Review Article

Cardioprotective Effects of Angiotensin Converting Enzyme II

Leta Melaku, Andualem Mossie[1]

Department of Biomedical Sciences, College of Health Sciences, Arsi University, Asella, [1]Department of Biomedical Sciences, College of Public Health and Medical Sciences, Jimma University, Jimma, Oromia, Ethiopia

Abstract

To circumvent the major threats of low blood volume and low blood pressure, animals need powerful mechanisms for salt and water conservation, which is renin–angiotensin system (RAS). Activation of RAS is therefore a useful response in many demanding situations. However, an increased activity of the RAS, especially in combination with other cardiovascular risk factors, may lead to a cascade of deleterious effects such as hypertension, atherosclerosis, myocardial remodeling, heart failure, ischemic stroke, and diabetes mellitus. Many of these pathophysiological actions of angiotensin II (Ang II) may still be viewed as being homeostatic in principle but harmful if carried to excess. Numerous experimental studies have indicated that angiotensin-converting enzyme II (ACE II) efficiently hydrolyzes the potent vasoconstrictor Ang II to Ang 1–7. Thus, the axis formed by ACE II/Ang 1–7/Mas appears to represent an endogenous counter-regulatory pathway within the RAS, the actions of which are in opposition to the vasoconstrictor/proliferative arm of the RAS consisting of ACE, Ang II, and Ang II Type 1 receptor (AT1R). Although most of the well-known cardiovascular and renal effects of RAS are attributed to ACE, an important enzyme in the generation of Ang II, much less is known about the functions of ACE II. This review summarizes recently published data on the basic properties of ACE II and Ang 1–7 and a summary of the evidence from experimental and clinical studies of various pathological conditions related to the biological roles of ACE II/Ang 1–7/Mas in the heart.

Keywords: Angiotensin 1–7, angiotensin-converting enzyme II, heart, Mas receptor, renin–angiotensin system

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Introduction

To circumvent the major threats of low blood volume and low blood pressure, animals and our ancestors, with a diet relatively poor in sodium, needed powerful mechanisms for salt and water conservation, and these organisms relied heavily on one of the oldest hormone systems called renin–angiotensin system (RAS).[1] In 2000, angiotensin-converting enzyme (ACE) II was serendipitously discovered by two independent research groups who had conducted a study upon characterization of cDNA genomic-based strategies.[2,3] ACE II (ACE-related carboxypeptidase or angiotensin-converting enzyme homolog) is a mono-carboxypeptidase Type I transmembrane protein that contains 805 amino acids and it has an extracellular (ecto) domain (amino acids 18–739), a transmembrane region (amino acids 740–768), and an intracellular tail. The extracellular part of ACE II [Figure 1] contains the catalytic domain (amino acids 147–555), which has a substrate-binding region (amino acids 273–345) and a typical HEMGH metalloproteinase zinc-binding site (amino acids 374–378).[4-7] The catalytic domain of ACE II is 42% identical to that of ACE.[3] The peptidase activity of ACE II depends on the C-terminus sequence of the substrate (sequence specificity). The C-terminal part of ACE II (614–805) is homologous (48% identity) to a transporter protein known as collectrin.[5,8] ACE II substrates generally have a hydrophobic or basic residue at the C-terminal end, preceded by a Pro-X-Pro motif, where either one of the two proline residues is sufficient to allow ACE II-dependent hydrolysis.[9] In this circumstance, ACE II displays potent peptidase activity to angiotensin II (Ang II) (Pro-Phe), Ang I (Pro-Phe-His-Leu), and des-Arg^9-bradykinin (BK) (Ser-Pro-Phe) but shows no activity toward Ang 1–9, Ang 1–7, or BK[10] [Figure 2].

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Address for correspondence: Mr. Leta Melaku, Department of Biomedical Sciences, College of Health Sciences, Arsi University, Arsi, Ethiopia.
E-mail: letamelaku@gmail.com

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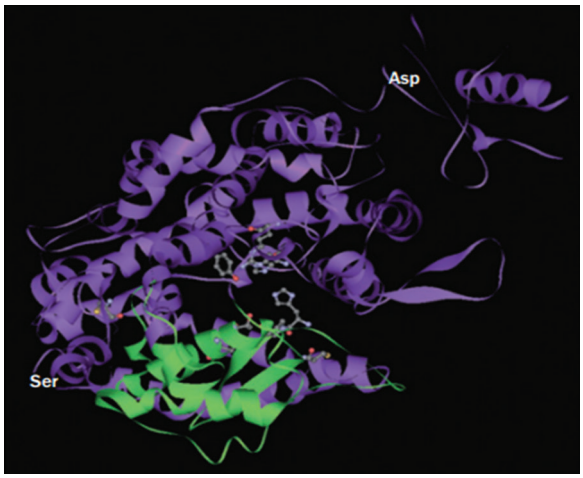

Figure 1: Structure of the extracellular domain (from Ser[19] to Asp[615]) of human ACE II. In this image, the extracellular domain is arbitrarily divided into two subdomains (shown in green and purple), forming a deep cleft that is proposed to be the active site for substrate binding and catalysis. The catalytic domain (amino acids 147–555) has a substrate-binding region (amino acids 273–345) and a typical HEMGH metalloproteinase zinc-binding site (amino acids 374–378). The regions shown in ball-and-stick figuration are proposed binding sites for the angiotensin-converting enzyme II inhibitor MLN-4760

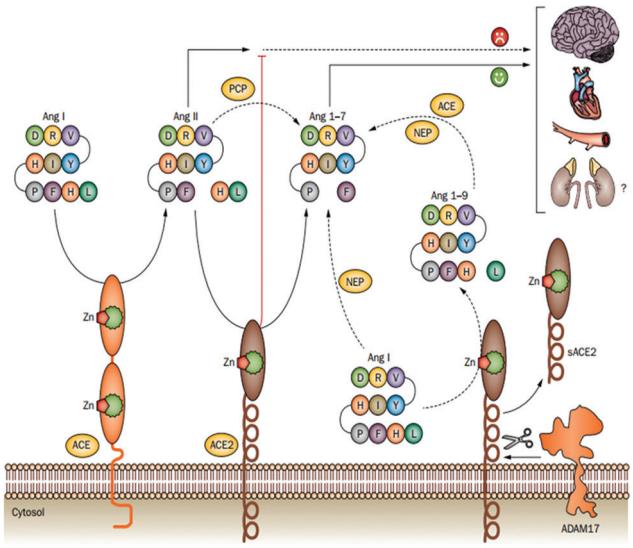

Figure 2: Overview of the angiotensin-converting enzyme II–angiotensin 1–7 pathway. Angiotensin-converting enzyme II and prolyl carboxypeptidase converts angiotensin II to angiotensin 1–7. Angiotensin-converting enzyme II can also convert angiotensin I to angiotensin 1–9, which is then cleaved by either neutral endopeptidase or angiotensin-converting enzyme to yield angiotensin 1–7. The membrane-bound angiotensin-converting enzyme II can be cleaved by the metalloproteinase ADAM 17, forming a soluble form of angiotensin-converting enzyme II. The physiological relevance of soluble angiotensin-converting enzyme II is not fully understood

ACE II can also hydrolyze other bioactive peptides, such as apelin-13, β-casomorphin, dynorphin A 1–13, and ghrelin.[10] Within the RAS, ACE II competes with ACE because it is capable of hydrolyzing the inactive decapeptide Ang I into the nonapeptide Ang 1–9, thus decreasing the amount of Ang I available for pressor Ang II generation by ACE. To the same extent, ACE II degrades the vasoconstrictor Ang II into Ang 1–7, which is the most important active product.[11,12] The Ang 1–7 can be primarily generated through two routes. First, both ACE II and prolyl carboxypeptidase can directly hydrolyze Ang II to yield Ang 1–7; second, this mono-carboxypeptidase can also remove the amino acid leucine from the C-terminus of Ang I to form the biologically active peptide Ang 1–9,[3,13] which is then cleaved by either neutral endopeptidase (NEP) or ACE to yield Ang 1–7[6,14,15] [Figure 2]. The heart, brain, and kidney are major sources of Ang 1–7 production.[14]

In the human coronary circulation, NEP seems to have a more prominent role in Ang 1–7 production than ACE II.[16] Pharmacokinetic experiments have determined that, in humans, Ang 1–7 has a short half-life of ~0.5 h.[17] Following subcutaneous injection, the peptide is quickly available in the blood and reaches its peak plasma concentration at ~1 h.[17] In rats, the plasma half-life of Ang 1–7 is only 9 s.[18] It has been established that Ang 1–7 binds to a non-AT1R/AT2R originally identified as the Mas oncogene receptor[19] and mediates vasodilatation; myocardial protection; antiarrhythmic at low concentration, antihypertensive, and positive inotropic effects; and inhibition of pathological cardiac remodeling within the cardiovascular system.[15,20,21] In addition, it is thought to have favorable effects on metabolism by lessening insulin resistance.[22-24] Although most effects are protective, some seem to be variable. Many of the diverse actions of Ang II, the major end-product of the RAS, can be viewed in a single conceptual framework as serving to prevent life-threatening shrinkage of intravascular volume (rapid actions of Ang, in combination with the sympathetic nervous system), to help maintain volume homeostasis by minimizing the changes in arterial pressure and fluid volumes required to achieve sodium balance (prevention of salt sensitivity), and to increase the efficiency of cardiovascular dynamics by promoting the growth of the heart and vessels and sensitizing blood vessels to vasoconstrictor agents (slowest actions of Ang) through its coordinated effects on the heart, blood vessels, kidneys, and nervous system.[25,26] Activation of the RAS is therefore a useful response in many demanding situations. However, an increased activity of the RAS, especially in combination with other cardiovascular risks factors, may lead to a cascade of deleterious effects such as hypertension, atherosclerosis, myocardial remodeling, heart failure (HF), ischemic stroke, and diabetes mellitus.[27,28] Many of these pathophysiological actions of Ang II may still be viewed as being homeostatic in principle but harmful if carried to excess. Thus, ACE II may have a role to counterbalance the action of ACE in producing the vasoconstrictor Ang II, leading to have protective effects in various tissues and to prevent overactive RAS-associated diseases, including hypertension.[29-33] The affinity of ACE II to Ang II (K_m = 2.0 μmol/l, which

represents the concentration of substrate required for the enzyme to achieve half maximum catalytic velocity, that is, the higher the K_m value, the lower the affinity) is higher than to Ang I (K_m = 6.9 μmol/l). The ACE II catalytic efficiency for Ang II is >300 times that for Ang I.[10]

A newly described RAS component, ACE II, has been characterized recently in humans[2,3] and in mice.[34] In humans, ACE II was found at various levels in 72 tissues that also express ACE mRNA[35] and it is highly expressed in kidneys, blood vessels, heart, lung, brain, and testis.[2,3,36,37] Interestingly, it has also been reported to be localized in glucose-regulating tissues such as pancreas, including β-cells[38-40] adipose tissue,[36] and liver.[41,42]

Cardiac Actions of ACE II

The components of local cardiac RAS

For a number of years, ACE and its main biologically active peptide Ang II have assumed a central position in the cardiac RAS.[43] With the discovery of ACE II, a new regulator entered within the established metabolic RAS pathways.[44] The presence and synthesis of RAS components in the heart suggest that locally produced bioactive Ang peptides modulate cardiac structure and function.[43,44] Components of the local cardiac RAS are heterologously distributed on different cell types within the heart.[45] For instance, angiotensinogen is primarily distributed in atrial muscle and the neuronal fibers of the conduction system, with small amounts in the subendocardial region of the ventricle.[46] In contrast, ACE is primarily expressed by coronary endothelial cells and cardiac fibroblasts.[46] In addition, ACE expression can be detected in all four heart valves, coronary blood vessels, aorta pulmonary arteries, endocardium, as well as epicardium.[47,48] However, ACE II is localized to the endothelium and smooth muscle cells of most intramyocardial vessels, including capillaries, venules, and medium-sized coronary arteries and arterioles.[49] Furthermore, ACE II protein expression was detected in cardiac myocytes from failing human hearts.[49] Ang I is extensively metabolized during a single pass through the coronary bed leading to the generation of Ang II, Ang III, Ang IV, and Ang 1–7 in isolated hearts from normal[50,51] and diabetic rats.[51] As a result of its affinity to Ang II is higher than to Ang I,[11,12] recent studies report that ACE II is an important regulator of cardiac pathophysiology.[52,53] However, it should be stressed that the role of ACE II in heart function and structure might depend on the species.[54] Interestingly, ACE II expression has been reported to be increased in failing human heart ventricle.[49,55,56] Nevertheless, there are contrasting findings in rat hearts. While an increase of both ACE and ACE II was found by Burrell *et al*.[49] in hearts from Sprague–Dawley rats after myocardial infarction (MI), Ishiyama *et al*.[57] observed an increase in ACE II expression only after AT1 blockade in Lewis normotensive rats. These divergent results further suggest that ACE II effects are strain dependent. ACE II gene transfection using lentiviral vectors significantly attenuated cardiac damage in spontaneously hypertensive rat (SHR)[58] and in Ang II-infused Sprague–Dawley rats.[59] In addition, the stage of the disease apparently influences the expression of ACE II.

At the early phase of MI, ACE II activity in plasma and left ventricles is increased in rats while the plasma and left ventricular (LV) ACE II activities and mRNA levels are lower than in controls at 8 weeks postinfarction.[13] Similar findings were observed regarding the cardiac expression of Mas, i.e., it changes depending on the nature and duration of the physiological and pathological stimuli.[60] Ang 1–7, which is also one of the components of RAS, is present within hearts. The localization and local generation of Ang 1–7 have been demonstrated within aortic root, coronary sinus, and right atrium of dogs at basal conditions, and its levels were markedly reduced following treatment with the ACE inhibitor CGS-14831.[61] In addition, immunohistochemical staining revealed that Ang 1–7 is expressed in rat cardiac myocytes[62] and sinoatrial node cells.[63] Of note, the Ang 1–7, Ang 1–7 receptor, Mas, mRNA, and protein of Ang 1–7 are localized in human cardiac tissues.[35,63,64] It is important to note that although all the components of RAS are present in the heart, not all of them are believed to be synthesized in heart. For example, the question whether renin is synthesized in heart or is derived primarily from circulation remains still unresolved.[65]

The role of ACE II on conductivity of the heart

In several published studies, Ang II has been implicated in conduction abnormalities although some results appear contradictory. Slowed conduction was associated with increased myocardial and plasma ACE activity. Moreover, administration of an ACE inhibitor improved conduction velocities in cardiomyopathy using a Syrian Hamster model.[66-68] These observations suggest that Ang II slows cardiac conduction. This conclusion is further supported by the findings of slowed ventricular conduction in mice overexpressing the AT1R.[69] However, in contrast, in cardiac myocyte cultures, Ang II stimulated an increase in connexion 43, a protein implicated in the upregulation of cardiac conduction,[70] implying that Ang II may accelerate cardiac conductance. Interestingly, in ACE II null mice, elevated levels of Ang II did not affect normal conductivity, and the mice appear to have a normal life span at least under nonstress laboratory conditions.[71]

The question whether cardiac conduction is, in fact, influenced by the RAS under physiological condition was examined and it has been demonstrated that Ang 1–7, a main product of ACE II enzymatic activity in the heart, decreases the incidence and duration of ischemia–reperfusion arrhythmias in isolated perfused rat hearts[63] apparently involving activation of the sodium pump.[72] These effects were abolished by ouabain.[72] In addition, Ang 1–7 decreased total (Na^+, K^+, Mg^{2+})-ATPase activity in sheep atrium.[73] Furthermore, the antiarrhythmogenic effect of Ang 1–7 was blocked by Ang 1–7 antagonist A-779 and by

cyclooxygenase inhibitor indomethacin.[74] This peptide also improved postischemic contractile function in isolated heart preparations by a mechanism involving Mas and the release of BK and prostaglandins.[75] However, at concentrations nearly 10,000-fold higher, Neves *et al*.[76] found that Ang 1–7 facilitated reperfusion arrhythmias in isolated perfused rat hearts. In keeping with these latter data, transgenic mice overexpressing ACE II in the heart presented sudden death due to cardiac arrhythmias.[71] These observations suggest that only very high local concentrations of Ang 1–7 exert deleterious effects in the heart possibly through activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase[77] or release of norepinephrine.[78] In fact, transgenic rats presenting a local increase of Ang 1–7 of up to 20-fold in the heart did not show any sign of arrhythmias.[79]

The role of ACE II on contractility of the heart

Although hearts from young ACE II-mutant mice are functionally normal, hearts of old ACE II-deficient mice in this particular mouse background display a reduction in cardiac contractility as demonstrated by 40% reduction in fractional shortening and velocity of circumferential shortening (heart rate corrected) with slight ventricular dilation.[52] The significance of ACE II in regulating cardiac function is further highlighted by the thinning of the LV wall in aged ACE II-mutant mice. This progressive cardiac dysfunction occurred without myocardial fibrosis or hypertrophy and in the absence of the myosin heavy chain isoform switches which is also typically found in other animal models of HF. Thus, one may speculate that the observed phenotype closely resembles the defective heart found in patients with cardiac stunning/hibernation.[80] Cardiac stunning and hibernation reflect adaptive responses to prolonged tissue hypoxia that occurs in coronary artery disease or after bypass surgery.[81]

In these human diseases and related animal models, chronic hypoxic conditions lead to compensatory changes in myocyte metabolism,[82] upregulation of hypoxia-induced genes,[83] and reduced heart function.[84] Accordingly, the hearts of ACE II null mice show upregulation of mRNA expression of hypoxia-inducible genes such as BNIP362 and PAI-1.[84] The magnitude of increased expression of these hypoxia-inducible genes resembles previously observed levels in other hypoxic models such as myocyte-specific vascular endothelial growth factor-mutant mice.[85] However, the link between cardiac stunning/hibernation and the heart defect observed in ACE II-knockout (KO) mice has to be investigated further. Whether ACE II expression levels indeed change under conditions of hypoxia remains to be demonstrated. ACE II-KO mice show also increased local heart Ang II levels.[52] Interestingly, both the cardiac phenotype and increased Ang II levels were completely reversed by additional deletion of ACE gene (i.e. ablation of ACE expression on an ACE II-mutant background abolished the cardiac dysfunction phenotype of ACE II single-KO mice).[52] The heart function of ACE/ACE II double-mutant mice was similar to that in ACE single-mutant and wild-type littermates. The normal cardiac functions of ACE/ACE II double-mutant mice suggest that the catalytic products of ACE account for the observed contractile impairment of old ACE II single-mutant mice. These observations for the first time demonstrated at the genetic level that ACE II counterbalances the enzymatic actions of ACE. It seems that increased local cardiac Ang II might have been the cause for the cardiac abnormalities in ACE II-deficient mice. However, it remains unclear why, despite the elevated plasma and heart Ang II levels, the heart of the ACE II-deficient mice did not show any evidence for cardiac hypertrophy. In fact, it is well established that cardiac myocytes express Ang II receptors and undergo hypertrophy in response to Ang II. However, *in vivo*, elevated cardiac Ang II levels alone do not directly induce cardiac hypertrophy but do increase interstitial fibrosis.[86] Thus, it is important to note that Ang II-independent pathways could also play an important role in ACE/ACE II-regulated heart function. Apparently, generation of Ang 1–7 directly from Ang II through the cleavage of the C-terminal amino acid phenylalanine by ACE II is physiologically and biochemically more relevant.[10] According to Loot *et al*.,[87] chronic infusion (8 weeks) of Ang 1–7 improved coronary perfusion and preserved cardiac function in an experimental rat model of HF induced by ligation of the left coronary artery. The vascular endothelial dysfunction observed in aortic rings from rats with MI was also reversed by chronic infusion of Ang 1–7.[87]

In addition, Ang 1–7 immunoreactivity was significantly increased in the tissue surrounding the infarct area of rat hearts with MI[62,88] published the first study, demonstrating that the compound AVE 0991 is a nonpeptide and orally active Ang 1–7 receptor agonist that mimics the Ang 1–7 effects in bovine endothelial cells. Pinheiro *et al*.[89] and Lemos *et al*.[90] reported that this compound acts as a Mas agonist in the kidney and isolated aortic rings, respectively. Another study also revealed that AVE 0991 preserved cardiac function and attenuated the development of hypertrophy and fibrosis in hearts from rats chronically treated with isoproterenol.[91] This nonpeptide Ang 1–7 analog also significantly improved the cardiac function in hearts subjected to MI and preserved the myocardium after ischemia.[92] Furthermore, long-term treatment with AVE 0991 prevented the end-organ damage in hearts from SHRs treated with N (G)-nitro-l-arginine methyl ester.[93] Recently, it has been shown that the inclusion of Ang 1–7 into the cavity formed by the oligosaccharide hydroxypropyl β-cyclodextrin (HPβCD) could protect the peptide during the passage through the gastrointestinal tract. Taking advantage of this formulation, Marques *et al*.[94,95] found that chronic oral administration of HPβCD/Ang 1–7 significantly attenuated the impairment of heart function and cardiac remodeling induced by isoproterenol treatment and MI in rats. The actions of Ang 1–7 in coronary vessels include biochemical and functional alterations leading to vasodilatation either directly in artery rings or indirectly through BK potentiation or by opposing Ang II actions.[96] In isolated canine coronary artery rings precontracted with the thromboxane A_2 analog, U46619, Ang 1–7 elicited a dose-dependent vasorelaxation, which was

completely blocked by the nonselective Ang II antagonist (Sar[1], Thr[8])-Ang II, but not by the selective AT1 or AT2 antagonists, CV11974 and PD 123319, respectively.[97] This heptapeptide induced a concentration-dependent dilator response in porcine coronary artery rings, which were markedly attenuated by nitric oxide (NO) inhibition.[98] However, Gorelik *et al*.[99] observed a vasodilator effect of Ang 1–7 only in BK-stimulated pig coronary artery rings. Furthermore, Ang 1–7 elicited an increase in the vasodilator effect of BK in isolated perfused rat hearts. This effect was dependent on Mas and NO and prostaglandin release.[100] Ang 1–7 also evoked vasodilation in isolated perfused mouse hearts involving interaction of Mas with AT1- and AT2-related mechanisms.[101] Together, these data suggest that Ang 1–7 is a vasorelaxant peptide in the coronary bed and that this effect involves coupling to Mas and release of NO and prostaglandins.

Nevertheless, because Neves *et al*.[76] found that, at high concentrations (>25 nM), Ang 1–7 induces a concentration-dependent decrease in coronary flow in isolated perfused rat hearts, it remains to be demonstrated whether Ang 1–7 directly causes vasodilation in the coronary bed. This effect was not accompanied by consistent changes in contraction force and heart rate. A similar finding was observed in isolated hamster hearts.[102] The other of the most important beneficial effects of Ang 1–7 is its ability to regulate the expression of extracellular matrix (ECM) proteins and cardiac remodeling. Iwata *et al*.[103] reported that Ang 1–7 binds to isolated adult rat cardiac fibroblasts, which play a critical role in cardiac remodeling. Treatment of these cells with Ang 1–7 inhibited Ang II-induced increases in collagen synthesis [Figure 3]. Importantly, deletion of Mas produced impairment of cardiac function associated with a significant increase in collagen Type I, III and fibronectin content in the heart.[104,105] On the other hand, Ang 1–7 also attenuated either fetal bovine serum- or endothelin 1-stimulated ^{3}H-leucine incorporation into isolated neonatal rat cardiac myocytes through a mechanism involving inhibition of serum-stimulated extracellular signal-regulated kinase 1/2 mitogen-activated protein kinase activity and activation of Mas.[106] Chronic administration of this peptide significantly attenuated LV hypertrophy and fibrosis in pressure-overloaded rats[107] and fibrosis in Ang II-infused and deoxycorticosterone acetate-salt rats.[108,109] In addition, these animals showed a slight, but significant, increase in daily and nocturnal dP/dt, more resistance to isoproterenol-induced cardiac hypertrophy, reduced duration of reperfusion arrhythmias, and improved postischemic function in isolated perfused hearts,[110] further supporting a beneficial role for Ang 1–7 in cardiac function at physiological concentrations. Altogether, these findings indicate that the ACE II/Ang 1–7/Mas axis is a functional cardioprotective arm of the RAS [Figure 4]. The signal transduction pathways following activation of Mas in the heart are not fully characterized but probably involve release of prostacyclin and/or NO release[74,100,101] since Ang 1–7 stimulated NO production and activated endothelial NO synthase and Akt in cardiomyocytes.[60] Of note, the antihypertrophic effects of Ang 1–7 on Ang II-treated cardiomyocytes were prevented by the blockade of the NO/cGMP pathway.[111] Moreover, amplification of the actions of BK[99,100] and decrease of Ang II levels in the heart[112,113] may also be possible mechanisms involved in the beneficial cardiac effects of Ang 1–7.

Application of Cardioprotective Effects of ACE II

Application of cardioprotective effects of ACE II in animal studies

Several observations and experimental evidence from animal models have suggested a beneficial role of the ACE II-Ang 1–7 axis on cardiovascular function.[114] Elevated ACE II expression appears to occur at the initial stage of several pathologic conditions and declines with disease progression.[115] Loss of ACE II enhances the susceptibility to myocardial dysfunction, while enhancing ACE II action

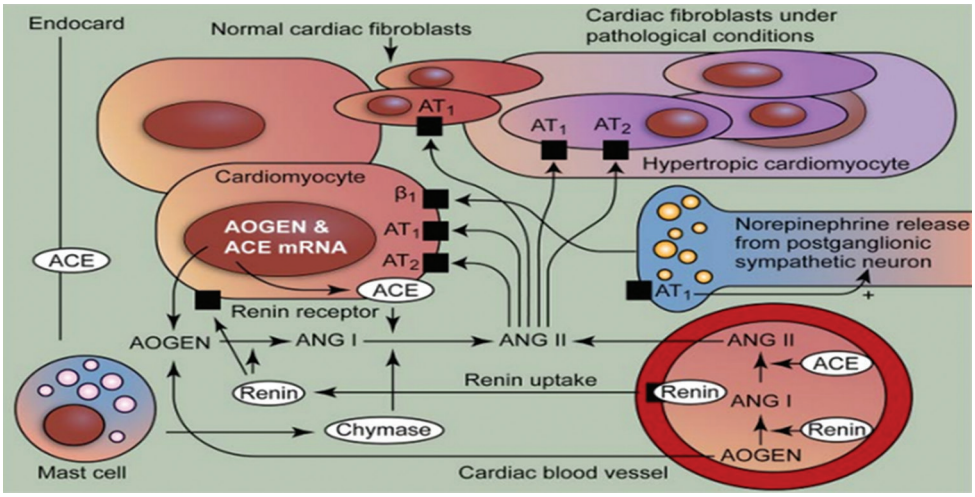

Figure 3: Angiotensin II-induced cardiac fibrosis and hypertrophy under pathological conditions. Angiotensin II acts on cell-specific receptors on cardiomyocytes and fibroblasts. Mast cell production of human heart chymase may present an alternative pathway

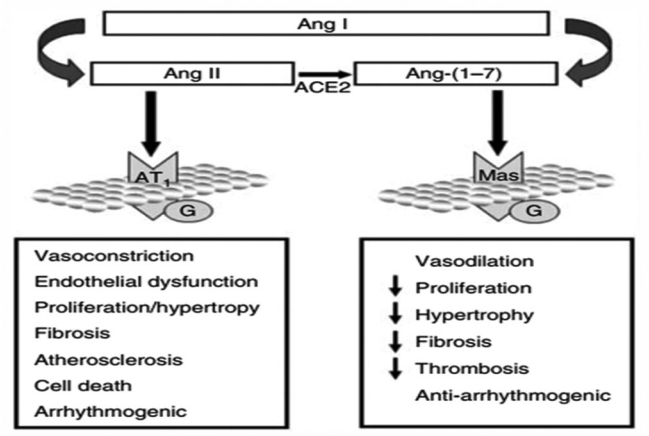

Figure 4: Opposing cardiovascular effects of the two major peptides of the renin–angiotensin system, angiotensin II and angiotensin 1–7. The intersection between these two arms of the system is the angiotensin-converting enzyme II since this enzyme can cleave the vasoconstrictor/proliferative peptide angiotensin II to form the vasodilator/antiproliferative fragment angiotensin 1–7

prevents adverse pathological remodeling and slows the progression to HF.[115,116] Mechanistically, loss of ACE II may also trigger activation of the myocardial NADPH oxidase system, increased production of superoxide, and activation of matrix metalloproteinases (MMPs), leading to further adverse myocardial remodeling and dysfunction.[117] Animal studies have directly demonstrated a potentially critical role of ACE II in counterbalancing the maladaptive pathophysiological effects of Ang II.[59,53] In the heart, ACE II appears to be the primary pathway for the metabolism of Ang II.[118] At the same time, excess Ang II may promote its increase conversion to Ang 1–7 in the presence of ACE II.[119] Hence, a deficiency of ACE II can lead to increased tissue and circulating levels of Ang II and reduced levels of Ang 1–7 as demonstrated in animal models, which result in early cardiac hypertrophy and fibrosis that is reversible with double knockout (DKO) mice of ACE and ACE II genes or following treatment with ACE inhibitors or angiotensin receptor blockers (ARBs).[115,116] Overexpression of ACE II prevents adverse cardiac remodeling,[59] and treatment with Ang 1–7 prevents cardiac fibrosis in animal models.[120] Consistent with a key role of ACE II in post-MI remodeling, overexpression of ACE II ameliorates LV remodeling and dysfunction in a rat model of MI.[121] On the other hand, loss of ACE II worsens the pathological remodeling and results in a rapid progression to reduced systolic function and HF in a pressure-overload mouse model.[53] These observations suggest that ACE II could be an important regulator of LV remodeling.[114]

Application of cardioprotective effects of ACE II in human

Role of ACE II in cardiac remodeling and systolic dysfunction in humans

Cardiac remodeling of the heart plays a key role in the progressive deterioration of cardiac function that leads to human HF.[114] In patients with HF, elevated levels of Ang II, and myocardial ACE, mRNA level activity has been reported.[122] On the other hand, the role of ACE II expression in the development of LV remodeling in human HF remained poorly understood.[123] The first evidence of ACE II-mediated formation of Ang 1–7 in human HF came from ACE II protein and substrate activity analyses of explanted human heart tissues.[124] They found that Ang 1–7-forming activity from both Ang I and Ang II was increased in failing human heart ventricles but was mediated by at least two different angiotensinases.[114] The first, which demonstrated substrate preference for Ang I, was NEP-like whereas ACE II appears to favor Ang II.[124] ACE II expression is increased regardless of etiology.[56] Meanwhile, the relationship between the expression of ACE II mRNA and the severity of LV remodeling was investigated in 14 patients with end-stage HF.[114] Interestingly, there was a strong relationship between the amount of ACE II gene expression and the severity of LV remodeling determined by LV dimensions,[125] suggesting that ACE II expression could be activated as an adaptive compensatory mechanism to militate against LV remodeling. However, ACE II expression did not correlate with either LV ejection fraction or plasma brain natriuretic peptide levels, which implies that such compensatory increase in expression of ACE II may be insufficient to counter pathologic processes as disease is progressed.[125] The clinical relevance of ACE II in the setting of systolic dysfunction was further demonstrated by the detection of soluble ACE II (sACE II) activity in patients with systolic HF.[126] Increasing sACE II plasma activity strongly correlated with a clinical diagnosis of HF regardless of etiology and tracked with worsening functional class and higher natriuretic peptide levels, while independent of other disease states and medication use.[126] In a separate cohort of 113 patients with chronic systolic HF with detailed echocardiographic analysis, higher sACE II activity was associated with a lower LV ejection fraction, more right ventricular systolic dysfunction, and larger LV end-diastolic diameter.[127]

Furthermore, sACE II was an independent predictor of adverse clinical events (death, cardiac transplant, and HF hospitalizations), independently of LV ejection fraction and natriuretic peptide levels.[127] These findings have now been substantiated in several independent confirmatory studies.[128,129] The impact of drug therapy on directly modulating ACE II activity and expression has not been well described. In the setting of acute decompensated HF in patients with advanced HF, circulating sACE II activity was found to increase following intensive medical therapy aiming to optimize hemodynamic derangements and relief congestion.[129] Interestingly, patients who experienced >50% increase in their sACE II activity were associated with better long-term outcomes, further supporting the potential counter-regulatory role of ACE II. Conventional drugs such as ARBs and mineralocorticoid receptor antagonists (MRAs) have been reported to increase ACE II-related beneficial effects, thus providing an additional rationale for their use in the setting

of HF. From this perspective, ARBs would appear to have an advantage since they tend to increase Ang II levels, an effect that along with its promotion of ACE II activity would raise the levels of the protective peptide Ang 1–7. However, superiority of the ARBs over ACE inhibitors in either MI survivors or HF patients has yet to be demonstrated in large clinical trials, suggesting that the perceived incremental benefit may not be as clinically relevant.[114] Meanwhile, MRA decreased ACE and increased ACE II. Each and both are capable of reducing Ang II level.[130] Studies demonstrating incremental benefit of MRAs in patients with systolic HF have supported its broad adoption, yet to our knowledge, no studies on long-term effects of MRA on ACE II or Ang 1–7 metabolism and effects in humans have been conducted.[114]

Role of ACE II in diastolic dysfunction and hypertension

Epidemiological studies have consistently demonstrated that HF with preserved ejection fraction (HFpEF) accounts for over 30% of all HF, particularly in elderly patients. The development of myocardial fibrosis and pathological hypertrophy has been the central hypothesis that contributes to the development of diastolic dysfunction and HFpEF due to increased myocardial stiffness.[114] Indeed, elevated sACE II levels have been observed in patients with HFpEF as well.[126] As previously discussed, ACE II negatively regulates the pathophysiological effects of a pressor and suppressor dose of Ang II on myocardial structure and function.[131]

ACE II is also a negative regulator of Ang II-induced myocardial hypertrophy, fibrosis, and diastolic dysfunction.[132] With the paucity of effective drug therapies, strategies to enhance ACE II effects in patients with HFpEF are promising although studies showing reversal and recovery of myocardial stiffness after its development are lacking. Several studies have demonstrated the modulatory effect of ACE II on blood pressure. ACE II is present in vascular endothelial walls and plays a major role in producing Ang 1–7.[114] Based on *in vitro* biochemical data and *in vivo* findings, a reduction in ACE II levels could lead to elevated Ang II levels, thus promoting increased blood pressure.[131,133] Overexpression of ACE II in the vasculature reduces blood pressure and improves endothelial function in hypertensive rats.[134] Moreover, interventions to augment the expression or activity of ACE II have been shown to significantly reduce blood pressure levels.[115] However, data associated with ACE II polymorphism to hypertension are controversial, with some studies conceding a possible association with LV mass, septal wall thickness, and hypertrophy, while others refute that association.[135-137] Meanwhile, the vasodilatory effects of Ang 1–7 are attributed to stimulating the production of NO, prostaglandins, and endothelium-derived relaxation factors.[118] In humans, Ang 1–7 elicited a direct vasodilation in the forearm circulation of both normotensive and hypertensive patients.[138] Interestingly, Ang 1–7 levels are elevated with treatment of ACE inhibitors and ARB, which might suggest the contribution of this peptide in their antihypertensive effects.[139] Localized ACE II/Ang 1–7 axis in the brain may also modulate centrally mediated hypertension.

ACE II as a potential therapy for heart failure

The negative regulatory role of ACE II in RAS has made it an important therapeutic target in the control of cardiovascular diseases (CVDs).[140] Oudit *et al*.[116] reported the loss of ACE II in Ace II$^{-/y}$ mutant mice lead to an age-dependent and progressive decline in cardiac function associated with ventricular dilation at 6 and 12 months of age. These results collectively demonstrated that loss of ACE II enhances the susceptibility to HF by Ang II-mediated injury through AT1R and PI3Kγ.[116] A similar result was recognized with the mice lacking ACE II (Ace II$^{-/y}$ mice) that exhibited reduced cardiac contractility and developed cardiac hypertrophy and dilatation in response to transverse aortic constriction.[53]

Localization of ACE II in the heart and significant activation of cardiac ACE II after MI suggest the importance of ACE II in cardiac function, and that ACE II may combat the adverse effects of a locally activated cardiac RAS.[141] Thus, the question is whether ACE II deficiency compromises the cardiac response to MI. To clarify this, ACE II-KO (Ace II$^{-/y}$) mice and wild-type littermates (ACE II$^{+/y}$) were used and were subjected to either left anterior descending coronary artery ligation or sham surgery. The results demonstrated that loss of ACE II is associated with increased mortality and infarct expansion with adverse ventricular remodeling and were associated with greater oxidative stress, MMP activation, and inflammation.[140] Collectively, these results support the hypothesis that enhancing ACE II activity would serve to minimize adverse post-MI ventricular remodeling.[142] This is in agreement with the study by Der Sarkissian *et al*., who showed that cardiac overexpression of ACE II exerts a protective influence on the heart during MI by preserving cardiac functions, LV wall motion, and contractility and by attenuating LV wall thinning.[143] Also, in order to understand mechanism of the adverse remodeling and increased propensity to develop HF, study by Parajuli *et al*[140] has examined the role of p47^{phox} subunit in the exaggerated oxidative stress response in pressure overloaded male ACE II-KO mice. The double-mutant mice (DKO) lacking ACE II and p47^{phox} genes (ACE II$^{2/y}$/p47$^{phox\ 2/2}$) were subjected to aortic constriction. The reversal of pathological hypertrophy and systolic dysfunction in the DKO mice indicated that the adverse remodeling of the myocardial ECM was minimized. These results showed that the adverse myocardial remodeling in ACE II-KO mice in response to biomechanical stress was clearly NADPH oxidase-dependent, with a dominant role being played by the p47 phox subunit.[117] ACE II also plays a pivotal role in the suppression of diabetes-associated cardiac complications. Cardiac and vascular structure and function, Ang II metabolism, signaling, and tissue-reactive oxygen species generation were investigated in insulin-deficient diabetic Akita mice in response to genetic ablation of ACE

II.[140] In this report, loss of ACE II-mediated disruption of the balance of the RAS in a diabetic state was observed. Further, it led to Ang II–AT1R axis-dependent systolic dysfunction and impaired vascular function in ACE II-disrupted mice, which demonstrated the protective mechanism elicited by ACE II against diabetes-induced cardiovascular complications.[144] The knowledge concerning ACE II, gained over a decade, emphasizes how it can be used for translational purposes.

The molecular and biological mechanisms mediated by ACE II can operate simultaneously within the context of any specific organ or cell. Importantly, the effects mediated by ACE II can be attributed to suppressed activation of the Ang II–AT1R axis and (or) stimulation of the Ang 1–7 Mas receptor (MasR) axis.[140] To understand the relative importance of both the axes, another study on pressure and volume overload model in ACE II null (Ace II-KO) mice and treated them with irbesartan or Ang 1–7 shows that there is functional redundancy in the cardioprotective effects of irbesartan as well as Ang 1–7, as shown by the suppression of NADPH oxidase activity, activation of MMP2 and MMP9, and pathological signaling, suggesting the equal importance of both the axes.[144] These data and other studies focusing on the Ang 1–7 MasR axis in cardiac diseases support the importance of activation of the Ang 1–7 MasR axis.[87,92,144,145] This could be achieved by Ang 1–7 supplementation and genetic delivery of MasR agonism using AVE 0991.[146-148] However, the comparative therapeutic benefits of ACE II treatment compared with activating the Ang 1–7 MasR axis need to be studied. The development of CVD is known to be gender specific as men are more likely to have a coronary event than women. The location of the ACE II gene on the X chromosome encompasses an area where genes are known to escape from X-chromosome inactivation and that could make the women less prone to coronary events.[149] Thus, when considering ACE II therapy for CVD, the gender of an individual should be considered. This is probably due to sex differences in the posttranscriptional and posttranslational mechanisms of gene regulation.[150] Considering formulations, the clinical use of ACE II is limited as it is a peptide that undergoes rapid proteolytic degradation in the circulation. Pharmacological and cell-based strategies aimed at increasing vascular ACE II bioavailability may represent a new therapeutic avenue in the treatment of CVD.[140] ACE II activators, for example, resorcinolnaphthalein or 1-([2-dimethylamino] ethylamino)-4-[hydroxymethyl]-7-([4-methylphenyl] sulfonyl oxy)-9H-xanthene-9-one, have shown promising results in animal models of diabetic cardiovascular complications.[151,152] In contrast, recombinant human ACE II (rhACE II) has also shown cardioprotective effects on different preclinical models of systolic as well as diastolic HF.[131] However, chronic administration of recombinant proteins has its own complications, and that needs to be tested for rhACE II.[140]

The adenoviral delivery of ACE II seems to be the most interesting approach for ACE II therapy as adenovirus-mediated Ace II gene delivery offers local ACE II delivery that is hypothesized to provide greater therapeutic benefits compared with systemic ACE II delivery. Thus, increased knowledge of the local and systemic RAS warrants comparison among the different modes of ACE II activation, particularly systemic administration, for example, rhACE II, ACE II activator, and local ACE II delivery, for example, adenoviral ACE II delivery.[140]

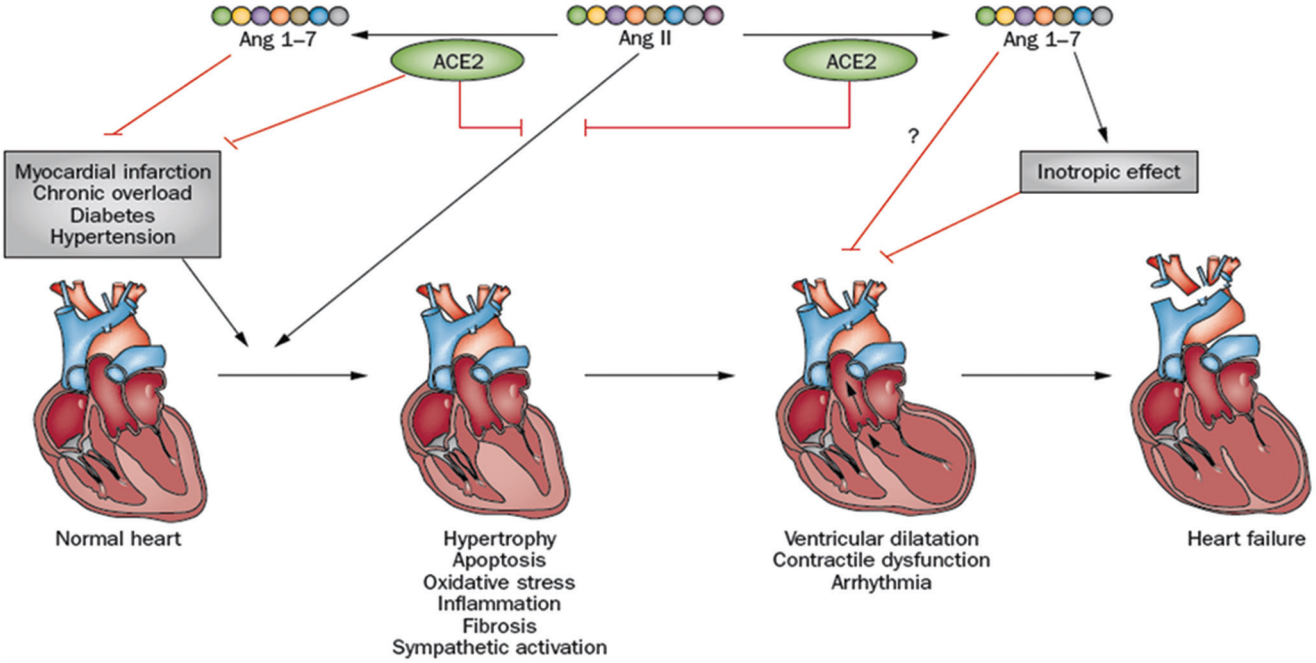

Figure 5: Potential protective effects of angiotensin-converting enzyme II and angiotensin 1–7 on pathological cardiac remodeling and heart failure. ACE II: Angiotensin-converting enzyme II, Ang: Angiotensin

Conclusion

ACE II is a new component of the RAS. This transmembrane protease has emerged as a negative regulator of the RAS that counterbalances the multiple functions of ACE. Because ACE II efficiently hydrolyzes the potent vasoconstrictor Ang II to Ang 1–7, this has changed overall perspective about the classical view of the RAS because it represents the first example of a feed-forward mechanism directed toward mitigation of the actions of Ang II. Ang 1–7 appears to play a central role in the RAS because it exerts a vast array of actions; many of them opposite to those attributed to the main effector peptide of the RAS, Ang II. It is now generally accepted that the RAS is dual and that, besides the well-known mainly deleterious arm if stimulated excessively (ACE/Ang II/AT1), there is a second beneficial axis consisting of ACE II, Ang 1–7, and Mas to recorrect the deleterious arm. A summary of the evidence from both experimental and clinical studies shows that the overall biological role of ACE II/Ang 1–7/Mas axis has a protective role within normal function of heart [Figure 5]. Therefore, the development of drugs that could activate ACE II function would allow extending treatment options in hypertension, HF, and other CVDs.

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Conflicts of interest

There are no conflicts of interest.

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