

Angiotensin-converting enzyme 2 and angiotensin 1–7: novel therapeutic targets

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Abstract | The renin–angiotensin system (RAS) has pivotal roles in the regulation of normal physiology and the pathogenesis of cardiovascular disease. Angiotensin-converting enzyme (ACE) 2, and its product angiotensin 1–7, are thought to have counteracting effects against the adverse actions of other, better known and understood, members of the RAS. The physiological and pathological importance of ACE2 and angiotensin 1–7 in the cardiovascular system are not completely understood, but numerous experimental studies have indicated that these components have protective effects in the heart and blood vessels. Here, we provide an overview on the basic properties of ACE2 and angiotensin 1–7 and a summary of the evidence from experimental and clinical studies of various pathological conditions, such as hypertension, atherosclerosis, myocardial remodelling, heart failure, ischaemic stroke, and diabetes mellitus. ACE2-mediated catabolism of angiotensin II is likely to have a major role in cardiovascular protection, whereas the relevant functions and signalling mechanisms of actions induced by angiotensin 1–7 have not been conclusively determined. The ACE2–angiotensin 1–7 pathway, however, might provide a useful therapeutic target for the treatment of cardiovascular disease, especially in patients with overactive RAS.

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Introduction

The importance of the renin–angiotensin system (RAS) in the regulation of normal physiology and pathogenesis of cardiovascular disease is well established.^{1–4} The best-known components of the RAS include angiotensinogen, angiotensin I and II, and the peptidases renin and angiotensin-converting enzyme (ACE). Renin is the rate-limiting enzyme in the RAS, whereas angiotensin II is the major effector molecule, which exerts its biological actions via the type-1 angiotensin II (AT1) and type-2 angiotensin II (AT2) receptors. Angiotensin II is generated in the circulation and within tissues (local RAS).^{1,3,5,6} RAS is implicated in the pathogenesis of various cardiovascular disorders, including hypertension, atherosclerosis, myocardial infarction, pathological cardiac remodelling, heart failure, and metabolic syndrome.^{1,2,4} ACE2 and its product angiotensin 1–7 were identified more recently than the aforementioned components of this system, and are thought to have counteracting effects against the adverse actions of these other RAS components (Figure 1),⁷ although their physiological and pathophysiological roles are incompletely understood. Findings from numerous experimental studies have suggested notable protective effects in the heart, blood vessels, kidney, and central nervous system. ACE2 and angiotensin 1–7 might, therefore, represent new therapeutic targets for cardiovascular disease.⁷ In this Review, we summarize the evidence from experimental and

clinical studies of the effects of ACE2 and angiotensin 1–7 in various pathological cardiovascular conditions.

Properties of ACE2

Biochemical characteristics

ACE2 (also known as ACE-related carboxypeptidase, angiotensin-converting enzyme homologue, or ACEH) is a monocarboxypeptidase that is mainly expressed in vascular endothelial cells and the renal tubular epithelium.⁸ The gene for ACE2 was cloned in 2000 by two independent groups as a homologue of human ACE.^{8,9} The ACE2 protein is an 805 amino-acid type-I transmembrane protein that contains an extracellular (ecto) domain (amino acids 18–739), a transmembrane region (amino acids 740–768), and an intracellular tail. The extracellular part of ACE2 (Figure 2) 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).^{8,10–12,13} The C-terminal part of ACE2 (614–805) is homologous (48% identity) to a transporter protein known as collectrin.^{11,14} The catalytic domain of ACE2 is 42% identical to that of ACE.⁸

The peptidase activity of ACE2 is dependent on the C-terminus sequence of the substrate (sequence specificity). ACE2 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 ACE2-dependent hydrolysis.¹⁵ In this circumstance, ACE2 displays potent peptidase

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Competing interests

The authors declare no competing interests.

Key points

- Angiotensin-converting enzyme (ACE) 2 and its product angiotensin 1–7 are thought to have effects that counteract the adverse actions of other, better-known renin–angiotensin system (RAS) components
- Numerous experimental studies have suggested that ACE2 and angiotensin 1–7 have notable protective effects in the heart and blood vessels
- ACE2-mediated catabolism of angiotensin II is likely to have a major role in cardiovascular protection, whereas the functional importance and signalling mechanisms of angiotensin-1–7-induced actions remain unclear
- New pharmacological interventions targeting ACE2 are expected to be useful in clinical treatment of cardiovascular disease, especially those associated with overactivation of the conventional RAS
- More studies, especially randomized controlled clinical trials, are needed to clearly delineate the benefits of therapies targeting angiotensin 1–7 actions

activity to angiotensin II (Pro-Phe), angiotensin I (Pro-Phe-His-Leu), and des-Arg⁹ bradykinin (Ser-Pro-Phe), but shows no activity towards angiotensin 1–9, angiotensin 1–7, or bradykinin.¹⁶ ACE2 can also hydrolyse other bioactive peptides, such as apelin-13, β-casomorphin, dynorphin A 1–13, and ghrelin.¹⁶ The most-important active product of ACE2 is angiotensin 1–7, which can be primarily generated via two routes. First, ACE2 can directly hydrolyse angiotensin II to yield angiotensin 1–7;

second, ACE2 can convert angiotensin I to angiotensin 1–9, which is then cleaved by either neutral endopeptidase (NEP) or ACE to yield angiotensin 1–7.^{12,17,18} In addition, angiotensin 1–7 can be produced by prolyl carboxypeptidase from angiotensin II or by NEP directly from angiotensin I.¹⁸ The biological functions of angiotensin 1–9 are poorly understood, although important effects in the cardiovascular system have been purported.^{19,20} The affinity of ACE2 to angiotensin II ($K_m = 2.0 \mu\text{mol/l}$, 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 angiotensin I ($K_m = 6.9 \mu\text{mol/l}$). The ACE2 catalytic efficiency for angiotensin II is >300 times that for angiotensin I.¹⁶ The angiotensin I–ACE–angiotensin II–ACE2–angiotensin 1–7 pathway (Figure 1) ought, therefore, to have a predominant role in the production of angiotensin 1–7 *in vivo*.

The membrane-bound ectodomain of ACE2 can be cleaved by the metalloproteinase ADAM17, which releases a soluble form of ACE2 (Figure 1).^{21,22} By use of serial deletion constructs of ACE2, Iwata and colleagues identified a juxtamembrane region (amino acids 720–729) that is essential for ADAM17-mediated ectodomain

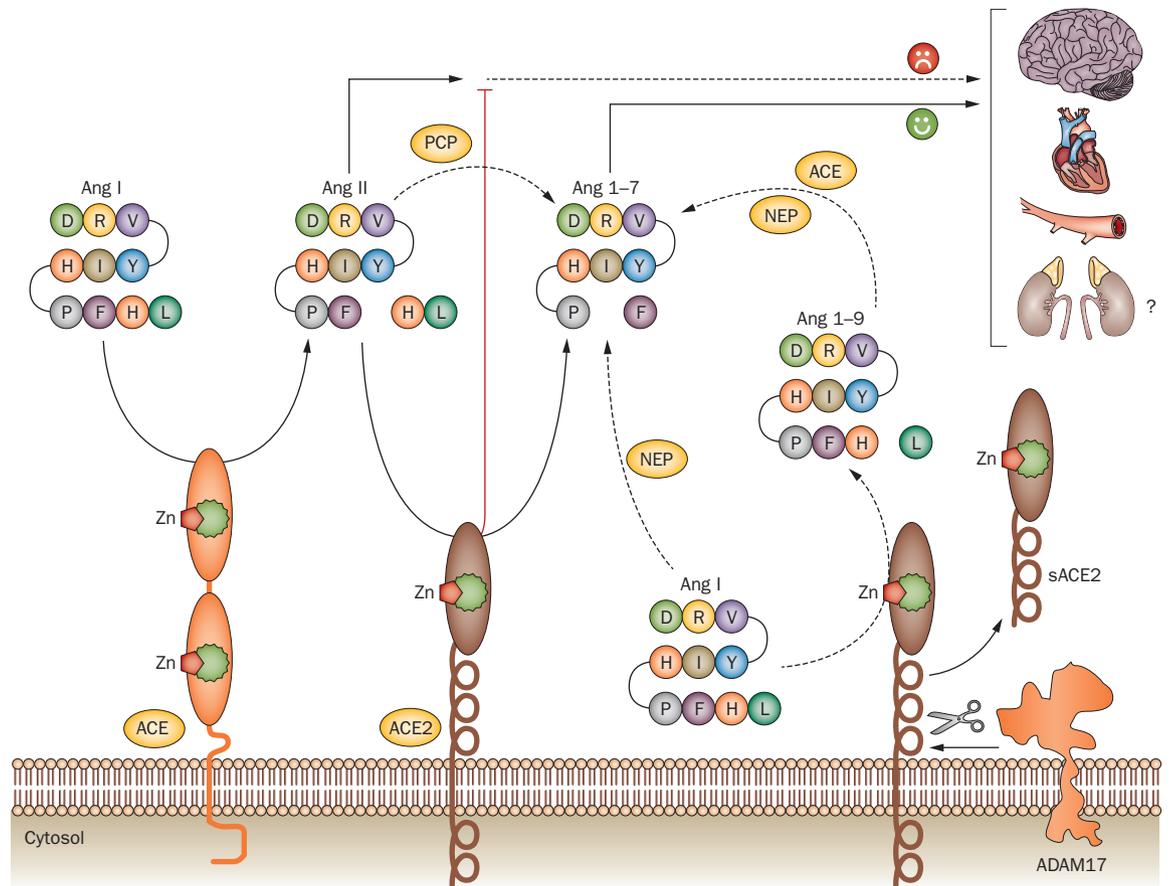


Figure 1 | Overview of the ACE2–Ang 1–7 pathway. ACE2 converts Ang II to Ang 1–7. ACE2 can also convert Ang I to Ang 1–9, which is then cleaved by either NEP or ACE to yield Ang 1–7. The membrane-bound ACE2 can be cleaved by the metalloproteinase ADAM17, forming a soluble form of ACE2. The physiological relevance of soluble ACE2 is not fully understood. Abbreviations: ACE, angiotensin-converting enzyme; Ang, angiotensin; NEP, neutral endopeptidase; PCP, prolyl carboxypeptidase.

shedding.²³ The physiological relevance of soluble ACE2 is not fully understood. Its activity in plasma is raised in patients with chronic heart failure²³ and is associated with increased cardiac dysfunction and poor clinical outcomes.²⁴ Moreover, soluble ACE2 activity is raised after myocardial infarction, which has been associated with an increased incidence of subsequent adverse left ventricular remodelling.²⁵ Findings from one study suggested that angiotensin II promotes ACE2 shedding by increasing ADAM17 activity in the myocardium. This action represents a positive feedback mechanism by which angiotensin II facilitates myocardial injury.²⁶

Pharmacological activators and inhibitors

Several compounds are known to affect ACE2 activity. MLN-4760 is a specific and potent ACE2 inhibitor with a half maximum inhibitory concentration (IC_{50}) of 0.4 nmol/l.²⁷ X-ray diffraction studies have revealed that the catalytic domain of ACE2 contains two subdomains linked by an α -helix hinge region, which forms a long and deep cleft. MLN-4760 binds to both of the subdomains (Figure 2) and causes the hinge to flex, which shrinks the cleft and prevents substrate binding.²⁸ ACE2 inhibition *in vivo* by MLN-4760 aggravated glomerular injury in a model of type 1 diabetes mellitus,²⁹ and exacerbated cardiac hypertrophy and fibrosis in transgenic hypertensive rats.³⁰

DX600 is a peptide inhibitor of ACE2 with a high affinity ($K_d = 10.8$ nmol/l; this value is the equilibrated concentration required to achieve 50% occupancy of the receptors in radioligand binding assays; that is, the higher the K_d value, the lower the affinity) and high efficacy ($IC_{50} = 10.1$ nmol/l), but has no obvious inhibitory effect toward ACE.³¹ In *in vitro* and *in vivo* experiments, DX600 has shown antagonistic effects on ACE2-mediated actions.^{32,33} Kinetic analyses have revealed that DX600 exhibits a mixed competitive and noncompetitive type of inhibition, which indicates that this peptide might act as a partial allosteric modulator of ACE2; this mode of action is analogous to that of some ACE inhibitors, such as captopril.³¹ Several studies reported that DX600 displayed different efficacy of inhibition for human and rodent ACE2.^{34,35}

Given the potential protective roles of ACE2 in the cardiovascular system, identification of novel ACE2 activators is an important avenue for drug discovery. Two small-molecule ACE2 enhancers, xanthone and resorcinolnaphthalein, have been discovered.³⁶ *In vitro*, these compounds enhanced ACE2 activity by around twofold at concentrations of 100 μ mol/l, but showed no effects on ACE activity. The pharmacological effects of xanthone were further explored *in vivo* in healthy, normal rats and spontaneously hypertensive rats (SHRs), and showed remarkable hypotensive and cardiac protective actions.^{36,37} Potent antithrombotic actions were reported for xanthone in a rat model of venous thrombosis, whereas DX600 led to opposite effects.³³ These beneficial effects should, however, be interpreted with caution, because xanthone can increase ACE2 expression.³⁷ Indeed, the specificity of xanthone for ACE2 has been questioned.³⁸

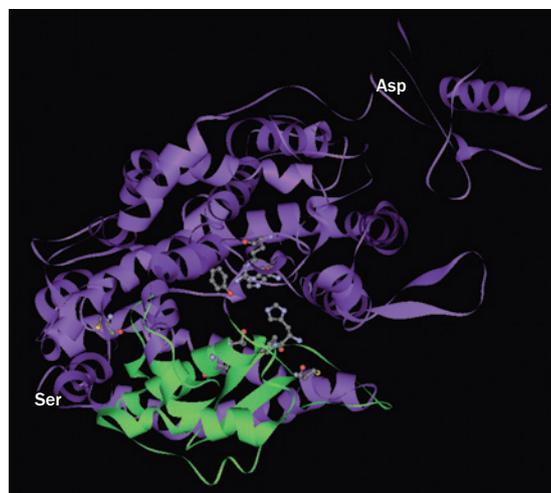


Figure 2 | Structure of the extracellular domain (from Ser19 to Asp615) of human ACE2. In this image, the extracellular domain is arbitrarily divided into two subdomains (shown in green and purple), forming a deep cleft that is proposed to be the active site for substrate binding and catalysis. The catalytic 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 ACE2 inhibitor MLN-4760. Abbreviation: ACE2, angiotensin-converting enzyme 2. Amino acid sequence annotations are derived from Towler, P. *et al. J. Biol. Chem.* **279**, 17996–18007 (2004).²⁸

Properties of angiotensin 1–7

Biochemical characteristics

In addition to ACE2-dependent routes, angiotensin 1–7 can be generated directly from angiotensin I by prolyl endopeptidase and NEP.¹⁸ The heart, brain, and kidney are major sources of angiotensin 1–7 production.¹⁷ In the human coronary circulation, NEP seems to have a more prominent role in angiotensin 1–7 production than ACE2.³⁹ Pharmacokinetic experiments have determined that, in humans, angiotensin 1–7 has a short half-life of ~0.5 h.⁴⁰ Following subcutaneous injection, the peptide is quickly available in the blood and reaches its peak plasma concentration at ~1 h.⁴⁰ In rats, the plasma half-life of angiotensin 1–7 is only 9 s.⁴¹ Angiotensin 1–7 is metabolized mainly by ACE in the lungs.⁴² In addition, aminopeptidase and NEP can degrade angiotensin 1–7 into peptide segments.⁴²

Angiotensin 1–7 has broad effects in the cardiovascular system, including vasodilatation; myocardial protection; antiarrhythmic, antihypertensive, and positive inotropic effects; and inhibition of pathological cardiac remodeling.^{18,43,44} In addition, angiotensin 1–7 is thought to have favourable effects on metabolism by lessening insulin resistance.^{45–47} Although most effects are protective, some seem to be variable. For example, effects on arrhythmia might be biphasic, with high concentrations of angiotensin 1–7 promoting cardiac arrhythmias and low concentrations having protective effects.^{48–50} Similarly, some experimental findings suggest that the

biological functions of angiotensin 1–7 in the kidney might be beneficial (diuretic or natriuretic, antifibrotic, and antiproliferative), whereas other findings suggest they are detrimental (antidiuretic, proinflammatory, and profibrotic).^{18,44}

Although the biological actions of angiotensin 1–7 are well described, the signalling mechanisms are still poorly understood. Treatment with angiotensin 1–7 activates the phosphatidylinositol-3-kinase–Akt pathway *in vitro*^{51–54} and *in vivo*.^{45,55} This action could at least partly explain the beneficial effects of angiotensin 1–7 on blood vessel functions and metabolism, given the pivotal roles of Akt in promoting endothelial nitric oxide synthase function and insulin signalling.¹⁸ By contrast, angiotensin 1–7 has inhibitory effects on activated mitogen-activated protein kinase (MAPK) pathways in endothelial cells,⁵⁶ smooth-muscle cells,^{57,58} cardiac myocytes,⁵⁹ and renal proximal tubular cells.⁶⁰ In these cells, angiotensin II induces oxidative, hypertrophic, and proliferative responses by activating MAPKs and, therefore, angiotensin 1–7 might counter-regulate these effects. The effects of angiotensin 1–7 on MAPKs, however, are highly cell specific; in some types of cells, angiotensin 1–7 augments basal or angiotensin-II-induced MAPK activation.^{61–63} Smooth-muscle depolarization via angiotensin 1–7-induced opening of potassium channels has been demonstrated, and seems to be dependent on activation of the cAMP–cAMP-dependent protein kinase (PKA) pathway.⁶⁴

Receptors and their agonists and antagonists

Early radioligand-binding studies indicated that angiotensin 1–7 could bind to the AT1 receptor in the brain and kidney,^{65,66} although in a study that used a more-homogenous assay system, virtually no binding was observed in HEK293 cells stably expressing AT1 receptors.⁶⁷ Angiotensin 1–7 binds to the AT2 receptor with a moderate affinity (five times lower than that for angiotensin II).⁶⁷ *In vitro*⁶⁸ and *in vivo*⁶⁹ studies have demonstrated that certain biological effects of angiotensin 1–7 can be attenuated by use of the AT2 receptor antagonist PD123319 and, therefore, angiotensin 1–7 binding to AT2 receptors is functionally relevant.

The Mas protein, which was originally cloned as an oncogene product,⁷⁰ is a seven-transmembrane G-protein-coupled receptor with a high affinity for angiotensin 1–7 ($K_d = 0.83$ nmol/l).⁷¹ The gene for this protein, *MAS1*, is mainly expressed in the brain, testis, kidney, and heart.^{72–76} The expression level of Mas is dynamically regulated by physiological and pathological stimuli in the heart.⁷⁷ Mas expression is also observed in vascular endothelial cells.^{53,73,78} Expression of Mas remains to be confirmed in vascular smooth-muscle cells, although angiotensin 1–7-induced effects have been readily observed in these cells in various functional experiments.^{58,79,80} *Mas1*-knockout animals show cardiovascular-related phenotypes, including myocardial contractile dysfunction, cardiac fibrosis, hypertension, endothelial dysfunction, renal fibrosis, glomerular dysfunction, insulin resistance, and dyslipidaemia.^{75,81–83} However, in two studies in which different models of

hypertension (the two-kidney, one-clip model and hypertension induced in response to high salt ingestion) were assessed, Mas deficiency had opposite effects on blood pressure.^{84,85}

Functional studies have suggested that most cardiovascular effects of angiotensin 1–7 are likely to be mediated by Mas.^{18,86–88} The precise angiotensin 1–7–Mas signalling mechanisms in the context of cardiovascular physiology have not, however, been firmly established. First, *MAS1* is an oncogene, and activation of Mas signalling induces cell transformation via the small GTPase p21-Rac1.⁸⁹ This effect is not reproduced by angiotensin 1–7. Rather, it has some antitumorigenic effects.^{90,91} Second, Mas is constitutively coupled to $G_{\alpha_{q/11}}$ and downstream phospholipase C–protein kinase C signalling.^{92,93} Evidence indicates, however, that angiotensin 1–7 does not stimulate G_{α_q} signalling in Mas-expressing cells.⁹³ Moreover, ectopic expression of Mas induces an increase in the intracellular concentration of Ca^{2+} , whereas angiotensin 1–7 treatment does not raise intracellular concentrations of Ca^{2+} .^{93–95} Third, the mechanistic link between Mas and the Akt pathway, which has been implicated in the cardioprotective actions of angiotensin 1–7, is still missing.

In many circumstances, the effects of angiotensin 1–7 are antagonistic to those induced by angiotensin II in the cardiovascular system,^{18,56,58,96} but the underlying signalling mechanisms remain to be defined. Mas binds directly to the AT1 receptor, but the interaction is unlikely to have a role in mediating angiotensin 1–7 effects, since administration of angiotensin 1–7 has no effects on this complex.⁹⁷ Despite the discovery of binding, Mas is still classified as a class A orphan receptor under the latest International Union of Basic and Clinical Pharmacology classification.⁹⁸

The widely used Mas antagonist (D-Ala⁷)-angiotensin-(1–7) is an angiotensin 1–7 analogue with the last proline residue substituted with D-Ala.⁹⁹ This agent is water soluble. Radioligand-binding experiments show that (D-Ala⁷)-angiotensin-(1–7) has a high efficacy in Mas inhibition (IC_{50} for angiotensin 1–7 binding = 0.3 nmol/l).⁷¹ By contrast, the binding affinity of (D-Ala⁷)-angiotensin-(1–7) to the AT1 and AT2 receptors is negligible.^{67,99} A similar peptide Mas antagonist is (D-Pro⁷)-angiotensin-(1–7). The pharmacological properties of this agent have not been fully characterized, but at 10 μ mol/l it exhibits similar effects to (D-Ala⁷)-angiotensin-(1–7) in competing for angiotensin 1–7 binding, and showed weak affinities for the AT1 and AT2 receptors.¹⁰⁰ Currently, no specific nonpeptide Mas antagonist is available.

AVE 0991 is a small-molecule agonist with high affinity and selectivity for Mas that competes for specific binding of angiotensin 1–7. It has an IC_{50} of $\sim 10^{-8}$ mol/l, and virtually no affinity to the AT1 or AT2 receptors.^{67,101,102} The maximum stimulating effect of AVE 0991 on release of endothelial nitric oxide is equivalent to that of angiotensin 1–7.¹⁰¹

CGEN-856S is a 24 amino acid peptide with no obvious homology to angiotensin fragments or other known endogenous GPCR ligands.¹⁰³ CGEN-856S at 0.1 μ mol/l

Table 1 | Experimental findings for the effects of ACE2 and Ang 1–7 on BP

Animal models	Interventions	BP effect	References
Effects of ACE2			
Hypertension induced by Ang II in mice	Ace2 gene deletion	↑	104
SHR and hypertension induced by Ang II in rats	Ace2 gene transfer in CNS	↓	105, 106
Hypertension induced by Ang II in mice	Ace2 neuron-specific gene transfer in CNS	↓	107, 108
Stroke-prone SHRs	Vascular smooth-muscle-cell-specific Ace2 gene transfer	↓	109
Hypertension induced by Ang II in mice, SHRs	Systemic infusion of human or murine recombinant ACE2	↓	35, 110, 111
SHRs	Systemic infusion of an ACE2 activator	↓	36
Effects of angiotensin 1–7			
Healthy, normal rats	Ang 1–7 infusion in CNS	Null	113
Rats with normal blood pressure and SHRs	Ang 1–7 infusion in CNS	↑	99, 100, 126
Hypertension induced by Ang II in mice	Systemic infusion with Ang 1–7	Null	110
Healthy, normal rats, SHR, RHRs	Systemic infusion with Ang 1–7	Null	112–114
Healthy, normal rats, SHRs, RHRs	Systemic infusion with Ang 1–7	↓*	112, 116
Dahl salt-sensitive rats, SHRs	Systemic infusion with Ang 1–7	↓	115, 117, 118
DOCA-salt hypertensive rats	Systemic infusion with Ang 1–7	Null	119
DOCA and aldosterone salt-hypertensive rats	Ang 1–7 infusion in CNS	↓	124, 125
2K1C hypertensive rats	Systemic infusion with Ang 1–7	Null†	120, 121
Subtotal-nephrectomy-induced hypertension in rats	Systemic infusion with Ang 1–7	↑	122
SHRs	Infusion of monoclonal antibody to Ang 1–7	↑	130
Rats with normal blood pressure and SHRs	Systemic infusion with (D-Ala ⁷)-Ang-(1–7)	Null	128
2K1C hypertensive rats	Systemic infusion with (D-Ala ⁷)-Ang-(1–7)	↑	113
Aldosterone salt-hypertensive rats	(D-Ala ⁷)-Ang-(1–7) infusion in CNS	↑	125
*Effects observed only in the presence of bradykinin. †Some beneficial effects observed in exercise-trained animals and for endogenous Ang 1–7. Abbreviations: 2K1C, two-kidney one clip; ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; BP, blood pressure; CNS, central nervous system; DOCA, deoxycorticosterone-acetate; RHR, renal hypertensive rat; SHR, spontaneously hypertensive rat.			

and 1.0 μmol/l is equally as potent as angiotensin 1–7 for Mas receptor binding. This agent has no binding activity for the AT1 receptor, but it shows a moderate binding affinity for the AT2 receptor (IC₅₀ ~5 μmol/l).¹⁰³

Blood-pressure regulation

ACE2 seems to have beneficial effects on the regulation of blood pressure (Table 1; Figure 3). ACE2-deficient C57BL/6 mice have raised basal blood pressure, with notable amplifications of angiotensin-II-induced

hypertension, and have increased accumulation of angiotensin II in plasma and the kidney after angiotensin II infusion.¹⁰⁴ Gain-of-function experiments in SHRs indicated that ACE2 expression in the central nervous system is associated with substantially decreased blood pressure. For example, overexpression of human ACE2 in the rostral ventrolateral medulla produced long-term hypotensive effects in SHRs.¹⁰⁵ Similarly, overexpression of ACE2 in the paraventricular nucleus ameliorated hypertension induced by angiotensin II in healthy, normal rats.¹⁰⁶ Moreover, pan-neuronal expression of ACE2 in the brain and delivery of ACE2-expressing adenoviruses into the cerebral ventricle have attenuated high blood pressure and improved baroreflex dysfunction induced by angiotensin II.^{107,108} In addition to these central actions, targeted expression of human ACE2 in vascular smooth muscle cells decreases blood pressure in stroke-prone SHRs.¹⁰⁹

Pharmacological intervention studies have revealed similar beneficial effects of ACE2 on high blood pressure. Systemic administration of human recombinant ACE2 counteracted pressor effects induced by angiotensin II, which was associated with accelerated clearance of circulating angiotensin II.^{110,111} Moreover, in SHRs, chronic ACE2 treatment resulted in a sustained decrease in blood pressure.¹¹¹ Murine recombinant ACE2 has similar anti-hypertensive effects to human ACE2 in hypertension induced by angiotensin II.³⁵ Likewise, chronic infusion of the ACE2-activating compound xanthenone slightly decreases blood pressure in SHRs.³⁶

In contrast to the reported protective effects of ACE2 in experimental hypertension, studies with angiotensin 1–7 have yielded rather heterogeneous results (Table 1). Most of the studies have shown that acute systemic infusion of angiotensin 1–7, in either rats with normal blood pressure or those with hypertension due to various aetiologies, does not change blood pressure.^{112–114} Decreases in mean blood pressure in Dahl salt-sensitive rats, however, have been reported.¹¹⁵ In anaesthetized mice, bolus administration of angiotensin 1–7 showed no effect on angiotensin-II-induced elevation in blood pressure or on blood-pressure recovery after injected angiotensin II was stopped.¹¹⁰ In healthy, normal rats, acute infusion of angiotensin 1–7 led to an enhancement of the hypotensive effects of bradykinin.^{112,116} Chronic treatment with angiotensin 1–7 was associated with transient or sustained reductions in blood pressure in various hypertensive models, including SHRs and Dahl salt-sensitive rats,^{117,118} but not in deoxycorticosterone-acetate (DOCA) salt-treated rats.¹¹⁹ Results in the two-kidney one-clip model of hypertension in rats have been inconsistent.^{120,121} Moreover, chronic angiotensin 1–7 treatment has been observed to further increase blood pressure in rats with hypertension induced by subtotal nephrectomy.¹²²

Administration of angiotensin 1–7 into the central nervous system of rats has induced null, hypertensive, and hypotensive effects. Intracerebroventricular infusion of angiotensin 1–7 showed no effects in rats with normal blood pressure,^{113,123} and lowered blood pressure in DOCA and aldosterone salt-hypertensive rats.^{124,125}

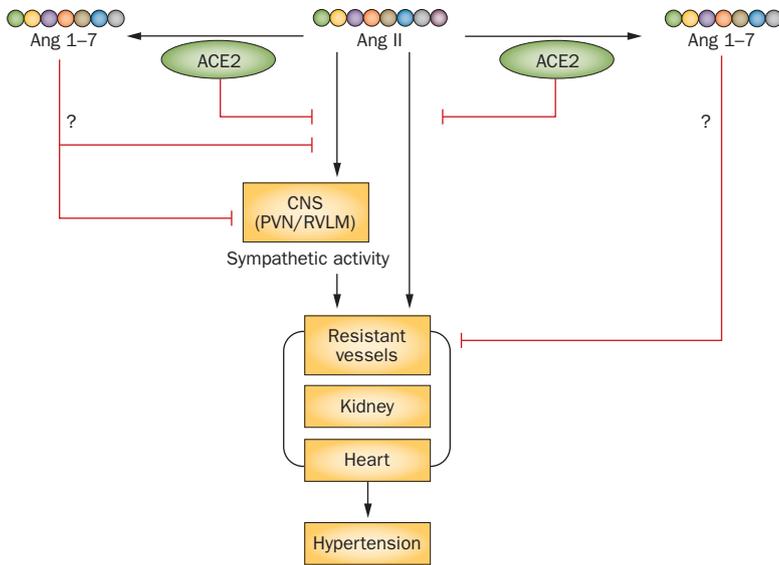


Figure 3 | Potential effects of ACE2 and Ang 1–7 on blood-pressure regulation. ACE2 is known to cause reductions in blood pressure, whereas various experimental evidence has indicated no effects, hypotensive effects, and hypertensive effects of Ang 1–7. Abbreviations: ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; CNS, central nervous system; PVN, paraventricular nucleus, RVLM, rostral ventrolateral medulla.

Conversely, injection of angiotensin 1–7 into the rostral ventrolateral medulla triggered an increase in blood pressure in rats with normal blood pressure and in SHR. ^{99,100,126}

In anaesthetized mice, acute treatment with (D-Ala⁷)-angiotensin-(1–7) showed no effect on basal or angiotensin-II-induced systolic hypertension. ¹¹⁰ Acute central administration of (D-Ala⁷)-angiotensin-(1–7) does not change mean arterial blood pressure ¹²⁷ and chronic treatment does not modify the blood pressure ¹²⁸ in rats with normal blood pressure or SHR. (D-Ala⁷)-angiotensin-(1–7) given centrally or systemically, however, antagonizes the hypotensive actions of ACE2 in transgenic mice ¹⁰⁸ and worsens hypertension, baroreflex dysfunction, or both in various models. ^{120,125,129} Acute infusion of a monoclonal antibody to angiotensin 1–7 in SHR notably raised blood pressure. ¹³⁰

In humans, infusion of angiotensin 1–7 induced notable vasodilator effects in control individuals with normal blood pressure and in patients with hypertension. ¹³¹ Yet, in another study, acute infusion of angiotensin 1–7 did not change the blood pressure in individuals with normal blood pressure. ¹³²

Taken together, these results suggest that ACE2 has potential blood-pressure-lowering effects via multiple mechanisms, among which catabolism of angiotensin II is likely to be essential (Figure 3). By contrast, the functional importance of ACE2-dependent angiotensin 1–7 production remains to be confirmed.

Atherosclerosis

ACE2 is expressed in atherosclerotic lesions in animal and human vessels. ^{133–135} The role of ACE2 in atherogenesis (Figure 4) has been investigated with use of various

in vivo models. ¹³⁶ Systemic overexpression of ACE2 suppressed atherogenesis in apolipoprotein-E-knockout mice. ¹³⁷ A similar antiatherosclerotic effect of ACE2 was seen in rabbits fed with a high-cholesterol diet. ¹³⁸ ACE2 overexpression also stabilized the atherosclerotic plaques via inhibition of inflammation and production of matrix metalloproteinases; this effect was reversed by (D-Ala⁷)-angiotensin-(1–7). ¹³⁹ Conversely, loss-of-function mutations in *Ace2* promoted plaque accumulation with increased expression of inflammatory adhesion molecules and cytokines. ^{140–142}

Similar to ACE2, angiotensin 1–7 has beneficial effects in experimental atherosclerosis (Figure 4). Long-term treatment with angiotensin 1–7 in apolipoprotein-E-knockout mice prevented progression of atherosclerotic lesions and improved endothelial function of nitric oxide. ¹⁴³ These effects seemed to involve both Mas and the AT2 receptor. Systemic administration of angiotensin 1–7 inhibited atherosclerotic lesion formation and enhanced plaque stability in mice in a dose-dependent manner. These effects were associated with decreased proliferation and migration of vascular smooth muscle cells, and decreased macrophage release of inflammatory cytokines. ¹⁴⁴ Activation of the angiotensin 1–7 receptor with AVE 0991 also inhibited atherogenesis in apolipoprotein-E-knockout mice. ^{145,146} The atheroprotective actions of angiotensin 1–7 could be related to its inhibitory effects on vascular inflammation and smooth-muscle-cell proliferation and migration, probably via Mas-mediated suppression of the nuclear factor κB or MAPK pathways, or both. ^{58,144,147} Although (D-Ala⁷)-angiotensin-(1–7) alone does not modulate early atherogenesis, it seems to aggravate destabilization of atherosclerotic plaques. ^{143,144}

Cardiac remodelling and heart failure

ACE2 was originally cloned as a heart-failure-associated gene from the heart tissue of a patient with idiopathic dilated cardiomyopathy, ⁸ but studies have not unequivocally confirmed that cardiac expression is changed in human heart failure. ^{148–151} The level and activity of soluble ACE2 in plasma seem to be increased in patients with heart failure. ^{23,24,152} Associations between ACE2 polymorphisms and hypertrophic cardiomyopathy have also been reported. ^{153,154}

ACE2 shows multiple beneficial effects in left ventricular remodelling and dysfunction in rat models of cardiac hypertrophy induced by angiotensin II, diabetic cardiomyopathy induced by streptozotocin, and myocardial infarction induced by coronary artery occlusion. ^{155–157} In mice with right ventricular dysfunction induced by pressure overload, treatment with recombinant human ACE2 reduces ventricular hypertrophy and improves systolic and diastolic functions. ¹⁵⁸ By contrast, *Ace2* knockout augments angiotensin-II-induced myocardial hypertrophy and interstitial fibrosis and accelerates the transition from hypertrophy to heart failure. ^{159–162} *Ace2* knockout also aggravates cardiac dysfunction induced by diabetes in mice. ¹⁶³ Although the cardioprotective mechanisms of ACE2 are not completely clear, mediation of

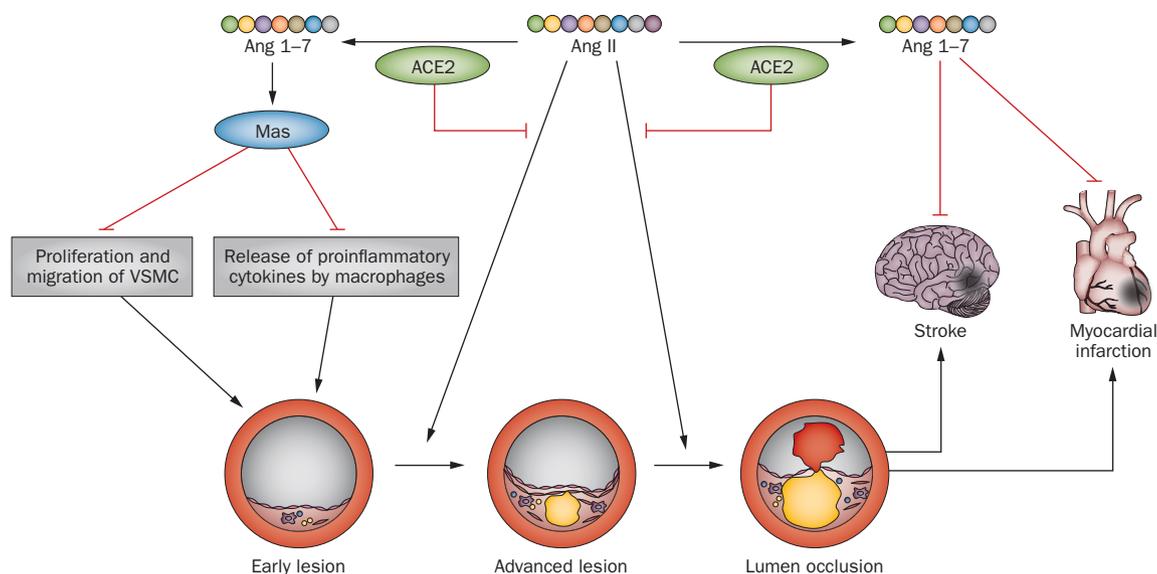


Figure 4 | Protective effects of ACE2 and Ang 1–7 on atherosclerosis and resultant myocardial infarction and stroke. Abbreviations: ACE2, angiotensin-converting enzyme 2; Ang, angiotensin; VSMC, vascular smooth muscle cell.

angiotensin II catabolism and subsequent inhibition of angiotensin-II-induced oxidative stress, inflammation, and interstitial fibrosis are thought to have important roles (Figure 5).^{156,160,163} In addition, reductions in sympathetic nerve activity might be attributable to ACE2-induced cardioprotection, as demonstrated in studies with central ACE2 overexpression (Figure 5).¹⁶⁴

Production of angiotensin 1–7 is substantially increased in heart failure induced by myocardial infarction.¹⁶⁵ To date, most studies support that angiotensin 1–7 has protective effects on pathological cardiac remodelling and heart failure (Figure 5). For instance, systemic infusion of angiotensin 1–7 prevents the development of heart failure following myocardial infarction in rats.¹⁶⁶ The angiotensin 1–7 receptor agonist AVE 0991 shows similar beneficial effects.¹⁶⁷ Infusion of angiotensin 1–7 also prevents cardiac remodelling induced by angiotensin II in rats,¹⁶⁸ and diabetes-induced diastolic dysfunction (with a preserved ejection fraction) in db/db mice.¹⁶⁹ Testis-specific angiotensin 1–7 in transgenic rats, which doubles the concentration of circulating angiotensin 1–7, has notable protective effects on myocardial hypertrophy and fibrosis induced by β -adrenoceptor agonists.^{170,171} Treatment with angiotensin 1–7 also produces equivalent protective actions on pressure-overload-induced cardiac hypertrophy and remodelling as AT1-receptor blockade in ACE2-null mice.¹⁷²

The cardiac protective actions of angiotensin 1–7 are unlikely to be primarily mediated by a local mechanism in the myocardium, since the effects of cardiomyocyte-restricted overexpression on pathological cardiac remodelling seem to be inconsistent.^{173,174,175} Additionally, the effects of angiotensin 1–7 on arrhythmia are biphasic; the beneficial effects on the myocardium associated with low concentrations arise through induction of hyperpolarization and increased the conduction velocity and refractoriness, whereas high concentrations have

arrhythmogenic actions.⁵⁰ Whether angiotensin 1–7 leads to cardiac dysfunction and remodelling in rodent models of renal disease is controversial.^{122,176}

Cerebral vessels and ischaemic stroke

Involvement of angiotensin 1–7 in modulation of cerebral vascular functions and the process of ischaemic stroke (Figure 4) is supported by several studies. In *ex vivo* preparations of canine middle cerebral arteries, Feterik *et al.*¹⁷⁷ demonstrated that angiotensin 1–7 induced endothelium-dependent relaxation sensitive to blockade of nitric oxide synthase and soluble guanylyl cyclase. Relaxation induced by angiotensin 1–7 is not affected by (D-Ala⁷)-angiotensin-(1–7) or the AT2 receptor antagonist PD123319, but is partially blocked by a bradykinin B2 receptor antagonist.¹⁷⁷ Similar vasodilatory effects have been observed in rat middle cerebral arteries.¹⁷⁸ Moreover, chronic intravenous infusion of angiotensin 1–7 reversed endothelial dysfunction in the middle cerebral artery in rats maintained on a high-salt diet.¹⁷⁸

In rats with acute ischaemic stroke, expression of ACE2 and Mas are upregulated, and concentrations of local and circulating angiotensin 1–7 are substantially increased.¹⁷⁹ In a rat model of stroke, induced by occlusion of middle cerebral arteries, pretreatment with angiotensin 1–7 by intracerebroventricular infusion was associated with reductions in infarct size and severity of neurological deficits; these effects were blocked by administration of (D-Ala⁷)-angiotensin-(1–7).^{180,181} The neural protective actions of angiotensin 1–7 in brain ischaemia, however, are unlikely to be related to alterations in cerebral blood flow. Rather, they seem to be mediated by an anti-inflammatory effect of angiotensin 1–7, as evidenced by reductions in the expression of inducible nitric oxide synthase and cyclo-oxygenase-2, activation of nuclear factor κ B, oxidative stress, and release of proinflammatory cytokines in the injured brain tissue.^{180–182}

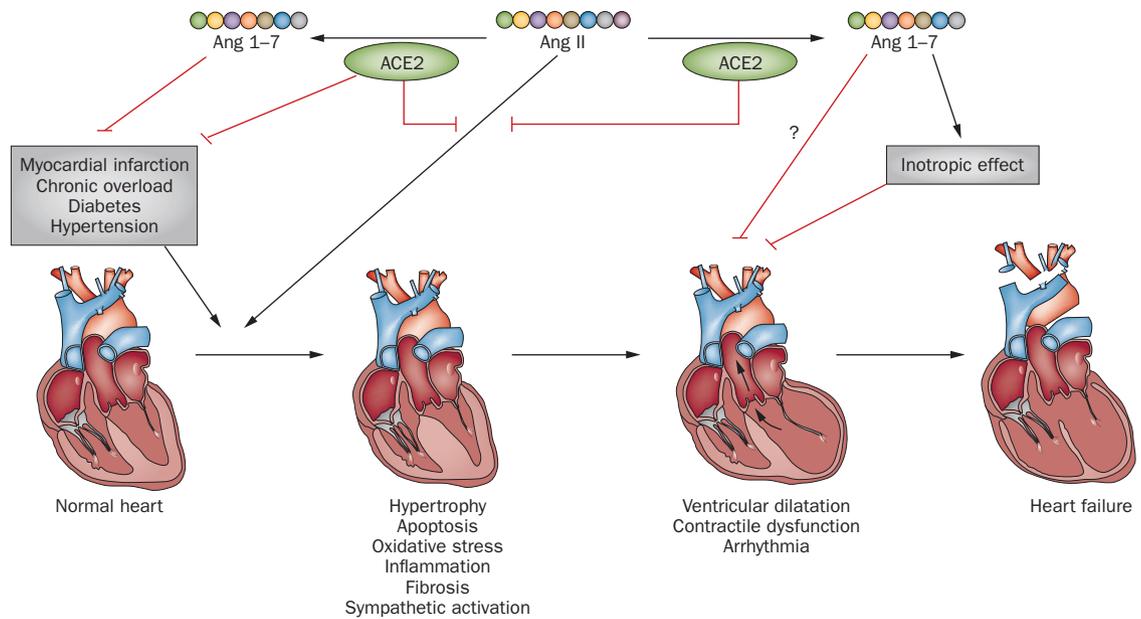


Figure 5 | Potential protective effects of ACE2 and Ang 1–7 on pathological cardiac remodelling and heart failure. Abbreviations: ACE2, angiotensin-converting enzyme 2; Ang, angiotensin.

Diabetes

Accumulating evidence has suggested that the functions of ACE2 and angiotensin 1–7 are affected by diabetes. Additionally, however, ACE2 and angiotensin 1–7 substantially affect glucose metabolism, which suggests they could have potential beneficial effects in patients with diabetes (Figure 6). Circulating ACE2 activity was increased in streptozotocin-treated rats with diabetes and in nonobese mice with diabetes.^{183,184} Likewise, ACE2 activity in serum was increased in humans with type 1 diabetes.¹⁸⁵ Ectopic expression of human ACE2 in the pancreas of young db/db mice was associated with significantly decreased hyperglycaemia, improved islet function, and reduced β -cell apoptosis.¹⁸⁶ These effects were prevented by administration of (D-Ala⁷)-angiotensin-(1–7).¹⁸⁶ Conversely, ACE2 deficiency aggravated impaired glucose tolerance and insulin sensitivity induced by diet.¹⁸⁷ Increased circulating concentrations of angiotensin 1–7, as a result of chronic infusion or transgenic expression, ameliorated hyperinsulinaemia, insulin resistance, and inflammatory responses in adipose tissue in overnourished rats,^{45,188,189} and increased insulin sensitivity and glucose tolerance in normoglycaemic rats.⁴⁷ Moreover, in a rat model of type 2 diabetes, chronic oral treatment with an angiotensin 1–7 formula induced a substantial hypoglycaemic effect, improved insulin sensitivity, and prevented hyperinsulinaemia.¹⁹⁰

The mechanisms underlying the beneficial actions of ACE2 and angiotensin 1–7 in glucose metabolism could be multifaceted. First, activation of the RAS, especially the local RAS, for instance in skeletal muscle, adipose tissue, and the pancreas, contributes notably to the development of insulin resistance and diabetes.^{1,191} Hence, increased degradation of angiotensin II by ACE2 might be important in glucose metabolism. Second, angiotensin 1–7 can activate the phosphatidylinositol-3-kinase–Akt

pathway^{45,51–55} and thereby facilitate insulin-induced signalling and glucose uptake. Angiotensin 1–7 might also antagonize dysfunction in insulin signalling induced by angiotensin II by preventing serine phosphorylation of insulin receptor substrate 1,⁹⁶ probably via inhibition of activation of MAPK pathways induced by angiotensin II.^{58,60,192} Moreover, evidence suggests that angiotensin 1–7 facilitates glucose uptake by upregulation of glucose transporter type 4 expression, which is the major glucose transporter in humans.¹⁸⁷

ACE2 and angiotensin 1–7 have shown remarkable protective actions against diabetic nephropathy in several studies. Clinical and experimental studies have revealed reduced ACE2 expression in the kidney in the setting of diabetes and associated kidney disease.^{183,193–195} ACE2 inhibition with MLN-4760 worsened glomerular injury in mice with streptozotocin-induced diabetes,²⁹ and *Ace2* knockout accelerated diabetes-induced kidney injuries.^{196,197} Increasing the ACE2 function in Akita mice with diabetes by systemic treatment with recombinant ACE2, however, ameliorated albuminuria and prevents glomerular proliferation, fibrosis, and oxidative stress.¹⁹⁸ In rats with diabetes, global overexpression of ACE2 has produced remarkable renal protection similar to that induced by the ACE inhibitor benazepril, but no synergistic actions between ACE inhibition and ACE2 have been observed.¹⁹⁹ Local overexpression of ACE2 in renal podocytes also seems to prevent kidney injuries in mice with diabetes.²⁰⁰ Additionally, chronic systemic treatment with angiotensin 1–7 reduced the risk of renal fibrosis, oxidative stress, and inflammation in the Zucker obese rat model of type 2 diabetes.²⁰¹

The gut

ACE2 is highly expressed in gut tissues, at least in rodents.²⁰² ACE2 in the gut has been shown to have a

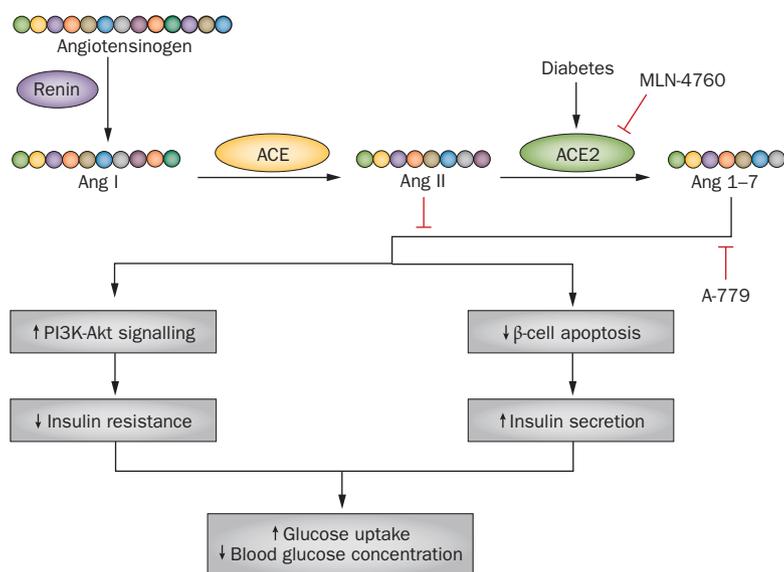


Figure 6 | Beneficial effects of ACE2 and Ang 1-7 on insulin resistance and diabetes. The conversion of Ang II to Ang 1-7 by ACE2 is increased in type 1 and in type 2 diabetes. Ang 1-7 increases insulin sensitivity and glucose uptake through activation of PI3K–Akt signalling and reduction of pancreatic β -cell apoptosis. Blockade of the effects of ACE2 by MLN-4760, or Ang 1-7 by A-779, might reverse the metabolic effects of ACE2 and Ang 1-7 and thereby worsen vascular complications. Abbreviations: ACE, angiotensin-converting enzyme; Ang, angiotensin; PI3K, phosphatidylinositol 3-kinase.

critical role in the regulation of intestinal innate immunity, amino acid homeostasis, and the ecology of the gut microbiota.²⁰³ ACE2 deficiency results in increased susceptibility to intestinal inflammation induced by epithelial damage.²⁰³ Of note, some evidence suggests that metabolism of dietary components by the intestinal microbiota might also have effects on the cardiovascular health,²⁰⁴ which raises the possibility that ACE2 expressed in the gut contributes to vascular protection indirectly by modulating the interactions between gut microbiota and the intestinal epithelium.

Targeted clinical therapies

The success of chemical ACE inhibitors and AT1-receptor blockers in clinical management of various cardiovascular diseases underscores the possibility that searching for chemical modulators that target other active components of RAS could be fruitful. In a first-in-human study, human recombinant ACE2 was administered intravenously to healthy volunteers to assess the pharmacokinetic, pharmacodynamic, and safety profiles of native ACE2.²⁰⁵ Bioavailability of recombinant ACE2 in the human body showed a linear dose-dependent increase and had a terminal elimination half-life of ~10 h. Strikingly, within 30 min of administration of ACE2 at 400 $\mu\text{g}/\text{kg}$ and 1,200 $\mu\text{g}/\text{kg}$, the plasma concentration of angiotensin II dropped and remained suppressed for more than 24 h. The level of angiotensin 1-7 varied in response to different doses of ACE2. Recombinant ACE2 was well tolerated, with no obvious effects on haemodynamic parameters.²⁰⁵ These results also highlight the possibility that, in humans, the potential cardiovascular protective actions of raised

ACE2 concentrations might largely be mediated by the accelerated catabolism of angiotensin II.

The basic pharmacokinetic and safety profiles of native angiotensin 1-7 were mainly obtained from several studies in patients with cancer.^{40,206} Native angiotensin 1-7 causes vasodilatation in healthy individuals and patients with hypertension,¹³¹ but it shows no effect in patients with heart failure.²⁰⁷ Angiotensin 1-7 is continuously catabolized by ACE, NEP, and aminopeptidases; therefore, decreased degradation seems to be a plausible contributor to the beneficial effects of ACE inhibitors.⁴² The value of angiotensin 1-7 as a therapeutic agent could be limited by the very short half-life of this peptide in the body. An ACE-resistant, cyclic angiotensin 1-7 analogue, in which a thioether bridge is introduced to the modified residues 4 and 7 of angiotensin 1-7, has been described.²⁰⁸ The bridged peptide was highly resistant to ACE activity, and showed a substantial (34-fold) reduction in plasma clearance in rats. Moreover, this agent induced a Mas-dependent vasodilator effect in isolated rat aorta.²⁰⁸ Thioether-bridged angiotensin 1-7 is stable at low (2.0) pH and is bioavailable after oral administration in rats.²⁰⁹ In a rat model of myocardial infarction, chronic infusion of thioether-bridged angiotensin 1-7 started 2 weeks after the infarction suppressed myocyte hypertrophy and improved cardiac and vascular functions.²¹⁰

A hydroxypropyl β -cyclodextrin-incorporated angiotensin 1-7 formulation (HP β CD/Ang1-7) designed to overcome the instability of native angiotensin 1-7 in the gastrointestinal tract has been created.²¹¹ Oral treatment with HP β CD/Ang1-7 has shown remarkable cardioprotective effects in rats with myocardial infarction and pathological remodelling after coronary artery occlusion.^{211,212} Moreover, orally administered HP β CD/Ang1-7 was associated with improved lipid metabolism and prevention of hepatic steatosis in mice fed a high-fat diet.²¹³ Similarly, long-term oral HP β CD/Ang1-7 treatment was found to reverse established hyperglycaemia, improve insulin sensitivity, and prevent diabetic nephropathy in a transgenic rat model of insulin resistance and type 2 diabetes.¹⁹⁰

Our understanding of the roles of ACE2 and angiotensin 1-7 is mainly derived from studies of experimental models,^{7,214–217} and evidence for biological actions in humans is sparse. Nevertheless, new drugs or pharmaceutical formulations targeting ACE2 and angiotensin 1-7 are expected to have strong clinical potential to treat cardiovascular diseases, especially those associated with increased activation of the conventional RAS. Research taking various directions,²¹⁸ including modifications of native ACE2 and angiotensin 1-7 and identification of compounds that can specifically modulate the functions of ACE2, is underway. The latter might include small-molecule ACE2 activators and angiotensin 1-7 mimetics. Clearly, randomized, controlled clinical trials are required to assess the therapeutic efficacy and safety of targeted interventions.

Conclusions

Numerous experimental studies have suggested that ACE2 has protective effects in the heart and blood vessels,

primarily by modulating the homeostasis of systemic neurohumoral factors and by improving cardiovascular risk factors. ACE2-mediated catabolism of angiotensin II is likely to have a major role in cardiovascular protection, especially in patients with overactive RAS. Interventions targeting ACE2, therefore, might represent a promising direction in the discovery of novel therapies for cardiovascular disease.

By contrast, the physiological and pharmacological actions of angiotensin 1–7 are complex and even paradoxical in certain circumstances, such as in the kidney. Similarly, the effects of angiotensin 1–7 on blood-pressure regulation are variable. Infusion of angiotensin 1–7 induces either vasodilatation or a null effect in normotensive humans. The effects of angiotensin 1–7 on cardiac dysfunction and remodelling caused by renal disease in rodents are contradictory in different studies. Moreover, angiotensin 1–7 shows biphasic actions on cardiac arrhythmia following myocardial injury. Therefore, more studies, especially controlled clinical trials, are needed to clearly delineate the benefits of angiotensin-1–7-based therapies.

Review criteria

The articles reviewed in this paper were selected from the PubMed database using the search terms: “angiotensin-converting enzyme 2 OR ACE2”, or “angiotensin 1–7” or “Mas”, plus other identifiers, including (but not limited to) “hypertension OR blood pressure”, “atherosclerosis”, “heart OR cardiac remodeling”, “diabetes OR insulin resistance OR hyperinsulinaemia”, and “stroke OR cerebral vascular”. Review articles were filtered by using the search term “review [pt]”. Articles relating to the biochemistry and pharmacology of ACE2/angiotensin 1–7/Mas were searched using terms including “biochemistry”, “inhibitor OR antagonist”, “agonist OR activator”, “binding affinity”, “ligand binding”, or “pharmacokinetics OR pharmacodynamics”. On some occasions, terms including “domain structure”, “domains”, or “crystallography” were also used for a general search in the internet. We applied no restrictions on date of publication or origin of the work, but only articles published in English were included. Most of the papers cited were full-text articles. Selected reference lists from retrieved papers were used to identify further relevant citations. During the revision stage we searched again to update the reference list with the latest published articles.

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Author contributions

All authors participated in literature research and data classification. F.J., J.Y., and C.Z. wrote the manuscript. F.J. and C.Z. reviewed and/or edited the manuscript before submission. F.J., M.D. S.W., Q.Z. and F.F.L. contributed to the creation of artworks.