Not just angiotensinases: new roles for the angiotensin-converting enzymes

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Received: 1 July 2009 / Revised: 27 August 2009 / Accepted: 27 August 2009 / Published online: 10 September 2009
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Abstract The renin-angiotensin system (RAS) is a critical regulator of blood pressure and fluid homeostasis. Angiotensin II, the primary bioactive peptide of the RAS, is generated from angiotensin I by angiotensin-converting enzyme (ACE). A homologue of ACE, ACE2, is able to convert angiotensin II to a peptide with opposing effects, angiotensin-(1-7). It is proposed that disturbance of the balance of ACE and ACE2 expression and/or function is important in pathologies in which angiotensin II plays a role. These include cardiovascular and renal disease, lung injury and liver fibrosis. The critical roles of ACE and ACE2 in regulating angiotensin II levels have traditionally focused attention on their activities as angiotensinases. Recent discoveries, however, have illuminated the roles of these enzymes and of the ACE2 homologue, collectrin, in intracellular trafficking and signalling. This paper reviews the key literature regarding both the catalytic and non-catalytic roles of the angiotensin-converting enzyme gene family.

Keywords ACE · ACE2 · Angiotensin · Signalling · Collectrin

The renin-angiotensin system

The renin-angiotensin system is a critical regulatory cascade which operates at both a systemic, circulatory level and at a local tissue level. The circulatory RAS plays a key role in maintaining cardiovascular function by regulating blood pressure and electrolyte homeostasis. The local tissue RAS, however, is thought to play an important part in regulating vascular tone and the function of major organs such as the heart and kidney. In addition, it is becoming increasingly apparent that aberrant functioning of the local tissue RAS contributes to carcinogenesis [1, 2].

The first step in the RAS is the conversion of the glycoprotein angiotensinogen to the decapeptide angiotensin I (Ang I), catalysed by the proteolytic prorenin-renin system [3]. Ang I is subsequently converted to the octapeptide, angiotensin II (Ang II), by angiotensin converting enzyme (ACE). Other enzymes, such as chymase, are also involved in this conversion in the local tissue RAS but will not be considered further in this review [4]. Ang II is a major bioactive peptide which mediates its effects by binding to two G protein-coupled receptors, AT1 and AT2, triggering downstream signalling cascades. Binding of Ang II to the AT1 receptor triggers pathways regulating vascular tone and cellular proliferation and migration; in addition, it influences pressor and tachycardic responses. It is also thought to play a role in the mitogenic effects of Ang II in tumour cells. The effects of Ang II binding to the AT2 receptor are less well-characterised but are thought generally to oppose those mediated by the AT1 receptor. It has recently become apparent that the conversion of Ang II to angiotensin-(1-7) (Ang 1-7) by the ACE homologue, angiotensin-converting enzyme 2 (ACE2), plays a critical role in regulating its activity. The heptapeptide Ang 1-7,
also formed by the action of a distinct metallopeptidase, neprilysin, on Ang I, is thought to oppose the actions of Ang II by binding to the Mas receptor and triggering signalling pathways leading to apoptosis, vasodilation and reduced proliferation [5].

**Angiotensin-converting enzyme**

ACE is an evolutionarily conserved zinc-metallopeptidase, with orthologues known to exist in organisms as diverse as *D. melanogaster*, *C. elegans* and bacteria [6, 7]. In vertebrates, it exists as two isoforms with distinct tissue distributions. Somatic ACE (sACE) is composed of two homologous catalytic domains each containing the HEMGH zinc-dependent active site motif and is expressed on the surface of endothelial and epithelial cells in a wide variety of tissues. Germinal ACE (gACE), by way of contrast, is composed of only a single catalytic domain, is transcribed from a distinct promoter and is confined in its expression to the testes, where it plays an essential role in fertility. The basis for the role of gACE in fertility has yet to be established; it has been suggested that it is not the dipeptidase activity of ACE that is responsible for its role in fertility but, rather, another intrinsic catalytic activity of ACE: its apparent ability to hydrolyse and release from membranes glycosylphosphatidylinositol (GPI)-anchored proteins [8, 9]. However, other independent studies both in cellular and animal models have cast substantial doubt on the ability of ACE to act as a “GPlase” [10, 11]. Both isoforms of ACE are type-I transmembrane glycoproteins with an extracellular amino-terminal ectodomain and short intracellular cytoplasmic tail (Fig. 1). This membrane localisation ideally positions them to hydrolyse peptides in the extracellular milieu. ACE is able to hydrolyse a wide variety of peptides, acting either as a peptidyl dipeptidase (carboxydipeptidase) in the case of substrates such as Ang I, or as an endopeptidase in the case of substance P or luteinising hormone-releasing hormone [12, 13]. Other physiologically relevant substrates of ACE are bradykinin and the haemoregulatory peptide N-acetyl-SDKP [14]. The two homologous catalytic domains of ACE, termed the N- and C-domains, have somewhat different substrate and inhibitor profiles and can be distinguished by selective inhibitors (N-domain: RXP407; C-domain: RXPA380; [15]). ACE inhibitors have been widely used in the clinic as anti-hypertensive agents with great success; however, the presence of undesirable side-effects (e.g. dry cough, angio-oedema) and continuing escalation of mortality from cardiovascular disease necessitates the development of novel therapeutic agents targeting enzymes of the RAS.

**Angiotensin-converting enzyme 2**

The discovery almost a decade ago of ACE2 by two groups concurrently but using distinct methodologies shed new light on angiotensin metabolism and regulation of the RAS [16, 17]. ACE2, like ACE, is a zinc-metallopeptidase, displaying approximately 42% identity with ACE in its catalytic domain (Fig. 1). However, unlike somatic ACE, ACE2 only contains a single catalytic site and functions as a carboxyynopeptidase, cleaving a single C-terminal residue from peptide substrates. In keeping with its distinct catalytic properties, ACE2 displays quite distinct substrate specificities and inhibitor profiles from those of ACE. Although both enzymes are able to cleave Ang I, the kinetics of this with respect to ACE2 (which would
hydrolyse it to Ang 1-9 are not favourable, making this an unlikely physiological substrate [18]. Unlike ACE, however, ACE2 is able to cleave Ang II to Ang 1-7, suggesting ACE2 may oppose the effects of ACE in the local RAS. ACE2, like ACE, is widely expressed [19] but in humans is only found at high levels in the heart, kidney and testes [17].

A balance of ACEs

Given the opposing effects of ACE and ACE2 with regard to the generation and hydrolysis of the vasopressor and mitogenic peptide Ang II, the balance of the levels of these two enzymes is postulated to be critical in maintaining correct functioning of the local tissue RAS. Many studies have therefore sought to analyse the levels of ACE and ACE2 in pathological conditions in the aetiology of which Ang II plays a role.

In the case of cardiovascular disease, the clinical significance of raised Ang II levels is highlighted by the efficacy of ACE inhibitors (which primarily prevent the formation of Ang II from Ang I), and AT1 receptor antagonists, as antihypertensive agents [20, 21]. The causes of hypertension are complex and multifactorial but generally result in elevated Ang II levels which promote vasoconstriction and renal sodium reabsorption. The importance of Ang II in the pathogenesis of hypertension raises the possibility that ACE2 may have a protective effect by degrading Ang II to Ang 1-7, particularly given the predominantly cardiac and renal expression of ACE2 [17]. Furthermore, the ace2 gene maps to a quantitative trait locus in three rat models of hypertension, and ACE2 expression levels are lower in these animals than in wild-type mice [22], although the significance of this observation is controversial [23]. Data from ACE2 knockout mice are somewhat inconsistent; Gurley et al. [24] showed an increase in blood pressure but no changes in cardiac function whereas Crackower et al. [22] did not detect altered blood pressure but did provide evidence of abnormal cardiac contractility, a defect reversed in mice lacking both ACE and ACE2, suggesting an Ang II-related cause. Attempts to delineate these findings using transgenic mice overexpressing ACE2 have been frustrated by unexpected cardiac arrhythmias in these mice [25]. While the reasons for these findings are unclear, a recent study in which ACE2 was overexpressed in the hearts of adult mice have indicated that this may be a non-specific effect of ACE2 and/or Ang 1-7 overexpression during development [26]. In the latter study, adult mice overexpressing cardiac ACE2, which did not display cardiac arrhythmias, were found to be resistant to damage caused by hypertension and Ang II infusion, suggesting a protective role for ACE2 in hypertension. In addition, a recent study has shown, using transgenic mice overexpressing ACE2 in vascular smooth muscle cells, a role for ACE2 in decreasing blood pressure in hypertensive rats [27]. The beneficial effects of ACE2 in cardiovascular disease may not be without caveat, however, as a study by Masson et al. [28] indicated that overexpression of ACE2 in the myocardium of spontaneously hypertensive rats resulted in cardiac fibrosis and the activation of profibrotic signalling cascades. The molecular mechanisms underlying this observation have yet to be established.

In the kidney, the RAS is a critical regulator of fluid and electrolyte homeostasis; elevated expression of both ACE and Ang II are associated with a variety of renal pathologies [29]. Both ACE and ACE2 are expressed on the apical surface of tubular epithelial cells suggesting they may have a role in regulating the interstitial levels of angiotensin peptides [30]. It would be a logical hypothesis that kidney disease may be a result of ACE upregulation and/or ACE2 downregulation resulting in increased Ang II levels and subsequent detrimental effects. In reality, the picture appears more complex than this. A number of studies of ACE2 expression in both diabetic and hypertensive models of renal disease have yielded conflicting data [31–34]; indeed, there exists controversy as to whether Ang II or Ang I is the primary substrate of ACE2 in kidney [35]. Some support for a protective role for ACE2 in renal disease is provided by a study by Oudit et al. [36] which demonstrated that ace2-deficient mice have increased susceptibility to Ang II-induced glomerulosclerosis; the precise role of ACE2 in modulating angiotensin peptide metabolism in the kidney, however, remains to be fully elucidated.

In the lung, Ang II is known to promote the development of pulmonary hypertension and pulmonary fibrosis [37, 38]. ACE levels are known to be increased in the lungs of rat models of pulmonary hypertension, a finding associated with elevated expression of AT1 receptors [39]. Furthermore, a positive association between the presence of the ACE D/D polymorphism, which increases circulating ACE levels, and severity of pulmonary hypertension, has been reported [40]. More recent studies have demonstrated that overexpression or activation of ACE2 can reduce experimentally-induced pulmonary hypertension, suggesting a critical protective role for ACE2 [41, 42]. Increased Ang II levels are also detected in the lungs of animals with experimentally-induced pulmonary fibrosis [43], and the severity of lung injury in these models can be reduced by AT1 receptor antagonism [44] and ACE inhibition [45], suggesting a central role for Ang II in pulmonary fibrogenesis. In acute lung injury, a positive association between the ACE D/D polymorphism and the development and mortality from acute respiratory distress
syndrome (ARDS), the most severe form of injury, has been identified, suggesting a role for the RAS in the development of this frequently fatal disease [46]. Recently, an elegant study by Imai et al. [47] demonstrated a critical role for ACE2 in the development of ARDS. This work revealed that ace2-deficient mice displayed dramatically more severe symptoms of ARDS, an effect attenuated by concomitant ablation of ACE expression or administration of AT1 receptor antagonists. The balance of ACE/ACE2 function may therefore be critical in the aetiology of lung disease.

Ang II has long been known to promote hepatic fibrogenesis by stimulating the release of pro-fibrotic cytokines and influencing extracellular matrix remodelling in response to tissue injury. Recently, it was shown that ACE2 levels are elevated in an animal model of obstructive biliary disease and in liver from patients suffering from liver cirrhosis [48]. It is postulated that ACE2 may therefore protect against the development of chronic liver disease by attenuating local Ang II levels and that the local, counterbalancing actions of ACE and ACE2 are critical in this regard [49, 50].

In recent years, a number of groups have investigated the role of ACE2, and that of the RAS in general, in the central nervous system (CNS). It has been known for some time that the local RAS in the brain is involved in regulating blood pressure, and that changes in the levels of components of the brain RAS are associated with hypertension [51–53]. The angiotensin metabolite, Ang III, generated from Ang II by aminopeptidase A, may also be an important effector peptide of the brain RAS, and hence inhibitors of this enzyme could constitute novel antihypertensive agents [54, 55]. Although not identified in the CNS in initial studies [17], more detailed analysis has revealed the presence of ACE2 in the brain, not only in the endothelial and smooth muscle cells of blood vessels [56] but also in neurons in the cardio-respiratory centre of the brainstem and, in mice, throughout the brain [57]. Indeed, a recent study by Elased et al. [58] revealed significantly higher levels of ACE2 than ACE activity in mouse CNS; it should be noted, however, that this is in contrast with the findings of Harmer et al. [59] who detected higher levels of ACE than ACE2 in human brains. Overexpression of ACE2 in the brain of mice reduced the pressor and drinking responses to Ang II administration and was associated with downregulation of the AT1 receptor [60]. Furthermore, overexpression of ACE2 in the CNS of hypertensive mice reduced blood pressure and drinking responses [61]. In the same mice, administration of AT1 receptor antagonists increased ACE2 expression and normalised blood pressure [61]. Together, these results suggest an important role for ACE2 in CNS-regulation of blood pressure. The CNS-specific effects of ACE2 may not be confined to its action on Ang II; ACE2 is known to cleave a number of non-RAS peptides with roles in blood pressure regulation by the CNS such as neurotensin and apelin-13 [62]. Intriguingly, Doobay et al. [57] demonstrated nuclear localisation of ACE2 in neurons in areas of the brain involved in cardiovascular regulation, hinting at the possibility of non-catalytic regulatory roles for ACE2 in the CNS.

Angiotensinases as signalling molecules

Whilst the majority of the physiological and pathophysiological effects of ACE are due to its processing of a variety of substrates, a number of observations suggested that ACE may also have effects not easily ascribed to its catalytic functions. The ability of ACE inhibitors to influence the functioning of the B2 kinin receptor and potentiate the effects of ACE resistant kinin analogues [63, 64] suggested that ACE may have non-catalytic roles. This hypothesis is strengthened by the observation that the cytoplasmic tail of ACE can be phosphorylated on specific serine residues in response to inhibitor binding, suggesting a possible outside-in signalling role [65]. Subsequently, it was demonstrated that inhibitor-mediated ACE phosphorylation triggers JNK (c-Jun NH2-terminal kinase) and CK2 (casein kinase-2) activation and c-jun phosphorylation leading to increased expression of ACE itself and of cyclooxygenase-2 (COX-2) in endothelial cells (Fig. 2) [66], and of cellular retinol binding protein (CRBP1) and adiponectin in adipocytes [67], a cascade triggered by ACE dimerisation [68].

In addition to modulating intracellular signalling events by directly activating kinases, phosphorylation of the ACE cytoplasmic tail is also known to influence shedding of its ectodomain, possibly by perturbing its interaction with calmodulin, a ubiquitous calcium binding protein which itself has signalling functions. Ectodomain shedding is a common mechanism by which the biological functions of a variety of cell surface proteins, including enzymes, growth factors, cytokines and molecules involved in neurodegeneration, are modified by their release into the extracellular milieu by other proteinases, commonly members of the MMP (matrix metalloproteinase) or ADAM (a disintegrin and metalloproteinase) families. Although the physiological role of the shed ACE remains to be fully established, a conditional knockout model in which ACE was ablated from vascular endothelium but retained in the circulation displayed no overt phenotype, suggesting circulating ACE may have an important role in maintaining cardiovascular homeostasis. Intriguingly, many proteins which are subject to ectodomain shedding are also cleaved within the membrane, releasing an intracellular fragment, a process known as regulated intramembrane proteolysis (RIP). It is
becoming increasingly apparent that these intracellular domains (ICDs) themselves often have signalling and gene regulatory functions, for example in the well-characterised case of the Alzheimer’s amyloid precursor protein (APP) [69]. Whether such a mechanism exists for ACE or ACE2 remains to be established.

The ability of ACE2 to act as an outside-in signalling molecule also requires investigation. It is known that the intracellular domain of ACE is known to dimerise, become phosphorylated and associate with CK2 (casein kinase-2) in response to inhibitor binding, triggering downstream signalling events involving JNK (c-Jun NH₂-terminal kinase) and c-Jun and culminating in transcriptional upregulation of ACE and COX-2 (cyclooxygenase-2) in endothelial cells. In adipocytes, inhibitor binding to ACE results in upregulation of CRBP (cellular retinol binding protein) and adiponectin expression (not shown). A role for ACE2 in intracellular signalling modulation is unclear.

Further evidence that ACE family members exert non-catalytic functions is seen in invertebrate species that lack one or more critical catalytic residues and are therefore enzymically incompetent. For example, the only ACE-like protein in the C. elegans genome, ACN-1 (ACE-like non-metallopeptidase), completely lacks the zinc motif HExxH of the MA clan of metallopeptidases, as well as some other key active site residues [76]. Nevertheless, this protein plays a key role in moulding and morphogenesis in the nematode, although the mechanisms involved are unknown. Four of the six ACE-like genes in D. melanogaster (Ace-2, -3, -4, -5) also lack one or more critical active site residues suggesting that their physiological functions reside in their residual peptide-binding capacity or ligand-independent cell signalling events [77]. A third ACE-like gene is also present in several mammalian genomes and has been termed ACE3 [78]. In the human, ACE3 appears to be a pseudogene but in some other species (cow, dog, rat) the gene is expressed but the catalytic glutamate in the HExxH motif is replaced by a glutamine [78].

ACE2’s double life as a virus receptor

In 2003, a hitherto undocumented and frequently fatal respiratory illness, causing atypical pneumonia and ARDS, emerged in south Asia and spread globally. This infection, termed severe-acute respiratory syndrome (SARS), was caused by a novel pathogenic coronavirus, SARS-CoV. Research into this pathogen proceeded at a breathtaking pace and a study by Li et al. [79] quickly identified ACE2 as its cellular receptor in vitro, a finding subsequently confirmed in vivo [80]. Structural studies indicate that SARS interacts with ACE2 via trimers of its spike protein which extend into a hydrophobic pocket [81]; the acquisition of a single mutation within the loop extending into this hydrophobic region is thought to have conferred on the virus the ability to cross from civets, believed to be the source of the infection, to humans [82]. As well as functioning as the receptor for the SARS virus, ACE2 is also proposed to play a critical role in its pathogenicity. Binding of SARS-CoV or its spike protein alone leads to down-regulation of ACE2 expression on the cell surface, most likely by a combination of increased internalisation and ectodomain shedding, producing locally elevated Ang II levels which contribute to the development of ARDS [80]. This hypothesis is given credence by the finding that AT1
receptor antagonists can ameliorate SARS-induced ARDS and that administration of spike protein to ace2 null mice with experimentally-induced ARDS does not worsen symptoms [80].

**ACE2 and its homologue collectrin: molecular chaperones**

In 2001, Zhang et al. [83] identified a protein upregulated in a rat model of partial nephrectomy which they termed collectrin (also known as TMEM27). A protein of unknown function, it excited interest due to its significant homology to the cytoplasmic tail of ACE2. Like its homologue ACE2, collectrin is also a type 1 transmembrane protein, which is subject to ectodomain shedding [84], but it has only a short extracellular domain, lacks any catalytic residues, and has no homology to ACE (Fig. 1).

ACE2, therefore, appears to resemble a chimera of an ACE-like catalytic domain and a collectrin C-terminal domain. Originally thought to be restricted to the kidney [83], collectrin expression has subsequently been detected within the pancreas, liver and brain, and has been implicated in both kidney and pancreatic development, where it is expressed during gestation and the neonatal period [84]. Apkinar et al. [84] also observed a potential role for collectrin during cellular proliferation by the observation of increased pancreatic cell mass in mice overexpressing collectrin. However, this change in pancreatic morphology has not been reported by all groups [85, 86]. The subsequent discovery by Zhang and colleagues that siRNA knockdown of collectrin resulted in absent or stunted primary cilia may imply another role for collectrin in kidney homeostasis [87].

The function of collectrin remained enigmatic for five years until Danilczyk et al. [88] made a serendipitous discovery. When storing the urine of collectrin knockout mice at 4°C, the group observed the formation of crystal structures, which upon high performance liquid chromatography (HPLC) analysis were revealed to be composed of tyrosine and phenylalanine [88]. Further investigation revealed a downregulation of members of the B0 family of solute carriers and excitatory amino-acid carrier 1 (EAAC1) in collectrin knockout mice, which exhibited a decrease in glutamine and phenylalanine uptake within the proximal tubule, and aminoaciduria [88]. Investigation of the role of collectrin in regulation of amino acid transporters revealed that there was no difference in transporter transcription rate between wild-type and collectrin knockout mice [86]. However, the amount of the Na+ dependent neutral amino acid transporter B0AT1 (and less predominantly the heterodimeric cystine transporters rBAT and B0–AT) which reached the plasma membrane was decreased [86]. Intracellular sequestering of EAAC1 was also evident from western blot analysis of collectrin-deficient mice [86]. These results were further supported by co-immunoprecipitation (co-IP) analysis which showed an association between collectrin and B0AT1 [88, 89]. In addition, it was recently shown that collectrin is necessary for the membrane trafficking of B0AT3, a homologue of B0AT1 [90]. Cumulatively, these results imply that collectrin exhibits a non-covalent association with certain amino acid transporters within proximal tubule cells where it regulates transporter expression on the plasma membrane. Recently, immunoblotting has also indicated a possible involvement of collectrin in vesicle trafficking where it shows association with SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor), actin-myosin II-A and polycystin-2-polaris complexes [87].

Intracellular sequestering of B0AT1 results in a loss of neutral amino acids characterised in Hartnup disease [91]. Hartnup disorder is caused by an R240Q mutation on the outer edge of B0AT1 [92] resulting in its failure to reach the plasma membrane. This causes a defect in amino acid uptake in the kidney and small intestine. It is therefore possible that the R240Q mutation disrupts an interaction required for correct trafficking to the plasma membrane. B0AT1 has recently also been shown to co-immunoprecipitate with ACE2 in the small intestine, where collectrin is not present. In addition, ACE2 knockout mice showed a 10-fold decrease in the activity of B0AT1 [89]. Combination of surface biotinylation and electrophysiology recording from ACE2 injected *X. laevis* oocytes showed an increased B0AT activity through increased surface expression [92]. This group, however, failed to co-immunoprecipitate the two proteins, which they suggest is due to weak complex formation [92]. Kowalczyk and colleagues [92] also showed an increased leucine uptake in the presence of ACE2, which was abolished by the R240Q mutation. Taken together, these data suggest that in Hartnup disease aminoaciduria results from failure of B0AT to reach the plasma membrane which is in turn due to loss of ACE2/collectrin accessory complex formation (Fig. 3). These observations imply that the closely related cytoplasmic domains of ACE2 and collectrin do mediate similar complex formation and physiological functions albeit in different tissues and may explain the absence of a disease phenotype within collectrin knockout mice by suggesting a compensatory role for ACE2 [87].

**Collectrin and insulin secretion**

Two groups simultaneously identified collectrin as a transcriptional target for hepatocyte nuclear factor α
Na\textsuperscript{+} channel, ENaC, and Na\textsuperscript{+}/K\textsuperscript{+} ATPase-\alpha 1 in Wistar-Kyoto (WKY) rats compared to spontaneously hypertensive rats (SHR) [95]. The identification of a putative calmodulin binding domain within collectrin suggests a possible role in calcium signalling, supporting the evidence for a role in exocytosis and insulin secretion [93, 95].

**Fig. 3** ACE2 is a critical regulator of amino acid transporter function in small intestine. ACE2 regulates the function of the amino acid transporters B\textsuperscript{0}AT1 and B\textsuperscript{0}AT3 in the small intestinal enterocyte brush border. Mutations in a number of residues in the B\textsuperscript{0}AT1 transporter block the association between ACE2 and the transporter and lead to the amino acid uptake defect observed in human Hartnup disorder. The ACE2 homologue, collectrin, carries out this role in the tubular cells of the kidney and is similarly influenced by mutations in B\textsuperscript{0}AT1 (not shown in this diagram).

Conclusions and perspectives

The discovery of ACE2 in 2000 uncovered hitherto unidentified complexity in the regulation of angiotensin peptide metabolism and triggered renewed interest in developing novel pharmacological agents targeting the RAS. Subsequent studies have identified non-catalytic roles for ACE2 and its homologue, collectrin, which have provided new insight into diseases of the kidney and intestinal tract and the pathogenicity of the SARS virus. In addition, identification of new signalling functions for an old enzyme, ACE, have begun to elucidate additional mechanisms underlying the actions of ACE inhibitors used globally in the clinic to treat cardiovascular disease. This may aid the development of more effective anti-hypertensive drugs with fewer side effects. Further study is required to understand fully the molecular mechanisms of the non-catalytic roles of the angiotensinsases and collectrin, and those resulting in the observed changes in the expression levels of both ACE and ACE2 in a variety of diseases. Investigation of the physiological functions of the little studied non-catalytic ACE species in *D. melanogaster*, the *C. elegans* ACN-1, or rodent ACE3 could help to unravel the mechanisms underlying the novel signalling actions of the ACE family.

Acknowledgments We thank the U.K. Biotechnology and Biological Sciences Research Council, the Medical Research Council and the British Heart Foundation for support.

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