

# Aging and Remodeling of the RAS and RAAS and Related Pathways: Implications for Heart Failure Therapy

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## Introduction: Statement of the Problem

Human aging is a natural biological process that is progressive and is associated with cardiovascular (CV) and other biological changes that impact disease expression and response to injury and therapy [1–10]. As expected, progress, prosperity, and improved health care have been accompanied by prolonged longevity with an increase in people aged  $\geq 65$  years in industrialized developed countries and most developing countries are following this pattern [11, 12]. As reviewed in the first two chapters of this book, the definition of elderly by the chronological age  $\geq 65$  years was driven by pension legislations in the Europe of the 1870s, presumably to reduce the socioeconomic burden and political pressure when very few people were expected to survive to that age. This nearly 150-year-old definition has persisted into the twenty-first century despite the changing demographics, increased longevity, progress in medical therapies, and prosperity [13–16] and certainly mandates upward adjustment in keeping with modern trends. Progress and prosperity over

these years have been accompanied by expansion of an elderly population burdened with cardiovascular disease (CVD) including hypertension (HTN) and coronary heart disease (CHD), comorbidities that impact the CV system, and heart failure (HF), the known ultimate result of CVD [1, 14, 17]. While medical progress and clinical trials since the mid-1970s have improved therapy of CVDs in the non-elderly (aged  $< 65$  years), there is an alarming knowledge gap about the pathobiology and pathophysiology of CVDs and their therapy in the elderly people aged  $\geq 65$  years. The negative impact of that knowledge gap is already clearly apparent. The burden of CVD and HF is known to be greatest in the elderly, with escalating morbidity/mortality and related healthcare costs [18–24].

One reason for this global problem is that therapies for the non-elderly may not be optimal for the elderly because aging may result in remodeling of major pathways leading to CVD [6, 10, 17, 25, 26]. This chapter focuses on the aging-related remodeling of the renin–angiotensin system (RAS) [27] and related pathways including the renin–angiotensin–aldosterone system (RAAS) which play central roles in the pathophysiology and pharmacotherapy of CVDs and HF.

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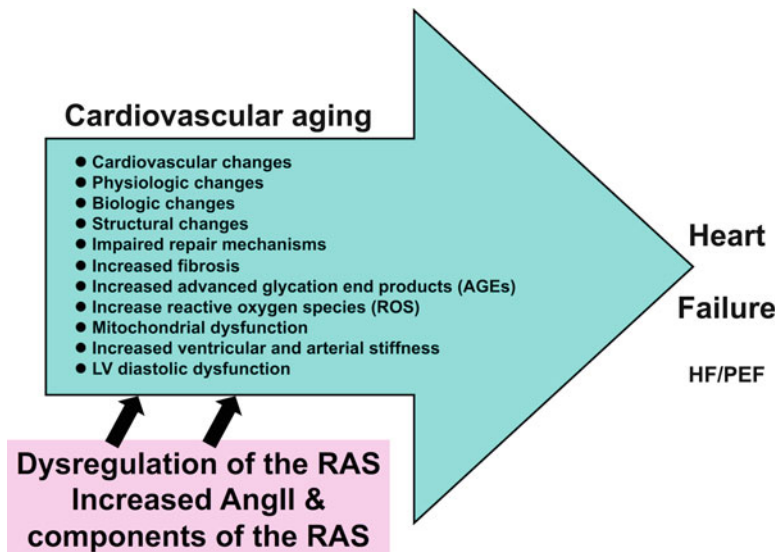
## Aging and the RAS

Population studies have shown that myocardial infarction (MI), HTN, and HF are more prevalent in the elderly [6, 11, 24, 28]. The healthcare costs

for post-MI HF in the elderly are high and increasing [6, 11, 17–19, 28]. While the RAS has critical functions in CV physiology, an upregulated RAS leads to CV pathology in both non-elderly and elderly populations [6]. Accordingly, components of the RAS have become important targets for CVD and HF pharmacotherapy since the mid-1980s [6, 28, 29]. Angiotensin II (AngII) is the primary effector molecule of the RAS. Since the 1990s, AngII inhibitors have formed the basis of therapy for both elderly and non-elderly HF patients [6, 29]. However, several clinical studies indicate that elderly post-MI patients are at higher risk for adverse events despite recommended therapy with RAS/AngII inhibitors compared to younger patients [6, 28]. Aging is associated with increased AngII and other components of the RAS [6, 30–33]. Emerging evidence suggests that the RAS may also become further upregulated and/or dysregulated during aging [6, 9, 29]. This adverse remodeling of the RAS may account for poor outcome and increased HF burden in the elderly despite conventional therapies [27].

## Aging and Remodeling of the RAS and Left Ventricle Post-MI and Post-HTN

Aging of the CV system is associated with fundamental physiological, biological, and structural changes that lead to increased extracellular matrix (ECM) and fibrosis, increased ventricular-arterial stiffening, left ventricular (LV) diastolic dysfunction, and HF associated with preserved ejection fraction (HF/PEF) [6, 7, 17] (Fig. 18.1). The aging process is progressive and marches in parallel with the CVD continuum towards HF [7, 17]. In this aging-HF continuum [14], CVD risk factors and comorbidities as well as the upregulated RAS [27] and other related pathways can fuel progression towards HF in the elderly (Fig. 18.1). It is important to recognize that the biological changes during aging lead to profound structural remodeling of the CV system that impact cardiac reserve. Even in the absence of CVD, the progressive changes during aging can themselves lead to HF. However, exposure to CV risk factors and interaction



**Fig. 18.1** Cardiovascular aging and role of the RAS in the march to heart failure. While age is considered to be a nonmodifiable risk factor, the age-related changes in cardiovascular vascular structure and function and associated physiological, biochemical, cellular/subcellular, and pathophysiological changes provide the substrate

for heart failure with preserved ejection fraction (HF/PEF). Dysregulation of the RAS and related pathways and other unrelated pathways influence the aging-related changes and can be modified by appropriate interventions. RAS renin-angiotensin system; AngII angiotensin II

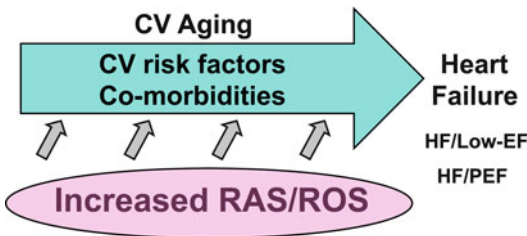
with comorbidities during aging together with the effect of increased RAS and reactive oxygen species (ROS) tend to accelerate progression to both categories of HF, namely, HF associated with low ejection fraction (HF/low EF) and HF/PEF in the elderly (Fig. 18.2).

Upregulation of the RAS is especially pertinent to the two major categories of HF (i.e., HF/low EF and HF/PEF) that contribute nearly equally to the HF burden in the elderly [7, 17] (Fig. 18.3). Some studies suggest that the prevalence of HF/PEF in the elderly is higher than 50 %, especially the very old [34–36]. Based on evidence suggesting that aging is associated with increased AngII and other components of the

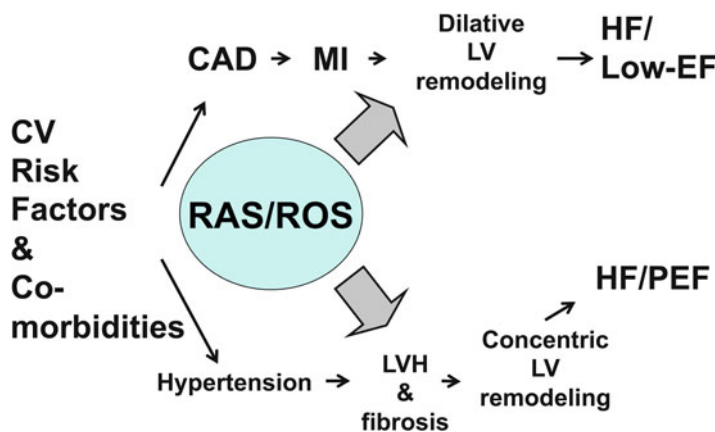
RAS [30–33], it is reasonable to speculate that remodeling of the RAS with aging may play a critical role in increased fibrosis in elderly hearts [37, 38] as well as hypertrophic remodeling and enhanced fibrosis in HF/PEF associated with hypertensive disease and enhanced dilative remodeling and HF/low-EF post-MI (Fig. 18.3).

Additionally, increased AngII with aging can also lead to amplified pro-inflammatory and pro-remodeling effects after injury. Aging has been shown to result in impaired repair mechanisms after reperfused MI [6–9]. Increased RAS/AngII and oxidative stress/ROS with aging [6] aggravate myocardial and matrix damage post-MI [9] and exacerbate adverse LV remodeling and dysfunction post-MI and progression to HF/low EF [7–10, 17]. Taken together, the aging-related increase in AngII, other components of the RAS, ROS, and oxidative stress may explain both the increased fibrosis in elderly hearts and HF/PEF and the enhanced postinfarction adverse LV remodeling and associated HF/low EF [6, 10].

It follows that there is need for a paradigm shift in HF management, going beyond targeting the RAS with respect to HF therapy for the elderly. There is need for identifying new therapeutic targets and strategies through translational research for optimizing HF therapy in the elderly. Because responses to major causes of HF such as



**Fig. 18.2** Role of risk factors, comorbidities, ROS, and RAS in heart failure. Interactions between cardiovascular (CV) risk factors, comorbidities, and increased ROS and RAS spur the march to heart failure during CV aging. RAS renin–angiotensin system; ROS reactive oxygen species



**Fig. 18.3** The two major categories of heart failure in the elderly. Role of risk factors, comorbidities, ROS, and RAS. Aging-related increase in RAS and ROS play major roles in dilative and concentric LV remodeling, leading to the two main categories of heart failure. CAD

coronary artery disease; CV cardiovascular; EF ejection fraction; HF heart failure; LV left ventricular; LVH LV hypertrophy; MI myocardial infarction; PEF preserved EF; RAS renin–angiotensin system; ROS reactive oxygen species

HTN and MI may be amplified in the elderly, different therapeutic strategies may be needed in the elderly compared to the young. Because biological changes with aging may alter responses to therapies that were tested in mostly young and non-elderly patients with HF, caution should be exercised and appropriate adjustments of medications (i.e., type, dosage, and timing) should be done when applying those in the elderly.

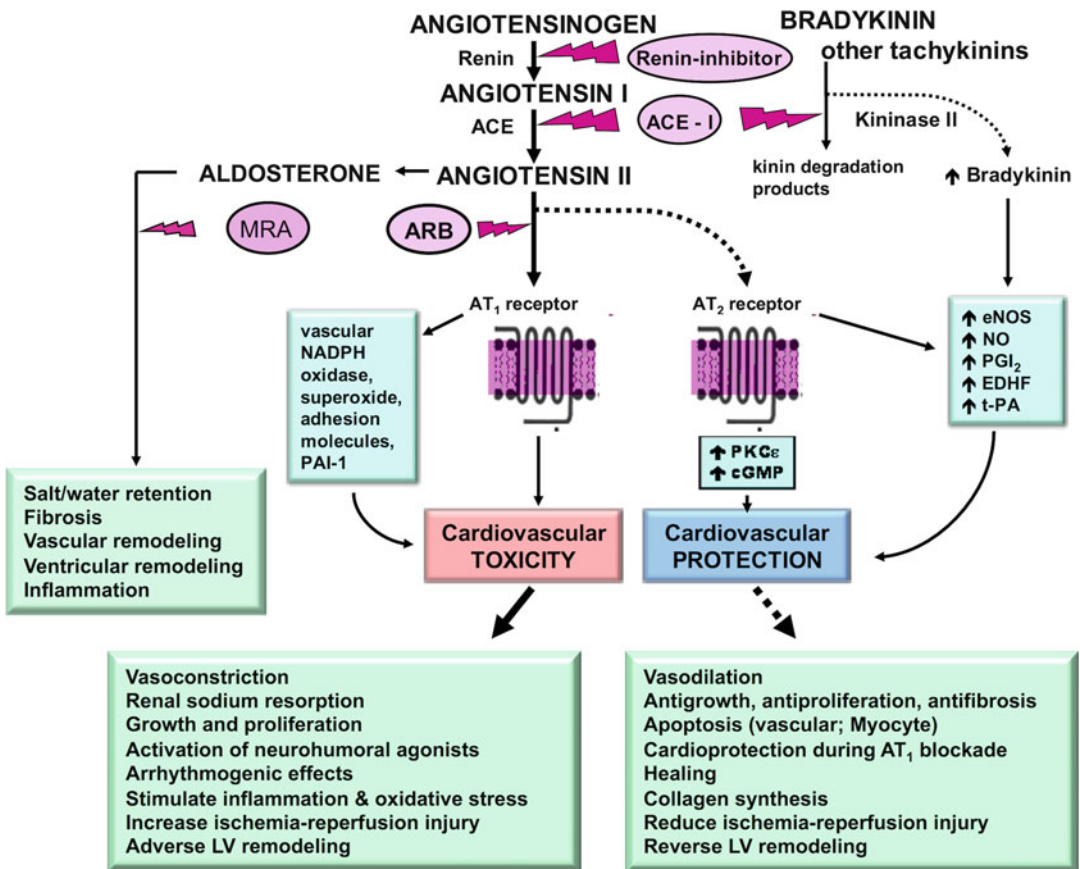
fundamental concepts and pathways are similar, there are some important differences with evidence of aging-related RAS dysregulation that may profoundly impact therapy of HF in the elderly.

### Aging and AngII, AT<sub>1</sub>/AT<sub>2</sub> Pathways

Traditional concepts of RAS inhibition have been based on research studies that were done in predominantly young animals and humans. While the

### Pharmacology of the RAS/RAAS in the Young and Non-elderly

The pertinent aspects of RAS/RAAS pharmacology have been reviewed elsewhere [39]. Briefly, the RAS/RAAS regulates blood volume and systemic vascular resistance, thereby modulating cardiac output and blood pressure (BP) through a cascade of events (Fig. 18.4). Renin, released primarily from the kidneys, cleaves circulating



**Fig. 18.4** The major pathways in the RAS and RAAS cascades. *ACE* angiotensin-converting enzyme; *ACE-I* ACE inhibitor; *ARB* angiotensin receptor blocker; *cGMP* cyclic guanosine 3' 5' monophosphate; *EDHF* endothelin-derived hyperpolarizing factor; *eNOS* endothelial

nitric oxide synthase; *MRA* mineralocorticoid receptor antagonist; *PAI-1* plasminogen activator inhibitor-1; *PGI<sub>2</sub>* prostacyclin; *PKCε* protein kinase Ce; *RAS* renin-angiotensin system; *RAAS* renin-angiotensin-aldosterone system; *t-PA* tissue plasminogen activator

angiotensinogen to the decapeptide angiotensin I (AngI), which is further cleaved by the angiotensin-converting enzyme (ACE), found primarily in pulmonary vascular endothelium, to the octapeptide molecule AngII. The latter is considered to be the major effector polypeptide molecule of the RAS/RAAS. It is also a pleiotropic cytokine that exerts several important physiological actions, including vasoconstriction, release of neurohumoral agonists (such as aldosterone, vasopressin, and norepinephrine), drinking, secretion of prolactin and adrenocorticotrophic hormone, and glycogenolysis. AngII also plays a critical role in the pathophysiology of CVD. Thus, chronically increased AngII induces increased vasoconstriction (leading to high BP and HTN), stimulates growth (leading to cardiac and vascular hypertrophy), contributes to LV dysfunction and progression of HF, mediates adverse structural cardiac and vascular remodeling [40], and causes deleterious activation of other neurohumoral agonists including endothelin. Sodium and water homeostasis becomes dysregulated, especially in the elderly. Since high AngII levels and low cardiac output stimulate thirst, the elderly patient with high AngII, HF, and low cardiac output is prone to develop hyponatremia despite reduced water intake.

While the RAS was initially regarded as an endocrine system, a steady flow of new evidence since the 1990s has expanded the paradigm into that of a multifunctional endocrine, paracrine, and intracrine system with circulating and local/tissue components [40–42]. While AngII is produced in the circulation and tissues and acts on the AngII type 1 (AT<sub>1</sub>) and AngII type 2 (AT<sub>2</sub>) receptors [39, 41], most of the effects of AngII are normally mediated through the AT<sub>1</sub> receptor. However, in the presence of CV pathology—such as cardiac hypertrophy, vascular injury, MI, HF, and wound healing—the AT<sub>2</sub> receptor is upregulated and may mediate some CV effects of AngII through AT<sub>2</sub>. Since there is a decrease in AT<sub>1</sub> and an increase in AT<sub>2</sub> receptors in HF, it was proposed that the antiproliferative and vasodilatory effects of AT<sub>2</sub> counterbalance the growth-stimulating and vasoconstricting effects of AT<sub>1</sub>. In that construct, it follows that an AT<sub>1</sub> receptor blocker (ARB) would completely block effects of AngII via AT<sub>1</sub>

and result in unopposed AT<sub>2</sub> receptor stimulation that might augment its beneficial effects [43]. Although, there is controversy about the role of AT<sub>2</sub> in humans [44], it may be important in the elderly. However, the role of AT<sub>3</sub> and AT<sub>4</sub> receptors with aging remains unclear and needs study.

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### **Cardioprotective Effect of RAS Inhibition: Role of Kallikrein–Kinin System**

Cumulative evidence supports the view that the cardioprotective effects of ACE inhibitors are due not only to the inhibition of AngII formation via ACE but also to inhibition of breakdown of bradykinin and other tachykinins related to ACE's kininase II activity. In this construct, ACE inhibition decreases the amount of AngII that is presented to both AT<sub>1</sub> and AT<sub>2</sub> receptors, at least initially, so that decreased but balanced AT<sub>1</sub> and AT<sub>2</sub> effects is expected. However, increased bradykinin during ACE inhibition leads to stimulation of nitric oxide (NO), prostaglandins such as prostacyclin (PGI<sub>2</sub>), endothelial-derived hyperpolarizing factor (EDHF) and tissue-thromboplastin activator (t-PA), thereby contributing to vasodilation, CV protection, and other favorable CV effects of ACE inhibitors [45]. The increased bradykinin also contributes to the hypotensive effect of ACE inhibitors which may be pertinent for the elderly.

In contrast to ACE inhibitors, the cardioprotective effect of ARBs is mediated through at least three pathways: (1) the primary pathway involves selective AT<sub>1</sub> receptor blockade, (2) a secondary pathway involves AT<sub>2</sub> receptor activation, and (3) a third pathway involves the release of kinins and stimulation of kinin B<sub>1</sub> or B<sub>2</sub> receptors [46–49] and/or direct AT<sub>2</sub>-mediated signaling via protein kinase C (PKCε), NO, and cyclic guanosine monophosphate (cGMP) [50–53]. Coupling between the AT<sub>2</sub> receptor and kallikrein during AT<sub>1</sub> blockade has been demonstrated in young mice with myocardial ischemia–reperfusion injury [49].

However, while kinins contribute to the protective effects of ACE inhibitors and ARBs

acting through the AT<sub>2</sub> receptor and NO contribute to myocardial and vascular protection, the kinin system is also involved in inflammation and the pathogenesis of inflammatory diseases such as arthritis ([54], for review). The kinins are known to act as mediators of inflammation by promoting maturation of dendritic cells which activate the body's adaptive immune system and thereby promote inflammation. This may also be pertinent in elderly HF patients.

### **Role of Chymase and Other Non-ACE Pathways in Angiotensin II Generation**

The discovery, in the 1990s, of non-ACE pathways that can generate AngII during ACE inhibition [55–60] has important implications for optimal therapy of HF in both non-elderly and elderly patients. Data in mostly non-elderly patients indicated that ACE inhibitors do not block all AngII formation. This includes AngII from AngI via the serine protease chymase and other non-ACE enzymes and/or that from angiotensinogen via non-renin pathways. Several studies showed that AngII levels persist during long-term ACE-inhibitor therapy, suggesting incomplete RAS blockade or the so-called ACE-inhibitor escape due to a reactive rise in active plasma renin [57–60]. This finding not only supported the use of ARBs for blocking the effects of AngII at the AT<sub>1</sub> receptor but also fueled the push for using combined ACE inhibition and AT<sub>1</sub> receptor blockade for more complete blockade of the deleterious effects of AngII and therefore anticipated greater benefits. However, support for the concept of dual ACE and AT<sub>1</sub> receptor blockade came from experimental [61] and clinical [62] studies that were done in mostly the young with HF. For example, in young rats with post-MI HF, the ACE inhibitor fosinopril combined with the ARB valsartan resulted in suppression of histopathologic evidence of remodeling and normalized collagen I, macrophages, and myofibroblasts [63].

Two additional findings deserve emphasis. First, the AngII-forming capacity of chymase

from AngI was found to be 20-fold higher than that for ACE [55, 56]. Second, chymase was also shown to activate the Kallikrein–kinin pathway [64, 65]. These findings imply that the combination of a chymase inhibitor with either an ACE inhibitor or an ARB may potentially double the beneficial effects through decrease of AngII and increased kinins. Dual chymase and ACE inhibition was proposed for HTN and HF to boost the efficacy of ACE inhibition and prevent *ACE-inhibitor escape*.

In fact, chymase inhibitors have been tested in young experimental animal models. They were shown to improve diastolic LV function and prevent fibrosis in a canine model of tachycardia-induced HF [66], reduce arrhythmias and LV dysfunction after MI [67, 68], attenuate LV remodeling in a mouse model of intermittent hypoxia [69], and decrease infarct size after ischemia–reperfusion through attenuation of matrix metalloproteinase (MMP)-9 and pro-inflammatory cytokines in a porcine model of reperfused MI [70]. In the mouse model of intermittent hypoxia, decrease in LV hypertrophy and fibrosis was associated with decrease in LV chymase, AngII, oxidative stress, interleukin (IL)-6, tumor necrosis factor (TNF)- $\alpha$ , and transforming growth factor (TGF)- $\beta$  [69]. In a hamster model of post-MI HF, combined ACE and chymase inhibition decreased infarct size and LV remodeling and dysfunction compared to ACE inhibition alone [71]. In the canine model of LV volume overload due to mitral regurgitation, the anti-remodeling effect of chymase inhibition appears to be in part due to inhibition of MMP and kallikrein activation and fibronectin degradation [72], thereby attenuating loss of ECM and cell-ECM connections, and cell death. The multiple actions of chymase on tissue remodeling support its role in adverse LV remodeling and HF post-MI [73–75] atherosclerosis [76–79].

Whether the AngII generation by non-ACE pathways is increased with aging and in elderly HF patients needs investigation. Whether chymase inhibitors might be beneficial in older animals and patients with HF needs study. Recent evidence indicates that chymase is upregulated in coronary and renal arteries of diabetic patients [80] and implicates chymase in the intracellular

formation of AngII in cardiomyocytes, fibroblasts, and renal mesangial and vascular smooth muscle cells under hyperglycemic conditions [81]. This finding may be especially pertinent in the context of aging as diabetes is a major comorbidity in elderly patients with HF.

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## Interactions Between the RAS and the Endothelin System

Extending the concept of dual inhibition one step further, the ARB valsartan combined with the endothelin (ET) blocker bosentan was shown to produce added benefits on neurohumoral activity and LV loading and performance end-points in pigs with HF induced by rapid atrial pacing [82]. Both the RAS acting through AngII and the endothelin system acting through ET-1 produce powerful vasoconstrictor and vasopressor effects that promote adverse LV remodeling and progression of HF ([83–85] for review). As AngII, both circulating and myocardial tissue ET levels are increased in animals and humans with HF, interactions between the RAS and ET system have been described [83]; AngII stimulates ET production through transcriptional regulation while ET inhibits renin synthesis and stimulates aldosterone secretion. Both AngII and ET stimulate matrix synthesis. In animal models of HF, both nonselective ET-A and ET-B antagonists and selective ET-A antagonists exert beneficial CV effects. However, ET-A antagonist therapy in experimental HF results in augmentation of the RAS and sustained sodium retention [86]. Furthermore, in patients with HF, short-term ET antagonist therapy improved hemodynamics but long-term therapy did not improve combined morbidity/mortality end-points [87]. In the EARTH trial, the ET antagonist darusentan as an add-on to an ACE inhibitor,  $\beta$ -adrenergic blocker, or aldosterone antagonist in patients with chronic HF failed to improve LV remodeling, clinical symptoms, or outcomes [87]. Long-term trials of the ET antagonist bosentan in HF were prematurely terminated due to increased adverse events [88]. Despite anecdotal evidence of success with bosentan in the subgroup of patients with pulmonary artery hypertension secondary to HF, a

recent trial showed no improvement [89]. Despite further anecdotal reports of short-term benefit in patients with severe pulmonary hypertension awaiting heart transplantation, the routine use of ET antagonists is not recommended by the World Health Organization (WHO) Pulmonary Hypertension group 2 [90]. Whether ET antagonists may have an application in elderly HF patients has not been studied.

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## Role of the Counter-Regulatory ACE2 and Ang-(1–7) Arm of the RAS

While the RAS became recognized as a central regulator of CV and renal function with a major role in pathophysiology of CVD and HF, the discovery of angiotensin-converting enzyme 2 (ACE2) in 2000 [91, 92] further modified the traditional concepts about the RAS. Subsequent studies viewed ACE2 as an essential regulator of cardiac function [93] and several studies underscored the importance of AngII degradation by the carboxypeptidase ACE2 to Ang-(1–7), a vasodilator, antitrophic and antifibrotic heptapeptide that functions as an endogenous inhibitor of AngII [94, 95]. Both ACE2 and Ang-(1–7) were demonstrated in rat and human cardiomyocytes. Later, ACE2/Ang-(1–7) was also demonstrated in other tissues, including blood vessels, kidneys, lungs, and brain, and was implicated in CV homeostasis.

Experimentally in rats, ACE inhibition decreased AngII formation and increased Ang-(1–7), while AT<sub>1</sub> blockade increased AngII and Ang-(1–7) [96]. The increase in Ang-(1–7) with ACE inhibition was attributed to increased AngI and inhibition of Ang-(1–7) metabolism, while the increase with AT<sub>1</sub> blockade was attributed to formation from increased AngI [96]. After MI in rats, AT<sub>1</sub> blockade was shown to upregulate ACE2 [97], which may contribute to its cardioprotective effect via Ang-(1–7) formation as verified by Ang-(1–7) infusion [98]. In the late phase of LV dysfunction after MI in rats, the ACE inhibitor enalapril was shown to attenuate downregulation of ACE2 [99]. In hypertensive rats, the ARB telmisartan attenuated aortic hypertrophy through modulation of ACE2 [100].

Preliminary data from our laboratory showed that the ARB candesartan and the vasopeptidase inhibitor omapatrilat attenuated LV remodeling and dysfunction during healing after reperfused MI in rats through modulation of ACE2 as well as MMP-9, inflammatory cytokine IL-6, TNF- $\alpha$ , TGF- $\beta$ , *N*-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), collagens, and fibrosis [101]. In that study, increased ACE2 and Ang-(1–7) levels, associated with enhanced AT<sub>2</sub> receptor signaling and suppression of TGF- $\beta$ <sub>1</sub> and smad-2 signaling, inflammatory cytokines, and AngII signaling via AT<sub>1</sub> receptors, effectively limited fibrosis, adverse remodeling, and dysfunction during healing after reperfused MI [101]. Recent evidence suggests that the ACE2-Ang-(1–7)-Mas receptor can counter-regulate remodeling effects of AngII and inhibit hypertrophy and fibrosis [102].

It appears that whereas the RAS through ACE-AngII-AT<sub>1</sub> receptor axis promotes adverse remodeling, the ACE2/Ang-(1–7) system through ACE2-Ang-(1–7)-Mas receptor axis is counter-regulatory ([96, 102, 103] for review) and prevents adverse remodeling. The increase in ACE2/Ang-(1–7) mediated by ACE inhibitors and ARBs may contribute to the anti-remodeling and other beneficial effects in HF ([102, 103] for review). However, ACE2/Ang-(1–7) is upregulated in human HF [104] and overexpression of ACE2 attenuates LV remodeling post-MI in young rats [105].

In human HF, plasma ACE2 activity is elevated and correlates with poor outcome [106, 107], suggesting a compensatory response to LV dysfunction. ACE2 expression in rat lung decreases with aging [108]. Responses to Ang-(1–7) differ in young, aged, and diabetic rabbit corpus cavernosum [109]. Whether a loss of ACE2 function with aging might lead to amplified activation of RAS and more adverse remodeling and explain the worse clinical outcome in the elderly with HF [6, 8, 14] needs study. Preliminary data from our laboratory showed that more severe age-related LV remodeling and dysfunction after acute reperfused ST-segment elevation MI was associated with downregulation of myocardial AT<sub>2</sub> receptors, ACE2, Ang-(1–7), Ac-SDKP, and smad-2 in the dog model [110].

Since Ang-(1–7) is a substrate for inactivation by ACE, it competes with AngI and bradykinin for degradation, thereby inhibiting AngII formation and augmenting bradykinin activity and its vasodilatory effects [111]. Increased Ang-(1–7) with ACE inhibition may further augment bradykinin activity. Later, AT<sub>1</sub> blockade was shown to increase bradykinin levels in hypertensive humans, probably due to decreased metabolism by ACE and neutral endopeptidase [112]. The authors warned that the increased bradykinin with ARBs may augment their therapeutic actions but may also lead to angioedema. Collectively, these findings indicated that ACE inhibitors and ARBs increase both Ang-(1–7) and bradykinin. Whether these effects are amplified in the elderly needs study. An alternative strategy needing study for slowing progression of HF with aging would be to increase levels of Ang-(1–7) as opposed to reducing levels of AngII with ACE inhibitors and ARBs, thereby avoiding side effects related to increased bradykinin such as coughing, dizziness, and angioedema.

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### **Role of the Chymase/Ang-(1–12) Axis in Renin-Independent Generation of AngII**

Recent evidence since 2006 suggests that Ang-(1–12), a propeptide cleaved from angiotensinogen, may represent an alternate substrate for the formation of angiotensins including AngII [113, 114]. Ang-(1–12) was shown to be increased in cardiomyocytes of adult spontaneously hypertensive rats [115]. Cardiac Ang-(1–12) as well as AngI and AngII was increased in myocardium of rats with bilateral nephrectomy and absence of circulating renin while plasma levels decreased [116]. In a rat model of ischemia–reperfusion injury, pro-angiotensin 12 (PA12) was suggested to act as a circulating substrate for a chymase-mediated AngII production [117]. Recently, this renin-independent mechanism of AngII generation was demonstrated in human left atrial tissue from patients undergoing the MAZE surgical procedure for chronic atrial fibrillation [118].



This pathway has also been demonstrated in the normal and diseased human LV tissue [119, 120].

Whether this system is augmented in the elderly needs study.

### RAS Inhibition for Heart Failure: ACE Inhibitors and ARBs

The introduction of RAS inhibition with ACE inhibitors and ARBs for the treatment of chronic HF represents one of the most significant advances in CV medicine during the latter half of the twentieth century. When the role of the RAS in CV disease was first recognized in the 1950s, the focus was on HTN and the neurohumoral paradigm. Several major large-scale, multicenter randomized clinical trials (RCTs) of ACE inhibitors since the mid-1980s helped to establish its use for improving the survival of patients with HF and acute MI [121–126] (Table 18.1). The rationale for using ACE inhibitors was to inhibit ACE and thereby decrease the formation of AngII and its adverse effects. Subsequently, RCTs since the mid-1990s investigated the benefits of using ARBs in patients with HF and MI [127–136] (Table 18.2). The main rationale for ARBs was to achieve specific and selective blockade of the effects of AngII via the AT<sub>1</sub> receptor [137].

Three points about those and subsequent RCTs need emphasis. First, most of the early RCTs recruited patients aged ≤65 or 18–65 years as reflected in the mean ages in Tables 18.1 and 18.2.

Elderly patients were excluded. Second, since the benefits of ACE inhibitors in hypertension, HF, and MI were already established when ARBs were introduced, it became necessary to demonstrate that ARBs were superior to them or equally effective in patients intolerant to them and receiving other background therapies in RCTs rather than relative to a true placebo group. Third, ACE inhibitors and ARBs were used on top of background contemporary therapy that often included β-blockers in patients with LV systolic dysfunction and HF, and β-blockers were known to reduce renin [138] and AngII [139] and produce effects additive to that of ACE inhibitors [140].

Additionally, three reasons were proposed as justification for using ARBs as an add-on or alternative to already established ACE inhibitors. First, compared to ACE inhibitors, ARBs were expected to provide more complete inhibition of AngII derived from all sources, including non-ACE and non-renin pathways, especially as the latter is increased during ACE inhibition [43, 55]; however, ARBs were subsequently found to increase renin, AngI, and AngII as well as Ang-(1–7) levels [94, 96]. Second, since ARBs do not inhibit kininase II, or via this mechanism increase systemic peptides of the inflammatory response such as bradykinin, substance P, and other tachykinins known to produce cough and angioedema side effects associated with ACE inhibitors [141, 142], these side effects would be avoided; however, as discussed before, ARBs can also increase release of kinins and stimulation of kinin B<sub>1</sub> or B<sub>2</sub>

**Table 18.1** Major trials of ACE inhibitors in heart failure

Year—trial [reference]	<i>N</i>	Drug	Comparator	Age (years)	Outcome
1987 CONSENSUS [121]	253	Enalapril	Placebo	70	↓ Mortality/morbidity
1989 Lisinopril [122]	189	Lisinopril	Captopril	60	↑ EF, function, and QOL
1991 SOLVD (symptomatic) [123]	2,569	Enalapril	Placebo	61	↓ Mortality/morbidity
1992 SOLVD (asymptomatic) [124]	4,228	Enalapril	Placebo	59	↓ Mortality (NS); ↓ morbidity
2000 ATLAS [125]	3,164	Lisinopril (high dose)	Lisinopril (low dose)	64	↓ Mortality/morbidity with high dose
2006 PEP-CHF [126]	850	Perindopril	Placebo	76	Short-term symptoms improvement; ↓ hospitalization

*Abbreviations:* ↓ decrease in, *EF* ejection fraction, *HF* heart failure, *N* number of patients, *NS* nonsignificant, *QOL* quality of life

**Table 18.2** Major trials of ARBs in heart failure

Year—trial [reference]	<i>N</i>	Drug	Comparator	Age (years)	Outcome
1997 ELITE [127]	722	Losartan	Captopril	73	Unexpected 46 % ↑ in mortality (2° end-point)
1999 RESOLVD [128]	768	Candesartan	Enalapril	63	Early trend in ↑ mortality and HF (2° end-point)
2000 ELITE II [129]	3,152	Losartan	Captopril	71.5	Not superior
2001 Val-HeFT [130]	5,010	Valsartan	ACE-Is	62.5	Not superior; ↓ composite end-point
2003 CHARM-Overall [131]	7,601	Candesartan	ACE-Is	66	Improved 1° outcome (mortality and morbidity)
2003 CHARM-Added [132]	2,548	Candesartan	ACE-Is	64	Improved 1° outcome (clinical, morbidity)
2003 CHARM-Alternative [133]	2,028	Candesartan	ACE-Is	66.5	Improved 1° outcome (mortality and morbidity)
2003 CHARM-Preserved [134]	3,023	Candesartan	ACE-Is	67	Similar 1° outcome (improved 2° outcome)
2005 VALIANT (MI+HF) [135]	14,703	Valsartan	Captopril	55, 70, 79, 88	Not superior; ↑ risk adverse events with combination
2008 I-PRESERVE [136]	4,128	Irbesartan	Placebo	72	Not superior

*Abbreviations:* ↑ increase in, ↓ decrease in, *ACE-I* angiotensin-converting enzyme inhibitor, *ARB* angiotensin receptor blocker, *HF*, heart failure, *MI* myocardial infarction, *N* number of patients

receptors [46–49]. Third, ARBs might produce unopposed AT<sub>2</sub> receptor stimulation resulting in added benefits, including long-term CV structural changes over that seen with ACE inhibitors [43]. These arguments led to RCTs on the effects of valsartan in post-MI LV systolic dysfunction and/or HF [130] and chronic HF [144], respectively. However, in that study (VALIANT), there was no upper age limit and valsartan was shown to be not superior to the ACE inhibitor captopril and the combination increased the risk of adverse events in elderly patients [135]. Importantly with increasing age (<65, 65–74, 75–84, and ≥85 years), the 3-year mortality increased fourfold (13 %, 26 %, 36 %, and 52 %, respectively), the composite end-point events increased more than twofold (25 %, 41 %, 52 %, and 67 %, respectively), and HF admissions increased threefold (12 %, 23 %, 31 %, and 35 %, respectively) in VALIANT [135]. These findings further underscore the fact that the elderly represent a high-risk group for acute MI and HF/low EF with disproportionately high mortality and morbidity in need of improved therapies.

## RAS Inhibitors for Hypertension: ACE Inhibitors and ARBs

The central role of RAS in the regulation of BP, fluid and electrolyte balance, and pathophysiology of CV disease is well recognized (Fig. 18.1) [24, 144–147]. AngII not only increases BP but also promotes vascular inflammation leading to endothelial dysfunction and atherosclerosis, stimulates vascular smooth muscle hypertrophy and vascular remodeling, and stimulates myocardial fibrosis and hypertrophy leading to cardiac remodeling [144–147]. Importantly, it also increases aldosterone which stimulates fibrosis and CV remodeling (Fig. 18.1). The fact that most of the effects of AngII are mediated via AT<sub>1</sub> receptors provides the rationale for ACE inhibition and AT<sub>1</sub> receptor blockade (Fig. 18.1). Since aging is associated with increased AngII and other RAS components which in turn contribute to increased CV remodeling and CV risk in the elderly [6], RAS inhibition with ACE inhibitors and ARBs is recommended in that group [24].

ACE inhibitors have also been studied for reducing CV risk. Indeed, they effectively control BP in patients with hypertension and have additional benefits on CHD, stroke, MI, HF, diabetes, or chronic kidney disease. Several RCTs have established that ACE inhibitors reduce rates of death, MI, and stroke in patients with HF [123], LV dysfunction [148], vascular disease [149–152], or high-risk diabetes [153]. The HOPE trial with the ACE inhibitor ramipril showed improved prognosis; decreased rate of death, MI, and stroke in high-risk patients for CV events and without low EF or HF; and decreased new-onset diabetes and complications of diabetes [154]. The EUROPA trial, which included lower risk patients than HOPE, showed improvement in the composite end-point of CV mortality, MI, and resuscitation [149]. However, QUIET which included low-risk patients showed no significant benefit [150]. The PEACE trial, which also included low-risk patients and used the ACE inhibitor trandolapril, showed no benefit [151].

Although the dose of ACE inhibitors in QUIET and PEACE may not have been optimal, a meta-analysis of the trials, with pooled data in 31,600 patients, showed that ACE inhibitors are effective in preventing CV events, with 26 % reduction in the risk of HF or stroke and 13–18 % reduction in total and CV mortality, and MI compared to placebo [152]. Other studies suggested that ACE inhibitors not only control BP and reduce stroke but also prevent renal complications of diabetes [155]. In the HOPE/The Ongoing Outcomes (TOO) study, development of diabetes in the follow-up phase decreased, suggesting an added benefit of long-term ramipril [156]. In the MICRO-HOPE substudy [153], ramipril was beneficial for CV events and overt nephropathy in patients with diabetes. In the ADVANCE trial, the ACE inhibitor perindopril together with the diuretic indapamide reduced the risks of major vascular events and death in type 2 diabetes [157].

As discussed before, the ability of ARBs to selectively block AngII at the AT<sub>1</sub> receptor resulting in more complete inhibition was considered advantageous (Fig. 18.4). ARBs also do not increase bradykinin by suppressing its degradation as ACE inhibitors do, thereby enhancing vasodilation but

also increasing cough and angioneurotic edema that troubles ~20 % of patients, especially women and Asians [29, 153, 154]. ARBs may result in unopposed AT<sub>2</sub> receptor activation and enhance vasodilation via downstream AT<sub>2</sub>-mediated signaling (Fig. 18.4). Apart from blocking deleterious effects of AngII and controlling BP, ARBs might have similar protective effects as ACE inhibitors. RCTs showed that ARBs effectively control BP in HTN and are well tolerated [158]. However, despite well-known arguments for using ARBs [29], whether ARBs are as effective as ACE inhibitors in reducing events such as stroke and MI has been questioned [159]. Moreover, ARBs can also release kinins and increase bradykinin levels in hypertensive patients [112, 158] and thereby mediate CV protection. Such ARB-induced increase in bradykinin can augment therapeutic actions but also lead to cough and angioedema [112, 158]. As discussed before, both ACE inhibitors and ARBs can increase Ang-(1–7) in the counter-regulatory arm of the RAS.

A complicating factor with the chronic use of ACE inhibitors in HF patients is that AngII levels increase and symptoms worsen [128]. However, studies in HTN have shown that ARBs such as losartan and valsartan are as effective as ACE-Is in lowering BP [160, 161]. In hypertensive patients with ACE-I-induced cough, this complication is less frequent with ARBs [162]. In patients with HF/low EF, an ARB was shown to reduce the rate of death or hospitalization relative to placebo in those patients who could not tolerate an ACE inhibitor [133] or were already receiving it [130, 132]. In the LIFE study, compared to  $\beta$ -blockers, ARBs reduced vascular events in high-risk patients with hypertension and LV hypertrophy (LVH) [163]. Taken together, these studies suggest that an ARB is an effective and well-tolerated alternative to an ACE inhibitor for CV protection.

Since ACE inhibitors preceded ARBs for treating HTN and HF, it has become necessary in clinical trials to demonstrate non-inferiority or superiority of an ARB over an ACE inhibitor as comparator. In patients with MI, two studies comparing an ARB with an ACE inhibitor produced different results. OPTIMAAL [164] and

VALIANT [143] compared the ARBs losartan and valsartan, respectively, to the captopril in patients with signs of HF within 10 days of MI. In OPTIMAAL, the ARB was not superior and the non-inferiority criteria were not met; in fact, there was an increase in CV mortality after a 2.7-year mean follow-up [164]. In VALIANT, the ARB was non-superior and non-inferior for mortality and the composite end-point of fatal and nonfatal events; the study established that valsartan was as effective as an ACE inhibitor in reducing mortality in high-risk survivors of MI [143]. A meta-analysis of 54,254 patients from 11 trials showed a potential 18 % increase in MI with ARBs compared to placebo and a possible increase compared with other active therapy [165]. In a separate meta-analysis of 55,050 patients from 11 trials that compared ARBs with either placebo or an active comparator, ARBs were found to reduce event rates for stroke, not to reduce event rates for global death, and to increase rates of MI by 8 % [159]. The cloud of doubt cast by these reports has been partly dispelled by studies with the ARB telmisartan [166–169].

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### **Aging, RAS Dysregulation, and AngII Inhibition with ACE Inhibitors and ARBs**

The contribution of the RAS to CV changes during aging has been confirmed in experimental studies. As mentioned previously, aging alters all RAS components and results in increased angiotensinogen, AngII, AT<sub>1</sub> and AT<sub>2</sub> receptors, and ACE in rat hearts [30, 31]. Increased AngII and other RAS components with aging may explain increased cytosolic and mitochondrial oxidant production, mitochondrial dysfunction, and increased ECM deposition associated with aging [32]. Short-term AngII was shown to downregulate AT<sub>1</sub> receptor mRNA in fibroblasts from aged rat myocardium [170]. A study with long-term AngII inhibition with the ACE inhibitor enalapril or the ARB losartan protected against CV effects of aging and prolonged life in rats [171], implying a harmful effect of increased RAS and AngII effects during aging. Furthermore, disruption of

the AT<sub>1</sub> receptor in aging mice was shown to protect from CV morbidity and mortality and promote longevity [33].

As discussed above, clinical studies of RAS inhibition with using ACE inhibitors and ARBs in predominantly non-elderly patients with HF/low EF produce undeniable benefits. However, evidence from some of these clinical studies has established that elderly patients with post-MI HF are at higher risk despite therapy and a dominant mechanism is persistent adverse LV remodeling [6, 8, 10]. While reperfusion is widely used in acute MI, data on healing and remodeling post-reperfused MI in the elderly is lacking [6]. Post-MI survivors who develop HF on therapy have a 10-fold greater risk of dying [172], and the risk is even greater in the elderly [28, 173, 174]. The survivors with persistent post-ischemic LV dysfunction after reperfused MI remain at risk for remodeling and its consequences including HF despite receiving optimal therapy [135, 175–182]. Evidence suggests that aging-related impaired or defective healing/repair may be a major culprit resulting in adverse remodeling and poor outcome [6, 181].

Recent evidence from our laboratory suggests that the RAS may become dysregulated with aging [6, 9]. Physiological, cellular, and molecular changes that occur with CV aging appear to negatively impact the healing/repair response to injury including reperfused MI [6]. Timed release of several factors after injury modulate healing/repair [6, 26, 38, 176, 177]; the factors include AngII, ROS, chemokines, inflammatory cytokines, growth factors, MMPs, and other matrix proteins such as healing-specific matrix and matricellular proteins (HSMPs) including secretory leukocyte protease inhibitor (SLPI), secreted protein acidic and rich in cysteine (SPARC), and osteopontin (OPN) [6]. Together, they orchestrate inflammation, ECM remodeling and fibrosis, and adverse LV remodeling [6, 175–177]. In 2008, Bujak et al. first reported defective infarct healing and increased adverse remodeling after reperfused MI in old versus young mice [7, 8]. Since therapy for optimizing healing is lacking [6, 8], our laboratory considered the hypothesis that in aging hearts, increased myocardial AngII,

through its pro-inflammatory, pro-oxidant, and pro-remodeling effects, may amplify increases in pro-inflammatory cytokines, MMPs, and oxidative markers and contribute to impaired healing/repair and adverse LV remodeling [6]. We postulated that aging results in a dysregulation of matrix, inflammation, and fibrosis pathways, leading to impaired healing and adverse LV remodeling post-reperfused MI (Fig. 18.3). In this construct, aging-related impaired or defective healing post-MI may be the major culprit leading to defective infarct fibrosis that in turn might result in amplified adverse maladaptive LV remodeling, increased progressive LV enlargement, and increased disability and/or death in older patients. Evidence from our laboratory supports the idea that aging-related adverse remodeling may be due in part to impaired healing/repair mechanisms after reperfused MI [6, 9, 182], but further research is needed.

Our preliminary data in dog, rat, and mouse models of post-reperfused MI suggest that concurrent upregulation of the three HSMPs (SLPI, SPARC, and OPN) may interact with concurrently upregulated ECM-proteolytic, inflammation, and fibrosis pathways and contribute to remodeling in young animals [9, 182] and upregulation of the HSMPs and proteolytic, inflammation, and fibrosis pathways is amplified in old animals that develop more severe LV remodeling and dysfunction [9, 182, 183]. Importantly, the ARB candesartan attenuated these changes across the old groups albeit with a trend towards lesser benefit in the oldest [9], implicating AngII and dysregulation of inflammatory and ECM-proteolytic pathways in the augmented remodeling.

Taken together, our data suggest that the HSMPs SLPI, SPARC, and OPN that are increased post-reperfused MI may interact with inflammation and fibrosis pathways and improve healing and LV remodeling in the young, but the pathways become dysregulated in the old [9]. Thus, aging amplifies responses in the critical cellular signaling and ECM-proteolytic pathways, and interaction with the AngII/AT<sub>1</sub> receptor pathway after reperfused MI in the clinically relevant dog model [182]. Our preliminary data during healing after reperfused MI in rats [183] confirm

that aging amplifies the increased expression of HSMPs, MMPs, and inflammatory and fibrogenic cytokines in infarct zones during healing after reperfused MI. In that study [183], SLPI, SPARC, and OPN were colocalized in macrophages and monocytes. Importantly, candesartan during healing suppressed these changes in the HSMPs and remodeling in young and old rats, implying regulation by AngII with aging. Candesartan also attenuated increases in MMPs, inflammatory and fibrogenic cytokines, and iNOS in the young and old rats, implying regulation by AngII during healing. The data also showed age-related increase in tissue myeloperoxidase (MPO, oxidant activity marker), and MPO-positive granulocytes and CD68 and MAC387-positive macrophages at day 25, implying persistent inflammation and granulation tissue with impaired healing in the late phase after reperfused MI.

In summary, our data suggest that aging upregulates two critical pathways: (1) increased AngII/ROS → increased iNOS-NO → peroxynitrite → MMP and HSMP activation → adverse remodeling and dysfunction and (2) increased AngII/ROS → increased inflammatory cytokines → MMP and HSMP activation → adverse remodeling and dysfunction.

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### **Dysregulation of AT<sub>2</sub> Pathway with Aging and Effect of ACE Inhibitors and ARBs**

Evidence also suggests that other components of the RAS are altered with aging. Normally in adults, most actions of AngII are mediated through AT<sub>1</sub> and AT<sub>2</sub> receptor expression is low. However, AT<sub>2</sub> is reexpressed in CV disease, and during AT<sub>1</sub> blockade, AngII induces AT<sub>2</sub>-mediated vasodilation through the bradykinin/NO/cGMP pathway [184]. Paradoxically with aging, AT<sub>2</sub> activation leads to vasoconstriction via ROS rather than vasodilation in old rats [185], which explains the aging-induced AT<sub>2</sub>R paradox in resistance arteries. This may also explain the so-called ARB-MI paradox suggesting that increased AT<sub>2</sub> receptor signaling during ARB therapy may prove harmful in elderly patients [159].

In the study by Pinaud et al. [185], the resistance arteries of the old rats had impaired flow- and NO-mediated vasodilation and reduced expression of endothelial NO synthase compared to young rats. Importantly, aging increased AT<sub>2</sub> expression in vascular smooth muscle rather than endothelial cells, and AT<sub>2</sub> receptor blockade improved flow-mediated dilation, implying AT<sub>2</sub>-receptor-mediated vasoconstriction [185]. In addition, treatment with the vasodilator hydralazine attenuated AT<sub>2</sub> receptor induction of ROS and direct vasoconstriction, thereby enhancing flow-mediated vasodilation [185]. In a study of microvascular AT<sub>2</sub> receptor expression in hypertensive patients with type 2 diabetes receiving therapy with an ARB, AT<sub>2</sub> appears to mediate vasodilation [186]. Whether aging in humans leads to (1) a similar molecular switch from AT<sub>2</sub>-receptor-mediated vasodilation to vasoconstriction, (2) impaired responsiveness of the bradykinin/NO/cGMP vasodilator cascade to AT<sub>2</sub> receptor activation, and a cell signaling switch that converts AT<sub>2</sub> receptor inhibition of phosphorylation of mitogen-activated protein kinase (extracellular signal-related kinase 1/2) into stimulation needs verification [184]. Whether AT<sub>2</sub> receptor blockers and hydralazine might be beneficial in the elderly HF patient needs study.

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### **Aging and Remodeling of ACE-2/Ang-(1–7) and Other Pathways**

Interestingly, besides AngII receptor remodeling with aging [184], in the counter-regulatory arm of the RAS, decrease of ACE-2 in null mice is associated with decreased cardiac function [93] and ACE-2 also decreases with aging [108–110]. Whether increasing the ACE2/Ang-(1–7) pathway might be beneficial in the elderly HF patient needs study. Our studies with ACE inhibition and AT<sub>1</sub> blockade in aging hearts with HF post-reperfused MI suggest that beneficial effect of RAS inhibition may be blunted in elderly [6, 8–10]. Remodeling of other RAS-related pathways with aging has also been described, including the inflammation and Kallikrein–kinin pathways [187], the

β-adrenergic pathway [188], and the AngII-aldosterone pathway [189]. It should be noted that precautions in using both ACE inhibitors and ARBs are needed in the elderly with MI and hypertension [24, 28, 159, 190–193].

Taken together, remodeling of RAS with aging may account for the reported poor outcome post-MI despite optimal use of evidence-based therapy in the elderly [6, 27, 28]. As discussed before, clinical studies indicate that elderly post-MI patients are at higher risk for adverse events despite contemporary therapy including RAS inhibitors. Since AngII is the primary effector molecule of the RAS and AngII inhibitors form the basis of therapy for both elderly and non-elderly HF patients, this aging-induced remodeling of the RAS may have important implications for therapy based on AngII inhibitors for the elderly with post-MI HF.

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### **RAS, Aldosterone, and the Mineralocorticoid Receptor Antagonists**

The RAS was expanded to RAAS in order to emphasize the importance of the AngII/AT<sub>1</sub>-receptor-mediated activation of aldosterone which promotes sodium retention, loss of magnesium and potassium, sympathetic activation, parasympathetic inhibition, myocardial and vascular fibrosis, baroreceptor dysfunction, vascular damage, and impaired arterial compliance. The rationale for using mineralocorticoid receptor (MR) antagonists (MRAs) is that AngII stimulates the release of aldosterone from the adrenal cortex, thereby activating MRs whose activation persists despite treatment with ACE inhibitors, ARBs, and β-adrenergic blockers. Several trials with MRAs have shown that MRAs effectively reduce total mortality in patients with HF/low EF [194–199] (Table 18.3). Based on evidence from two of the first two RCTs [194, 196], the addition of an MRA is reasonable in patients with moderate to severe HF/low EF provided renal function (serum creatinine ≤2.5 mg/dL in men and ≤2.0 mg/dL in women) and serum potassium (≤5.0 mEq/L) are carefully

**Table 18.3** Some major trials of mineralocorticoid receptor antagonists (MRAs) in heart failure

Year—trial [reference]	<i>N</i>	Drug	Comparator	Age (years)	Outcome
1999 RALES [194]	1,663	Spirolactone	Placebo	65	↓ Mortality/morbidity
2000 RALES substudy [195]	261	Spirolactone	Placebo	69	↓ Mortality/morbidity; benefit highest in patients with high baseline collagen synthesis marker (PIIINP)
2003 EPHEUS (MI+HF) [196]	6,632	Eplerenone	Placebo	64	↓ Mortality/morbidity
2005 EPHEUS substudy [197]	6,632	Eplerenone	Placebo	64	↓ All-cause mortality at 30 days
2009 Diastolic HF [198]	44	Eplerenone	Control	80	↓ Collagen turnover marker (PIIINP); no clinical benefit
2011 Systolic HF [199]	2,737	Eplerenone	Placebo	69	↓ Mortality; ↓ hospitalization

Abbreviations: ↓ decrease in, HF heart failure, MI myocardial infarction, *N* number of patients

monitored. In RALES [194], the MRA spironolactone as an add-on to background therapy with ACE inhibitor,  $\beta$ -adrenergic blocker, diuretic, and digoxin in patients with moderate to severe HF (EF < 35 %) was prematurely terminated due to an early finding of a 30 % reduction in all-cause mortality and reduced morbidity and hospitalization. However, gynecomastia occurred in 10 % and hyperkalemia in 2 % of treated patients. In EPHEUS [196], the MRA eplerenone that selectively blocks the MR but not glucocorticoid, progesterone, or androgen receptors was assessed as an add-on to optimal medical therapy in post-MI HF patients with EF  $\leq$  40%. EPHEUS [196] reported a 14 % reduction in all-cause mortality, 17 % in CV mortality, and 15 % in risk of hospitalization. However, serious hyperkalemia occurred in 5.5 % and increased to 10 % in patients with baseline renal dysfunction defined as creatinine clearance < 50 mL/min [196]. In a recent trial in patients with systolic HF and mild symptoms, eplerenone reduced both the risk of CV death and HF hospitalization but the trial was terminated prematurely according to prespecified rules [199]. In that study [199], serum potassium was > 5.5 mmol/L in 11.8 % of patients receiving eplerenone compared to 7.2 % receiving placebo ( $P < 0.001$ ).

In a substudy of RALES [195], the MRA spironolactone increased levels of markers of collagen synthesis, suggesting that limitation of excessive extracellular matrix (ECM) turnover contributed to the benefits. In a substudy of EPHEUS [197], early initiation of eplerenone

was reported to reduce the 30-day all-cause mortality after acute MI. In a study of HF/PEF patients, eplerenone prevented progressive increase in procollagen type-III aminopeptide but had no impact on other markers of collagen turnover or diastolic function [198]. A recent meta-analysis of RCTs with MRAs spironolactone, canrenoate, and eplerenone demonstrated that MRAs exert beneficial effects on reversal of LV remodeling and dysfunction [200].

Close monitoring of renal function and serum potassium should be done when using MRAs, especially in the elderly. Both RALES and EPHEUS excluded patients with serum creatinine > 2.5 mg/dL. Since use of aldosterone antagonists in patients with renal dysfunction (creatinine clearance < 50 mL/min) increases the risk of hyperkalemia and this risk is greater in elderly patients and those receiving an ACE inhibitor or ARB concurrently, spironolactone or eplerenone should be used at a low dose under those circumstances and avoided in those with creatinine clearance < 30 mL/min. In patients already receiving a long-term diuretic and potassium supplements, the latter should be reduced or discontinued.

### Aging, RAAS, and Mineralocorticoid Receptor Antagonists

Whereas AngII is increased AngII [30, 31] with aging and AngII stimulates aldosterone secretion, aging in healthy humans results in decreased

plasma aldosterone levels [201]. While this finding implies a dysregulated response in the AngII–aldosterone axis with aging and would suggest a reduced need for MRAs in elderly patients with HF, several lines of evidence suggest that aging may in fact result in enhanced activation of the MR. First, in a study of steroid hormone metabolites in hypertensive patients aged 18–84 years, aging was associated with decreased 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (11 $\beta$ HSD2) activity [202]. This might explain the rising prevalence of HTN in the elderly. Importantly, the enzyme 11 $\beta$ HSD2 has been shown to convert cortisol and corticosterone to cortisone and 11-dehydrocorticosterone which are MR-inactive 11-keto derivatives [203, 204]. More importantly, cortisol not only activates MR but has greater affinity for MRs than aldosterone [205]. Since 11 $\beta$ HSD2 in renal tubular epithelium converts most of the cortisol into cortisone, aldosterone is the main endogenous agonist and activator of MRs in renal tubules normally and in the young [205]. Normally, cortisol seems to occupy epithelial and nonepithelial cells in tonic inhibitory fashion but, in the presence of tissue damage, becomes an MR agonist; this may explain both vascular and myocardial MR activation in HTN and HF and efficacy of MRAs despite low aldosterone levels [206]. Moreover, in the elderly with HTN [202] and non-elderly patients with essential HTN aged 40–60 years [207] with low aldosterone levels, decreased renal 11 $\beta$ HSD2 activity and increased cortisol may explain enhanced MR activation and justify use of MRAs. While data from mostly non-elderly patients show that the gene encoding aldosterone synthase (CYP11B2) is associated with high aldosterone and HTN and the adjacent gene (CYP11B1) encoding 11 $\beta$ -hydroxylase is associated with altered adrenal 11-hydroxylation efficiency (deoxycortisol to cortisol), more research is needed to determine their relative roles in the elderly and very old with HTN.

Second, while MRs are also present in myocardium, most cardiac aldosterone seems to come from the adrenals via the circulation, and glucocorticoids and aldosterone may serve as endogenous cardiac MR agonists [208]. As discussed

before, low myocardial 11 $\beta$ HSD2 and increased cortisol in the elderly may lead to enhanced myocardial MR activation. In addition, increased AngII, ROS, and oxidative stress in the elderly and HF may act in synergy and contribute to increased MR activation, inflammation, fibrosis, myocardial hypertrophy and apoptosis, and thence HF progression [6, 189].

Third, a study in rats showed that aging is also associated with increased MR activity in vascular smooth muscle cells which promotes inflammation via extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase and epidermal growth factor receptor-dependent pathways [209]. Fourth, since enhanced MR activation in the elderly leads to increased expression of tissue ACE and upregulation of AT<sub>1</sub> receptors [210], combined AngII and aldosterone blockade or inhibition has been proposed for increasing benefits [211]. Both these studies [210, 211] used telomere length in white blood cells (WBCs), an index of CV aging and the burden of oxidative stress. Fifth, the three early clinical trials of MRAs in HF/low EF included the elderly and showed efficacy in both young and old patients with [194–196]. The mean age was 65 years in RALES with spironolactone [194] and 64 and 69 years, respectively, in EPHEBUS [196] and EMPHASIS-HF [199] with eplerenone. Importantly, the decrease in total mortality and HF hospitalizations was similar in the elderly and non-elderly groups.

Sixth, MRAs may be especially effective in elderly patients with HF/PEF, where the dominant pathology is HTN associated with LV hypertrophy and myocardial fibrosis. Therapy with ACE inhibitors, ARBs, and  $\beta$ -adrenergic blockers fail to reduce mortality in that group. Specific therapy for diastolic dysfunction is lacking in all age groups and myocardial fibrosis is a major cause of diastolic dysfunction in the elderly even in the absence of HTN. Since RAAS inhibitors are powerful antifibrotic agents, it is reasonable to hypothesize that elderly patients might benefit from them unless the pathways are dysregulated [6].

As discussed before, the emerging paradigm is that aldosterone-induced MR activation leads to myocardial and vascular fibrosis in the non-elderly,



while amplified cortisol-induced MR activation leads to amplified stimulation of fibrosis in the elderly and patients with essential HTN. In a sub-study of RALES in patients with HF/low EF, spironolactone limited fibrosis and reduced procollagen I and III levels [195] and the mortality benefit was mainly in patients with continuing collagen turnover [189]. In the 4E trial of older adult patients with essential HTN, LV hypertrophy, and mean ages between 59 and 60 years [212], combination therapy with the ACE inhibitor enalapril and the MRA eplerenone was more effective than monotherapy in decreasing LV mass and albuminuria despite normal plasma aldosterone levels, endorsing the hypothesis that the MRA may have blocked cortisol-induced MRA activation in the face of decreased  $11\beta$ -HSD2. In the small study of elderly patients with HF/PEF ( $N=44$ ; mean age  $80\pm 8$  years), eplerenone prevented progressive increase in collagen turnover assessed by procollagen III aminoterminal peptide and despite background therapy with ACE inhibitors in 64 %, ARBs in 34 %, and  $\beta$ -adrenergic blockers in 68 % [198]. In a small study of older adult patients with HF/PEF and mean age  $62\pm 6$  years, spironolactone limited diastolic dysfunction [213]. In another small study of patients with HTN aged between 30 and 70 years, eplerenone reduced vascular stiffness more than the  $\beta$ -adrenergic blocker atenolol [214]. However, the mean ages in the three groups in that study (controls 51, eplerenone 54, atenolol 44 years, respectively) suggested that most patients were non-elderly [214]. In a small study of patients with HTN, regression of LV mass correlated with the decrease in plasma aldosterone, and the ARB losartan reduced LV mass more than the calcium channel blocker amlodipine by reducing plasma aldosterone in addition to BP [215]. In the Aldo-DHF multicenter, prospective, randomized, double-blind, and placebo-controlled 6-year (2007–2012) trial of spironolactone in HF/PEF patients aged  $\geq 50$  years (mean  $67\pm 8$  years), spironolactone improved diastolic function without improving exercise capacity or quality of life [216].

In the ongoing 6-year (2006–2013) phase 3 treatment of preserved cardiac function heart

failure with an aldosterone antagonist (TOPCAT) trial of spironolactone on CV mortality and HF hospitalization in 3,445 older adult and elderly patients with HF/PEF (age  $\geq 50$  years; mean  $69\pm 10$  years), 91 % have HTN [217]. This trial is also multicenter, prospective, randomized, double-blind, and placebo controlled. Comorbidities typically include coronary artery disease (57 %), atrial fibrillation (35 %), chronic kidney disease (38 %), and diabetes mellitus (32 %) [217].

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### MRA in Very Old Elderly Patients with HF/PEF

Elderly and especially very old (aged  $\geq 80$  years) patients with HF/PEF frequently have concomitant comorbidities including obesity, sleep apnea, coronary artery disease, atrial fibrillation, chronic renal disease, and diabetes that may benefit from MRAs [189, 217]. Several lines of evidence provide justification for blockade of effects of aldosterone and MR activation in epithelial and nonepithelial tissues of these patients. First, evidence from studies of telomere length in white blood cells (WBCs) suggests that aldosterone accelerates CV aging through mechanisms that generate ROS and telomere length may serve as an index of the burden of oxidative stress. In a study of normotensive and mild hypertensive males, plasma aldosterone was inversely related to telomere length, suggesting that aldosterone is not only pro-oxidant but increased levels might be linked to accelerated telomere shortening and increased biological aging [211].

Second, other studies suggest that aldosterone and MR activation exert CV and renal pleiotropic effects that extend well beyond the classical renal regulation of sodium balance. Mechanisms include oxidative stress, inflammation, impaired vascular reactivity, and endothelial-mediated vasorelaxation, downregulation of proteins in insulin metabolic pathways, and impaired renal podocyte and mesangial cell integrity. Elevated plasma aldosterone in patients with metabolic syndrome [218], kidney fibrosis [219], sleep apnea [220], and CHD [221, 222] has been implicated in the pathophysiology of these conditions.

Interestingly in the latter study [222], plasma aldosterone was directly related to BMI (body mass index), HTN, and NYHA class besides mortality and ischemic events and inversely to age, creatinine clearance, and use of  $\beta$ -blockers.

Third, adipocytes which protect against urinary protein loss were shown to release Rac-1 and other proteins that stimulate adrenal release of aldosterone [223]. Interestingly, while obese patients with metabolic syndrome often have salt-sensitive HTN and only ~33 % have elevated plasma aldosterone, MRAs prevent salt-induced cardiorenal damage suggesting that high salt and aldosterone contribute to the damage [224]. This is explained by the discovery of an alternative pathway of MR activation through the small GTP-binding protein Rac-1 which is activated by both high salt and hyperglycemia [224]. Fourth, evidence from patients with primary aldosteronism also supports the hypothesis that aldosterone suppresses pancreatic beta-cell function leading to insulin resistance with hyperglycemia and diabetes [225]. Fifth, aldosterone was shown to impair vascular reactivity by reducing glucose-6-phosphate dehydrogenase (G6PD) expression thereby decreasing glutathione, antioxidant reserve, nitric oxide (NO) generation, and NO availability [226].

Sixth, aldosterone was shown to increase signaling via nuclear factor  $\kappa$ B (NF $\kappa$ B) and transcription factor activator protein-1 (AP-1) pathways, inflammation, and cytokine expression in vascular smooth muscle cells, thereby contributing to endothelial dysfunction and atherosclerosis [227, 228]. These and other studies of the aldosterone  $\rightarrow$  oxidative stress  $\rightarrow$  inflammation  $\rightarrow$  endothelial dysfunction axis and the aldosterone  $\rightarrow$  oxidative stress  $\rightarrow$  hypertrophic remodeling/fibrosis axis suggest that many of the effects of aldosterone are mediated by genomic and non-genomic pathways in MR-dependent or independent manner [227]. Seventh, macrophages in the vascular wall and atherosclerotic plaques have been shown to express MRs [229]; aldosterone also stimulates vascular NADPH oxidase and p38MAP kinase and release of MMPs that mediate progression of atherosclerosis and plaque rupture [189, 227, 228]. Eighth, other studies in monocyte-derived macrophages from

patients with congestive HF suggested that the protective role of MRAs is partly due to increased generation of Ang-(1-7) and ACE2 and decreased AngII formation, and the effects were mediated by NADPH oxidase [230]. Interestingly, aldosterone and/or cortisol-induced of MR is associated with upregulation of the AT<sub>1</sub> receptor as well as downregulation of ACE2 [231].

Ninth, in the EMPHASIS-HF study of elderly patients with systolic HF and mild symptoms, eplerenone not only reduces mortality and hospitalization [199] but also reduces the incidence of new atrial fibrillation/flutter likely via attenuation of atrial remodeling and fibrosis [232]. Tenth, MRAs also block the aldosterone-induced renal podocyte injury associated with decreased NADPH oxidase, increased oxidative stress, and enhanced aldosterone effector kinase Sgk1, thereby decreasing podocyte damage, albuminuria, and mesangial fibrosis [219, 233]. MRAs also inhibit MR-mediated kidney DNA damage in DOCA-salt hypertensive rats [234]. Eleventh, MRAs also attenuate LV hypertrophy and vascular stiffness in chronic kidney disease [223].

Twelfth, although many studies show that MRAs limit remodeling and fibrosis in various models of chronic HF, the mechanisms of benefit are not completely clear. In one study of MI in rats, angiotensin and aldosterone blockade inhibited osteopontin expression, LV remodeling, and fibrosis [235]. Enhanced MR signaling induced by transgenic expression of 11 $\beta$ HSD2 to drive cardiac hypertrophy and HF results in severe cardiomyopathy, fibrosis, and increased mortality that are partially improved by eplerenone treatment [236]. Transgenic overexpression of aldosterone synthase in cardiomyocytes causes coronary dysfunction but prevents harmful effects of diabetes by preserving capillary density [237]. Ablation of MRs in cardiomyocytes but not cardiac fibroblasts preserves cardiac function and limits cardiac dilation in chronic pressure overload [238]. Studies of selective ablation of MR expression in different cardiac cell types may help to clarify mechanisms of cardioprotection by MRAs [238]. More systematic studies of MRAs in the old and very old versus young groups are needed to uncover ancillary pathways.

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## **MRAs and Hyperkalemia in the Elderly and Very Old Elderly Patients with HF**

While MRAs are considered beneficial in elderly and very old patients, the risk of hyperkalemia is greater in very old elderly HF patients [189] and those with chronic kidney disease and/or diabetes [239]. It is therefore prudent to closely monitor serum potassium and reduce the dose of MRAs in these patients. In TOPCAT, spironolactone was initiated at a lower dose of 15 mg daily, with escalation to 45 mg daily provided serum potassium is <5 mEq/L [217]. Hyperkalemia can be treated by the new potassium binding polymer RLY5016 [240]. This was evaluated in patients with mean age of 68 years, chronic kidney disease, and a history of hyperkalemia resulting in discontinuation of RAAS therapy in the PEARL-HF trial [240]. The patients received 30 g/day of RLY5016 on top on spironolactone 25–50 mg/day; adverse drug reactions (ADRs) with RLY5016 were seen in 7 % (versus 6 % in placebo) while hypokalemia ( $K^+ < 3.5$  mEq/L) occurred in 6 % (versus 0 % in placebo) [240]. More cardioselective MRAs with better  $Na^+/K^+$  ratio than spironolactone and eplerenone may be safer in elderly and very old patients [189].

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## **Novel RAAS Therapies in Hypertension: Aldosterone Synthase Inhibition**

A meta-analysis of RCTs of RAAS inhibitors in hypertensive patients suggested that in patients with HTN, ACE-inhibitor treatment, but not ARB treatment, resulted in further reduction of all-cause mortality [241]. However, the authors did not discuss impact of age. Control of BP and end-organ damage with therapy of HTN with conventional RAAS inhibitors can be challenging but a number of novel approaches hold promise for treatment of resistant HTN [242] and need evaluation in the elderly. Resistant or uncontrolled HTN despite three antihypertensive agents of different classes [243] is considered a trigger of cardiac decompensation in very old patients with HF/PEF and comorbidities such as diabetes

and chronic kidney disease [189]. While there are proponents of triple therapy with combined RAAS inhibition using combined ACE inhibitor, ARB, and MRA for resistant HTN, such triple therapy is not recommended for all patients along the cardiorenovascular continuum; rather, in congestive HF patients with incomplete neuroendocrine blockade evidenced by repeated bouts of cardiac decompensation, dual therapy can be tried with close attention to patient safety [244].

The rationale for aldosterone synthase (CYP11B2) inhibitors is to inhibit aldosterone formation and thereby prevent increase in aldosterone levels and their MR-independent effects. Evidence suggested that the aldosterone synthase inhibitor LCI99 was modestly effective in patients with primary aldosteronism (mean age 50 years), decreased BP, corrected hypokalemia, and produced latent inhibition of cortisol synthesis [245]. Its development was stopped in favor of the search for more specific inhibitors [242]. The aldosterone synthase inhibitor FAD286 was shown to reduce mortality, cardiac hypertrophy, albuminuria, cell infiltration, and matrix deposition in the heart and kidney of double transgenic renin and angiotensinogen (dTGR) rats without profound effect on BP [246]; reduce cardiac and renal fibrosis induced by AngII and high salt in uninephrectomized rats [247]; and improve LV hemodynamics, remodeling, function, and redox status in rats with HF [248]. Data on the aldosterone synthase inhibitors in older groups is lacking.

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## **Novel RAAS Therapies in Hypertension: Renin Inhibition**

Renin as a target has been debated at least since the 1990s, since it catalyzes the key rate limiting step in the RAAS cascade. The selective renin inhibitor aliskiren has been shown to reduce AngI and AngII levels [249] and attenuate BP comparable to  $\beta$ -blockers [250], diuretics [251], ACE inhibitors [252, 253], and ARBs [254]. It attenuates plasma renin activity that is increased by ACE inhibitors and ARBs [255]. However, high renin levels due to escape during aliskiren therapy is a concern [256] as aliskiren does not prevent binding of renin to pro(renin) and

activation of pro(renin) receptors [257]. Aliskiren, in a dose that did not reduce BP, improved LV dysfunction and remodeling after MI in mice and decreased apoptosis [258]. In patients with symptomatic HF, HYHA class II–IV, a history of HTN, elevated BNP, and mean age of 68 years, aliskiren on top of an ACE inhibitor or ARB and  $\beta$ -blocker had favorable neuro-humoral effects [259]. The ASTRONAUT study will test whether aliskiren on top of standard therapy will reduce post-discharge mortality and rehospitalization in patients with worsening HF/low EF [260]. The ATMOSPHERE study will determine whether aliskiren added to or as an alternative to ACE inhibition in patients with chronic systolic HF improves outcomes [261]. In the SPIRE study, adding aliskiren to standard therapy including a RAAS inhibitor in high-risk post-MI patients with LV systolic dysfunction did not result in further attenuation of LV remodeling and was associated with more adverse effects [262]. The SPIRE HIGHER program should provide data on protection from target organ damage and CV morbidity/mortality in a range of cardiorenal conditions including HF, post-MI, and diabetic nephropathy [263]. The AVOID study showed that aliskiren may be renoprotective and reduced albuminuria in patients with type 2 diabetes, kidney disease, and HTN [264]. Several of those studies included the elderly. The ALTITUDE study which aimed to determine whether aliskiren on top of an ACE inhibitor or ARB therapy delays cardiorenal complications in patients with type 2 diabetes at high risk for cardiorenal events was stopped in 2012 because of no apparent benefit and an increase in adverse events including hyperkalemia (aliskiren 11 % versus placebo 7 %) and hypotension (12 % versus 8 %) [265]. Whether aliskiren might benefit the elderly with HTN and HF/PEF remains to be addressed.

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### Dual-Action Molecules in Elderly Patients with HF/PEF

Dual inhibition of ACE and neutral endopeptidase (NEP) pathways in a single molecule such as omapatrilat (OMA) was studied in patients

with HTN, but despite superior antihypertensive efficacy over ACE inhibition and equal anti-remodeling efficacy in HF patients [266], the Federal Drug Administration (FDA) bureau did not approve OMA for patients with HTN because of troubling angioedema. The concept of dual-action molecules was recently revisited with LCZ696, which combines neprilysin (NEP) and the ARB valsartan in a phase 2 trial and was shown to be beneficial in patients with HF/PEF [267] and is being evaluated in patients with HF/low EF [267, 268]. Whether dual pathway inhibition may be more effective in elderly HF patients needs study.

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### Conclusion

The RAS/RAAS has critical functions in CV physiology and CV pathophysiology. Evidence over three decades since the 1980s indicates that RAS/RAAS upregulation plays a major role in CV pathophysiology. Thus, the RAS/RAAS play critical roles in both post-MI dilative remodeling associated with HF/low EF and hypertrophic remodeling and fibrosis associated with hypertensive disease and HF/PEF. Aging is associated with enhanced dysregulation of the RAS evidenced by increased AngII and several components of the RAS. Evidence suggests that dysregulation of the RAS may contribute to CVD and the RAS dysregulation may be further amplified with aging. This enhanced remodeling of the RAS may account for the poor outcome in elderly post-MI patients. Emerging evidence suggests aging-related dysregulation in the RAAS with reduced plasma aldosterone and enhanced MR activation related to cortisol and other pathways that may benefit from MRAs. Enhanced RAS/RAAS remodeling may have important implications for therapy based on AngII inhibitors and MRAs in elderly patients with post-MI HF. It is important to remember that these therapies were tested in mostly non-elderly patients. More research into the biology of aging-induced remodeling of the RAS/RAAS and related pathways may lead to discovery and development of improved therapies for post-MI HF and post-HTN HF in different age groups and tailored for the young, adult, and elderly patient.

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