Cell. Mol. Life Sci. (2010) 67:89–98
DOI 10.1007/s00018-009-0152-x

Cellular and Molecular Life Sciences

REVIEW

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

Daniel W. Lambert · Nicola E. Clarke · Anthony J. Turner

Received: 1 July 2009 / Revised: 27 August 2009 / Accepted: 27 August 2009 / Published online: 10 September 2009

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 focussed 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

D. W. Lambert
Oral and Maxillofacial Pathology, Faculty of Medicine, Dentistry and Health, University of Sheffield, S10 2TA Sheffield, UK
e-mail: d.w.lambert@sheffield.ac.uk

N. E. Clarke · A. J. Turner (✉)
Institute of Molecular and Cellular Biology, Faculty of Biological Sciences, University of Leeds, LS2 9JT Leeds, UK
e-mail: a.j.turner@leeds.ac.uk

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, AT_1 and AT_2, triggering downstream signalling cascades. Binding of Ang II to the AT_1 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 AT_2 receptor are less well-characterised but are thought generally to oppose those mediated by the AT_1 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 "GPIase" [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.

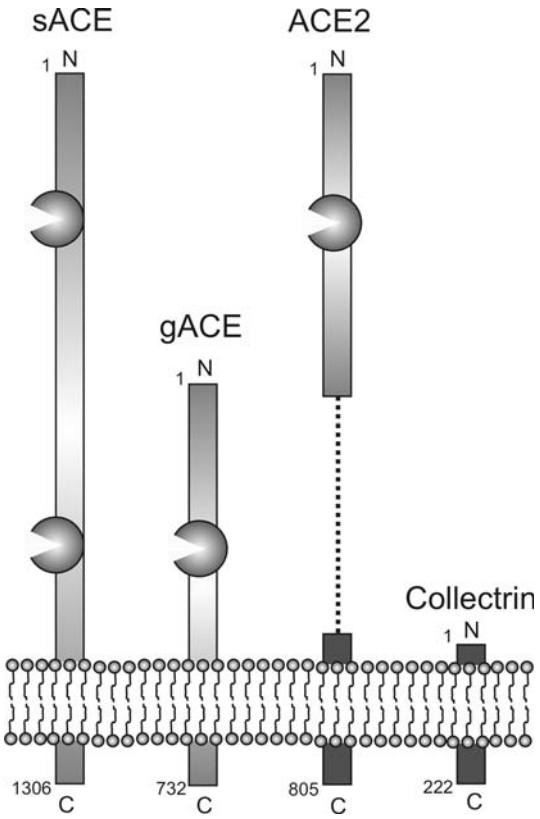

Fig. 1 Membrane topology and homology between ACE, ACE2 and its homologue collectrin. The ACE isoforms somatic ACE (sACE) and germinal ACE (gACE), ACE2 and its homologue collectrin, are type I transmembrane proteins with an intracellular C-terminal domain and an extracellular N-terminal domain. In the case of the ACE isoforms and ACE2, the N-terminal extracellular domains contain HEMGH zinc-dependent catalytic domains (denoted as 'Pacman' symbols); two in ACE and one in both gACE and ACE2. Collectrin contains no catalytic residues. Germinal ACE is entirely homologous to the C-terminal domain of sACE except for an *O*-glycosylated region at its N-terminus. ACE2 shares homology in its ectodomain with the N-terminal domain of sACE but has no homology with its C-terminal cytoplasmic domain; instead, it shares a number of residues with the intracellular domain of collectrin

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 carboxymonopeptidase, 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 vasopressive 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 AT_1 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 AT_1 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 AT_1 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 AT_1 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 AT_1 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 NH_2-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

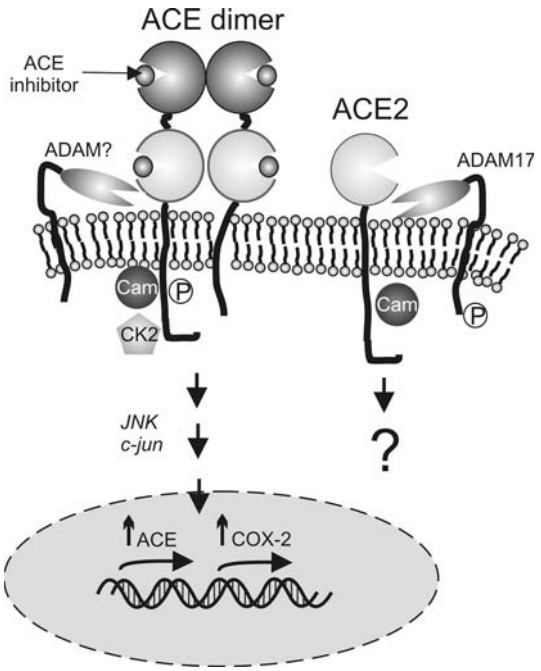

Fig. 2 ACE and ACE2 as outside-in signalling molecules. Both ACE and ACE2 are subject to regulated ectodomain shedding involving members of the ADAM family of proteinases. The identity of the ACE sheddase remains to be established. Shedding of both enzymes is regulated by calmodulin (Cam) and may generate intracellular signals. 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 NH_2-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

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 cytoplasmic domain contains a number of putative phosphorylation sites and interacts with calmodulin (Fig. 2). ACE2 is also subject to ectodomain shedding by a member of the ADAM family of proteinases, ADAM17, a process regulated by phorbol esters and, as for ACE, influenced by interaction with calmodulin [70–72]. Ironically, the identity of the ACE sheddase has still not been identified and is distinct from either ADAM 10 or 17 [73]. In addition, ACE2 has been shown to interact with a critical fibronectin receptor, integrin $\beta 1$, in the failing human heart [74]. Integrin $\beta 1$ is involved in a number of regulatory signalling cascades (for review, see [75]) and has a role in the pathogenesis of a number of diseases including those of the cardiovascular system. The ability of ACE2 to modulate integrin signalling is currently under investigation.

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 HE*XX*H of the MA clan of metallopeptidases, as well as some other key active site residues [76]. Nevertheless, this protein plays a key role in moulting and morphogenesis in the nematode, although the mechanisms involved are unknown. Four of the six ACE-like genes in *D. melanogaster* (Ance-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 HE*XX*H 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 chimaera 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 B^0 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 B^0AT1 (and less predominantly the heterodimeric cystine transporters rBAT and $b^{0+}AT$) 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 B^0AT1 [88, 89]. In addition, it was recently shown that collectrin is necessary for the membrane trafficking of B^0AT3, a homologue of B^0AT1 [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 B^0AT1 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 B^0AT1 [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. B^0AT1 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 B^0AT1 [89]. Combination of surface biotinylation and electrophysiology recording from ACE2 injected *X. laevis* oocytes showed an increased B^0AT 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 B^0AT 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

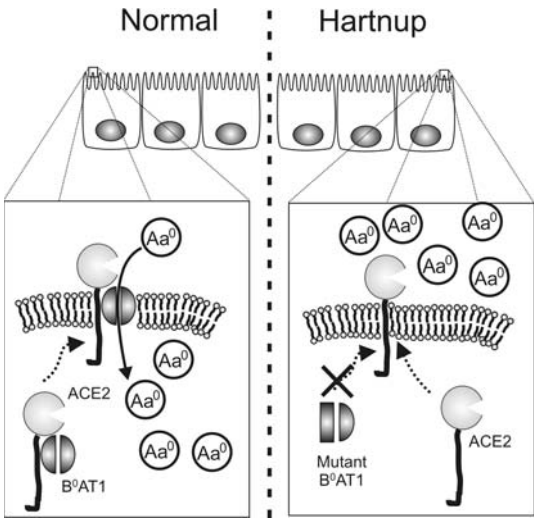

Fig. 3 ACE2 is a critical regulator of amino acid transporter function in small intestine. ACE2 regulates the function of the amino acid transporters B^0AT1 and B^0AT3 in the small intestinal enterocyte brush border. Mutations in a number of residues in the B^0AT1 transporter block the association between 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^0AT1 (not shown in this diagram)

(HNF-1α) [84, 93]. HNF-1α is known to be mutated in maturity onset diabetes in the young (MODY) [94]. To this end, a regulatory role for collectrin in the pancreas has been explored [84, 93]. Within the pancreas, collectrin exists as a dimer [84]. Studies in vitro by Fukui et al. [93] using siRNA knockdown of collectrin resulted in a reduction of insulin exocytosis in insulin-secreting INS-1 cells. In vivo, overexpression of collectrin under a rat promoter led to significant increases in insulin secretion [93]. However, knockout mice revealed no difference in insulin secretion from wild-type, only a decrease in insulin sensitivity [85].

Collectrin was therefore implicated in the insulin secretory pathway through an association between collectrin and snapin, part of the SNARE complex [87, 93, 95]. The role of collectrin in renal hypertension has been demonstrated by investigating salt-induced upregulation of ion/water channels [95]. A high salt diet increased expression of collectrin and the rate of vesicle to membrane trafficking of aquaporin-2, α ENac (an epithelial Na^+ channel), H^+ATPase and Na^+/K^+ ATPase-α 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].

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 angiotensinases 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.

References

1. Lever AF, Hole DJ, Gillis CR, McCallum IR, McInnes GT, MacKinnon PL, Meredith PA, Murray LS, Reid JL, Robertson JW (1998) Do inhibitors of angiotensin-I-converting enzyme protect against risk of cancer? Lancet 352:179–184
2. Khakoo AY, Sidman RL, Pasqualini R, Arap W (2008) Does the renin-angiotensin system participate in regulation of human vasculogenesis and angiogenesis? Cancer Res 68:9112–9115
3. Nguyen G, Danser AH (2008) Prorenin and (pro)renin receptor: a review of available data from in vitro studies and experimental models in rodents. Exp Physiol 93:557–563
4. Lambert DW, Hooper NM, Turner AJ (2008) Angiotensin-converting enzyme 2 and new insights into the renin-angiotensin system. Biochem Pharmacol 75:781–786
5. Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SV, Lopes MT, Bader M, Mendes EP, Lemos VS, Campagnole-Santos MJ, Schultheiss HP, Speth R, Walther T (2003) Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci USA 100:8258–8263
6. Riviere G, Michaud A, Corradi HR, Sturrock ED, Ravi Acharya K, Cogez V, Bohin JP, Vieau D, Corvol P (2007) Characterization of the first angiotensin-converting like enzyme in bacteria: ancestor ACE is already active. Gene 399:81–90
7. Macours N, Poels J, Hens K, Francis C, Huybrechts R (2004) Structure, evolutionary conservation, and functions of angiotensin- and endothelin-converting enzymes. Int Rev Cytol 239:47–97

8. Kondoh G, Tojo H, Nakatani Y, Komazawa N, Murata C, Yamagata K, Maeda Y, Kinoshita T, Okabe M, Taguchi R, Takeda J (2005) Angiotensin-converting enzyme is a GPI-anchored protein releasing factor crucial for fertilization. Nat Med 11:160–166
9. Kondoh G, Watanabe H, Tashima Y, Maeda Y, Kinoshita T (2009) Testicular angiotensin-converting enzyme with different glycan modification: characterization on glycosylphosphatidylinositol-anchored protein releasing and dipeptidase activities. J Biochem 145:115–121
10. Leisle L, Parkin ET, Turner AJ, Hooper NM (2005) Angiotensin-converting enzyme as a GPIase: a critical reevaluation. Nat Med 11:1139–1140
11. Fuchs S, Frenzel K, Hubert C, Lyng R, Muller L, Michaud A, Xiao HD, Adams JW, Capecchi MR, Corvol P, Shur BD, Bernstein KE (2005) Male fertility is dependent on dipeptidase activity of testis ACE. Nat Med 11:1140–1142
12. Skidgel RA, Engelbrecht S, Johnson AR, Erdos EG (1984) Hydrolysis of substance p and neurotensin by converting enzyme and neutral endopeptidase. Peptides 5:769–776
13. Skidgel RA, Erdos EG (1985) Novel activity of human angiotensin I converting enzyme: release of the NH2- and COOH-terminal tripeptides from the luteinizing hormone-releasing hormone. Proc Natl Acad Sci USA 82:1025–1029
14. Rieger KJ, Saez-Servent N, Papet MP, Wdzieczak-Bakala J, Morgat JL, Thierry J, Voelter W, Lenfant M (1993) Involvement of human plasma angiotensin I-converting enzyme in the degradation of the haemoregulatory peptide N-acetyl-seryl-aspartyl-lysyl-proline. Biochem J 296:373–378
15. Georgiadis D, Beau F, Czarny B, Cotton J, Yiotakis A, Dive V (2003) Roles of the two active sites of somatic angiotensin-converting enzyme in the cleavage of angiotensin I and bradykinin: insights from selective inhibitors. Circ Res 93:148–154
16. Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, Donovan M, Woolf B, Robison K, Jeyaseelan R, Breitbart RE, Acton S (2000) A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. Circ Res 87:E1–E9
17. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ (2000) A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem 275:33238–33243
18. Rice GI, Thomas DA, Grant PJ, Turner AJ, Hooper NM (2004) Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. Biochem J 383:45–51
19. Gembardt F, Sterner-Kock A, Imboden H, Spalteholz M, Reibitz F, Schultheiss HP, Siems WE, Walther T (2005) Organ-specific distribution of ACE2 mRNA and correlating peptidase activity in rodents. Peptides 26:1270–1277
20. Pals DT, Masucci FD, Denning GS Jr, Sipos F, Fessler DC (1971) Role of the pressor action of angiotensin II in experimental hypertension. Circ Res 29:673–681
21. Carini DJ, Christ DD, Duncia JV, Pierce ME (1998) The discovery and development of angiotensin II antagonists. Pharm Biotechnol 11:29–56
22. Crackower MA, Sarao R, Oudit GY, Yagil C, Kozieradzki I, Scanga SE, Oliveira-dos-Santos AJ, da Costa J, Zhang L, Pei Y, Scholey J, Ferrario CM, Manoukian AS, Chappell MC, Backx PH, Yagil Y, Penninger JM (2002) Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 417:822–828
23. Hamming I, Cooper ME, Haagmans BL, Hooper NM, Korstanje R, Osterhaus AD, Timens W, Turner AJ, Navis G, van Goor H (2007) The emerging role of ACE2 in physiology and disease. J Pathol 212:1–11
24. Gurley SB, Allred A, Le TH, Griffiths R, Mao L, Philip N, Haystead TA, Donoghue M, Breitbart RE, Acton SL, Rockman HA, Coffman TM (2006) Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. J Clin Invest 116:2218–2225
25. Donoghue M, Wakimoto H, Maguire CT, Acton S, Hales P, Stagliano N, Fairchild-Huntress V, Xu J, Lorenz JN, Kadambi V, Berul CI, Breitbart RE (2003) Heart block, ventricular tachycardia, and sudden death in ACE2 transgenic mice with downregulated connexins. J Mol Cell Cardiol 35:1043–1053
26. Der Sarkissian S, Grobe JL, Yuan L, Narielwala DR, Walter GA, Katovich MJ, Raizada MK (2008) Cardiac overexpression of angiotensin converting enzyme 2 protects the heart from ischemia-induced pathophysiology. Hypertension 51:712–718
27. Rentzsch B, Todiras M, Iliescu R, Popova E, Campos LA, Oliveira ML, Baltatu OC, Santos RA, Bader M (2008) Transgenic angiotensin-converting enzyme 2 overexpression in vessels of SHRSP rats reduces blood pressure and improves endothelial function. Hypertension 52:967–973
28. Masson R, Nicklin SA, Craig MA, McBride M, Gilday K, Gregorevic P, Allen JM, Chamberlain JS, Smith G, Graham D, Dominiczak AF, Napoli C, Baker AH (2009) Onset of experimental severe cardiac fibrosis is mediated by overexpression of Angiotensin-converting enzyme 2. Hypertension 53:694–700
29. Mezzano SA, Ruiz-Ortega M, Egido J (2001) Angiotensin II and renal fibrosis. Hypertension 38:635–638
30. Warner FJ, Lew RA, Smith AI, Lambert DW, Hooper NM, Turner AJ (2005) Angiotensin-converting enzyme 2 (ACE2), but not ACE, is preferentially localized to the apical surface of polarized kidney cells. J Biol Chem 280:39353–39362
31. Tikellis C, Bialkowski K, Pete J, Sheehy K, Su Q, Johnston C, Cooper ME, Thomas MC (2008) ACE2 deficiency modifies renoprotection afforded by ACE inhibition in experimental diabetes. Diabetes 57:1018–1025
32. Tikellis C, Cooper ME, Bialkowski K, Johnston CI, Burns WC, Lew RA, Smith AI, Thomas MC (2006) Developmental expression of ACE2 in the SHR kidney: a role in hypertension? Kidney Int 70:34–41
33. Wakahara S, Konoshita T, Mizuno S, Motomura M, Aoyama C, Makino Y, Kato N, Koni I, Miyamori I (2007) Synergistic expression of angiotensin-converting enzyme (ACE) and ACE2 in human renal tissue and confounding effects of hypertension on the ACE to ACE2 ratio. Endocrinology 148:2453–2457
34. Wysocki J, Ye M, Soler MJ, Gurley SB, Xiao HD, Bernstein KE, Coffman TM, Chen S, Batlle D (2006) ACE and ACE2 activity in diabetic mice. Diabetes 55:2132–2139
35. Li N, Zimpelmann J, Cheng K, Wilkins JA, Burns KD (2005) The role of angiotensin converting enzyme 2 in the generation of angiotensin 1–7 by rat proximal tubules. Am J Physiol 288:F353–F362
36. Oudit GY, Herzenberg AM, Kassiri Z, Wong D, Reich H, Khokha R, Crackower MA, Backx PH, Penninger JM, Scholey JW (2006) Loss of angiotensin-converting enzyme-2 leads to the late development of angiotensin II-dependent glomerulosclerosis. Am J Pathol 168:1808–1820
37. Morrell NW, Morris KG, Stenmark KR (1995) Role of angiotensin-converting enzyme and angiotensin II in development of hypoxic pulmonary hypertension. Am J Physiol 269:H1186–H1194
38. Wynn TA (2008) Cellular and molecular mechanisms of fibrosis. J Pathol 214:199–210
39. Chassagne C, Eddahibi S, Adamy C, Rideau D, Marotte F, Dubois-Rande JL, Adnot S, Samuel JL, Teiger E (2000) Modulation of angiotensin II receptor expression during development and regression of hypoxic pulmonary hypertension. Am J Respir Cell Mol Biol 22:323–332

40. Kanazawa H, Okamoto T, Hirata K, Yoshikawa J (2000) Deletion polymorphisms in the angiotensin converting enzyme gene are associated with pulmonary hypertension evoked by exercise challenge in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 162:1235–1238
41. Ferreira AJ, Shenoy V, Yamazato Y, Sriramula S, Francis J, Yuan L, Castellano RK, Ostrov DA, Oh SP, Katovich MJ, Raizada MK (2009) Evidence for angiotensin-converting enzyme 2 as a therapeutic target for the prevention of pulmonary hypertension. Am J Respir Crit Care Med 179:1048–1054
42. Yamazato Y, Ferreira AJ, Hong KH, Sriramula S, Francis J, Yamazato M, Yuan L, Bradford CN, Shenoy V, Oh SP, Katovich MJ, Raizada MK (2009) Prevention of pulmonary hypertension by Angiotensin-converting enzyme 2 gene transfer. Hypertension 54:365–371
43. Marshall RP, Gohlke P, Chambers RC, Howell DC, Bottoms SE, Unger T, McAnulty RJ, Laurent GJ (2004) Angiotensin II and the fibroproliferative response to acute lung injury. Am J Physiol 286:L156–L164
44. Otsuka M, Takahashi H, Shiratori M, Chiba H, Abe S (2004) Reduction of bleomycin induced lung fibrosis by candesartan cilexetil, an angiotensin II type 1 receptor antagonist. Thorax 59:31–38
45. Wang R, Ibarra-Sunga O, Verlinski L, Pick R, Uhal BD (2000) Abrogation of bleomycin-induced epithelial apoptosis and lung fibrosis by captopril or by a caspase inhibitor. Am J Physiol 279:L143–L151
46. Marshall RP, Webb S, Bellingan GJ, Montgomery HE, Chaudhari B, McAnulty RJ, Humphries SE, Hill MR, Laurent GJ (2002) Angiotensin converting enzyme insertion/deletion polymorphism is associated with susceptibility and outcome in acute respiratory distress syndrome. Am J Respir Crit Care Med 166:646–650
47. Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B, Yang P, Sarao R, Wada T, Leong-Poi H, Crackower MA, Fukamizu A, Hui CC, Hein L, Uhlig S, Slutsky AS, Jiang C, Penninger JM (2005) Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 436:112–116
48. Herath CB, Warner FJ, Lubel JS, Dean RG, Jia Z, Lew RA, Smith AI, Burrell LM, Angus PW (2007) Upregulation of hepatic angiotensin-converting enzyme 2 (ACE2) and angiotensin-(1–7) levels in experimental biliary fibrosis. J Hepatol 47:387–395
49. Herath CB, Lubel JS, Jia Z, Velkoska E, Casley D, Brown L, Tikellis C, Burrell LM, Angus PW (2009) Portal pressure responses and angiotensin peptide production in rat liver are determined by relative activity of angiotensin converting enzyme (ACE) and ACE2. Am J Physiol 297:G98–G106
50. Warner FJ, Smith AI, Hooper NM, Turner AJ (2004) Angiotensin-converting enzyme-2: a molecular and cellular perspective. Cell Mol Life Sci 61:2704–2713
51. Gyurko R, Wielbo D, Phillips MI (1993) Antisense inhibition of AT1 receptor mRNA and angiotensinogen mRNA in the brain of spontaneously hypertensive rats reduces hypertension of neurogenic origin. Regul Pept 49:167–174
52. Ambuhl P, Gyurko R, Phillips MI (1995) A decrease in angiotensin receptor binding in rat brain nuclei by antisense oligonucleotides to the angiotensin AT1 receptor. Regul Pept 59:171–182
53. Tamura K, Umemura S, Nyui N, Yamakawa T, Yamaguchi S, Ishigami T, Tanaka S, Tanimoto K, Takagi N, Sekihara H, Murakami K, Ishii M (1996) Tissue-specific regulation of angiotensinogen gene expression in spontaneously hypertensive rats. Hypertension 27:1216–1223
54. Bodineau L, Frugiere A, Marc Y, Inguimbert N, Fassot C, Balavoine F, Roques B, Llorens-Cortes C (2008) Orally active aminopeptidase A inhibitors reduce blood pressure: a new strategy for treating hypertension. Hypertension 51:1318–1325
55. de Mota N, Iturrioz X, Claperon C, Bodineau L, Fassot C, Roques BP, Palkovits M, Llorens-Cortes C (2008) Human brain aminopeptidase A: biochemical properties and distribution in brain nuclei. J Neurochem 106:416–428
56. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis GJ, van Goor H (2004) Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 203:631–637
57. Doobay MF, Talman LS, Obr TD, Tian X, Davisson RL, Lazartigues E (2007) Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system. Am J Physiol 292:R373–R381
58. Elased KM, Cunha TS, Marcondes FK, Morris M (2008) Brain angiotensin-converting enzymes: role of angiotensin-converting enzyme 2 in processing angiotensin II in mice. Exp Physiol 93:665–675
59. Harmer D, Gilbert M, Borman R, Clark KL (2002) Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett 532:107–110
60. Feng Y, Yue X, Xia H, Bindom SM, Hickman PJ, Filipeanu CM, Wu G, Lazartigues E (2008) Angiotensin-converting enzyme 2 overexpression in the subfornical organ prevents the angiotensin II-mediated pressor and drinking responses and is associated with angiotensin II type 1 receptor downregulation. Circ Res 102:729–736
61. Xia H, Feng Y, Obr TD, Hickman PJ, Lazartigues E (2009) Angiotensin II type 1 receptor-mediated reduction of angiotensin-converting enzyme 2 activity in the brain impairs baroreflex function in hypertensive mice. Hypertension 53:210–216
62. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Baronas E, Hsieh F, Acton S, Patane M, Nichols A, Tummino P (2002) Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J Biol Chem 277:14838–14843
63. Minshall RD, Tan F, Nakamura F, Rabito SF, Becker RP, Marcic B, Erdos EG (1997) Potentiation of the actions of bradykinin by angiotensin I-converting enzyme inhibitors. The role of expressed human bradykinin B2 receptors and angiotensin I-converting enzyme in CHO cells. Circ Res 81:848–856
64. Benzing T, Fleming I, Blaukat A, Muller-Esterl W, Busse R (1999) Angiotensin-converting enzyme inhibitor ramiprilat interferes with the sequestration of the B2 kinin receptor within the plasma membrane of native endothelial cells. Circulation 99:2034–2040
65. Kohlstedt K, Brandes RP, Muller-Esterl W, Busse R, Fleming I (2004) Angiotensin-converting enzyme is involved in outside-in signaling in endothelial cells. Circ Res 94:60–67
66. Kohlstedt K, Busse R, Fleming I (2005) Signaling via the angiotensin-converting enzyme enhances the expression of cyclooxygenase-2 in endothelial cells. Hypertension 45:126–132
67. Kohlstedt K, Gershome C, Trouvain C, Hofmann WK, Fichtlscherer S, Fleming I (2009) Angiotensin-converting enzyme (ACE) inhibitors modulate cellular retinol-binding protein 1 and adiponectin expression in adipocytes via the ACE-dependent signaling cascade. Mol Pharmacol 75:685–692
68. Kohlstedt K, Gershome C, Friedrich M, Muller-Esterl W, Alhenc-Gelas F, Busse R, Fleming I (2006) Angiotensin-converting enzyme (ACE) dimerization is the initial step in the ACE inhibitor-induced ACE signaling cascade in endothelial cells. Mol Pharmacol 69:1725–1732
69. Belyaev ND, Nalivaeva NN, Makova NZ, Turner AJ (2009) Neprilysin gene expression requires binding of the amyloid precursor protein intracellular domain to its promoter: implications for Alzheimer disease. EMBO Rep 10:94–100

70. Lambert DW, Clarke NE, Hooper NM, Turner AJ (2008) Calmodulin interacts with angiotensin-converting enzyme-2 (ACE2) and inhibits shedding of its ectodomain. FEBS Lett 582:385–390
71. Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, Hooper NM, Turner AJ (2005) Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). J Biol Chem 280:30113–30119
72. Haga S, Yamamoto N, Nakai-Murakami C, Osawa Y, Tokunaga K, Sata T, Sasazuki T, Ishizaka Y (2008) Modulation of TNF-alpha-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-alpha production and facilitates viral entry. Proc Natl Acad Sci USA 105:7809–7814
73. Allinson TM, Parkin ET, Condon TP, Schwager SL, Sturrock ED, Turner AJ, Hooper NM (2004) The role of ADAM10 and ADAM17 in the ectodomain shedding of angiotensin converting enzyme and the amyloid precursor protein. Eur J Biochem 271:2539–2547
74. Lin Q, Keller RS, Weaver B, Zisman LS (2004) Interaction of ACE2 and integrin beta1 in failing human heart. Biochim Biophys Acta 1689:175–178
75. Moser M, Legate KR, Zent R, Fassler R (2009) The tail of integrins, talin, and kindlins. Science 324:895–899
76. Brooks DR, Hooper NM, Isaac RE (2003) The Caenorhabditis elegans orthologue of mammalian puromycin-sensitive aminopeptidase has roles in embryogenesis and reproduction. J Biol Chem 278:42795–42801
77. Coates D, Isaac RE, Cotton J, Siviter R, Williams TA, Shirras A, Corvol P, Dive V (2000) Functional conservation of the active sites of human and Drosophila angiotensin I-converting enzyme. Biochemistry 39:8963–8969
78. Rella M, Elliot JL, Revett TJ, Lanfear J, Phelan A, Jackson RM, Turner AJ, Hooper NM (2007) Identification and characterisation of the angiotensin converting enzyme-3 (ACE3) gene: a novel mammalian homologue of ACE. BMC Genomics 8:194
79. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426:450–454
80. Kuba K, Imai Y, Rao S, Gao H, Guo F, Guan B, Huan Y, Yang P, Zhang Y, Deng W, Bao L, Zhang B, Liu G, Wang Z, Chappell M, Liu Y, Zheng D, Leibbrandt A, Wada T, Slutsky AS, Liu D, Qin C, Jiang C, Penninger JM (2005) A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. Nat Med 11:875–879
81. Li W, Choe H, Farzan M (2006) Insights from the association of SARS-CoV S-protein with its receptor, ACE2. Adv Exp Med Biol 581:209–218
82. Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, Luo S, Wong SK, Huang IC, Xu K, Vasilieva N, Murakami A, He Y, Marasco WA, Guan Y, Choe H, Farzan M (2005) Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. EMBO J 24:1634–1643
83. Zhang H, Wada J, Hida K, Tsuchiyama Y, Hiragushi K, Shikata K, Wang H, Lin S, Kanwar YS, Makino H (2001) Collectrin, a collecting duct-specific transmembrane glycoprotein, is a novel homolog of ACE2 and is developmentally regulated in embryonic kidneys. J Biol Chem 276:17132–17139
84. Akpinar P, Kuwajima S, Krützfeldt J, Stoffel M (2005) Tmem27: a cleaved and shed plasma membrane protein that stimulates pancreatic beta cell proliferation. Cell Metab 2:385–397
85. Malakauskas SM, Kourany WM, Zhang XY, Lu D, Stevens RD, Koves TR, Hohmeier HE, Muoio DM, Newgard CB, Le TH (2009) Increased insulin sensitivity in mice lacking collectrin, a downstream target of HNF-1α. Mol Endocrinol 23:881–892
86. Malakauskas SM, Quan H, Fields TA, McCall SJ, Yu MJ, Kourany WM, Frey CW, Le TH (2007) Aminoaciduria and altered renal expression of luminal amino acid transporters in mice lacking novel gene collectrin. Am J Physiol 292:F533–F544
87. Zhang Y, Wada J, Yasuhara A, Iseda I, Eguchi J, Fukui K, Yang Q, Yamagata K, Hiesberger T, Igarashi P (2007) The role for HNF-1ß-targeted collectrin in maintenance of primary cilia and cell polarity in collecting duct cells. PLoS ONE 2:e414
88. Danilczyk U, Sarao R, Remy C, Benabbas C, Stange G, Richter A, Arya S, Pospisilik JA, Singer D, Camargo SM (2006) Essential role for collectrin in renal amino acid transport. Nature 444:1088–1091
89. Camargo SM, Singer D, Makrides V, Huggel K, Pos KM, Wagner CA, Kuba K, Danilczyk U, Skovby F, Kleta R, Penninger JM, Verrey F (2009) Tissue-specific amino acid transporter partners ACE2 and collectrin differentially interact with Hartnup mutations. Gastroenterology 136:872–882
90. Singer D, Camargo SM, Huggel K, Romeo E, Danilczyk U, Kuba K, Chesnov S, Caron MG, Penninger JM, Verrey F (2009) Orphan transporter SLC6A18 is renal neutral amino acid transporter B0AT3. J Biol Chem 284:19953–19960
91. Seow HF, Broer S, Broer A, Bailey CG, Potter SJ, Cavanaugh JA, Rasko JEJ (2004) Hartnup disorder is caused by mutations in the gene encoding the neutral amino acid transporter SLC6A19. Nat Genet 36:1003–1007
92. Kowalczuk S, Broer A, Tietze N, Vanslambrouck JM, Rasko JEJ, Broer S (2008) A protein complex in the brush-border membrane explains a Hartnup disorder allele. FASEB J 22:2880–2887
93. Fukui K, Yang Q, Cao Y, Takahashi N, Hatakeyama H, Wang H, Wada J, Zhang Y, Marselli L, Nammo T (2005) The HNF-1 target collectrin controls insulin exocytosis by SNARE complex formation. Cell Metab 2:373–384
94. Sian E (2000) Hepatocyte nuclear factor 1 alpha (HNF-1α) mutations in maturity-onset diabetes of the young. Hum Mutat 16:377–385
95. Yasuhara A, Wada J, Malakauskas SM, Zhang Y, Eguchi J, Nakatsuka A, Murakami K, Kanzaki M, Teshigawara S, Yamagata K (2008) Collectrin is involved in the development of salt-sensitive hypertension by facilitating the membrane trafficking of apical membrane proteins via interaction with soluble n-ethylmaleiamide-sensitive factor attachment protein receptor complex. Circulation 118:2146–2155