/6 N mice for 4 weeks using osmotic pumps	Generating a mouse model of CSE conditional knockout in T cells by hybridizing CSE loxp/loxp mice with CD4cre mice	Deletion of CSE in T cells elevates Ang II-induced hypertension via reduced sulfhydration of liver kinase B1 and lower differentiation and proliferation of T regulatory cells	Cui et al. [46]
Spontaneously hypertensive rat	Upregulation of renal mRNA expression of renin, ACE, and AT1R	IP injections with NaHS (14 μmol/kg/day) from 4 to 8 weeks of age	H_2S reduces LNMMA, and increases NO bioavailability, and inhibits the activation of RAS in the kidney	Tain et al. [47]

(continued)

Table 24.1 (continued)

Animal model of CVD	Induction of RAS activation	Alteration of H_2S signals	Target signaling pathway	References
Hypertensive rat model induced by high-salt diet	Dahl rats fed with high-salt diet (8% NaCl) for 8 weeks	IP injection with NaHS (90 μmol/kg) daily for 8 weeks	Down-regulated CBS/H_2S pathway, reduced HIF-1α, and increased PHD2 as well as enhanced adenylyl cyclase activity and intracellular cAMP level in renal tissue	Huang et al. [48, 49]
Hypertensive rat model induced by high-salt diet	Dahl rats fed with high-salt diet (8% NaCl) for 6 weeks leading to higher renal RAS	2% taurine in drinking water for 6 weeks induces CBS/H2S signals in kidney	Taurine reduces the renin, Ang II, and aldosterone contents and the levels of oxidative stress in renal tissues	Huang et al. [50]
Spontaneously hypertensive rats exposed to high-salt intake	SHRs received 1% NaCl in drinking water for 8 weeks	Supplement of l-cysteine (8 mmol/kg body weight/day) via gastric intubation between 4 and 6 weeks of age	Cysteine promotes H_2S generation, reduces Ang I and Ang II, decreases mRNA expression of renin, and increases protein levels of AT2R in kidney tissues	Hsu et al. [51]
Renovascular hypertensive rat model by 2K1C	Left renal artery clipped resulting in partial occlusion of renal perfusion for 4 weeks	IP injection with NaHS (56 μmol/kg/day) for 4 weeks	H_2S suppression of vascular oxidative stress by inhibiting Ang II-AT1R action, downregulating NADPH oxidases, and upregulating antioxidant enzyme SOD	Xie et al. [52]; Liu et al. [53]; Xue et al. [54]
The offspring from fetal-programmed renovascular hypertensive rats	Renovascular hypertensive rats by 2-kidney-1-clip for 5 weeks	Downregulation of endogenous H_2S generation in the brain	Inhibition of the Ang II/AT1R pathway and upregulation of antioxidant enzymes in the nucleus tractus solitarii	Guo et al. [55, 56]; Feng et al. [57]

(continued)

Table 24.1 (continued)

Animal model of CVD	Induction of RAS activation	Alteration of H_2S signals	Target signaling pathway	References
Hypertensive rat model induced by Ang II	Ang II (200 ng/kg/min) infusion to SD rats for 4 weeks using osmotic pumps	Chronic intracerebroventricular infusion of NaHS (30–60 nmol/h) for 4 weeks using osmotic pumps	H_2S alleviates Ang II-induced microglial cell number in the paraventricular nucleus of the hypothalamus and attenuates neuroinflammation	Ayaz et al. [58]
Spontaneously hypertensive rats with homocysteine	A methionine-rich diet with 1.7% L-methionine for 8 weeks	Lower serum H_2S level and renal CSE expression	Homocysteine induces expressions of renal ACE and AT1R, suppresses CSE mRNA expression, and serum H_2S	Shi et al. [59]
In vitro cell model of myocardial hypertrophy induced by Ang II	Rat neonatal cardiomyocyte incubated with Ang II (100 nM) for 24 h	NaHS (50 μM) or GYY4137 (50 μM, incubation for 24 h	H_2S reverses Ang II-induced cardiomyocyte hypertrophy, mitochondrial dysfunction, oxidative stress in a SIRT3-dependent manner and Sp1 *S*-sulfhydration-mediated KLF5 transcription activity	Meng et al. [60, 61]
Rat cardiac hypertrophy model by abdominal aortic constriction	SD rats with abdominal aortic coarctation for 5 weeks	IP injection with NaHS (14 μmol/kg/day) daily for 5 weeks	H_2S downregulates cardiac Ang II levels and suppresses the development of left ventricle hypertrophy	Huang et al. [62]
Mouse cardiac hypertrophy model by transverse aortic constriction	C57BL/6 mice with transverse aortic constriction for 4 weeks	IP injection with NaHS (15 μmol/kg/day) daily for 4 weeks	H_2S protects against TAC-induced Ang II level and cardiac hypertrophy by attenuating oxidative stress	Shao et al. [63]

(continued)

Table 24.1 (continued)

Animal model of CVD	Induction of RAS activation	Alteration of H_2S signals	Target signaling pathway	References
Rat cardiac hypertrophy model by isoproterenol–caffeine	WKY rats were given 5 subcutaneous injections of isoprenaline of 5 mg/kg at 72 h intervals and caffeine 62 mg/L in the drinking water for a period of 2 weeks	IP injection with NaHS (56 μM) daily for 5 weeks	H_2S reduces plasma Ang II levels and inhibits the progression of left ventricular hypertrophy	Ahmad et al. [64]
Rat cardiac hypertrophy model by Ang II	Ang II (435 ng/kg/min) infusion to Sprague Dawley rats for 3 weeks using osmotic pumps	IP injections with NaHS (5.6 mg/kg/day) or sodium thiosulfate (1 g/kg/day) twice a day for 3 weeks	H_2S inhibition of Ang II-induced atrial natriuretic peptide mRNA, cardiac fibrosis and increased oxidative stress	Snijder et al. [65]
Rat model of cardiac fibrosis by ligation of coronary artery	SD rats with ligation of left anterior descending coronary artery for 14 days	IP injection with NaHS (56 μM/kg/day) daily for 14 days	H_2S inhibits Ang II, the expressions of α-smooth muscle actin, connective tissue growth factor, and type I collagen and upregulates expression of HO-1	Pan et al. [66]
Mouse model of cardiac fibrosis by transverse aortic constriction-	WT mice and SIRT3 KO mice with transverse aortic constriction for 2 weeks	IP injection with NaHS (50 μM/kg/day) daily for 14 days	H_2S attenuates blood Ang II level and cardiac fibroblast proliferation via regulation of SIRT3	Liu et al. [67]
In vitro cell culture model of cardiac fibrosis by Ang II	Neonatal rat cardiac fibroblasts incubated with Ang II (0.1 μM) for 24 h	Incubation with GYY4137 (12.5–50 μM) for 28	H_2S inhibits Ang II-induced expressions of collagen I, collagen III, α-smooth muscle actin, transforming growth factor-β1 as well as Smad2 phosphorylation	Meng et al. [68]

(continued)

Table 24.1 (continued)

Animal model of CVD	Induction of RAS activation	Alteration of H_2S signals	Target signaling pathway	References
Rat model of cardiac fibrosis by NO deficiency	SD rats with L-NNA (40 mg/kg/day) in food for 2 weeks	Sodium thiosulfate (2 g/kg/day) administered via the drinking water for 2 weeks	H_2S improves hypertension, left ventricular hypertrophy, interstitial fibrosis via inhibition of oxidative stress	Nguyen et al. [69]
Rat model of heart failure by isoproterenol	SD rats with IP injection of isoproterenol (150 mg/kg)	NaHS (0.056 mg/kg/day) administered before and 2 weeks after isoproterenol injection	H_2S inhibits local renin activity, reduces collagen deposition and degranulated mast cells in cardiac tissues	Liu et al. [70]
Mouse model of heart failure by pressure overload	C57BL/6 J mice with transverse aortic constriction for up to 18 weeks	IP injection of JK-1 (100 mg/kg/day) daily at 3 or 10 weeks following TAC	H_2S attenuates circulating levels of renin, Ang II, and aldosterone and efficiently ameliorates heart failure severity	Li et al. [71]
Ex vivo rat model of heart failure induced by perfusion of Ang II	Langendorff-perfused rat hearts with Ang II (0.1 μM) for up to 40 min	Perfusion with iminothioethers (300 μM) for up to 40 min	H_2S inhibits Ang I-caused reduction of the coronary flow	Barresi et al. [72]
Mouse model of atherosclerosis by carotid artery ligation	ApoE knockout mice with partial ligation of left carotid artery followed by being fed a high-fat diet for 4 weeks	IP injection of NaHS (1 mg/kg/day) or DL-propargylglycine (10 mg/kg/day) for 4 weeks	H_2S upregulates carotid expression of ACE2 and attenuates the severity of atherosclerosis,	Lin et al. [73]
Mouse model of aortic dissection with β-aminopropionitrile diet	C57BL/6 mice with 0.25% (w/w) β-aminopropionitrile in drinking water for 4 weeks	IP injection of NaHS (56 μmol/kg/day) for 3 weeks	H_2S attenuates Ang II-induced aortic dissection associated with moderated inflammation and oxidative stress through a NO-dependent pathway	Lu et al. [74]

Decreased H_2S levels were often found in SHR rats with the activation of RAS. Tain et al. showed that NaHS treatment significantly increased nitric oxide (NO) bioavailability and decreased renal mRNA expression of renin, ACE, and AT1R, thus preventing the development of hypertension in SHRs [47]. In cultured SMCs, H_2S inhibited Ang II-induced cell proliferation and collagen generation as demonstrated by the lower incorporation of [3H]TdR and [3H]proline. Mechanically, H_2S dose-dependently decreased Ang II-induced MAPK activation and the binding affinity of the AT1R. Moreover, the inhibitory effect of H_2S on SMC proliferation and collagen generation was stronger in the SHR than in the Wistar Kyoto (WKY) group [47].

In another model of high-salt-induced hypertension in Dahl rats, Huang et al. observed that the endogenous CBS/H_2S pathway in renal tissues was suppressed by a high salt diet, and administration of H_2S donor markedly decreased the level of renin, angiotensin and aldosterone in serum and renal tissues, improved aortic structural remodeling and salt-sensitive hypertension in Dahl rats [48]. H_2S could directly inhibit the expression, activity, and release of renin in renal tissues and also indirectly suppress renin degranulation via attenuation of adenylyl cyclase activity and intracellular cAMP level. Consistently, Huang et al. further found that a supplement of taurine (an amino sulfonic acid) increased the renal H_2S content and enhanced CBS expression and activity in Dahl rats fed a high-salt diet. In contrast, taurine reduced the renin, Ang II, and aldosterone contents and the levels of oxidative stress in Dahl rat renal tissues [50]. The increased H_2S production via the induction of CBS activity by taurine could inhibit RAS activation and oxidative stress damage in the kidney, thereby attenuating renal damage and high blood pressure in salt-sensitive Dahl rats. Consistent with these studies, Hsu et al. also observed supplement of cysteine promoted H_2S generation, reduced renal Ang I and Ang II, decreased mRNA expression of renin, and increased protein levels of AT2R in kidney tissues, therefore preventing hypertension and kidney injury in adult SHRs exposed to high salt consumption [51].

In a renovascular hypertensive rat model with 2 kidneys 1 clip (2K1C), several groups observed that the plasma Ang II level was significantly higher but plasma H_2S level was significantly lower [52, 53]. Treatment of renovascular hypertensive rats with H_2S donor lowered plasma Ang II level, improved ventricular dysfunction and myocardial remodeling, and restored blood pressure to the basal level as seen in the control rats. Preincubation with H_2S reversed the protein level of AT1R, oxidative stress, and vasocontraction induced by Ang II in isolated thoracic aorta of 2K1C rats [53]. It was concluded that inhibition of Ang II-AT1R action by H_2S likely contributes to the inhibition of ROS production and higher blood pressure. In another study, Huang et al. discovered that H_2S was able to inhibit Ang II-induced renal tubular epithelial-mesenchymal transition, which is a pivotal cellular event leading to renal damage and cardiac dysfunction [49]. Xue et al. also observed that H_2S inhibited high glucose-induced expressions of angiotensinogen, ACE and AT1R in renal mesangial cells via blockage of ROS generation, suggesting that H_2S may protect diabetic nephropathy by suppressing RAS activation [54].

Renovascular hypertension can also be induced by partial renal artery stenosis with increased Ang II level and AT1R activation via autonomic control of neurohumoral

pathways in the central nervous system. Guo et al. demonstrated the downregulation of endogenous H_2S generation and upregulation of Ang II/AT1R pathway were found in the nucleus tractus solitarius and rostral ventrolateral medulla, two hindbrain nuclei involved in blood pressure regulation, in the offsprings from renovascular hypertensive rats [55, 56]. Feng et al. further reported that intralateroventricular microinjection of Ang II stimulated the expression of AT1R and oxidative stress-related proteins and induced greater sympathetic responses in the offspring of hypertensive rats, which were abolished by prenatal or postnatal administration of H_2S donors [57]. The same group further discovered that H_2S increased methylation of AT1R gene promoter and then downregulated AT1R protein expression, therefore, epigenetic modification of H_2S contributed to the reduction in blood pressure of offspring from renovascular hypertensive rats [56]. These studies point to the importance of H_2S signals in preventing fetal programmed hypertension by counteracting RAS, which needs to be further explored by clinical studies with maternal hypertensive mothers or programmed hypertensive offspring. Another group also observed that chronic intra-cerebroventricular infusion of NaHS alleviated Ang II-induced increase in microglial cells in the paraventricular nucleus of the hypothalamus and attenuated neuroinflammation, all of which contribute to the anti-hypertensive effect of H_2S in Sprague Dawley rats [58].

In a hyperhomocysteinemia rat model, Shi et al. reported that blood pressure was significantly higher with increased serum homocysteine level, induced expressions of renal ACE1 and AT1R but lower serum H_2S level and renal CSE expression [59]. The authors speculated that elevated homocysteine could induce the RAS system, suppress CSE mRNA expression, and decrease serum H_2S, all of which would result in gradually increased blood pressure. Given all the evidence, it could be feasible for targeting H_2S signals for the prevention and treatment of hypertension via inhibition of the classical way of RAS activation.

Cardiac Hypertrophy

Cardiac hypertrophy is characterized by increased myocardial cell volume, greater ventricular wall thickness, and enhanced myocardial contractility. People with an enlarged heart are at higher risk for developing heart complications. Activation of RAS has been associated with cardiac myocyte hypertrophy. Meng et al. first observed that H_2S concentration and the expression of CSE were lower but Ang II level was significantly higher in myocardium from the patients exhibiting myocardial hypertrophy than in those without hypertrophy [60]. The administration of GYY4137, a slow-releasing H_2S donor, inhibited myocardial hypertrophy in SHR rats, as evidenced by the improvement in cardiac structural parameters, heart mass, size of cardiac myocytes, and expression of ANP (an indicator of myocardial hypertrophy) [60]. In an in vitro cell culture model with neonatal rat cardiomyocytes, H_2S reversed Ang II-induced cardiomyocyte hypertrophy, mitochondrial function impairment, permeability potential dysfunction, along with increased oxidative stress

in a SIRT3-dependent manner, since siRNA-mediated knockdown of SIRT3 abolished the protective role of H_2S against myocardial hypertrophy [60]. These findings were further supported by the in vivo animal model of cardiac hypertrophy induced with transverse aortic constriction in SIRT3 knockout mice [60]. Besides these, the same group further reported that H_2S prevented the development of myocardial hypertrophy by increasing KLF5 transcription activity via Sp1 *S*-sulfhydration [61].

In another rat model of left ventricle hypertrophy induced by abdominal aortic constriction, Huang et al. observed that the concentrations of Ang II were significantly increased but H_2S levels were reduced within the ventricle when compared with the sham-operated groups [62]. The authors further revealed that exogenous administration of H_2S significantly downregulated cardiac Ang II levels and suppressed the development of cardiac hypertrophy induced by pressure overload. Similarly, in a mouse model of cardiac hypertrophy with transverse aortic constriction, Shao et al. also observed lower H_2S levels in the myocardium than in the sham group [63]. H_2S would protect Ang II-induced neonatal rat cardiomyocyte hypertrophy by activating PI3K/Akt pathway, inducing nuclear accumulation of Nrf2 and the expressions of antioxidant genes (HO-1 and GCLM), and then attenuating ROS production [63].

With another animal model of cardiac hypertrophy induced by isoproterenol and caffeine in WKY rats, Ahmad et al. determined that exogenous administration of H_2S reduced plasma Ang II levels and inhibited the progression of left ventricular hypertrophy [64]. Snijder et al. further provided evidence that Ang II-induced cardiac hypertrophy in rats could be directly reversed by treatment with thiosulfate and NaHS, two different H_2S donors [65]. All these data point to the crucial role of H_2S in suppressing RAS for the possible treatment of myocardial hypertrophy in the future.

Cardiac Fibrosis

Cardiac hypertrophy is often accompanied by the excess deposition of extracellular matrix in the cardiac muscle, leading to cardiac fibrosis and also abnormal thickening of the heart valves due to inappropriate proliferation of cardiac fibroblasts. The expression of AT1R is substantially greater on cardiac fibroblasts than on cardiomyocytes, suggesting that the cardiac fibroblasts act as the predominant target for the RAS during cardiac fibrosis [76]. Pan et al. found exogenous administration of H_2S lowered cardiac Ang II level and improved fibrotic response during the development of myocardial infarction by ligation of coronary artery in rats [66]. Further by using cardiac fibroblast cells, Pan et al. showed that H_2S treatment inhibited Ang II-induced fibrotic responses, as demonstrated by the reduced expressions of α-smooth muscle actin, connective tissue growth factor, and type I collagen and upregulated expression of HO-1 [66]. In addition, Liu et al. found that NaHS would significantly inhibit Ang II-induced proliferation of neonatal rat cardiac fibroblasts by suppressing intracellular ROS production [67]. Similarly, Meng et al. also observed that another H_2S

donor GYY4137 effectively inhibited Ang II-induced neonatal rat cardiac fibroblast proliferation and the expressions of collagen I, collagen III, α-smooth muscle actin, transforming growth factor-β1 as well as Smad2 phosphorylation [68]. Liu et al. provided mechanism data that H_2S would attenuate Ang II-induced cardiac fibroblast proliferation via regulation of SIRT3 [67].

Indirectly, Nguyen et al. observed that orally administered H_2S donor thiosulfate improved cardiomyocyte hypertrophy and interstitial fibrosis in a rat model of chronic NO deficiency to the same extent as an ACE inhibitor Lisinopril [69]. H_2S has also been shown to inhibit CCl_4-induced hepatic fibrosis by suppressing the expression of AT1R [77]. These observations are consistent with evidence that H_2S would be a great target for RAS inhibition and myocardial fibrosis prevention and treatment.

Heart Failure

Heart failure is a serious condition that disturbs heart functions leading to interrupted blood flow in the body. The cardiac local RAS system is often activated in heart failure, especially marked by the increase of Ang II levels. Liu et al. reported that inhibition of local renin activity in rat hearts and blood by H_2S contributed to its protective role against isoproterenol-induced heart failure, as characterized by reduced collagen deposition and less degranulated mast cells in cardiac tissue [70]. In an in vitro cell study, H_2S treatment inhibited forskolin-induced renin degranulation in HMC-1.1 mast cells by lowering intracellular cAMP levels [70]. JK-1, a novel synthetic H_2S donor, attenuated circulating levels of renin, Ang II, and aldosterone in mice subjected to transverse aortic constriction, and efficiently ameliorated heart failure severity [71]. In Langendorff-perfused rat hearts, Ang II perfusion caused a significant reduction of the coronary flow, which could be restored to a normal level by co-incubation of an H_2S donor iminothioethers [72]. In consideration of the beneficial effects of H_2S in heart cardiac hypertrophy and fibrosis, it is conceivable that H_2S represents a promising therapeutic target for preventing heart failure and its comorbidities.

Atherosclerosis

Atherosclerosis, a life-threatening cardiovascular complication, is caused by endothelial damage, SMC proliferation, and the formation of lipid plaques in the large- and medium-sized arteries. Both H_2S signals and RAS contribute to the pathogenesis of atherosclerosis. Deficiency of either CSE or ACE2 in mice exacerbated the development of atherosclerosis [18, 78]. In a murine model of atherosclerosis with high-fat feeding and partially left common carotid artery ligation, Lin et al. detected significantly lower expressions of both CSE and ACE2 in carotid tissues

from apolipoprotein knockout mice [73]. Application of NaHS considerably upregulated carotid expression of ACE2 and attenuated the severity of atherosclerosis, while the supplement of an ACE2 inhibitor MLN-4760 dramatically abolished the anti-atherosclerotic effect of H_2S [73]. In cultured human umbilical vein endothelial cells (HUVECs), H_2S was capable of inducing ACE2 expression and stimulating the conversion of Ang II to Ang(1–7) but did not affect ACE expression [73]. Moreover, H_2S also reversed lipopolysaccharide-inhibited endothelial ACE2-Ang(1–7) expression and cytokine release in a dose-dependent manner [73]. These findings propose a protective role of H_2S against inflammatory response and atherosclerosis by reducing Ang II level via enhanced ACE2 expression in the endothelium. The intracellular pathways involved in H_2S-stimulated ACE2 expression within endothelial cells remain to be established. It is well studied that SARS-CoV-2 requires ACE2 for infecting the host organism [79]. A recent study by Pozzi et al. found that H_2S donor GYY4137 had no effect on the expression of ACE2 protein in airway epithelial cells, indicating that H_2S may regulate ACE2 expression in a cell-specific manner [80].

Aortic Aneurysms

Aortic aneurysm, a serious health problem due to its relation to CVDs, leads to aortic expansion, dissection, rupture, and sudden death. No effective treatment options are available for aortic aneurysms. The endogenous H_2S level and CSE expression were significantly lower in the human sample of the abdominal aortic aneurysm as compared to the healthy aorta [81]. In a mouse model of Ang II-induced aortic aneurysms, Lu et al. observed that the administration of H_2S inhibited the accumulation of inflammatory cells in the aortic wall and the related expression of inflammatory genes, and eventually retarded the development of aortic aneurysms [74]. These results were further confirmed by a recent study on a rat model of aortic aneurysms fed with a β-aminopropionitrile diet, in which H_2S supply improved aortic remodeling by alleviating irregular tissue arrangement and vascular fibrosis, increasing the expression of elastin fibers, decreasing collagen deposition and the expression of TGF-β1 and matrix metalloproteinase 2/9 [82]. In addition, H_2S inhibited NLRP3 inflammasome activation and decreased the level of IL-1β by disrupting TGF-β1 signaling in cultured human aortic vascular SMCs [82]. All these studies strongly suggest that H_2S signal would be a target for preventing RAS-associated medial degeneration and the development of aortic aneurysms.

Endothelial Dysfunctions

Endothelial injury acts as an initial trigger causing a plethora of CVDs, and Ang II is a critical factor contributing to endothelial dysfunctions [8, 83]. In cultured HUVEC,

Ang II incubation inhibited CSE expression and endogenous H_2S production in a time-dependent manner [84, 85]. Mechanistically, the high level of superoxide anion derived from Ang II-induced CSE ubiquitination and degradation [84]. Li et al. showed that H_2S significantly ameliorated Ang II-induced cellular impairment, NLRP3 inflammasome activity and ROS generation in cultured HUVECs [86]. Hu et al. also found that H_2S supplementation protected against Ang II-induced cytotoxicity in HUVECs via the inhibition of endothelin-1 generation and NFκB expression as well as upregulation of the Akt/eNOS signaling pathway [85, 87]. Monti et al. showed that ACE inhibitor zofenoprilat induced cell proliferation, maintained endothelial integrity, and inhibited inflammatory response by stimulating CSE/H_2S system in HUVECs [88]. The same group further observed that increased H_2S generation also contributed to the protective role of Zofenoprilat on doxorubicin-induced endothelial damage [89]. All these findings indicate that H_2S could improve Ang II-induced endothelial dysfunctions by inhibiting the vicious cycle of oxidative stress and inflammation in CVD.

H_2S as ACE Inhibitors

The ACE inhibitors are widely used in the clinic for the prevention and treatment of CVDs, including hypertension, atherosclerosis, and cardiac failure [8]. Accumulated evidence demonstrated that H_2S would act as an ACE inhibitor either by indirectly mediating the functions of already existing ACE inhibitors or directly inhibiting ACE activity (Fig. 24.3).

There are three main groups of ACE inhibitors, sulfhydryl-, dicarboxylate-, and phosphonate-containing agents. The sulfhydryl-containing ACE inhibitors display a greater extent than another two types in improving CVDs. Several studies demonstrated that H_2S mediated the beneficial effects of sulfhydryl-containing ACE inhibitors, i.e., captopril and zofenopril. In patients with hypertension, captopril treatment improved microvascular endothelium-dependent vasodilation via augmentation of both H_2S and NO generation [90]. Captopril alone increased the rate of NO release from S-nitrosoglutathione in a test tube, but significantly inhibit H_2S-induced NO release in a pH-dependent manner, which could be related to some of the biological effects of captopril via regulating sulfide-nitroso signaling pathways [91]. Donnarumma et al. examined the effect of zofenopril on H_2S bioavailability and cardiac damage in mouse and swine models of myocardial ischemia/reperfusion injury [92]. It was found that zofenopril significantly augmented both plasma and myocardial H_2S levels in mice and pigs. The release of H_2S from zofenopril would scavenge ROS directly and/or indirectly via upregulation of antioxidant defense, resulting in the prevention of ischemia-induced cardiac damage and heart failure. The administration of zofenopril to SHR restored tissue/plasma H_2S levels to WKY values, thus H_2S could account for the peripheral vascular effects of zofenopril. The elevations in tissue and plasma H_2S levels were primarily derived from zofenopril since zofenopril did not affect the protein expressions of all the 3 H_2S-generating

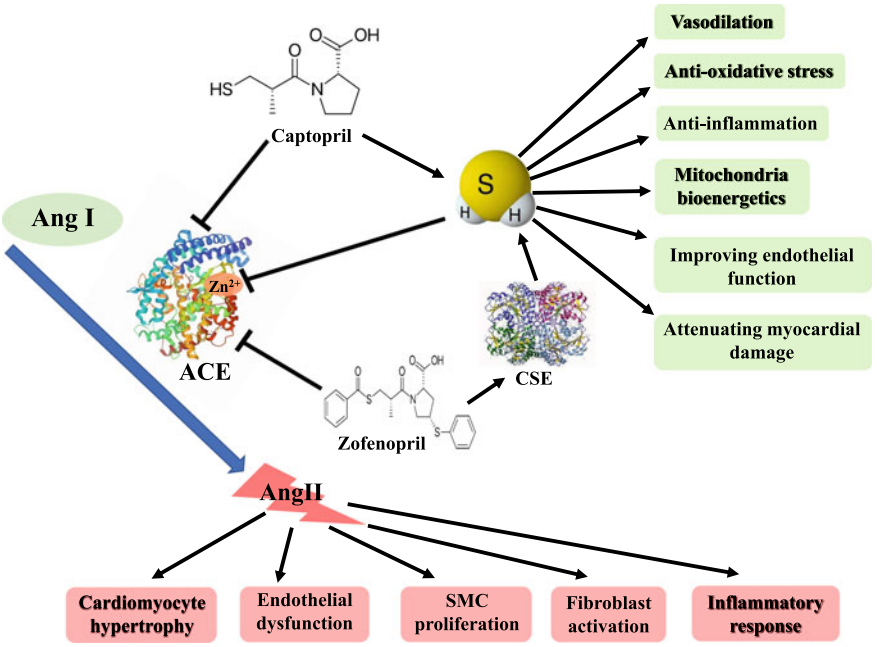

Fig. 24.3 H_2S as potential ACE inhibitors. H_2S would act as ACE inhibitor either by indirectly mediating the functions of already existing ACE inhibitors (captopril and zofenopril) or directly inhibiting ACE activity by reacting with Zn^{2+} in the active center of the enzyme. Abbreviation used: ACE, angiotensin-converting enzyme; Ang I, angiotensin-I; Ang II, angiotensin-II; CSE, cystathionine γ-lyase; SMC, smooth muscle cell

enzymes, including CBS, CSE, and MST [92]. Bucci et al. also observed that zofenoprilat (an active metabolite of zofenopril) could dose-dependently release H_2S in a cell-free assay [93]. In terms of the possible mechanisms underlying zofenoprilat stimulation of H_2S release, Monti et al. proposed that zofenoprilat could act as a substrate of CSE to produce a certain amount of H_2S since DL-propargylglycine inhibition of CSE completely reversed zofenoprilat effects on stimulating H_2S generation [88, 89]. These data suggest that the cardioprotective effects of sulfhydrylated ACE inhibitors are in part via H_2S-dependent mechanisms.

H_2S is a reducing agent and often interacts with metals in the target proteins for modulating enzyme activity [94]. ACE is a zinc (Zn^{2+})-containing enzyme. It is possible that H_2S may alter ACE activity by directly interfering with the Zn^{2+} in the active center of the enzyme. Indeed, Laggner et al. observed ACE activity was significantly inhibited by H_2S in both cultured HUVECs and ex-vivo umbilical veins, which would be reversed by the addition of Zn^{2+} but no other metals (Cd^{2+}, Ca^{2+} or Mg^{2+}) [95]. These data indicate that the direct inhibition of ACE activity by H_2S may contribute to the vasorelaxant effect of H_2S in the vasculature. H_2S also inhibits the release of renin from mast cells and juxtaglomerular cells, thus

protecting cardiac remodeling and renovascular hypertension [70]. In combination, the evidence indicates that H_2S would act as a potential inhibitor of ACE.

Conclusion and Perspectives

Recent advances have expanded our understanding of the biomedical importance of H_2S in the cardiovascular system via its anti-oxidative, anti-inflammatory, and vasodilatory actions. Overstimulation of the classical pathway of RAS is implicated in a chain of events that contribute to the pathogenesis of CVDs. As opposed to RAS activation, H_2S signals are often downregulated during the development of CVDs. In this review, we discussed the importance of H_2S signals and RAS in cardiovascular functions and summarized the current knowledge on the interplay of H_2S and RAS activation in various animal models of CVDs. H_2S as a potential RAS inhibitor was also highlighted. Despite the promising preclinical findings, a better understanding of the reciprocal relation between H_2S signals and RAS in the cardiovascular system from human trials is highly expected. Consequently, more research needs to be undertaken to develop drugs or strategies for simultaneously boosting H_2S generation but inactivating the classical pathway of RAS for the therapeutic interventions against CVDs.

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Chapter 25
Emerging Role of ACE-2 in Cerebrovascular and Neurological Disorders: Lessons Learnt from COVID-19

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Abstract The renin angiotensin system (RAS) plays a vital role in the maintenance of cardiovascular function and physiology. Direct renin inhibitors, angiotensin receptor blockers, and angiotensin-converting enzymes are widely used to treat a wide range of cardiovascular diseases. Identification and decoding of the role of ACE2 in physiology made the RAS interesting and complex as well. The role of ACE2 was found to counteract the effect of angiotensin I and angiotensin II, producing beneficial effects. Very few studies were present on the role of ACE2 in human physiology until 2020. COVID-19 pandemic becomes one of the biggest threats of the twenty-first century. Aggressive research to understand the pathophysiology of coronavirus highlighted the involvement of ACE2 receptors. Spike proteins of coronavirus target ACE2 receptors particularly in the lungs which lead to lung fibrosis and ultimately respiratory failure. The role of ACE2 in COVID-19 disease forces the researcher to rethink the prominent role of ACE2 in human physiology. Till now, the role of ACE2 in cardiovascular, cerebrovascular, neurological, and nephrological disorders has been evaluated. This chapter discusses the role of ACE2 in cardiovascular and neurological disorders along with the involvement of the ACE2 pathway in COVID-19 which paves the road to develop drugs, vaccines, and antibodies to treat COVID-19 as well as other disorders.

Keywords Renin angiotensin system · ACE2 · Cardiovascular disorders · Cerebrovascular disorders · Neurological disorders · COVID-19

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Introduction

The renin angiotensin system (RAS) is a well-known hormone system that plays a pivotal part in the homeostasis of several physiological functions viz. electrolyte balance, body fluid regulation, blood vascular engagements in the systemic circulation. Renin is an enzyme, secreted from the juxtaglomerular cells of kidney which acts on angiotensinogen, a precursor of angiotensin peptides to release angiotensin. One more enzyme is secreted from the kidney i.e. angiotensin-converting enzyme (ACE) which is responsible for conversion of Ang I (inactive decapeptide) to Ang II (active octapeptide). Though persistent stimulation of RAS and the upsurge level of Ang II may act on Angiotensin II type 1 receptor (AT1R) and lead to many pathophysiological processes, such as vasospasm, inflammation, renal sodium absorption increased [1–3]. Ang II is also recognized as the effector peptide and most characteristic bioactive peptide in the RAS as the final mediator. Ang II is reported to have a multiplying role in the development of cardiovascular diseases, such as coronary heart disease, restenosis, myocardial infarction, hypertension, and heart failures [4, 5].

ACE2 is described as a homolog of ACE which is responsible for the hydrolysis of Ang II to Ang (1–7). The metabolite Ang (1–7) shows opposite actions to those of Ang II and maintains the homeostasis in ACE2-Ang (1–7) axis. Moreover, synthesis of Ang (1–7) level generally depends on the level of Ang II and is mostly present in the viable myocardium. Therefore, the role of the ACE2-Ang (1–7) axis is incoherent as compared to the Ang II axis associated with cardiac functions [4]. RAS is one of the best-studied enzymes-neuropeptide component in the cardiovascular system, but its substantial role in the brain and associated organs are also comparable and can accomplish as a model for the accomplishment of peptides on neuronal functions. It is now known that the brain can maintain its its own RAS system with all its functional components [6]. Recent findings on brain RAS deliberating the roles of Ang IV and Ang-(1–7) in cognition and also support the existence of complex site-specific communications in the facilitation of key central roles of the brain RAS. In a study, it is reported that Angiotensin IV, which binds with AT1 and AT2, has been associated with several physiological functions *i.e.* cerebral blood flow, exploratory behavior, learning and memory, and neuronal growth [7]. Therefore, the RAS of the brain is actively tangled in the modulation of numerous additional tasks in the brain, including courses of motor and sensory communication, regulation of cognitive function and emotional responses along with regulation of blood pressure [6, 7].

Because of the COVID-19 pandemic, it is considered a priority for global public health management systems. As per the recent reports, SARS-CoV-2 is more specific to hACE2 in comparison to SARS-CoV because of more stabilized presence of hot spot-31 and hot spot-353 on hACE2 [8, 9]. After SARS-CoV-2 infection, the pathogenesis starts binding of SARS-CoV-2 spike glycoprotein with to ACE2, downregulation of ACE2 which produce an imbalance between the RAS and ACE2/angiotensin-(1–7)/MAS leads to multiple organ failures in patients suffering from COVID-19. Remarkably, the same SARS-CoV-2 spike glycoprotein which binds to ACE2 for the

pathogenesis of COVID-19 and also overlays the road to develop drugs, antibodies, and vaccines [9].

Cardiovascular Disorders and ACE-2

The renin–angiotensin–aldosterone system (RAAS) is at the heart of cardiovascular and renal pathophysiology. The classical pathway of RAAS starts from the renal juxtaglomerular cells which secrete renin. Renin acts on angiotensinogen which is present in the circulatory system and gets converted into angiotensin I. Angiotensin I is to angiotensin II by angiotensin-converting enzyme (ACE). Ang II is the main molecule that exerts its effect on different organs. Angiotensin II has pro-inflammatory, pro-fibrotic, and vasoconstrictive properties. A recent discovery of the angiotensin-converting enzyme (ACE2) made the RAAS complex a little bit complex. ACE2 was found to be involved in the cleavage of angiotensin I (1–10) into angiotensin (1–9) which further cleaved into angiotensin (1–7). Similarly, ACE2 cleaves angiotensin II (1–8) into angiotensin (1–7). The most intrigued finding of Angiotensin (1–7) was that angiotensin (1–7) has opposite effects such as vasodilation, apoptotic and anti-proliferative properties while ACE and ACE2 maintain the balance between angiotensin II and angiotensin (1–7). ACE2 was first identified in 2000 and since then various studies have pointed out the widespread abundance of ACE2 in the kidney, heart, lungs, and brain.

A homolog of ACE or ACE2 was first identified in 2000 by two research groups [10, 11]. ACE2 is a type 1 transmembrane glycoprotein having 805 amino acids with a molecular weight of 120 kDa [12]. The first major finding of involvement of ACE2 in the heart was presented by Burrell et al. [13] which showed that level of ACE2 was increased in infarct and border zone immediately after myocardial infarction and in the myocardium after 28 days. ACE2 was also found on a higher side in infiltrating mononuclear cells, endothelial cells, and myocytes. The study concluded that increased expression of ACE2 after MI may be beneficial and counter the adverse impact imparted by angiotensin I and angiotensin II. Another study showed that ACE2 knock-out mice demonstrated cardiac dysfunction. The myocardium of knock-out mice showed increased levels of angiotensin II suggesting that ACE and ACE2 maintain cardiac function and are involved in abnormalities of the heart [14]. To support the claim, another study used ACE2 knock-out mice and observe its effect on blood pressure. The study found that C57BL/6 knock-out mice, showed little increase in blood pressure compared to normal mice while 129/SvEv knock-out mice showed no significant increase in blood pressure compared to baseline blood pressure. After acute infusion of angiotensin II, plasma concentrations of angiotensin II raised three-fold in ACE2 knock-out mice than in controls. The authors suggest that ACE2 may be involved in metabolizing angiotensin II and thereby controlling blood pressure [15]. In a spontaneous hypertensive rat model, overexpression of ACE2 showed a protective effect on cardiac function and high blood pressure [16]. Overexpression of ACE2 in transgenic spontaneously hypertensive stroke-prone (SHRSP) rats showed

reduced hypertension and improvement in endothelial functions in vessels probably due to a decrease in the vasoconstrictive effect of angiotensin II [17]. Moreover, an increase level of ACE2 was detected in also found in the isolated human heart having ischaemic and idiopathic cardiomyopathy [13, 18, 19]. In a rat model of diabetic cardiomyopathy, overexpression of ACE2 effectively decreases the accumulation of myocardial collagen and improves LV functioning [20].

Many studies point out that overexpression of the RAS system in the heart particularly angiotensin II may contribute to the progression of heart failure. Based on the protective role of ACE2, several studies were carried out to evaluate the co-relation between the role of ACE2 and cardiovascular disease. One study observed that overexpression of cardiac ACE2 in myocardial infarction showed improved LV function and contractility and decreased wall thinning [21]. Another study also supports the claim that overexpression of ACE2 improves LV remodeling by decreasing levels of angiotensin II and increasing angiotensin (1–7) in myocardial infarction [22]. These findings indicate that increased levels of ACE2 may impart a protective response to the ischemic injury and the vasodilatory effect of Angiotensin (1–7) produce cardioprotective effects to oppose the effects of Angiotensin II.

An initial clinical study conducted to evaluate the role of ACE2 in cardiac function found that ACE2 level was detectable only in a patient with cardiovascular disease [23]. Although follow-up study showed contradict results and found that ACE2 level was dateable in healthy subjects [24]. A clinical study conducted by Epelman et al. presented ACE2 levels in different patient populations and levels of ACE2 was found overexpressed in a patient with heart failure [25]. Follow-up studies indicated that plasma level of ACE2 was overexpressed in left ventricular systolic dysfunction which is mostly involved in many cardiac diseases. The same study also found that overexpression of ACE2 was an independent factor of cardiac death, heart transplantation and heart failure in a hospitalized patient indicates the importance of ACE2 in heart failure [26]. Another study involving ST-elevation myocardial infarction (STEMI) patients showed that expression of ACE2 was increased in the acute phase of STEMI and had a direct correlation with infarct size [27]. Similarly, Uri et al. measured serum levels of ACE2 at different time points in patients who transit from hypertension to heart failure in a clinical setup. The study concluded that levels of ACE2 increase with time and a serum level of ACE2 can be used as serum biomarker in patients with cardiac dysfunction [28].

Cerebrovascular Disorders and ACE-2

The cerebrovascular disorder can be defined as any condition affecting blood vessels and blood flow in any region of the brain. The most common form of cerebrovascular complication is stroke. Angiotensin II activates NADPH oxidases (NOX) which further activates reactive oxygen species (ROS) and superoxide levels in the vasculature [29]. Superoxide further utilizes nitric oxide (NO) which results in decreased bioavailability of NO and endothelial dysfunctions [30]. Angiotensin II was found

to be involved in the impairment of endothelial function in cerebral blood vessels and microcirculation [31].

The neuroprotective effect of ACE2 had been evaluated in the pre-clinical model of cerebral ischemia. Endothelin-1 induced ischemia of the middle cerebral artery was effectively attenuated by giving ACE2 activator in rats [32] while another study showed improvement of neurological function after permanent middle cerebral artery occlusion by possible inhibition of NF-kB in rats [33]. Another study on ischemic stroke rats indicated that levels of ACE2 and angiotensin (1–7) were upregulated after an incidence of stroke and ACE2 plays an important role to protect the brain from ischemic insult [34]. A subsequent study also found that ACE2 was effective in protecting the brain from ischemic injury through increasing angiotensin (1–7) and upregulating the NADPH/eNOS pathway [35]. Overexpression of ACE2 in response to angiotensin II in ischemic stroke decreases ROS production, apoptosis, and swelling of ischemic tissues in mice overexpressing human renin and angiotensinogen (R+A+) [36]. An oral formulation of angiotensin (1–7) showed a neuroprotective effect in ischemic stroke in rats [37]. Another comprehensive study evaluating the role of Ang-(1–7) in stroke showed that direct administration into the brain resulted in salvage of tissue followed by tissue reperfusion although, the study did not find any change in inflammatory levels or expression of oxidative stress genes. The study concluded that conversion of angiotensin (1–7) from angiotensin II via ACE2 activity had a little beneficiary effect on stroke [38].

Serum level of ACE2 was found to be decreased in patient with an acute ischemic stroke which indicated the protective role of ACE2 in cerebrovascular complication. A clinical trial involving cerebral stroke patients having type 2 diabetes mellitus showed that ACE2 G8790A polymorphism in T2DM patients was directly involved with cerebral stroke [39].

Neurological Disorders and ACE-2

Angiotensinogen, angiotensin-converting enzyme, angiotensin II and their receptors serves as several components of the RAS, are also found in the brain suggesting the possibility of various pathophysiology and drug targets related neurological disorders [40]. Furthermore, within the brain RAS, different neuroglial cells possess the expression of different receptor subtypes viz. AT1R, AT2R, AT4R, MasR etc. [41, 42]. Several reported studies support the key involvement of excessive brain ACE/Ang-II/AT1R axis in neurodegeneration in respective brain disorders via oxidative stress overactivation, apoptosis, and neuroinflammation [43–45]. Several *in-vivo* and *in-vitro* studies are now in support of the presence of a RAS in the hypothalamus, basal ganglia, substantia nigra pars compacta, and striatum where brain RAS are actively involved in neuroglial cell defense mechanism and modulation of consequences leads to neurodegeneration [46–50]. The brain RAS is a self-regulating

form of RAS which is majorly concerned in important brain functions and also regulates various neurological disorders [51–53]. Interestingly, a crosstalk amongst the RAS and neurohumoral transmission has been established [41].

Several studies also support the crucial role of transformed RAS in several neurodegenerative diseases [54–56]. Some reported studies have recommended that stimulation of local brain RAS influences the pathological changes in harming the neuroglial cells [36, 50, 57]. Increased level of Ang II is responsible for cognitive dysfunctions via AT1R mediated brain inflammation [58] although ACE inhibitors showed improvement in the cognitive function temporarily by navigating across the blood–brain barrier without interfering with blood pressure [59]. Elevated Ang II levels are detected to upsurge ROS and support propagation of inflammatory biomarkers associated with neuroinflammation, although many findings have proven effective neuroprotective sound effects by hindering AT1R in these brain ailments such as Angiotensin receptor II blockers (ARBs) avert numerous risk factors for AD and protect neuronal cell [60, 61]. Due to enhanced activity of ACE and surge in Ang II, the occurrence of vasoconstriction in the brain is prominent and causes cognitive dysfunctions, and inflammation-mediated neuroglial cell death through the activation of AT1R [43, 62, 63].

Numerous findings and reports are in support of the availability of various components of brain RAS which have been found and involved in various physiological functions and disorders of the brain [43, 56, 64–67]. Therefore, targeting the RAS components provide a good opportunity to better understand disorders of the brain. Various cellular and molecular studies reported conflicting results regarding the functional role of brain RAS components [68]. As per Hermann et al. the promoter regions of RAS genes are effective in primary neuronal and glial cells [69], while other accepted facts showed the *de-novo* synthesis of RAS components [69, 70]. The organization of RAS in the brain is also aided by many pharmacological inhibition findings with the help of angiotensin analogs for the management of the various brain disorders [71]. Additionally, the diminished activity of the RAS in brain are responsible for neurogenic hypertension which is also regulated by other components viz. brain-specific isoform of renin and hormonal and neurotransmitter [72–74].

The RAAS also endures in the mammalian brain and plays an important neuromodulator role where it interrelates with different neurotransmitters such as acetylcholine, catecholamines, serotonin, and other peptides [6, 75]. In a study, Qi et al. have demonstrated the activation of RAS in the hypothalamic paraventricular nucleus which increases Ang II-induced neurogenic hypertension along with other biomarkers viz. proinflammatory cytokines and neurotransmitters [76]. In an independent experimental study with zebrafish Gaucher disease model and Drosophila pink1-deficient PD model, authors have demonstrated that RAAS inhibitors play a significant neuroprotective role and revealed the therapeutic potential and mechanisms of targeting the RAAS pathway for neuroprotection [77]. Therefore, as per the suggestion from preclinical and clinical trials, pharmacological manipulation of RAAS pathway may be beneficial in the treatment and management of cognitive dysfunctions associated with vascular dementia, Alzheimer's disease, and related neurodegenerative disorders [75, 78]. There is strong supportive data that provide

sufficient information for a major involvement of RAAS inhibitors causing neurodegeneration in several brain disorders such as zebrafish and *Drosophila pink1*-deficient Parkinsonism model. Therefore, targeting RAAS and its components may offer an exciting and novel drug development for neurodegenerative diseases [77].

In a review by Labandeira-Garcia et al. authors have reviewed the shreds of evidence based on published studies and recommended that the RAS may play a significant role in neuroinflammation and ROS formation which is responsible for the degeneration of dopaminergic neurons in Parkinson's disease [46, 79]. Hence, controlling brain RAS may constitute an effective neuroprotective strategy for Parkinson's disease. Furthermore, connection among dopaminergic neurons and RAS have been described [47]. In animal models, depletion of dopamine provokes compensatory overactivation of the local RAS, which primes microglial responses and dopaminergic neuronal damage via activation of the NOX system and can be inhibited by angiotensin receptor blockers and inhibitors of ACE [29, 46, 47, 80, 81].

Angiotensinogen (AGT) is a well-known precursor peptide in brain RAS and various studies stated that astrocytes is the main neuroglial cell where more than 90% of AGT peptides are synthesized [82, 83] and relatively, their products has also been described in neurons and glial cells [52, 84, 85]. Besides AGT, brain ACE is the main component of RAS and is identified in the cerebral vasculature (endothelial), even though it is highly expressive in the choroid plexus, organum vasculosum of the lamina terminalis, subfornical organ, and area postrema [75]. In an independent study, Jouquey et al. demonstrated brain ACE activity in spontaneously hypertensive rats after chronic treatment with trandolapril or enalapril noticed a connection between hypertension and brain ACE expression [86]. As concluded by Pena-Silva et al. ACE2 deficiency may reduce endothelial functions in cerebral arteries and augmented endothelial dysfunction during aging in comparison to adult mice. Therefore, ACE2 deficiency and aging might be responsible for oxidative stress which leads to cerebrovascular dysfunction [87]. The use of angiotensin analogs has been also reported in the treatment and management of clinical illnesses associated with neurological disorders and comorbidities [53, 88]. Brain RAS might regulate oxidative stress in the paraventricular nucleus (PVN) of the hypothalamus in the development of hypertension. In the brain, ANG II is supposed to increase sympathetic outflow by rising oxidative stress and stimulating local inflammation in the PVN [89–91]. In a study reported by Su et al. with salt-induced hypertensive rats, it is concluded that increased synthesis of Ang II due to stimulation of RAS in hypertensive rats promotes intracellular ROS formation, oxidative damage, apoptosis and cause neuronal dysfunction [1, 92]. It has been well established that neurotrophic factors (NFs) are a protein used by discrete neuronal cell types for their survival and maintenance and provide neuroprotection [93]. Some studies agree with the modulatory connection between NFs and brain RAS and recommended brain angiotensin peptides as a potential drug candidate in neurodegenerative disorders [41, 50]. Ang II is envisaged the main troublemaker that promotes inflammation via surge of ROS and associated with neurodegenerative diseases [94]. Though, ARBs play a beneficial role to safeguard the cerebral vasculature and prevent other cerebrovascular pathologies by selectively blocking the AT1R [95, 96]. Moreover, few studies reported neuroprotective

potentials of ARBs antioxidant effect via inhibition of NOX in neuroglial cells and circulating immune cells to improve neurological disease conditions [1, 97]. Thus, treatment with ARB may be beneficial to neurological brain disorders by modulation of brain RAS [50]. Another study also demonstrated the key role of Mitochondrial ATP-sensitive potassium channels which increases angiotensin-induced oxidative injury, neuroinflammation, and dopaminergic neuron degeneration in the substantia nigra in rats [98]. Sarro et al. suggested the involvement of RAS in the pathogenesis of neurological and psychiatric disorders such as seizures [99]. On the other hand, there are other reports which demonstrated the potential neuroprotective role of RAS in neurodegenerative disease via various mechanisms such as oxidative stress [33, 87], apoptosis [57, 100], and neurotrophic factors [41]. However, the precise physiological function and pathological roles of RAS in CNS are still needing further studies to explicate their functions in the brain. Therefore, enhanced understanding of brain RAS would be potential targets to shield neurodegenerative disorders.

COVID-19 and ACE-2

The SARS-CoV-2 uses the resembling receptor, ACE2 as that for SARS-CoV for cell entry, however, the SARS-CoV-2 binding is more condensed by a four-residue motif viz. glycine-valine/glutamine-glutamate/threonine-glycine to hACE2, thus facilitates higher binding affinity of SARS-CoV-2 compared to SARS-CoV for hACE [8, 101]. Based on the fact that pathophysiology of SARS-CoV mediates through ACE2, Hamming et al. reported the essential role of ACE2 and its impact in the pathophysiology of SARS disease manifestations [102]. The first genetic proof was reported by Kuba et al. by way of an *in-vivo* study with SARS-CoV infected Ace2 knockout and control wild-type mice [103]. Authors have concluded that ACE2 is an essential receptor for SARS-CoV to facilitate their pathophysiology and viral entry. After infection with SARS-CoV the Spike protein of the SARS-CoV, which binds to ACE2 reduces the ACE2 expression. Also, it was found that ACE2 plays central role in SARS-CoV induced induced lung injury [104]. After injection of SARS-CoV Spike protein, one of the major structural proteins, into mice worsens acute lung failure which can be decreased by blocking the RAS pathway [103]. The expression of ACE2 is predominantly present on neuroglial cells, it is deliberated as potential target for the drug development for the treatment and management of COVID-19 infection and problems associated with long COVID-19 [105, 106].

In a study, protective RAS pathways have been established which continue to be medicated through overexpression of ACE2 and cause decreased neurogenic hypertension [107]. As a beneficial point of view, Ang-II to angiotensin-(1–7) conversion as a hydrolytic product is triggered by ACE2 and reduces the negative effects of the RAS but on the other hand ACE2 act as an entry point for SARS-CoV and SARS-CoV-2 [9, 108, 109]. Nevertheless, in the case of COVID-19 there is a significant downregulation of ACE2 and fail to show a beneficial compensatory role and thus responsible for severe organ injuries via cytokines storm and upregulation of macrophage induced

chemokines and involved in multiple organ failures in the patients suffering from COVID-19 [9, 101, 103, 107, 110, 111]. Several findings agreed and now ratified that ACE2 is the entry point for SARS-CoV-2 same as SARS-CoV [11, 112, 113]. Internalization of ACE2 and downregulated expression of ACE2 is the characteristics of SARS-CoV-2 infected cells. Consequently, there is a decrease in Ang II to the Ang (1–7) conversion, which is responsible for the adverse effect through Ang II/AT1 complex in COVID-19 patients [114, 115]. Summary of studies involving ACE 2 is provided in Table 25.1.

Conclusions

Based on the clinical and pre-clinical pieces of evidence, the role of ACE2 was well established in human physiology. Spike proteins of coronavirus targeting ACE2 receptors in lungs have established a prominent role of ACE2 in different disorder than previously thought. ACE2 was found to be countering the effect of angiotensin I and angiotensin II by cleavage of angiotensin I and II into angiotensin (1–7). ACE2 is considered the counterpart of ACE. It is also established that ACE and ACE2 maintain the critical balance between angiotensin II and angiotensin (1–7) thereby maintaining the physiological processes. Downregulation of ACE2 receptor in COVID-19 leads to an inequality amongst Ang-II-angiotensin-(1–7) conversion and ultimately producing lung fibrosis and pneumonia. As rigorous efforts are currently underway to target ACE and ACE2 receptors in the treatment of COVID-19, the same approach can be useful to treat other physiological disorders such as stroke, hypertension, myocardial infarction, and neurological diseases. As the role of ACE2 is now established in the arena of disorders including cerebrovascular and neurological diseases, it can be targeted for novel treatment strategies. The research in coming years will be focused on targeting ACE2 for bringing out effective management of cardiovascular, cerebrovascular, and neurological disorders.

Table 25.1 Summary of studies involving ACE 2

Sr. No.	Complication	Disorder	Type of study	Key output	References
1	Cardiovascular	Myocardial infarction	Clinical	Role of ACE2 is beneficial in MI	[13]
		Cardiac function	Pre-clinical	ACE2 involves in normal cardiac function	[14]
		Hypertension	Pre-clinical	Metabolism of angiotensin II by ACE2 maintains blood pressure	[15]
			Pre-clinical	Spontaneous Hypertension in rats was decreased by overexpression of ACE2	[16]
		Hypertension stroke	Pre-clinical	transgenic spontaneously hypertensive stroke-prone (SHRSP) rats showed reduced hypertension and improvement in endothelial functions by overexpression of ACE2	[17]
		Cardiomyopathy	Clinical	Increased levels of ACE2 was detected in Cardiomyopathy showed possible protective role of ACE2	[13, 18, 19]
		LV function	Pre-clinical	ACE2 improved LV function and contractility and decreased wall thinning	[21, 22]
		Heart failure	Clinical	ACE2 was found to be overexpressed in heart failure patient and may have a protective role in cardiac failure	[25–28]
2	Cerebrovascular	cerebral ischemia	Pre-clinical	ACE2 attenuate cerebral ischemia and improves neurological function	[32–34]

(continued)

Table 25.1 (continued)

Sr. No.	Complication	Disorder	Type of study	Key output	References
		Ischemic stroke	Pre-clinical	Levels of ACE2 was overexpressed in ischemic stroke and treatment with ACE2 attenuate stroke	[35, 37, 38]
			Clinical	Levels of ACE2 was decreased in acute stroke which gradually increases showed protective role of ACE2	[39]
3	Neurological	Cognitive function	Pre-clinical	ACE2 increases cognitive function by inhibiting angiotensin II	[58, 59]
		Aging	Pre-clinical	ACE2 concentration was decreased in aged animals showed that ACE2 deficiency leads to ROS generation which aggravate the aging	[87]
4	COVID-19	COVID-19	Pre-clinical and clinical	Spike proteins of SARS-CoV-2 interact with ACE2 which leads to lung fibrosis, pneumonia and respiratory failure	[102, 104, 105, 108, 109, 111, 112]

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