Pflugers Arch - Eur J Physiol (2013) 465:79–85
DOI 10.1007/s00424-012-1120-0

INVITED REVIEW

ACE2, angiotensin-(1–7), and Mas: the other side of the coin

Michael Bader

Received: 2 May 2012 / Revised: 25 May 2012 / Accepted: 25 May 2012 / Published online: 10 June 2012

Abstract The renin–angiotensin system (RAS) has recently been extended by the addition of a novel axis consisting of the angiotensin-converting enzyme 2 (ACE2), the heptapeptide angiotensin (1–7) (Ang-(1–7)), and the G protein-coupled receptor Mas. ACE2 converts the vasoconstrictive and pro-oxidative peptide angiotensin II (Ang II) into Ang-(1–7) which exerts vasodilatory and antioxidative effects via its receptor Mas. Thereby, ACE2 regulates the local actions of the RAS in cardiovascular tissues and the ACE2/Ang-(1–7)/Mas axis exerts protective actions in hypertension, diabetes, and other cardiovascular disorders. Consequently, this novel RAS axis represents a promising therapeutic target for cardiovascular and metabolic diseases.

Introduction

Angiotensin-(1–7) [Ang-(1–7)] was discovered 1988 as a product of angiotensin I (Ang I) degradation by enzymes from the brainstem [69]. Angiotensin-converting enzyme (ACE), which classically generates Ang II from Ang I, was shown to degrade Ang-(1–7) into inactive peptides, in particular Ang-(1–5) [86] (Fig. 1). Ang-(1–7) binds and activates the Ang II AT1 receptor only at supraphysiological concentrations, and an interaction with the AT2 receptor has been shown but is still controversial [46, 90]. Thus, this heptapeptide represented the first molecule of a novel axis of the renin–angiotensin system (RAS). However, before the ACE homologue ACE2 and Mas as receptor were described, it was not clear how the peptide was generated and by which pathways it signals, not to speak about its physiological functions. This review tries to give a timely appraisal of the large body of evidence suggesting that the new RAS axis, ACE2/Ang-(1–7)/Mas, is important for cardiovascular physiology and beyond.

This article is published as part of the special issue on the Renin-Angiotensin System.

M. Bader (✉)
Max-Delbrück-Center for Molecular Medicine (MDC),
Robert-Rössle-Str. 10,
13125 Berlin, Germany
e-mail: mbader@mdc-berlin.de

Ang-(1–7)-generating enzymes

Ang-(1–7) can be generated from Ang I or II. Neprilysin (also known as neutral endopeptidase 24.11), thimet oligopeptidase, or prolylendopeptidase release the last three amino acids from Ang I [93] and ACE2 [16, 84] or prolylcarboxypeptidase (PRCP) [78] remove the C-terminal phenylalanine from Ang II, all liberating the heptapeptide (Fig. 1). ACE2 can also first generate Ang-(1–9) from Ang I followed by the action of ACE which releases the last two amino acids. It has to be noted that all these enzymes are not specific for the angiotensin peptides. For example, ACE2 also metabolizes kinins, apelins, and neurotensin [88], and PRCP degrades kinins, alpha melanocyte-stimulating hormone [89] and activates plasma prekallikrein [102]. Furthermore, ACE2 and its homologue collectrin are involved in amino acid transport in the kidney and gut and have, thus, additional functions beyond proteolysis [43]. Importantly, ACE2 and PRCP (also called angiotensinase C) not only generate Ang-(1–7) but at the same time degrade the potent effector peptide of the RAS, Ang II. Therefore, any alteration in expression or activity of these enzymes switches the net effect of the RAS between the two sides of the coin, leave alone their other functions. The relative contribution of the different Ang-(1–7)-generating enzymes may vary from tissue to tissue [18, 86]. Nevertheless, in the

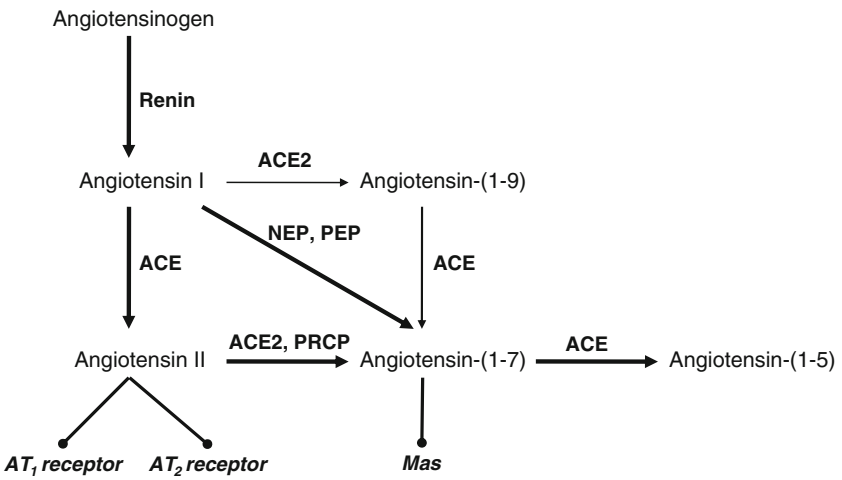

Fig. 1 Metabolic pathways of angiotensin peptides. Angiotensin peptides are metabolized by several subsequent enzymatic steps: First, renin cleaves angiotensinogen, into angiotensin I (Ang I). Ang I can be metabolized by angiotensin-converting enzyme (*ACE*) resulting in the production of the bioactive octapeptide angiotensin II (Ang II), which interacts with AT1 and AT2 receptors. Alternatively, it can be processed first by ACE2 to the inactive peptide Ang-(1–9) and then by ACE to Ang-(1–7) or by neutral endopeptidase 24.11 (*NEP*) or prolylendopeptidase (*PEP*) directly to Ang-(1–7). Ang-(1–7) can also be generated by ACE2 from Ang II and interacts with its receptor Mas

brain and kidney, Ang-(1–7) may even be the major product of angiotensin metabolism [18, 86].

In the following chapters, I will reduce the enzymes generating Ang-(1–7) to ACE2, since most studies have been done with this protein, but it is by no mean excluded that other enzymes, in particular PRCP, may be even more important in certain tissues or situations. A major problem to distinguish the enzymes and their functional relevance is the lack of specific inhibitors.

Mas

In 2003, Santos et al. finally solved two problems, by describing the long-sought receptor for Ang-(1–7) and deorphanizing Mas [74]. Before this discovery of Mas as Ang-(1–7) receptor, it had been shown to be a receptor for Ang II [41], but this was soon unveiled to be an artifact of the frog oocytes used. Probably the known interactions between Mas and the AT1 receptor [9, 42, 73] had changed the response of the oocytes to Ang II when Mas was expressed [2]. Originally, Mas had been discovered as proto-oncogene, but also the transforming activity could later not be confirmed and may have been due to a unique genetic rearrangement in the transformed cells which may have affected the imprinting of the neighboring genes [1, 53]. Alternatively, these cells may have dramatically overexpressed Mas and then its described ligand-independent activity may have transformed them [99]. However, this issue has never been totally solved. Interestingly, Mas agonism is now even discussed as antitumor strategy [29].

Mas is predominantly expressed in brain and testis, but was also detected in the kidney, heart, and vessels [56]. Mas belongs to the family of G protein-coupled receptors with seven transmembrane domains; however, its G protein coupling is still debated. At high concentrations in cells using synthetic agonists and antagonists, a coupling to Gq proteins has been described [99]. However, Ang-(1–7) does not elicit such a response. At more physiological receptor concentrations and with Ang-(1–7) as ligand, Mas induces arachidonic acid release from cells and intracellular Akt phosphorylation [67, 74]. A recent phosphoproteomic study identified several signaling pathways induced by Ang-(1–7) in human endothelial cells again including Akt [87]. However, the complete signaling pathways employed by Mas still await clarification. It is even not clear whether Ang-(1–7) has more receptors or whether Mas binds other natural ligands.

ACE2/Ang-(1–7)/Mas in vessels

Ang-(1–7) has been reported to be vasodilatory [47, 63], antithrombotic [27, 44], and antiproliferative [45, 79]. Most of these actions are mediated by changes in the redox balance in the vascular wall initiated by Ang-(1–7) via Mas [96]. Ang-(1–7) triggers NO release by Akt phosphorylation inducing the activation of endothelial NO synthase and inhibits Ang II-induced reactive oxygen species (ROS) production in endothelial cells [66, 67]. Accordingly, vessels of *Mas*-deficient mice produce more ROS and less NO leading to an impaired in vivo endothelial function and increased blood pressure [62, 96]. In the opposite, an improved endothelial function was observed in stroke-prone spontaneously hypertensive rats (SHRSP) expressing a human ACE2 transgene in vascular smooth muscle cells [64].

Increased Ang-(1–7) generation in the vascular wall appears to be the main mediator of this effect since also an improved endothelial function in the renal artery could be elicited in diabetic SHR by chronic treatment with Ang-(1–7) again by the reduction of oxidative stress [5].

Furthermore, activation of bradykinin signaling [49, 60] and attenuation of Ang II actions have been implicated in the vasculoprotective actions of Ang-(1–7). The known interactions between Mas and the AT1 receptor may have contributed to the latter effects [9, 42, 73].

In atherosclerosis, the ACE2/Ang-(1–7)/Mas axis was also shown to be protective. The genetic ablation of ACE2 significantly increases [81, 83], and transgenic vascular ACE2 overexpression decreases [51, 100] plaque formation in atherosclerotic apolipoprotein E or LDL receptor-deficient mice. In one study, the transfer of ACE2-deficient bone marrow into LDL receptor-deficient mice was already sufficient to aggravate plaque formation indicating that the enzyme on leukocytes is particularly beneficial in the atherosclerotic process [82]. Moreover, long-term Ang-(1–7) treatment induces protective effects in such animals [80]. In these cases again, an improvement of the redox balance by Ang-(1–7) has been reported to be pivotal for the anti-atherogenic effect.

ACE2/Ang-(1–7)/Mas in kidney

ACE2 and Mas are expressed in the kidney. ACE2 was found in endothelial cells of vessels, but the mesangium and glomerular endothelium were negative for ACE2. However, ACE2 is most highly expressed in the brush border of proximal tubular cells, while epithelial cells from other parts of the nephron showed weak cytoplasmic staining [36]. Mas is localized to the proximal and distal tubules, but also found in the glomerulus [11]. The majority of studies describe ACE2, Ang-(1–7), and Mas as protective factors in different kidney diseases [22]: Ang II-induced kidney damage and diabetic nephropathy are aggravated in ACE2-deficient mice, and the Ang II effects are ameliorated by recombinant ACE2 in wild-type animals [94, 101]. Ang-(1–7) infusion reverts diabetic renal damage in mice and rats [5, 33, 57], and Mas agonists protect the kidney from ischemia/reperfusion damage [3]. The mechanism involved in most of these cases seems to be a reduction in oxidative stress and reduced fibrosis by the components of the ACE2/Ang-(1–7)/Mas axis. Accordingly, Mas-deficient mice develop a spontaneous nephropathy with microalbuminuria [61]. Nevertheless, there have also been reports about aggravation of renal damage by Ang-(1–7) and Mas [7, 19]. The reasons for this discrepancy are not yet clarified.

Ang-(1–7) has also been shown to be involved in the normal function of the kidney by influencing sodium reabsorption. The effects of the peptide seem to be biphasic with an antidiuretic action at low concentration and diuretic effects at high levels [22, 30].

ACE2/Ang-(1–7)/Mas in heart

In the heart, ACE2 is mainly localized to the vascular endothelium and smooth muscle but was also detected in cardiomyocytes [8], and Mas was mainly described on cardiomyocytes [70]. Mice deficient for Mas and ACE2 show a reduced cardiac contractile function aggravating with age [10, 70]. Infusion of Ang-(1–7) rescues this phenotype in mice lacking ACE2 indicating that the loss of this peptide is an important component of the pathophysiology [59]. Diabetic cardiomyopathy is also exacerbated in the absence of ACE2, again based on increased oxidative stress [58]. In the opposite, local overexpression of ACE2 in the heart by lentiviral gene transfer elicited cardioprotective actions in several disease models [13, 15, 37]. A cardioprotective role of Ang-(1–7) was also observed in cardiac damage models, such as isoproterenol or Ang II-induced hypertrophy or ischemia/reperfusion injury, when the peptide was either infused or overexpressed from transgenes [4, 24, 35, 55, 72]. Besides NO-releasing, antioxidative, NO-increasing, and direct antihypertrophic effects on cardiomyocytes, the main actions of Ang-(1–7) in the heart seem to be the regulation of genes involved in fibrosis in cardiac fibroblasts via Mas [14, 31, 34, 40, 58, 59]. Accordingly, Mas agonists attenuate heart failure after myocardial infarction [50, 54].

ACE2/Ang-(1–7)/Mas in lung

ACE2 was found in type I and type II alveolar epithelial cells of normal lungs [36]. However, the cellular localization of Mas in the lung is not yet reported [85]. ACE2-deficient mice are more prone to lung injury in several disease models [38, 39], and recombinant ACE2 ameliorates the symptoms in the bleomycin-induced lung injury model [65]. Since also a lentivirally delivered Ang-(1–7) release construct has the same effect, the generation of this peptide by ACE2 seems to be of major importance [77] and not only the degradation of Ang II.

ACE2/Ang-(1–7)/Mas in brain

All components of the ACE2/Ang-(1–7)/Mas axis are expressed in the brain [56, 95, 97]. For Mas, the brain is even the organ with the highest expression, in particular in the hippocampus and the piriform cortex [56]. Therefore, it came to no surprise that Mas affects behavior and electrophysiology of the hippocampus [48, 91, 92]. Concerning cardiovascular actions, Ang-(1–7) and Mas have been shown to enhance

baroreflex sensitivity and influence blood pressure in different directions depending on the brain area studied [12, 25, 95]. Local overexpression of ACE2 by viral transfection in the medulla of SHRSP resulted in a decrease in blood pressure [98]. When the same technology was applied in the subfornical organ of mice, a significant reduction in the pressor effect of infused Ang II was observed [21]. Local administration of Ang-(1–7) did not have the same effect, indicating that in this case, the degradation of Ang II may be a major action of ACE2. Accordingly, also transgenic mice in which ACE2 was targeted to the brain were protected from Ang II-induced neurogenic hypertension [20].

ACE2/Ang-(1–7)/Mas in metabolism

The role of Ang-(1–7) and Mas in metabolic regulation has become increasingly clear in recent years. We have demonstrated that *Mas* deficiency in mice induces a metabolic syndrome-like state, with dyslipidemia, lower glucose tolerance and insulin sensitivity, hyperinsulinemia, decreased glucose uptake in white adipose cells, and an increase in adipose tissue mass [71]. In accordance, chronically increased Ang-(1–7) levels in transgenic rats reduce the amount of fat tissue and plasma lipid levels and enhance glucose tolerance and insulin sensitivity [68]. Insulin sensitivity is increased by an enhancing effect on its intracellular signaling by Ang-(1–7) leading to an increased Akt phosphorylation and GLUT4 translocation to the plasma membrane in different tissues [32, 33]. ACE2 is also involved in the regulation of insulin secretion in the pancreas [6]. Taken together, these observations provide strong evidence that the components of the ACE2/Ang-(1–7)/Mas axis have an important role in metabolic regulation.

Therapeutic perspectives of the ACE2/Ang-(1–7)/Mas axis

Based on the mainly protective actions of the ACE2/Ang-(1–7)/Mas axis described in this review, first attempts are under way to exploit the novel branch of the RAS for therapeutic purposes [23]. At present, recombinant human ACE2 is clinically tested for the treatment of lung and heart diseases. Moreover, ACE2 activating substances have been discovered and may also be used for pharmacological applications in the near future [26]. The third group of substances interfering with the ACE2/Ang-(1–7)/Mas axis are Mas agonists. These include just the peptide Ang-(1–7) itself in oral formulations [52], chemically slightly changed versions including cyclic peptides [17], or peptides with different sequences [76]. Most of these substances have already shown beneficial effects in animal models of lung diseases, hypertension, and diabetes [26, 28, 54, 75]. However, the first clinical trials are still under way and will finally clarify whether the other side of the RAS coin can be a successful therapeutic target.

References

1. Alenina N, Baranova TV, Smirnov E, Bader M, Lippoldt A, Patkin EL, Walther T (2002) Imprinting of the murine Mas protooncogene is restricted to its antisense RNA. Biochem Biophys Res Commun 290:1072–1078
2. Ambroz C, Clark AJL, Catt KJ (1991) The mas oncogene enhances angiotensin-induced [Ca^{2+}]i responses in cells with pre-existing angiotensin II receptors. Biochim Biophys Acta 1133:107–111
3. Barroso LC, Silveira KD, Lima CX, Borges V, Bader M, Rachid M, Santos RA, Simoes e Silva AC, Souza DG, Teixeira MM (2012) Renoprotective effects of AVE0991, a non-peptide Mas receptor agonist, in experimental acute renal injury. Int J Hypertens 2012:808726
4. Benter IF, Yousif MH, Al-Saleh FM, Raghupathy R, Chappell MC, Diz DI (2011) Angiotensin-(1–7) blockade attenuates captopril- or hydralazine-induced cardiovascular protection in spontaneously hypertensive rats treated with NG-nitro-L-arginine methyl ester. J Cardiovasc Pharmacol 57:559–567
5. Benter IF, Yousif MH, Dhaunsi GS, Kaur J, Chappell MC, Diz DI (2008) Angiotensin-(1–7) prevents activation of NADPH oxidase and renal vascular dysfunction in diabetic hypertensive rats. Am J Nephrol 28:25–33
6. Bindom SM, Hans CP, Xia H, Boulares AH, Lazartigues E (2010) Angiotensin I-converting enzyme type 2 (*ACE2*) gene therapy improves glycemic control in diabetic mice. Diabetes 59:2540–2548
7. Burns KD (2007) The emerging role of angiotensin-converting enzyme-2 in the kidney. Curr Opin Nephrol Hypertens 16:116–121
8. Burrell LM, Risvanis J, Kubota E, Dean RG, MacDonald PS, Lu S, Tikellis C, Grant SL, Lew RA, Smith AI, Cooper ME, Johnston CI (2005) Myocardial infarction increases ACE2 expression in rat and humans. Eur Heart J 26:369–375
9. Castro CH, Souza Dos Santos RA, Ferreira AJ, Bader M, Alenina N, de Almeida AP (2005) Evidence for a functional interaction of the angiotensin-(1–7) receptor Mas with AT1 and AT2 receptors in the mouse heart. Hypertension 46:942
10. 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
11. da Silveira KD, Pompermayer Bosco KS, Diniz LR, Carmona AK, Cassali GD, Bruna-Romero O, de Sousa LP, Teixeira MM, Santos RA, Simoes e Silva AC, Ribeiro Vieira MA (2010) ACE2-angiotensin-(1-7)-Mas axis in renal ischaemia/reperfusion injury in rats. Clin Sci (Lond) 119:385–394
12. de Moura MM, Dos Santos RA, Campagnole-Santos MJ, Todiras M, Bader M, Alenina N, Haibara AS (2010) Altered cardiovascular reflexes responses in conscious Angiotensin-(1-7) receptor Mas-knockout mice. Peptides 31:1934–1939
13. Der SS, 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
14. Dias-Peixoto MF, Santos RA, Gomes ERM, Alves MNM, Almeida PWM, Greco L, Rosa M, Fauler B, Bader M, Alenina

N, Guatimosim S (2008) Molecular mechanisms involved in angiotensin-(1-7)/Mas signaling pathway in cardiomyocytes. Hypertension 52:542–548
15. Diez-Freire C, Vazquez J, Correa de Adjounian MF, Ferrari MF, Yuan L, Silver X, Torres R, Raizada MK (2006) ACE2 gene transfer attenuates hypertension-linked pathophysiological changes in the SHR. Physiol Genomics 27:12–19
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. Durik M, Veghel R Van, Kuipers A, Rink R, Haas Jimoh AM, Moll G, Danser AH, Roks AJ (2012) The effect of the thioether-bridged, stabilized Angiotensin-(1-7) analogue cyclic ang-(1-7) on cardiac remodeling and endothelial function in rats with myocardial infarction. Int J Hypertens 2012:536426
18. 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
19. Esteban V, Heringer-Walther S, Sterner-Kock A, de Bruin R, van den Engel S, Wang Y, Mezzano S, Egido J, Schultheiss HP, Ruiz-Ortega M, Walther T (2009) Angiotensin-(1-7) and the g protein-coupled receptor MAS are key players in renal inflammation. PLoS One 4:e5406
20. Feng Y, Xia H, Cai Y, Halabi CM, Becker LK, Santos RA, Speth RC, Sigmund CD, Lazartigues E (2010) Brain-selective overexpression of human Angiotensin-converting enzyme type 2 attenuates neurogenic hypertension. Circ Res 106:373–382
21. 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
22. Ferrario CM, Varagic J (2010) The ANG-(1-7)/ACE2/mas axis in the regulation of nephron function. Am J Physiol Renal Physiol 298:F1297–F1305
23. Ferreira AJ, Bader M, Santos RA (2012) Therapeutic targeting of the angiotensin-converting enzyme 2/angiotensin-(1-7)/Mas cascade in the renin–angiotensin system: a patent review. Expert Opin Ther Pat 22(5):567–574
24. Ferreira AJ, Castro CH, Guatimosim S, Almeida PW, Gomes ER, Dias-Peixoto MF, Alves MN, Fagundes-Moura CR, Rentzsch B, Gava E, Almeida AP, Guimaraes AM, Kitten GT, Reudelhuber T, Bader M, Santos RA (2010) Attenuation of isoproterenol-induced cardiac fibrosis in transgenic rats harboring an angiotensin-(1-7)-producing fusion protein in the heart. Ther Adv Cardiovasc Dis 4:83–96
25. Ferreira AJ, Santos RA (2005) Cardiovascular actions of angiotensin-(1-7). Braz J Med Biol Res 38:499–507
26. Ferreira AJ, Shenoy V, Qi Y, Fraga-Silva RA, Santos RA, Katovich MJ, Raizada MK (2011) Angiotensin-converting enzyme 2 activation protects against hypertension-induced cardiac fibrosis involving extracellular signal-regulated kinases. Exp Physiol 96:287–294
27. Fraga da Silva RA, Pinheiro SVB, Goncalves ACC, Alenina N, Bader M, Santos RA (2008) The NO-mediated antithrombotic effect of angiotensin-(1-7) is abolished in *mas*-knockout mice. Mol Med 14:28–35
28. Fraga-Silva RA, Costa-Fraga FP, Sousa FB, Alenina N, Bader M, Sinisterra RD, Santos RA (2011) An orally-active formulation of angiotensin-(1-7) produces antithrombotic effect. Clinics 66:837–841
29. Gallagher PE, Cook K, Soto-Pantoja D, Menon J, Tallant EA (2011) Angiotensin peptides and lung cancer. Curr Cancer Drug Targets 11:394–404
30. Garcia NH, Garvin JL (1994) Angiotensin 1-7 has a biphasic effect on fluid absorption in the proximal straight tubule. J Am Soc Nephrol 5:1133–1138
31. Gava E, de Castro CH, Ferreira AJ, Colleta H, Melo MB, Alenina N, Bader M, Oliveira LA, Kitten GT, Santos RA (2012) Angiotensin-(1-7) receptor Mas is an essential modulator of extracellular matrix protein expression in the heart. Regul Pept 175:30–42
32. Giani JF, Gironacci MM, Munoz MC, Pena C, Turyn D, Dominici FP (2007) Angiotensin-(1 7) stimulates the phosphorylation of JAK2, IRS-1 and Akt in rat heart in vivo: role of the AT1 and Mas receptors. Am J Physiol Heart Circ Physiol 293:H1154–H1163
33. Giani JF, Mayer MA, Munoz MC, Silberman EA, Hocht C, Taira CA, Gironacci MM, Turyn D, Dominici FP (2009) Chronic infusion of angiotensin-(1-7) improves insulin resistance and hypertension induced by a high-fructose diet in rats. Am J Physiol Endocrinol Metab 296:E262–E271
34. Gomes ERM, Lara AL, Almeida WM, Guimaraes D, Resende ER, Campagnole-Santos MJ, Bader M, Santos RA, Guatimosim S (2009) Angiotensin-(1-7) prevents cardiomyocyte pathological remodeling through a NO/cGMP dependent pathway. Hypertension 55:153–160
35. Grobe JL, Mecca AP, Lingis M, Shenoy V, Bolton TA, Machado JM, Speth RC, Raizada MK, Katovich MJ (2007) Prevention of angiotensin II-induced cardiac remodeling by angiotensin-(1-7). Am J Physiol Heart Circ Physiol 292:H736–H742
36. 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
37. Huentelman MJ, Grobe JL, Vazquez J, Stewart JM, Mecca AP, Katovich MJ, Ferrario CM, Raizada MK (2005) Protection from angiotensin II-induced cardiac hypertrophy and fibrosis by systemic lentiviral delivery of ACE2 in rats. Exp Physiol 90:783–790
38. Imai Y, Kuba K, Penninger JM (2008) The discovery of angiotensin-converting enzyme 2 and its role in acute lung injury in mice. Exp Physiol 93:543–548
39. 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
40. Iwata M, Cowling RT, Gurantz D, Moore C, Zhang S, Yuan JX, Greenberg BH (2005) Angiotensin-(1-7) binds to specific receptors on cardiac fibroblasts to initiate antifibrotic and antitrophic effects. Am J Physiol Heart Circ Physiol 289:H2356–H2363
41. Jackson TR, Blair AC, Marshall J, Goedert M, Hanley MR (1988) The mas oncogene encodes an angiotensin receptor. Nature 335:437–440
42. Kostenis E, Milligan G, Christopoulos A, Sanchez-Ferrer CF, Heringer-Walther S, Sexton PM, Gembardt F, Kellett E, Martini L, Vanderheyden P, Schultheiss HP, Walther T (2005) G-protein-coupled receptor Mas is a physiological antagonist of the angiotensin II type 1 receptor. Circulation 111:1806–1813
43. Kuba K, Imai Y, Ohto-Nakanishi T, Penninger JM (2010) Trilogy of ACE2: a peptidase in the renin-angiotensin system, a SARS receptor, and a partner for amino acid transporters. Pharmacol Ther 128:119–128
44. Kucharewicz I, Pawlak R, Matys T, Pawlak D, Buczko W (2002) Antithrombotic effect of captopril and losartan is mediated by angiotensin-(1-7). Hypertension 40:774–779
45. Langeveld B, Van Gilst WH, Tio RA, Zijlstra F, Roks AJ (2005) Angiotensin-(1-7) attenuates neointimal formation after stent implantation in the rat. Hypertension 45:138–141
46. Lara LS, Cavalcante F, Axelband F, De Souza AM, Lopes AG, Caruso-Neves C (2006) Involvement of the Gi/o/cGMP/PKG

pathway in the AT2-mediated inhibition of outer cortex proximal tubule Na^+-ATPase by Ang-(1–7). Biochem J 395:183–190
47. Lavrentyev EN, Malik KU (2009) High glucose-induced Nox1-derived superoxides downregulate PKC-betaII, which subsequently decreases ACE2 expression and ANG(1-7) formation in rat VSMCs. Am J Physiol Heart Circ Physiol 296:H106–H118
48. Lazaroni TL, Raslan AC, Fontes WR, de Oliveira ML, Bader M, Alenina N, Moraes MF, Dos Santos RA, Pereira GS (2012) Angiotensin-(1-7)/Mas axis integrity is required for the expression of object recognition memory. Neurobiol Learn Mem 97:113–123
49. Li P, Chappell MC, Ferrario CM, Brosnihan KB (1997) Angiotensin-(1-7) augments bradykinin-induced vasodilation by competing with ACE and releasing nitric oxide. Hypertension 29:394–400
50. Loot AE, Roks AJ, Henning RH, Tio RA, Suurmeijer AJ, Boomsma F, Van Gilst WH (2002) Angiotensin-(1-7) attenuates the development of heart failure after myocardial infarction in rats. Circulation 105:1548–1550
51. Lovren F, Pan Y, Quan A, Teoh H, Wang G, Shukla PC, Levitt KS, Oudit GY, Al-Omran M, Stewart DJ, Slutsky AS, Peterson MD, Backx PH, Penninger JM, Verma S (2008) Angiotensin converting enzyme-2 confers endothelial protection and attenuates atherosclerosis. Am J Physiol Heart Circ Physiol 295: H1377–H1384
52. Lula I, Denadai AL, Resende JM, de Sousa FB, de Lima GF, Pilo-Veloso D, Heine T, Duarte HA, Santos RA, Sinisterra RD (2007) Study of angiotensin-(1-7) vasoactive peptide and its beta-cyclodextrin inclusion complexes: complete sequence-specific NMR assignments and structural studies. Peptides 28:2199–2210
53. Lyle R, Watanabe D, te Vruchte D, Lerchner W, Smrzka OW, Wutz A, Schageman J, Hahner L, Davies C, Barlow DP (2000) The imprinted antisense RNA at the *Igf2r* locus overlaps but does not imprint *Mas1*. Nat Genet 25:19–21
54. Marques FD, Ferreira AJ, Sinisterra RD, Jacoby BA, Sousa FB, Caliari MV, Silva GA, Melo MB, Nadu AP, Souza LE, Irigoyen MC, Almeida AP, Santos RA (2011) An oral formulation of angiotensin-(1-7) produces cardioprotective effects in infarcted and isoproterenol-treated rats. Hypertension 57:477–483
55. Mercure C, Yogi A, Callera GE, Aranha AB, Bader M, Ferreira AJ, Santos RA, Walther T, Thouyz RM, Reudelhuber TL (2008) Angiotensin 1-7 blunts hypertensive cardiac remodeling by a direct effect on the heart. Circ Res 103:1319–1326
56. Metzger R, Bader M, Ludwig T, Berberich C, Bunnemann B, Ganten D (1995) Expression of the mouse and rat *mas* proto-oncogene in the brain and peripheral tissues. FEBS Lett 357:27–32
57. Moon JY, Tanimoto M, Gohda T, Hagiwara S, Yamazaki T, Ohara I, Murakoshi M, Aoki T, Ishikawa Y, Lee SH, Jeong KH, Lee TW, Ihm CG, Lim SJ, Tomino Y (2011) Attenuating effect of angiotensin-(1-7) on angiotensin II-mediated NAD(P)H oxidase activation in type 2 diabetic nephropathy of KK-A(y)/Ta mice. Am J Physiol Renal Physiol 300:F1271–F1282
58. Patel VB, Bodiga S, Basu R, Das SK, Wang W, Wang Z, Lo J, Grant MB, Zhong J, Kassiri Z, Oudit GY (2012) Loss of angiotensin-converting enzyme-2 exacerbates diabetic cardiovascular complications and leads to systolic and vascular dysfunction: a critical role of the angiotensin II/AT1 receptor axis. Circ Res 110:1322–1335
59. Patel VB, Bodiga S, Fan D, Das SK, Wang Z, Wang W, Basu R, Zhong J, Kassiri Z, Oudit GY (2012) Cardioprotective effects mediated by angiotensin II type 1 receptor blockade and enhancing angiotensin 1-7 in experimental heart failure in angiotensin-converting enzyme 2-null mice. Hypertension 59:1195–1203
60. Paula RD, Lima CV, Britto RR, Campagnole-Santos MJ, Khosla MC, Santos RA (1999) Potentiation of the hypotensive effect of bradykinin by angiotensin-(1-7)-related peptides. Peptides 20:493–500
61. Pinheiro SVB, Ferreira AJ, Kitten GT, da Silveira KD, da Silva DA, Santos SHS, Gava E, Castro CH, Magalhaes JA, da Mota RK, Botelho-Santos GA, Bader M, Alenina N, Santos RA, Simoes e Silva AC (2009) Genetic deletion of the angiotensin (1-7) receptor Mas leads to glomerular hyperfiltration and microalbuminuria. Kidney Int 75:1184–1193
62. Rabelo LA, Xu P, Todiras M, Sampaio WO, Buttgereit J, Bader M, Santos RA, Alenina N (2008) Ablation of angiotensin (1-7) receptor Mas in C57Bl/6 mice causes endothelial dysfunction. J Am Soc Hypertens 2:418–424
63. Rakusan D, Burgelova M, Vaneckova I, ourkova Z, Huskova Z, Skaroupkova P, Mrazova I, Opocensky M, Kramer HJ, Netuka I, Maly J, Alenina N, Bader M, Santos RA, Cervenka L (2010) Knockout of angiotensin 1-7 receptor mas worsens the course of two-kidney, one-clip goldblatt hypertension: roles of nitric oxide deficiency and enhanced vascular responsiveness to angiotensin II. Kidney Blood Press Res 33:476–488
64. Rentzsch B, Todiras M, Iliescu R, Popova E, Campos LA, Oliveira ML, Baltatu OC, Santos RA, Bader M (2008) Transgenic ACE2 overexpression in vessels of SHRSP rats reduces blood pressure and improves endothelial function. Hypertension 52:967–973
65. Rey-Parra GJ, Vadivel A, Coltan L, Hall A, Eaton F, Schuster M, Loibner H, Penninger JM, Kassiri Z, Oudit GY, Thebaud B (2012) Angiotensin converting enzyme 2 abrogates bleomycin-induced lung injury. J Mol Med (Berl) 90(6):637–647
66. Sampaio WO, Henrique de Castro C, Santos RA, Schiffrin EL, Touyz RM (2007) Angiotensin-(1-7) counterregulates angiotensin II signaling in human endothelial cells. Hypertension 50:1093–1098
67. Sampaio WO, Souza dos Santos RA, Faria-Silva R, Mata Machado LT, Schiffrin EL, Touyz RM (2007) Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. Hypertension 49:185–192
68. Santos SHS, Braga JF, Mario EG, Porto LCJ, Botion LM, Alenina N, Bader M, Santos RA (2010) Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1-7). Arterioscler Thromb Vasc Biol 30:953–961
69. Santos RAS, Brosnihan KB, Chappell MC, Pesquero J, Chernicky CL, Greene LJ, Ferrario CM (1988) Converting enzyme activity and angiotensin metabolism in the dog brainstem. Hypertension 11 (Suppl I):I-153–I-157
70. Santos RA, Castro CH, Gava E, Pinheiro SVB, Almeida AP, Paula DR, Cruz JS, Ramos AS, Rosa KT, Irigoyen MC, Bader M, Alenina N, Ferreira AJ (2006) Impairment of in vitro and in vivo heart function in angiotensin-(1-7) receptor Mas knockout mice. Hypertension 47:996–1002
71. Santos SHS, Fernandes LR, Mario EG, Ferreira AVM, Porto LCJ, Alvarez-Leite JI, Botion LM, Bader M, Alenina N, Santos RA (2008) Mas deficiency in FVB/N in mice produces marked changes in lipid and glycemic metabolism. Diabetes 57:340–347
72. Santos RA, Ferreira AJ, Nadu AP, Braga AN, Almeida AP, Campagnole-Santos MJ, Baltatu O, Iliescu R, Reudelhuber TL, Bader M (2004) Expression of an angiotensin-(1-7)-producing fusion protein produces cardioprotective effects in rats. Physiol Genomics 17:292–299
73. Santos EL, Reis RI, Silva RG, Shimuta SI, Pecher C, Bascands J-L, Schanstra JP, Oliveira L, Bader M, Paiva AC, Costa-Neto CM, Pesquero JB (2007) Functional rescue of a defective angiotensin II AT1 receptor mutant by the Mas protooncogene. Regul Pept 141:159–167
74. Santos RA, Simoes e Silva AC, Maric C, Silva DMR, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SVB, 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
75. Savergnini SQ, Beiman M, Lautner RQ, de Paula-Carvalho V, Allahdadi K, Pessoa DC, Costa-Fraga FP, Fraga-Silva RA, Cojocaru G, Cohen Y, Bader M, de Almeida AP, Rotman G, Santos RA (2010) Vascular relaxation, antihypertensive effect, and cardioprotection of a novel peptide agonist of the Mas receptor. Hypertension 56:112–120
76. Shemesh R, Toporik A, Levine Z, Hecht I, Rotman G, Wool A, Dahary D, Gofer E, Kliger Y, Soffer MA, Rosenberg A, Eshel D, Cohen Y (2008) Discovery and validation of novel peptide agonists for G-protein-coupled receptors. J Biol Chem 283:34643–34649
77. Shenoy V, Ferreira AJ, Qi Y, Fraga-Silva RA, Diez-Freire C, Dooies A, Jun JY, Sriramula S, Mariappan N, Pourang D, Venugopal CS, Francis J, Reudelhuber T, Santos RA, Patel JM, Raizada MK, Katovich MJ (2010) The angiotensin-converting enzyme 2/angiogenesis-(1-7)/Mas axis confers cardiopulmonary protection against lung fibrosis and pulmonary hypertension. Am J Respir Crit Care Med 182:1065–1072
78. Skidgel RA, Erdos EG (1998) Cellular carboxypeptidases. Immunol Rev 161:129–141
79. Tallant EA, Clark MA (2003) Molecular mechanisms of inhibition of vascular growth by angiotensin-(1-7). Hypertension 42:574–579
80. Tesanovic S, Vinh A, Gaspari TA, Casley D, Widdop RE (2010) Vasoprotective and atheroprotective effects of angiotensin (1-7) in apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 30:1606–1613
81. Thatcher SE, Gupte M, Hatch N, Cassis LA (2012) Deficiency of ACE2 in bone-marrow-derived cells increases expression of TNF-alpha in adipose stromal cells and augments glucose intolerance in obese C57BL/6 mice. Int J Hypertens 2012:762094
82. Thatcher SE, Zhang X, Howatt DA, Lu H, Gurley SB, Daugherty A, Cassis LA (2011) Angiotensin-converting enzyme 2 deficiency in whole body or bone marrow-derived cells increases atherosclerosis in low-density lipoprotein receptor$^{-/-}$ mice. Arterioscler Thromb Vasc Biol 31:758–765
83. Thomas MC, Pickering RJ, Tsorotes D, Koitka A, Sheehy K, Bernardi S, Toffoli B, Nguyen-Huu TP, Head GA, Fu Y, Chin-Dusting J, Cooper ME, Tikellis C (2010) Genetic *Ace2* deficiency accentuates vascular inflammation and atherosclerosis in the *ApoE* knockout mouse. Circ Res 107:888–897
84. 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
85. Uhal BD, Li X, Xue A, Gao X, Abdul-Hafez A (2011) Regulation of alveolar epithelial cell survival by the ACE-2/angiotensin 1-7/Mas axis. Am J Physiol Lung Cell Mol Physiol 301:L269–L274
86. Velez JC, Ierardi JL, Bland AM, Morinelli TA, Arthur JM, Raymond JR, Janech MG (2012) Enzymatic processing of angiotensin peptides by human glomerular endothelial cells. Am J Physiol Renal Physiol (in press)
87. Verano-Braga T, Schwammle V, Sylvester M, Passos-Silva DG, Peluso AA, Etelvino GM, Santos RA, Roepstorff P (2012) Time-resolved quantitative phosphoproteomics: new insights into angiotensin-(1-7) signaling networks in human endothelial cells. J Proteome Res (in press)
88. 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
89. Wallingford N, Perroud B, Gao Q, Coppola A, Gyengesi E, Liu ZW, Gao XB, Diament A, Haus KA, Shariat-Madar Z, Mahdi F, Wardlaw SL, Schmaier AH, Warden CH, Diano S (2009) Prolyl-carboxypeptidase regulates food intake by inactivating alpha-MSH in rodents. J Clin Invest 119:2291–2303
90. Walters PE, Gaspari TA, Widdop RE (2005) Angiotensin-(1-7) acts as a vasodepressor agent via angiotensin II type 2 receptors in conscious rats. Hypertension 45:960–966
91. Walther T, Balschun D, Voigt JP, Fink H, Zuschratter W, Birchmeier C, Ganten D, Bader M (1998) Sustained long term potentiation and anxiety in mice lacking the Mas protooncogene. J Biol Chem 273:11867–11873
92. Walther T, Voigt J-P, Fink H, Bader M (2000) Sex specific behavioural alterations in *Mas*-deficient mice. Behav Brain Res 107:105–109
93. Welches WR, Brosnihan KB, Ferrario CM (1993) A comparison of the properties and enzymatic activities of three angiotensin processing enzymes: angiotensin converting enzyme, prolyl endopeptidase and neutral endopeptidase 24.11. Life Sci 52:1461–1480
94. Wong DW, Oudit GY, Reich H, Kassiri Z, Zhou J, Liu QC, Backx PH, Penninger JM, Herzenberg AM, Scholey JW (2007) Loss of angiotensin-converting enzyme-2 (*Ace2*) accelerates diabetic kidney injury. Am J Pathol 171:438–451
95. Xia H, Lazartigues E (2008) Angiotensin-converting enzyme 2 in the brain: properties and future directions. J Neurochem 107:1482–1494
96. Xu P, Goncalves ACC, Todiras M, Rabelo LA, Sampaio WO, Moura MM, Santos SS, Luft FC, Bader M, Gross V, Alenina N, Santos RA (2008) Endothelial dysfunction and elevated blood pressure in Mas gene-deleted mice. Hypertension 51:574–580
97. Xu P, Sriramula S, Lazartigues E (2011) ACE2/ANG-(1-7)/Mas pathway in the brain: the axis of good. Am J Physiol Regul Integr Comp Physiol 300:R804–R817
98. 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
99. Zhang T, Li Z, Dang H, Chen R, Liaw C, Tran TA, Boatman PD, Connolly DT, Adams JW (2012) Inhibition of Mas G-protein signaling improves coronary blood flow, reduces myocardial infarct size, and provides long-term cardioprotection. Am J Physiol Heart Circ Physiol 302:H299–H311
100. Zhang C, Zhao YX, Zhang YH, Zhu L, Deng BP, Zhou ZL, Li SY, Lu XT, Song LL, Lei XM, Tang WB, Wang N, Pan CM, Song HD, Liu CX, Dong B, Zhang Y, Cao Y (2010) Angiotensin-converting enzyme 2 attenuates atherosclerotic lesions by targeting vascular cells. Proc Natl Acad Sci USA 107:15886–15891
101. Zhong J, Guo D, Chen CB, Wang W, Schuster M, Loibner H, Penninger JM, Scholey JW, Kassiri Z, Oudit GY (2011) Prevention of angiotensin II-mediated renal oxidative stress, inflammation, and fibrosis by angiotensin-converting enzyme 2. Hypertension 57:314–322
102. Zhu L, Carretero OA, Xu J, Wang L, Harding P, Rhaleb NE, Yang JJ, Sumners C, Yang XP (2012) Angiotensin II type 2 receptor-stimulated activation of plasma prekallikrein and bradykinin release: role of SHP-1. Am J Physiol Heart Circ Physiol (in press)