Review

ACE2 of the heart: From angiotensin I to angiotensin (1–7)

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Received 28 June 2006; received in revised form 7 September 2006
Available online 19 September 2006
Time for primary review 22 days

Abstract

Angiotensin II (Ang II), a bioactive peptide of the renin–angiotensin system (RAAS), plays an important role in the development of cardiovascular diseases (CVD). Pharmacological inhibition of angiotensin-converting enzyme (ACE), the Ang II forming enzyme, or specific blockade of Ang II binding to angiotensin type 1 receptor (AT1R) through which it exerts its deleterious effects, were shown to provide some protection against progression of CVD.

The ACE-Ang II-AT1R axis has been challenged over the last few years with RAAS components able to counterbalance the effects of the main axis. The ACE homologue ACE2 efficiently hydrolyses Ang II to form Ang (1–7), a peptide that exerts actions opposite to those of Ang II. In contrast to the Ang II axis, the role of the ACE2-Ang (1–7) axis in cardiac function is largely obscure.

Ang (1–7) is present in the viable myocardium, and its formation depends on Ang II as a substrate. The expression of this peptide is associated with cardiac remodeling: it is lost in the infarcted area and significantly increased in the border area. Low doses of Ang (1–7) improve cardiac output and antagonize Ang II-induced vasoconstriction. The type of Ang (1–7) biological activity is tissue specific and dose dependent. These findings point to a possible protective role for Ang (1–7) in abating the Ang II-induced actions. The elevated expression of Ang (1–7) in failing heart tissue paralleled the expression of its forming enzyme, ACE2.

Several observations and experimental evidence suggest a beneficial role for ACE2 in cardiovascular function. Elevated ACE2 expression at the initial stage of several pathologies which decline with progression of disease might indicate a protective role for ACE2. Genetic manipulation of ACE2 expression, either targeted disruption or overexpression, point to the possible significance of this enzyme in cardiac function.

Based on the above, a therapeutic approach that will amplify the ACE2-Ang (1–7) axis could provide further protection against the development of CVD. It turns out that the merits of currently used drugs – ACE inhibitors, AT1R blockers and mineralocorticoid receptor blockers (MRB) – lay beyond their direct effects on suppression of the ACE-Ang II-AT1R axis as they also increase cardiac ACE2 and Ang (1–7) significantly.

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Keywords: Angiotensin II; Angiotensin (1–7); ACE; ACE2; Heart

1. Introduction

The renin–angiotensin–aldosterone system (RAAS) is a major regulator in human physiology. It controls blood pressure, volume and electrolytes, and affects the heart, vasculature and kidney.

Angiotensin processing begins with hydrolysis of angiotensinogen by renin to form the decapeptide angiotensin I (Ang I). The removal of two amino acids from the carboxy-terminal by the angiotensin-converting enzyme (ACE) results in the formation of the biologically active peptide angiotensin II (Ang II). The increased level of Ang II in pathological conditions and the detrimental effects of this peptide were reported in numerous biological and cellular...
systems and reducing the levels of Ang II is of a paramount clinical importance.

This main axis, in which Ang II and its forming enzyme ACE were playing major roles, has been challenged over the last few years. Another bioactive angiotensin [1], Ang (1–7), and an ACE homologue, ACE2 [2,3], add further complexity to this axis. A growing body of evidence [4,5] points to a possible promising role for these two members of the RAAS in opposing the action of the main axis.

This review will focus on the cardiac role of the new members of the RAAS: the active peptide Ang (1–7) and its forming enzyme, ACE2. The altered expression and activity of these components as pronounced in cardiovascular diseases as well as the results of their deliberate manipulation may offer an insight into their role in pathogenesis and possible therapeutic management of heart failure of a different etiology.

1.1. Angiotensin processing: “the Ang II highway”

Following the removal of two amino acids from the carboxy-terminal, the inactive peptide Ang I turns into the potent vasoconstrictor Ang II. This conversion is efficiently mediated by the activity of dipeptidylcarboxipeptidase, ACE. In the human heart, however, the majority of Ang I conversion to Ang II is mediated through alternative pathways, namely chymase [6,7]. The contribution of ACE and chymase to Ang II formation varies significantly in different species or tissues within the same species [8].

Ang II exerts its diverse biological activities via binding to one of three known receptor types. While these receptors share a similar high affinity to Ang II in the nanomolar range, similar to its circulating concentration, the consequences of their binding are opposite. The type 1 receptor (AT1R) is highly expressed in adults and plays an important role in cardiovascular diseases. AT1R mediates AngII-induced vasoconstriction [9], proliferation [10], inflammation [11], coagulation and extracellular matrix remodeling [12,13]. However, Ang II binding to the type 2 receptor (AT2R) counteracts both short- and long term AT1R-mediated effects [14,15,16], providing a protective role. Once Ang II is present, the selective functional outcome will be determined by the relative tissue/cellular distribution of AT1 and AT2, cross-talk between subunits, and availability of secondary messengers. In the failing heart, regardless of etiology (nonischemic cardiomyopathy-NICM or ischemic cardiomyopathy-ICM), Ang II concentration is increased with association to progression of heart failure (HF) [17] with no change in density of AT1R in the failing myocytes. In spontaneously hypertensive heart-failing rats, AT1R expression was upregulated in the compensated hypertrophy phase and returned to control values with progression to failure [18] while AT2R was unchanged at the initial stage and decreased following deterioration of heart failure.

Breakdown of Ang II through removal of the phenylalanine at the carboxy-terminal of Ang II results in the formation of Ang (1–7). This conversion is efficiently catalyzed by the recently discovered ACE homologue, ACE2 [19]. Yet, hydrolysis of Ang II by ACE2 is inferior to binding to its receptor, which has a three orders of magnitude higher affinity [20].

1.2. Bypassing Ang II: formation of Ang (1–7)

Another path, though kinetically less efficient [21], involves alternating roles for ACE and ACE2. Ang I is first hydrolyzed by ACE2 to form Ang (1–9) [2], a peptide which is not known to have any biological activity, and then converted by ACE to Ang (1–7). Although hydrolysis of Ang I to form Ang (1–9) was the first reported activity of ACE2, this activity was hardly measured in recombinant human ACE2 widely used in kinetic analysis of angiotensin peptide metabolism. Ang (1–9) forming activity was measured in non-solubilized fractions of membrane preparations from failing heart ventricle [22]. ACE2-dependent Ang I hydrolysis was measured in human monocyte-derived macrophages obtained from patients with CHF [23]. In myocardial infarcted rats, ACE2 correlated with Ang (1–9) rather than Ang (1–7) [24]. In rat isolated renal proximal tubule, Ang I rather than Ang II contributes to ACE2-dependent formation of Ang (1–7) [25] whereas in sheep proximal tubule Ang II, but not Ang I, is the substrate for ACE2 [26].

In addition, a direct conversion of Ang I to Ang (1–7) was shown to be mediated by several enzymes such as: prolyl-endopeptidase in vascular endothelial cells [27], neutral endopeptidase (nephrilysin) [28] in the circulation or kidney [26], and thimet oligopeptidase [29] in vascular smooth muscle cell.

Generation of Ang (1–7) through paths that bypass the formation of Ang II might be beneficial as these two angiotensin peptides play opposing roles. Yet, Ang I will preferably enter the highway rather than follow the bypass, as both endopeptidases and ACE2, have lower affinity to Ang I compared to ACE [21].

Ang (1–7) was recently reported to be the endogenous ligand for the G protein-coupled receptor Mas [30]. Genetic deletion of Mas abolished binding and activity of Ang (1–7) in mouse kidneys. Mas transfection increased Ang (1–7) activity, which was blocked by the specific Ang (1–7) antagonist, A-779. While binding of Ang (1–7) is not affected by Ang II receptor blockers [30], there is evidence for a functional interaction [31] of the Ang (1–7) receptor Mas with AT1R and AT2R in the mouse heart and kidney [32,33].

1.3. Vascular actions of Ang (1–7)

Ang (1–7) was shown to cause a vasodilating response in canine and porcine coronary arteries, rat aortas, and rabbit renal arterioles [34,35,36]. In rats, infusion of Ang (1–7) at doses in the fmol range reduced vascular resistance [36] whereas opposite effects were observed with a 100-fold higher concentration.
In human resistant vessels, a high dose of Ang (1–7) alone, at 1000 pmol/min, caused weak vasoconstriction [37], whereas at lower doses Ang (1–7) antagonized the Ang II-induced vasoconstriction. However, in chronic heart failure patients on ACEi therapy [38], brachial arterial infusion of a high dose of Ang (1–7) could not produce forearm vasodilatation. Neither was potentiation of bradykinin effect observed.

The Ang (1–7) blockade of Ang II-induced contractions could be accomplished in different ways: (1) by antagonism of AT1 receptors [39], (2) by release of NO [40,41] or other vasorelaxing factors (such as prostaglandins, e.g., PG12), or (3) by affecting other biologically active peptides (such as bradykinin [42,43]).

In addition, Ang (1–7) was also shown to inhibit human plasma, atrial, and arterial ACE and antagonizes Ang II-induced contractions in human arteries [44].

To summarize, Ang (1–7) can produce either vasodilatation or vasoconstriction, depending on dose and vascular bed [45,36] via different mechanisms. The roles of Ang (1–7) and its forming enzyme, ACE2, might be greater in reducing Ang II-actions rather than having beneficial effects by their own.

1.4. Protective effects of Ang (1–7) in the heart

In the intact human heart [46], processing of infused AngI to form Ang (1–7) is dependent on Ang II as a substrate, and is largely inhibited by the addition of ACE inhibitors (ACEis).

Ang (1–7) presence in the canine heart was demonstrated in the aortic root, coronary sinus and right atrium [47]. Ang (1–7) is distributed throughout cardiac myocytes in healthy cardiac tissue of the Lewis rat strain [48].

At doses in the fmol range, Ang (1–7) was shown to increase cardiac output in rats [36] whereas opposite effects were observed with 100-fold higher concentrations. After coronary artery ligation, Ang (1–7) immunoreactivity within the infarcted area is lost while an apparent increased expression of the peptide in the zones bordering the infarcted region of the left ventricle [48] was observed. The expression of the peptide is associated with the remodeling of cardiac tissue. An intravenous infusion of Ang (1–7) reduced left ventricular end-diastolic pressure, preserved coronary flow and endothelial function [49]. Genetic deletion of Mas, the Ang (1–7) receptor, resulted in impaired cardiac function and structure [50].

1.5. Altered ACE2 in heart failure

ACE2, the first known human homologue of ACE, was identified from 5' sequencing of a human heart failure ventricle cDNA library [2]. Analysis of gene expression in the development of heart failure reveals that nonischemic and ischemic cardiomyopathy share 41 genes involved in cell growth and signal transduction compared to the non-failing heart [51] whereas ACE2 gene upregulation was observed in NICM but not ICM.

Parameters of left ventricular hypertrophy association with ACE2 polymorphism was reported in the third MONICA (Monitoring of trends and determinants in cardiovascular disease) Augsburg survey [52]. Four single nucleotide polymorphisms were associated with higher left ventricular mass index, higher septal wall thickness and increased odds ratio for left ventricular hypertrophy.

Increased ACE and ACE2 immunoreactivity were observed in cardiac tissue of patients with ischemic heart failure compared to normal subjects [53]. ACE2 activity was also shown to increase in failing human heart ventricles obtained from patients with either idiopathic dilated cardiomyopathy or primary pulmonary hypertension [22].

Myocardial infarction in Sprague-Dawley rats, induced by ligation of the left coronary artery, increased cardiac ACE and ACE2 mRNA compared to control [53]. Expression of both ACEs in the border/infarcted area was significantly higher than in the viable area. Four weeks after MI, ACE2 mRNA expression at the viable area was significantly elevated compared to control. While in normal cardiac tissue, ACE2 is localized to myocytes and vascular endothelial cells, increased ACE2 protein following MI was strongly associated with infiltrating inflammatory monocyte/macrophage cells.

Despite the above, some reports do not demonstrate differences in the expression of ACE2 between failing and non-failing heart. In an experimental model of MI, in Lewis rat, Ang (1–7) immunostaining was reported to be significantly increased compared to control animals [48], although no changes in myocardial ACE or ACE2 were observed [54]. The distribution or intensity of protein expression in sections from failing and non-failing human heart samples showed no observable difference [2].

The role ACE2 might play in cardiac function is further investigated by deliberate genetic manipulation of its expression or via pharmacological interference.

1.6. Disruption of ACE2

Mice with targeted disruption of ACE2 develop abnormal heart function which progressed with age and was more pronounced in males [55]. Loss of ACE2 severely impaired cardiac function. Values of LV fractional shortening and velocity of circumferential fiber shortening were half of those measured in age-matched wild-type mice. Although displaying a slight wall thinning and increased chamber dimensions, there was no evidence of cardiac hypertrophy, dilated cardiomyopathy or fibrosis to accompany the reduced cardiac contractility.

In another model of disrupted ACE2 [56], mice exhibited normal cardiac function and had morphologically normal hearts. However, in response to chronic pressure overload induced by transverse aortic constriction, mice developed cardiac hypertrophy and dilatation with decreased cardiac contractility. Mice lacking ACE2 developed pulmonary congestion and increased incidence of cardiac death compared
with WT mice. Moreover, activation of hypoxia-induced response to Ang II was greater in cardiomyocytes isolated from ACE2(−/y) mice than in those isolated from WT mice.

In both models of ACE2 disruption, Ang II level was increased and hypoxia-induced genes were upregulated. Impaired cardiac function, spontaneous or induced by pressure overload, was probably related to the Ang II accumulation. In the first model, ablation of ACE in addition to ACE2 completely abolished the cardiac dysfunction phenotype of ACE2 deficient mice. In the second model, inhibition of the Ang II type 1 receptor attenuated the hypertrophic response. These results suggest an important role for ACE2 in counteracting the effects of accumulating Ang II.

ACE2 specific inhibitors were developed [57,58] and are widely used to inhibit the activity of the enzyme in-situ. Surprisingly, their effect on the cardiovascular system was not reported.

1.7. ACE2 overexpression

If loss of ACE2 impairs cardiac function, would excess of ACE2 play a protective role against development of heart failure? In several diseases, at initial stages of RAAS activation, a significant increase of ACE2 occurs followed by a marked decrease thereafter. In the spontaneously hypertensive rat ACE2 is highly expressed in the kidney at birth [59] and tubular expression falls with onset of hypertension and remains so compared to control rats. At initial stage of diabetes, ACE2 is highly expressed [60] and with development of nephropathy is significantly reduced. The control of the initial upregulation and the ensuing shut down are unknown.

Conflicting results were obtained in two different models of genetic ACE2 overexpression. Surprisingly, in the first described model, ACE2 overexpression did not provide evidence to being beneficial. ACE2 transgenic mice had a high incidence of sudden death correlated with level of transgene expression [61]. Young transgenic mice developed severe conduction and rhythm disturbances with complete AV block. The decreased expression of connexin43, a gap junction protein, was inversely related to ACE2 transgene expression. Surviving older mice recovered normal ventricular conduction, exhibited reduced ACE2 transgene expression and concomitant upregulation of connexin43. A possible weakness of this model might be that soluble form of ACE2 is over-expressed. Absence of the cytoplasmatic tail of ACE2 which contains an integrin-binding domain [62] could affect

Fig. 1. From angiotensin I to angiotensin (1–7): effect of drugs. This scheme illustrates the interactions between angiotensins, their forming enzyme and receptors. Solid and dashed lines indicate a positive or negative influence, respectively. Angiotensin-converting enzyme (ACE) and its homologue ACE2, AT1R and AT2R are angiotensin II type 1 and type 2 receptors, Mas is Ang (1–7) receptor, ACEi is ACE inhibitor and ARB is AT1R blocker.
integrin-dependent signaling related to connexin. In addition, while ACE2 production is specifically increased in the heart, increased serum ACE2 activity indicate that it is present and active at remote sites. Thus, observation in the transgenic mice hearts, combined with the significant reduced blood pressure might reflect secondary/compensatory effects such as volume/pressure control.

The second reported model, however, is in line with the beneficial ACE2 hypothesis. Using lentiviral vector, tissue over-expression of mouse ACE2 gene in the hearts of Sprague-Dawley rats protected animals against angiotensin II-induced cardiac hypertrophy or fibrosis [63].

1.8. Modulation of ACE2 and Ang (1–7): beyond the direct effects of anti-RAAS drugs

Blocking the formation and action of Ang II has long been a therapeutic target in the treatment of cardiovascular diseases. Both ACE inhibition (ACEI) and AT1 receptor blockade (ARB) are widely used. An alternative or complementary therapeutic approach would be to elevate the ‘good’ RAAS components.

Administration of a selective AT1R blocker to patients with cardiovascular diseases result in increased Ang II [64]. Under inhibition of AT1R, this excess of Ang II may have beneficial therapeutic outcome through either selective stimulation of cardiac AT2R or accelerated processing of Ang II resulting in increased Ang (1–7) [65].

Blockade of the AT1R with Olmesartan attenuated MI-induced cardiac hypertrophy and improved ventricular contractility. While abating the increased plasma aldosterone following MI, Olmesartan further increased plasma angiotensins concentrations and concomitantly increased ACE2 mRNA expression in the myocardium 3 fold [54].

Cardiac ACE2 mRNA expression was significantly increased following continuous administration of either Lisinopril or Losartan by 5- and 3-fold, respectively [66], independent of the hemodynamic effects of these drugs. However, only Losartan was shown to significantly increase cardiac Ang (1–7) concentration which paralleled the elevation of ACE2 activity.

ACE2 mRNA and immunostaining as well as Ang (1–7) were shown to increase in the aorta but not carotid arteries of spontaneously hypertensive rats following administration with Olmesartan [67]. These observations related to significantly reduced media thickness, indicating reversal of hypertrophic remodeling in an AT1R-dependent manner which is unrelated to pressure lowering.

We have recently shown [23] that mineralocorticoid receptor blocker therapy decreased ACE and increased ACE2, each and both capable of reducing Ang II level. Thus, drugs directed against aldosterone, downstream to Ang II, affected the balance of the two ACEs mRNA and activity, suggesting a feedback control.

Based on the above, the level of ACE2 and/or Ang (1–7) in patients with heart failure should be interpreted cautiously as these patients are on drug regimen that may alter these activities. Nevertheless, the merits of treatment with antagonists of the RAAS (Fig. 1), ACEi, ARB and MRB, lay beyond their direct effects on suppression of Ang II synthesis or activity as they also increase ACE2.

References


