



# ACE2 in Brain Physiology and Pathophysiology: Evidence from Transgenic Animal Models

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## Abstract

Angiotensin-converting enzyme 2 (ACE2) is a protein consisting of two domains, the N-terminus is a carboxypeptidase homologous to ACE and the C-terminus is homologous to collectrin and responsible for the trafficking of the neutral amino acid transporter B(0)AT1 to the plasma membrane of gut epithelial cells. The carboxypeptidase domain not only metabolizes angiotensin II to angiotensin-(1–7), but also other peptide substrates, such as apelin, kinins and morphins. In addition, the collectrin domain regulates the levels of some amino acids in the blood, in particular of tryptophan. Therefore it is of no surprise that animals with genetic alterations in the expression of ACE2 develop a diverse pattern of phenotypes ranging from hypertension, metabolic and behavioural dysfunctions, to impairments in serotonin synthesis and neurogenesis. This review summarizes the phenotypes of such animals with a particular focus on the central nervous system.

**Keywords** Angiotensin · Serotonin · Hypertension · SARS · Transgenic mice · Knockout mice

## Introduction

In the last century, the renin angiotensin system (RAS) was thought to just consist of renin, metabolizing angiotensinogen to angiotensin (Ang) I which in turn is cleaved by angiotensin-converting enzyme (ACE) to the active peptide Ang II interacting with two receptors, AT1 and AT2 [1]. Other peptides of the system were known, such as Ang-(1–7), but the enzymes producing them and their receptors were elusive. While only in 2003 the receptor for Ang-(1–7), MAS, was unveiled [2], already in 2000, the main enzyme generating this heptapeptide was discovered, ACE2. ACE2 was cloned twice independently by Anthony Turner's group from

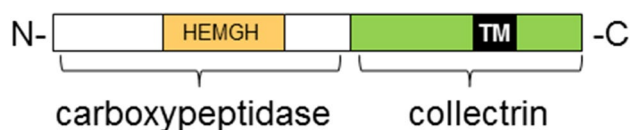
a human lymphoma cDNA library [3] and by a group at the company Millennium using a human heart library [4]. The gene consists of 18 exons and was mapped to the X chromosome (Xp22). ACE2 is an 805 amino acid type-1 transmembrane protein with a molecular weight of ~120 kDa. The amino-terminal domain of ACE2 has approximately 42% sequence identity with ACE, and its cytoplasmic and transmembrane domains show 48% homology to a more recently characterized protein, collectrin (Fig. 1). This, in turn, plays a critical role in the amino acid absorption of the kidney [5, 6]. Obviously, the *Ace2* gene is the result of a fusion of the *Ace* and the collectrin gene early in evolution [7, 8].

ACE2 is an ectoenzyme with an extracellular catalytic domain that predominantly localizes at the plasma membrane [9] and is thereby able to hydrolyze circulating peptides. Like ACE [10], it is also the subject of juxtamembranic cleavage, releasing the catalytically active ectodomain into the extracellular milieu [11]. This process is stimulated by a number of different factors (including phorbol esters) and involves the transmembrane protease ADAM17 [11, 12]. Similar to ACE, the physiological role of ectodomain cleavage of ACE2 is still unclear and the function of the circulating enzyme has not yet been identified. However, cleavage in the brain has been observed in neurogenic hypertension and is thought to be a mechanism for downregulating local ACE2 activity [12–14]. ACE2 is predominantly expressed

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**Fig. 1** Structure of ACE2 with two domains, a carboxypeptidase with homology to ACE at the N-terminus with one active site (HEMGH) and a collectrin-homologous domain at the C-terminus with a transmembrane (TM) domain

in the gastrointestinal tract, heart, kidney, lung and testes [3, 4]. With lower levels of expression, it is also found in the brain [15].

Unlike ACE, ACE2 has only one active site (Fig. 1) and acts as a carboxypeptidase, removing the most carboxy-terminal amino acid from each peptide substrate [3]. In the RAS ACE2 converts Ang I to Ang-(1–9), whose function is still unknown, and Ang II to Ang-(1–7) [16]. However, the affinity of ACE2 for Ang I is much weaker compared to ACE. Furthermore, the catalytic efficiency of ACE2 for Ang II is 400-fold greater than that for Ang I [17]. This suggests that the primary role of ACE2 is to inhibit the action of Ang II by its inactivation and the simultaneous formation of the antagonistic peptide Ang-(1–7) [1, 18, 19].

In addition to the hydrolysis of angiotensin peptides, ACE2 is also capable of cleaving various other peptides mainly with a proline residue at the penultimate position. These include apelin-13 and -36, the kinin metabolites (des-Arg<sup>9</sup>)-bradykinin and (des-Arg<sup>10</sup>)-kallidin, neurotensin and the closely related kinetensin, as well as opioid peptides such as dynorphin A-(1–13) [17]. In contrast to ACE, however, ACE2 has no effect on bradykinin, which underlines its substrate specificity.

More recently a completely independent function of ACE2 based on its collectrin domain was discovered (Fig. 1). It is responsible for the trafficking of the neutral amino acid transporter B(0)AT1 (SLC6A19) to the plasma membrane of gut epithelial cells. Thereby it regulates the uptake of several amino acids and its deficiency leads to significant reductions in tryptophan and glycine in the blood and to an inflammatory bowel disease [20, 21]. Of note, ACE2 was also chosen by the severe acute respiratory syndrome (SARS) coronavirus as receptor for its entry into cells, which represents a third, however solely pathophysiological function of the protein [22, 23].

Several observations suggest a role of ACE2 in the regulation of systemic blood pressure [24, 25]. Thus, an inverse correlation of the *Ace2* mRNA and the protein level with the occurrence of elevated blood pressure was found. In various rat strains with hypertension, such as spontaneously hypertensive rats, spontaneously hypertensive rats prone to stroke or Sabra (salt-sensitive rats prone to hypertension) rats, the *Ace2* gene maps to a quantitative trait locus for hypertension

on the X-chromosome [24]. Furthermore, a reduced *Ace2* transcription and protein expression could be detected in these rat models. These data are a strong indication of the involvement of ACE2 in blood pressure regulation. However, it remains to be elucidated in which tissue and by which mechanism ACE2 exerts its most relevant actions in cardiovascular physiology. As shown below the brain is a most prominent candidate.

In neurochemistry, transgenic and knockout animal models are invaluable tools to study neurohormonal systems, since they reveal effects of changes in single components of these systems on the whole physiology, as has been exemplified for the RAS [26–29]. This is particularly relevant for ACE2 with its multitude of functions and substrates. However, in each animal model with alterations in ACE2 expression the hormonal system involved in the observed phenotype needs to be sorted out. This review will summarize how animals with targeted alterations in ACE2 expression have helped to reveal its physiological and pathophysiological functions in particular in the central nervous system. The same and additional models have also been used to discover that ACE2 is a major determinant in cardiac [24, 30–32] and vascular function [33–35], atherosclerosis [36–39], metabolism [37, 40], kidney [41–44] and lung diseases [45, 46], to only name a few. However, these peripheral actions of ACE2 have previously been reviewed and are therefore not subject of this paper [19, 47–50].

### Transgenic Animal Models for the Study of the Central Actions of ACE2

In several experiments mentioned below, mice with targeted deletion of the *Ace2* gene were employed. Such animals have been independently generated by several groups and different methods including classical homologous recombination in embryonic stem cells [24, 51, 52], TALENs [53] and CRISPR/Cas9 technology [46]. For some of these different mouse models phenotypes have been reported, but the results concerning blood pressure and cardiac dysfunction were not fully consistent [19]. This may be due to the different genetic backgrounds on which the mice were generated. Recently, also ACE2-deficient rats have been generated using TALENs [54]. These animals have however not yet been analyzed for central nervous system alterations, but may be very suitable in the future for this purpose due to the advantages of rats over mice in the analysis of brain physiology and behaviours [55]. Of note, due to the X-chromosomal localization of the *Ace2* gene, male animals with a hemizygous deletion of the gene (*Ace2*<sup>-ly</sup>) are already deficient in the enzyme.

In other studies human ACE2 was overexpressed in all neurons of transgenic mice using the synapsin promoter in either a constitutive [56] or switchable manner by flanking the transgene with loxP sites which allows cell-type specific

ablation of the transgene by Cre recombinase expression [57].

Cell type-specific activation of ACE2 expression was achieved in another transgenic model. To this purpose, the mouse *Ace2* coding region was inserted into the Rosa26 locus with a stop-lox cassette in front of it inhibiting its expression. When a Cre-recombinase expressing mouse is bred with these animals ACE2 will be activated in the cells expressing the Cre-recombinase. Expression of Cre-recombinase in the germline results in ubiquitously ACE2 overexpressing mice [58, 59].

### Central Cardiovascular Regulation

Since ACE2 is a major determinant of Ang II levels, animals with human ACE2 overexpression in the brain exhibited a protective phenotype in several cardiovascular diseases including hypertension, cardiac hypertrophy, and chronic heart failure. When Ang II was infused peripherally by minipumps blood pressure and cardiac hypertrophy were significantly reduced in the central ACE2 overexpressing mice compared to controls [60]. Peripheral Ang II activates neurons in the circumventricular organs with a fenestrated endothelium, which in turn increase central Ang II and elicit cardiovascular effects [61]. This may be the reason that ACE2 overexpression in the brain which reduces central Ang II levels blunts the cardiovascular effects of peripheral Ang II. The same authors also reported that ACE2 overexpression in the brain attenuates the development of deoxycorticosterone acetate (DOCA)–salt hypertension, a neurogenic hypertension model with enhanced brain RAS and sympathetic activity [56]. In this model, ACE2 overexpression in neurons significantly increased nitric oxide (NO) synthase (NOS) and NO levels in the brain and blunted the Ang-II-mediated decrease in NOS expression and sympathetic activity. Moreover increased oxidative stress and cyclooxygenase mediated neuroinflammation were attenuated in transgenic mice [62]. A reduction in sympathetic nerve activity was also observed when the central ACE2 overexpressing mice were subjected to a chronic heart failure model based on permanent coronary artery ligation. This resulted in improved cardiac function in the transgenic animals [63]. Based on the use of a MAS antagonist the authors postulate that the mechanism for these effects involved a shift in the balance from central Ang II–AT1 to Ang-(1–7)/MAS signaling.

Using the model, in which the *ACE2* transgene is flanked by loxP sites the role of the enzyme in the paraventricular nucleus of the hypothalamus and the subfornical organ for the antihypertensive effect after DOCA/salt treatment could be studied. The authors injected Cre-recombinase expressing adeno-associated viruses into these regions and thereby ablated transgenic ACE2 expression only there, while in other areas of the brain it remained unchanged [57]. These

experiments revealed that ACE2 in both regions is important but also other areas contribute to the beneficial effect of the enzyme.

Accordingly, ACE2-deficient mice showed increased oxidative stress in the brain and autonomic dysfunctions compared to controls [64]. After Ang-II infusion and in old animals these changes were exacerbated. When ACE2 expression was restored in *Ace2*<sup>-/-</sup> mice by adenovirus mediated gene transfer into the paraventricular nucleus of the hypothalamus these phenotypes were attenuated [64].

Taken together these results confirm a strong antihypertensive and sympatholytic action of ACE2 in the hypothalamus which is driven by a reduction in Ang II and in increase in Ang-(1–7) levels.

### Stroke and Brain Injury

In a stroke model triggered by middle cerebral artery occlusion ACE2 overexpression in the brain resulted in a decreased stroke volume and improved neurological scores in mice [65, 66]. This was true for single transgenic mice [65] and for mice additionally overexpressing human renin and angiotensinogen with increased Ang II levels in the brain [66]. Infusion of the MAS antagonist A779 abrogated the beneficial effect of the *ACE2* transgene. Moreover, the human RAS transgenic mice showed increased tissue swelling and cell death in a brain slice model with oxygen and glucose deprivation which again could be prevented by additional transgenic expression of ACE2 in neurons [67]. Using MAS antagonists, the authors revealed that ACE2 exerts its beneficial effects in ischemic brain injury by shifting the balance between Ang II and Ang-(1–7) in favor of the latter, thereby reducing local reactive oxygen species production. These results concur with numerous reports showing that the Ang-(1–7)/MAS axis exerts beneficial effects in brain injury and stroke models [67–73].

### SARS

In mice transgenic for ACE2 the brain is a major target organ for SARS infection [74, 75]. The virus enters the brain primarily via the olfactory bulb resulting in rapid, transneuronal spread to other areas of the brain and finally death of the animal [76]. ACE2 is also expressed in human brain, which may be the reason that central nervous system infection by SARS is regularly observed [77].

### Cognition and Memory

ACE2-deficient mice exhibit significant impairment in memory and cognition in the Morris water maze and the Y-maze assays [78]. These deficiencies could be partially rescued by administration of an AT1 receptor antagonist and

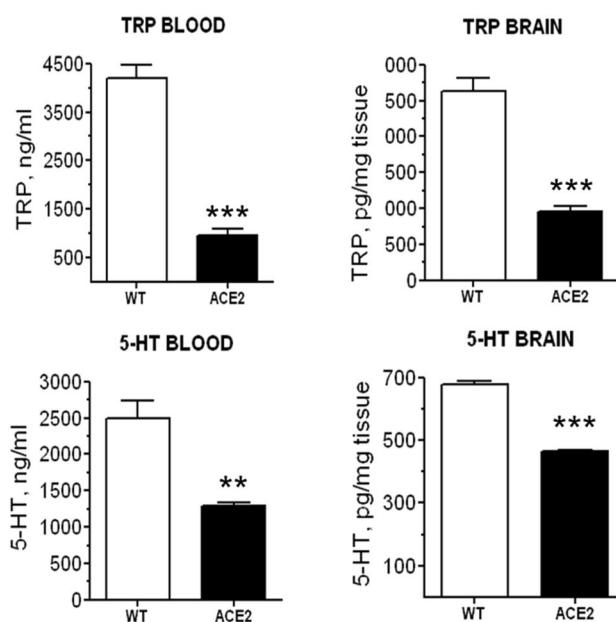
of Ang-(1–7) arguing again for a shift in the balance between Ang II and Ang-(1–7) as major mechanism involved. This leads to a downregulation of brain-derived neurotrophic factor (BDNF) expression and an increased generation of reactive oxygen species, which both contribute to the phenotype [78]. Accordingly, Ang-(1–7) and its receptor MAS has recently been shown to be essential for memory processing in the hippocampus [79].

### Stress Response and Anxiety

Mice which ubiquitously overexpress ACE2 by germline-deletion of a stop-lox cassette were employed to study the effect of the enzyme on stress response and anxiety. Such mice spend more time on the open arms of the elevated plus maze than controls, suggesting reduced anxiety [59]. This effect was probably mediated by the Ang-(1–7)/MAS axis since it was abolished by the MAS antagonist, A779, but the authors have not tested for contributions of other ACE2 substrates or serotonin to the phenotype. Since anxiety is often accompanied by a dysregulation of the hypothalamus–pituitary–adrenal axis involved in stress response, the authors analyzed parameters of this axis in a recent follow-up study [80]. They found reduced plasma corticosterone and proopiomelanocortin (POMC) expression in the pituitary of ubiquitously ACE2 overexpressing mice. In the same study, ACE2 overexpression was restricted to corticotropin releasing hormone (CRH) synthesizing cells by breeding the stop-lox ACE2 animals with a corresponding Cre-recombinase expressing mouse line [80]. Targeted overexpression of ACE2 in these cells resulted in decreased corticosterone response to restraint stress as well as decreased CRH mRNA in the hypothalamus and POMC mRNA in the pituitary. Moreover, these mice also displayed decreased anxiety-like behavior in the elevated plus maze. Together these findings indicate that ACE2 in the hypothalamus suppresses CRH synthesis and thereby the stress response and anxiety-related behavior. The mechanism may again involve the Ang-(1–7)/MAS axis since animal models with alterations in this axis also exhibit changes in anxiety-related behaviours [81–84].

### Serotonin and Neurogenesis

As mentioned above, ACE2-deficient mice present an impaired amino acid uptake in the gut leading to markedly reduced tryptophan levels in the blood [20, 21]. Serotonin is a monoamine that acts as an autacoid in the periphery and as a neurotransmitter in the brain. Since tryptophan is the precursor of serotonin synthesis, the impaired tryptophan uptake resulted in decreased serotonin synthesis and reduced levels in blood and brain [85] (Fig. 2). Besides other numerous functions in the central nervous system, serotonin is essential for exercise-induced hippocampal neurogenesis



**Fig. 2** Levels of tryptophan (Trp) and serotonin (5-HT) in blood and brain of wildtype (WT) and ACE2-knockout (ACE2) mice. \*\* $p < 0.01$ , \*\*\* $p < 0.001$  versus WT. Reproduced with permission from [85]

[86]. Therefore, we analyzed ACE2-deficient mice for this phenotype and discovered that exercise-induced cell proliferation in the dentate gyrus is abolished in these animals [85]. However, when we replaced serotonin in the brain by oral administration of glycyl-L-tryptophan running-induced neurogenesis was not rescued. Since we also present evidence that Ang II and Ang-(1–7) are not involved in this effect, the downstream mediator of ACE2 in the regulation of neurogenesis remains elusive [85].

### Conclusions and Future Directions

Transgenic mouse models with increased or depleted ACE2 in the whole brain or certain hypothalamic nuclei were instrumental to reveal the central functions of this enzyme. Marked beneficial effects on blood pressure, cardiac hypertrophy, stress response, anxiety, cognition, brain injury and neurogenesis could be observed rendering ACE2 activation a valuable therapeutic option for several disorders. However, based on the pleiotropic actions of this protein, not all effects are necessarily due to alteration in the relative amounts of Ang II and Ang-(1–7). Changed levels of other substrates, such as apelin, neurotensin, as well as kinin and opioid peptides may also be involved in the observed consequences of alterations in central ACE2. Moreover, our discovery of reduced tryptophan and serotonin levels in the brain of ACE2-deficient mice revealed a new pathway by



which ACE2 may influence brain function. Therefore, future therapeutic applications should be carefully evaluated for possible unwanted effects mediated by other hormone systems than the RAS.

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