The renin-angiotensin system: going beyond the classical paradigms

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INTRODUCTION

The renin-angiotensin system (RAS) is one of the most potent cardiovascular regulators and an important target for therapeutic drugs. From 120 yr ago, when renin was discovered (148), until 30 yr ago, only one peptide was thought to be active in this system: angiotensin II (ANG II). It was shown to be produced by successive digestion of angiotensinogen by the enzymes renin and angiotensin-converting enzyme (ACE), with ANG I as an intermediate, and to interact with a receptor, now called the ANG II type 1 receptor (AT1R). Physiologically, AT1R activation by ANG II elicits vasoconstriction, water intake, and Na+ retention. Pathophysiologically, activation of ACE/ANG II/AT1R signaling is associated with oxidative stress, hypertrophy, fibrosis, and inflammation. This view of the RAS began to change when, in 1988, Santos et al. (125) discovered the formation of ANG-(1–7), a peptide with a focus on noncentral nervous system-related tissues and the therapeutic opportunities based on these findings were discussed.

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that ANG-(1-7) via its receptor, Mas (127), as well as ANG II via AT\textsubscript{2}R counteract the classical RAS resulting in vasodilation, anti-inflammation, antifibrosis, and antiapoptosis, conferring beneficial effects in the settings of cardiovascular diseases (19, 128, 144). A central role in regulating the relative activities of the RAS arms in a tissue is played by the local levels of the enzyme ACE2, which transforms ANG II into ANG-(1-7) (31, 149). It was only a logical consequence of these findings that compounds were developed to activate the two protective arms of the RAS, which are undergoing clinical evaluation at the moment (5). However, the full complement of angiotensin peptides is still not completed, because Lautner et al. (74) and Jankowsky et al. (60) recently discovered a novel class, which we suggest to call “alatensins,” with an alanine at the amino-terminal position instead of the aspartate in the classical angiotensin peptides. Although Ala\textsuperscript{1}-ANG-(1-7) interacts with the same receptors as ANG II, AT\textsubscript{1}R, and AT\textsubscript{2}R, Ala\textsuperscript{1}-ANG-(1-7) (alamandine) has its own receptor, Mas-related G protein-coupled receptor D (MrgD) (74).

This review will summarize novel findings on the physiological and pathophysiological actions of the new beneficial RAS components, which were presented at the symposium on “The Renin-Angiotensin System: Going Beyond the Classical Paradigms” at the International Union of Physiological Sciences 2017 Rhythms of Life Congress in Rio de Janeiro, Brazil.

**ANG-(1-7) AS A CRITICAL MEDIATOR OF THE ACE2-RELATED PROTECTIVE AXIS OF THE RAS**

ACE2 is a monocarboxypeptidase that cleaves away phenylalanine from ANG II, converting it to ANG-(1-7), and leucine from ANG I, converting it to ANG-(1-9) (Fig. 1A) (104, 109). Together, ACE2, ANG-(1-9), and ANG-(1-7) act as endogenous negative regulators of the RAS. ANG-(1-7) is a biologically active heptapeptide exerting cardioprotective effects in the settings of cardiovascular diseases by antagonizing maladaptive signaling attributed to ANG II (1, 4, 88, 97). ANG-(1-7) primarily acts via the endogenous receptor Mas because the Mas antagonist A779 blocks the majority of ANG-(1-7) effects (1, 124, 127, 145, 171). More importantly, ANG-(1-7) effects are not observed in Mas-deficient animals (53, 127, 128, 164). MrgD can also mediate some ANG-(1-7) actions (127, 147). The AT\textsubscript{2}R has been linked to cardioprotective actions of ANG-(1-9) (36, 37, 94, 95). The cardioprotective effects of ACE2 are a combination of 1) the degradation of ANG I to ANG-(1-9), thereby limiting the availability of the substrate for ACE, and 2) the degradation of ANG II to cardioprotective ANG-(1-7). Therefore, loss of ACE2 activity results in loss of protection from maladaptive signaling of the ANG II/AT\textsubscript{1}R axis, promoting progression of cardiovascular diseases, whereas increased ACE2 activity leads to activation of ACE2/ANG-(1-7) and ACE2/ANG-(1-9) axes mediating cardiovascular protection (Fig. 1B).

**Biochemistry and Regulation of ACE2**

ACE2 is a type I transmembrane protein consisting of an extracellular amino-terminal domain containing the catalytic site and an intracellular carboxy-terminal tail (31, 149). ACE2 functions as a carboxymonopeptidase with the Arg\textsuperscript{273} residue being critical for substrate binding and enzymatic activity (49). As a carboxymonopeptidase, ACE2 degrades ANG II to generate the heptapeptide ANG-(1-7) and ANG I to generate the nonapeptide ANG-(1-9) by hydrolyzing the carboxy-terminal amino acid (31, 121, 149). ANG-(1-9) can be further converted to ANG-(1-7) by ACE. In the same manner, ACE2 can also cleave and partially inactivate pyr-apelin-13 and apelin-17 peptides (155). In addition to being an enzyme, ACE2 is a transmembrane protein that also acts as an amino acid transporter and is targeted by severe acute respiratory syndrome coronavirus (109). ACE2 is widely expressed in the cardiovascular system, kidneys, lungs, and brain to protect these organs from excessive ANG II signaling (32, 42, 54, 71, 99, 161). In the heart, both ACE2 and Mas are expressed in cardiomyocytes, cardiofibroblasts, and the coronary vasculature (58, 103, 108, 123, 124, 126). ACE2 activity is regulated by a disintegrin and metalloproteinase domain-containing protein 17 (ADAM-17; also known as TNF-\alpha-converting enzyme) and apelin. ADAM-17 acts as a negative regulator of ACE2 tissue activity by proteolytically cleaving ACE2, resulting in the shedding of ACE2 into the interstitium, leading to decreased ACE2 activity in the tissue and elevated circulating ACE2 activity (103, 109). Deletion of this negative regulator (ADAM-17 or TNF-\alpha-converting enzyme) protects the heart and vasculature in conditions of myocardial infarction, hypertension, pressure overload, and aortic aneurysm (34, 35, 43, 135, 136). Unlike ADAM-17, apelin regulates ACE2 expression. In mice, knockout of apelin is associated with lower ACE2 expression levels and exacerbated ANG II-induced cardiac hypertrophy and dysfunction (172). Treatment of apelin knockouts with pyr1-apelin-13 normalized ACE2 expression and counteracted ANG II-induced cardiac hypertrophy and dysfunction (172). Interestingly, ACE2 can also degrade and inactivate apelin peptides, creating a self-regulating negative feedback loop (155).

**Generation and Metabolism of ANG-(1-7)**

Recombinant human ACE2 (rhACE) produces both ANG-(1-7) and ANG-(1-9) (from ANG II and ANG I, respectively), whereas recombinant murine ACE2 generates predominantly ANG-(1-7) from ANG II (6, 169). Studies using rhACE2 and ACE2 purified from sheep tissues showed that ANG II is the preferred substrate for ACE2 (48, 107, 134, 149, 153, 162, 173). In rats, changes in ACE2 correlated with plasma ANG-(1-9) levels (93), whereas rhACE2 efficiently converted infused ANG II into ANG-(1-7) (80). In mice, rhACE2 counteracts detrimental ANG II signaling, and these therapeutic effects are mediated via ANG-(1-7) (102, 107, 173). Also, in human studies, rhACE2 clearly lowered plasma ANG II levels and increased plasma ANG-(1-7) levels (6, 109). In the heart, ANG-(1-7) is primarily generated by ACE2 (41, 173), whereas plasma ANG-(1-7) is primarily produced by neprilysin from ANG I because inhibition of neprilysin reduced ANG-(1-7) levels and increased ANG II levels (111). The degradation of ANG-(1-7) is governed predominantly by the amino-terminal catalytic domain of ACE, which degrades ANG-(1-7) to ANG-(1-5) (28). Inhibition of ACE increases plasma levels of ANG-(1-7) in both rodents and humans (3, 6, 20).
Role of ACE2 and ANG-(1−7) in Heart Failure

Heart failure (HF) is driven by activation of multiple neurohumoral and signaling pathways resulting in pathological hypertrophy and maladaptive ventricular remodeling. Activation of the RAS and increased ANG II levels play a pivotal role in adverse myocardial remodeling and disease progression (6, 85, 87) contributing to systolic and diastolic dysfunction in patients with HF (13, 57). Use of ACE inhibitors does not necessarily result in lower ANG II formation because ANG II levels can remain elevated in optimally treated patients with HF. In fact, ~50% of patients using current ACE inhibition therapies exhibit elevated levels of ANG II, which is probably the result of increased ANG I levels and activity of mast cell chymase (6, 62, 78, 113, 158). This ACE-independent ANG II production highlights a need for a better understanding of the contribution of various parts of the RAS to HF progression. Our current understanding of the role of various parts of the RAS in the development of HF is shown in Fig. 1B. In models of HF with reduced ejection fraction, loss of ACE2 worsened the HF phenotype (67, 102). Under the conditions of elevated ANG II levels, loss of protective ACE2 activity worsens cardiac dysfunction, hypertrophy, and fibrosis, leading to greater diastolic dysfunction (2, 173). Supplying rhACE2 decreased plasma and myocardial ANG II levels and increased plasma ANG-(1−7) levels, resulting in attenuated pathological remodeling and corrected diastolic dysfunction (173). Even partial loss of ACE2 (in heterozygous female mice) is sufficient to enhance the susceptibility to heart disease, which is a clinically relevant observation because ACE2 is downregulated in human hearts with dilated cardiomyopathy (156). Increase in soluble ACE2, which reflects loss of tissue-bound ACE2, is associated with severity of HF and serves as an independent
predictor of major adverse cardiac events (6, 33, 119). Because shedding of ACE2 is mediated via ADAM-17 (59, 103, 163), it suggests a possible involvement of ADAM-17 in the development of HF. Involvement of ADAM-17 in HF development has been shown primarily in relation to the TNF-α signaling pathway in humans (129, 130) and in mouse models of heart disease (34, 66). However, in light of the findings that ANG II induces ADAM-17-dependent cleavage of ACE2, it becomes clear that ANG II, ADAM-17, and ACE2 create a potentially dangerous positive feedback loop conducive to the development of HF (103). Conversely, loss of ANG-(1−7) actions exacerbated heart disease in an ACE2-deficient state (102), whereas inhibition of ANG-(1−7)/Mas signaling prevented rhACE2-mediated cardioprotection (107), confirming the importance of ANG-(1−7) contribution to the cardioprotective effects of ACE2. Supplying ANG-(1−7) directly was also cardioprotective in preclinical models of heart disease (81, 88, 118, 145). ANG-(1−7) suppressed cardiomyocyte growth and myocardial infarction-induced ventricular hypertrophy and decreased myocardial levels of proinflammatory cytokines, leading to a reduction in myocardial inflammation (118, 145). ANG-(1−7) activation of Mas results in the activation of phosphatidylinositol 3-kinase (PI3K)-Akt-endothelial nitric oxide (NO) synthase (eNOS), upregulation of MAPK phosphatase (84), inhibition of PKC-p38 MAPK and reactive oxygen species production (50, 102, 173), and suppression of collagen expression (reducing myocardial fibrosis) (29, 44, 124), thereby antagonizing pathological effects of ANG II (88, 109, 127). In addition to ANG-(1−7), ANG-(1−9), the product of ACE2 degradation of ANG I, is antihypertrophic, antifibrotic, and antihypertensive in models of hypertension and myocardial infarction (36, 37, 94, 95).

ACE2 and ANG-(1−7) also play important roles in vascular disease and hypertension (109). For example, rhACE2 pretreatment partially reversed ANG II-mediated hypertension because of decreased plasma ANG II and increased plasma ANG-(1−7) levels (80, 162), whereas loss of ACE2 exacerbates ANG II-mediated hypertension (108). Cyclodextrin-encapsulated ANG-(1−7) and Mas agonists (AVE0091 and CGEN856S) have demonstrated antihypertensive effects in preclinical models (146). These antihypertensive effects of ACE2/ANG-(1−7) suggest new possible therapeutic options against hypertensive heart disease.

Role of ACE2/ANG-(1−7) in Obesity-Associated Cardiomyopathy

Diabetes and obesity are major causes of cardiovascular morbidity and mortality worldwide and result in microvascular and macrovascular complications, including hypertension (174). Diabetic heart disease, linked to systolic and diastolic dysfunctions, and HF (39, 47, 70, 174) are associated with activation of the RAS (11, 12, 56, 90). Obesity, the major metabolic precursor to type 2 diabetes, is an independent risk factor for the development of HF with preserved ejection fraction (HFpEF) (68, 69, 98, 166). Emerging preclinical and clinical data strongly support a key pathogenic role for ACE2 and ANG-(1−7) in the progression of cardiovascular diseases (109, 132, 142). In a model of type 2 diabetes (db/db), ANG-(1−7) rescued diastolic dysfunction and reduced cardiac hypertrophy, fibrosis, lipotoxicity, and adipose inflammation (89, 100). Conversely, in the settings of high-fat diet-induced obesity, loss of ACE2 worsened heart disease because of increased epicardial adipose tissue inflammation, myocardial lipotoxicity, and worsened cardiac insulin resistance (105). ANG-(1−7) prevented these changes and rescued HFpEF in ACE2 knockout mice (105). These findings, coupled with the protective effects of ACE2 and ANG-(1−7) in the vasculature and adipose tissue, support inflammation and microvascular dysfunction as key mediators of HFpEF (110, 116). These results suggest that in patients with HFpEF, epicardial adipose tissue inflammation may be related to cardiac dysfunction and adverse remodeling (105, 106). In patients with obesity, administration of ANG-(1−7) improved insulin-stimulated endothelium-dependent vasodilation and blunted endothelin-1-dependent vasoconstrictor tone (132). Therefore, enhancing the ACE2 and ANG-(1−7) pathways represents a potential therapy for HFpEF, a condition with an adverse prognosis that lacks effective therapy.

ALATENSINS

In 2008, Jankowski and coworkers (60) described a novel octapeptide derived from ANG II, which they called ANG A. This peptide can be formed from ANG II by decarboxylation of the Asp residue to Ala. The affinity of Ala1-ANG II for AT1Rs and AT2Rs, as determined by displacement of 125I-Sar1-Ile8-ANG II, was similar to ANG II. However, the pressor effect induced by ANG A was smaller than ANG II, suggesting the involvement of other mechanisms on this effect. This observation led to the hypothesis that reduced pressor effect was because of the formation of an ANG-(1−7)-like peptide [Ala1-ANG-(1−7)] from ANG A. In pursuing this hypothesis, Santos’ group identified and characterized the heptapeptide Ala1-ANG-(1−7), which they called alamandine (74). Differing from ANG A, which stimulates the same receptors as its precursor, alamandine did not act through Mas. Actually, alamandine stimulates MrgD (74). This receptor was initially believed to be an IB4-nociceptive neuron-specific receptor (30, 76). However, there is growing evidence that MrgD is present in other tissues/cells, including cardiomyocytes and endothelial cells (51, 61, 96). It is not clear yet if the lower pressor effect of ANG A compared with ANG II is because of the formation of alamandine.

Although only two Ala1-ANG-related peptides have been reported so far (i.e., ANG A and alamandine), we believe that this peptide family is larger than anticipated [probably containing Ala1-ANG I, Ala1-ANG-(1−9), Ala1-ANG-(1−5), etc.]. To avoid confusion related to angiotensin peptides, we are introducing the term “alatensins” to refer to the Ala1-ANG family. We are currently studying the physiological presence of these peptides using a LC-MS/MS platform with promising results that shall be published elsewhere soon.

Physiological Effects of Alamandine

Alamandine has been reported to reverse hyperhomocysteinemia-induced vascular dysfunction. Activation of PKA appears to be involved in this effect (117). In adipose tissue, an alamandine-induced reduction of leptin has been described through a mechanism involving Src/p38 MAPK (150). As previously described for ANG-(1−7), an inclusion compound of alamandine/2-hydroxypropyl (HP)-β-cyclodextrin reduced blood pressure in nonanesthetized spontaneously hypertensive...
Moreover, ANG-(1-7) has been described in an experimental model of sepsis (79). In keeping with these effects, it has been reported that knockdown of MrgD in mice leads to marked dilated cardiomyopathy (96). Centrally, the action of alamandine resembles those described for ANG-(1-7) in the rostral ventrolateral medulla, caudal ventrolateral medulla, and hypothalamus (38, 74, 137, 140). These observations suggest that, as described for ANG II and ANG-(1-7), alamandine may act as a neuronal excitatory molecule in the brain. However, striking differences between these two peptides are becoming progressively evident (61, 82). For instance, in the insular cortex, alamandine but not ANG-(1-7) promotes excitatory cardiovascular effects, including increases in blood pressure and heart rate associated with increased renal sympathetic activity (82). Moreover, ANG-(1-7) [and ANG II/AT2Rs (112, 168)] promotes NO release mainly by activating the PI3K/Akt/eNOS pathway (124), whereas alamandine leads to NO release by a mechanism not involving this pathway (Fig. 2). AMP-activated protein kinase (AMPK) appears to be the main target for alamandine-induced NO formation (61).

It was well demonstrated by Jankowski and colleagues that ANG A binds and produces biological effects through interactions with AT1Rs (60). This finding was confirmed by other authors (23, 167). However, the relationship of ANG A and AT1Rs apparently is not as straightforward as it appears. The vasoconstrictor effect of ANG A is smaller than that of ANG II, despite the fact that the affinity of both peptides for AT1Rs is remarkably similar (60). This could be because of the formation of alamandine, as we have previously hypothesized (74). However, in isolated cardiomyocytes, ANG A was essentially ineffective in promoting electrically stimulated Ca2+ influx (23). In the same preparation, ANG II was active. Accordingly, a 10-fold difference between ANG II and ANG A for intracellular Ca2+ release in vascular smooth muscle cells was observed by Jankowski et al. (60). These observations suggest that ANG A could be a biased agonist of AT1Rs. This possibility has not been explored yet.

### Novel Findings on Alamandine and AT1R Interactions

Canta et al. (17) compared the effects of alamandine and ANG-(1-7) in nonanesthetized normotensive Sprague-Dawley rats. In contrast to ANG-(1-7), which has no effect on blood pressure, alamandine produced a dose-response U-shaped decrease in blood pressure. The maximal decrease in blood pressure was achieved by intravenous administration of 20 pg/animal. To test whether the U-shaped dose-response was because of stimulation of AT1Rs, the experiments were repeated in losartan-treated rats. In this condition, a magnification of the hypotensive response was observed (Fig. 3). However, the U-shaped form was still evident. These results suggest that alamandine is more potent than ANG-(1-7) as a vasodilator and that AT1Rs are involved in alamandine effects. A similar conclusion was drawn by Soltani Hekmat et al. (141) in pentobarbital-anesthetized rats. However, a more general conclusion about the modulatory role of AT1Rs on the cardiovascular effects of alamandine cannot be drawn in other rat strains (Wistar and spontaneously hypertensive stroke-prone rats), because losartan abolished rather than increased alamandine vasorelaxant action (75). In addition, in the caudal ventrolateral medulla, blockade of AT1Rs did not alter the hypotensive effect of alamandine (140).
NOVEL DOWNSTREAM PLAYERS OF ANG-(1−7)/MAS AND ALAMANDINE/MRGD SIGNALING NETWORKS IDENTIFIED BY PHOSPHOPROTEOMICS

Cell signaling is a communication process governing cellular actions and is mainly driven by reversible phosphorylation of downstream effectors. In the past decade, phosphoproteomics has emerged as a powerful approach to study phosphorylation dynamics. Figure 4 shows a general phosphoproteomic workflow to study cell signaling dynamics. This technology has been used to study signaling of ANG-(1−7)/Mas in human endothelial cells (152) and alamandine/MrgD signaling in Chinese hamster ovary MrgD and cancer cells (138).

ANG-(1−7)/Mas Signaling

Because ANG-(1−7) was identified as the endogenous ligand of Mas in 2003 (127), Western blot-based studies have contributed to build a solid knowledge of ANG-(1−7)/Mas signaling. For example, using this method, it was reported that ANG-(1−7)-induced NO production is dependent on the activation of the PI3K/Akt/eNOS pathway (124) and that ANG-(1−7) activates Src homology region 2 domain-containing phosphatase (SHP)-2 to counterregulate ANG II/AT1R signaling (123).

Phosphoproteomics has been used to build a comprehensive time-resolved signaling network of ANG-(1−7) in human endothelial cells (152). ANG-(1−7) stimulation led to differential regulation of 121 phosphosites from 79 proteins, including dephosphorylation of Ser256 on the transcription factor FOXO1, leading to its activation. FOXO1 is an important regulator of tumor suppression and cell metabolism (46, 72). FOXO1 activation by ANG-(1−7) seems to be an important molecular event on the antitumoral effect attributed to this heptapeptide. One could not anticipate that ANG-(1−7) would induce FOXO1 activation before this phosphoproteomic study because Akt1, a crucial player of ANG-(1−7) signaling (124), induces FOXO1 phosphorylation and inhibition (15). Thus, this is a fine example of how phosphoproteomics can help one building an unbiased and comprehensive signaling network. Nevertheless, it is clear that other upstream players yet to be identified are responsible for FOXO1 dephosphorylation upon Mas activation.

ANG-(1−7)/Mas and Alamandine/MrgD Induce NO Formation Via Different Signaling Pathways

NO formation is a shared outcome of ANG-(1−7) and alamandine treatment. This event is associated with many beneficial effects observed for these peptides (45, 74). However, as mentioned above in ALATENSINS, ANG-(1−7) and alamandine do not trigger a common pathway to activate NOS (Fig. 2). Although ANG-(1−7)/Mas induces NO production via the PI3K/Akt/eNOS pathway on endothelial cells and cardiomyocytes (29, 124), alamandine increases NO levels in a LKB1/AMPK-dependent manner in cardiomyocytes. Using ventricular cardiomyocytes isolated from hearts of 10- to 12-wk-old male C57BL/6 mice, de Jesus and collaborators (61) showed that alamandine induced phosphorylation of AMPK-α (Thr172) and its upstream effector LKB1 (Ser428). Although ANG-(1−7) induced phosphorylation of Akt at its activation site (Ser473), the authors did not observe its phosphorylation using alamandine. Using cardiomyocytes from Mas-deficient mice, the authors ruled out the contribution of Mas in LKB1 and AMPK-α phosphorylation induced by alamandine (61).

Although alamandine and ANG-(1−7) have 86% sequence identity (six of seven amino acid residues are the same), the replacement of the negatively charged residue (Asp) to the neutral one (Ala) in position 1 changes the physicochemical feature of alamandine, influencing its receptor affinity as alamandine activates MrgD but not Mas (74). As reviewed here, by activating different receptors, alamandine and ANG-(1−7) induce the activation of different signaling pathways, adding new therapeutic clues and insights for the understanding of the RAS.
AT2Rs: FROM ENIGMA TO THERAPEUTICS

In the late 1980s, the development of new angiotensin receptor ligands by some pharmaceutical companies resulted in the realization that some of these ligands, such as Dupont compound DUP753 (later losartan) or Ciba-Geigy compound CGP42112A, were able to distinguish between two different angiotensin receptor subtypes because of different affinity (21, 159). It soon became the consensus to name the receptor to which DUP753 bound with high affinity and CGP42112A with low affinity the ANG II type 1 receptor (AT1R) and the receptor to which DUP753 bound with low affinity and CGP42112A with high affinity the ANG II type 2 receptor (AT2R) (25).

Despite these new experimental tools for distinguishing between AT1R and AT2R, it took more than 5 more years until the scientific community began to really understand that AT1Rs and AT2Rs generally mediate opposing actions. There were several reasons as to why this process took so long. For example, 1) CGP42112A was initially regarded to be an antagonist, which led to wrong interpretation of experimental data (159), 2) for some time, several researchers regarded the AT2R as binding site without function (139), and 3) as discussed in detail below, the G protein coupling of the AT2R is quite unusual (170), which makes studies on its signaling and function difficult.

Signaling of the AT2R and Cross-Talk With Mas

The mid-1990s brought a real breakthrough in the understanding of the AT2R. Four groups independently discovered that activation of phosphatases seemed to be a major signaling mechanism of the AT2R, whereas the AT1R signals mainly through kinase-driven signaling cascades. Although Bottari et al. (9) found in 1992 that, in general, AT2R activation leads to tyrosine dephosphorylation, in subsequent years, the groups of Summers, Dzau, and Nahmias identified protein phosphatase 2 (PP2A) (63), MAPK phosphatase 1 (165), and SHP-1 (92) as specific AT2R-stimulated phosphatases. At the same time, it was excluded that the AT2R couples to “conventional” G proteins such as Gq or Gi proteins (10). However, coupling to Gq proteins was demonstrated and shown to be involved in the modulation of ion channel currents and activation of PP2A (55, 63). The lack of conventional G protein coupling of the AT2R was elegantly explained by a Nature publication from 2017, which reported the crystalline structure of the AT2R (170). Although this study confirmed that the AT2R displays all characteristics of a 7-transmembrane, class A G protein-coupled receptor, the authors unexpectedly discovered that upon activation of the AT2R, intracellular helix 8 changes its orientation in a way that it interacts with intracellular helices III, V, and VI, thereby sterically blocking binding of conventional G proteins and β-arrestins.

Although these newest findings explain the lack of conventional G protein coupling of the AT2R, the actual signaling mechanisms of the receptor are still only incompletely understood. This holds true, in particular, for the initiation of signaling upon receptor activation. What is known, however, is that the third intracellular loop and COOH-terminal end of the AT2R seem crucial for AT2R signaling (114, 115). In fact, some initial signaling molecules, such as SHP-1, PP2A, and AT2R-interacting protein, interact directly with the AT2R upon receptor activation: SHP-1 and PP2A (probably under involvement of Gi) with the third intracellular loop (64, 133) and AT2R-interacting protein with the COOH-terminal end (8). Certain kinases may be involved, too, in this early initiation of signaling, such as tyrosine kinase c-Src (133).

From a functional perspective, the lack of conventional G protein signaling and the activation of Gi and phosphatases, which again interfere with kinase-driven signaling in an inhibitory way, make sense and are in accordance with known AT2R actions, which oppose actions of cytokines, growth factors, and classical G protein-couple receptors such as the AT1R (22, 24, 25, 122, 151, 157).

Signaling mechanisms of the AT2R and Mas have many similarities like, e.g., involvement of SHP-1/SHP-2 and signaling through PI3K/Akt/eNOS (Fig. 2). Moreover, the AT2R and Mas form heterodimers, at least in certain tissues, which may explain the shared signaling pathways and also the phenomenon that often effects of ANG-(1–7) can be inhibited by an AT2R antagonist and effects of an AT1R agonist by A779 (77, 101, 154). Interestingly, when the AT2R and Mas dimerize, they seem to depend on each other functionally, because knockout of one of the receptors leads to loss of function of the other receptor in the respective cell or tissue (77).

Physiological and Pathophysiological Actions of the AT2R

In the physiological situation, the AT2R is usually expressed at low levels and in most tissues appears to be dormant (25). Exceptions seem to be a role in the central regulation of blood pressure (143), a weak vasodilation (160), a natriuretic effect (52), and an impact on cell differentiation, e.g., in neurons (86), uterus (26), or fetal tissue (18).

In the pathophysiological situation, the AT2R mediates a variety of tissue protective actions, which again very much resemble the actions of ANG-(1–7) through Mas and which comprise, for example, anti-inflammation, immune modulation, antifibrosis, inhibition of sympathetic outflow, antiapoptosis, and neuregeneration (91). In the context of HF, several of these actions work together in a well-orchestrated way. For example, in rats with HF caused by myocardial infarction, AT2R stimulation acts in an anti-inflammatory manner by reducing cytokine synthesis and in an antifibrotic manner by inhibition of transforming growth factor-β generation, thus ameliorating peri-infarct remodeling, which again results in improved cardiac function (65, 73). An antifibrotic effect was also seen in the right ventricles of rats with pulmonary hypertension (14). Right ventricular fibrosis leading to HF is in fact a major pathomechanism determining mortality in patients with pulmonary hypertension. In another model of ischemia-induced HF (coronary ligation model), central administration of the AT2R agonist C21 for 7 days by intracerebroventricular infusion significantly reduced sympathetic outflow and improved baroreceptor sensitivity, both mechanisms with a proven beneficial effect on HF (40).

The protective effects of AT2R stimulation have been observed and associated with improved outcome in multiple other disease models, including cardiovascular disease, diabetic end-organ damage, autoimmune disease, neurological disease, etc. For more details on the protective actions of the AT2R in a...
broad spectrum of diseases, the reader is referred to recent review articles (22, 24, 25, 27, 122, 151, 157).

**Targeting the AT2R in Drug Development**

As a result of the better understanding of AT2R actions and the realization that the receptor promotes tissue protection, repair, and regeneration in the context of several different pathologies, drug development projects have been initiated for the development of AT2R agonists. In the meantime, some of these projects have reached the clinical phases of development. The nonpeptide AT2R agonist compound 21 (C21), a proprietary molecule of Vicore Pharma (https://vicorepharma.com/), has successfully undergone phase I clinical testing and will soon be forwarded into a phase IIa clinical study in patients with idiopathic pulmonary fibrosis. The cyclic peptide MOR107 (Morphosys, https://www.morphosys.com/; previously LP-2 by Lanthio Pharma) has been successfully tested in a phase II trial for pulmonary fibrosis. The cyclic peptide MOR107 (Morphosys, https://vicorepharma.com/; previously Spinifex) has been successfully tested in a phase II trial for the treatment of neuropathic pain (120). More phase II studies with EMA401 are currently being initiated.

**CONCLUSIONS AND FUTURE DIRECTIONS**

The numerous beneficial actions of the novel RAS arms summarized in this review warrant clinical verification and therapeutic exploitation.

ACE2 has emerged as the dominant mechanism for negative regulation of the RAS by metabolizing ANG II into the beneficial peptide ANG-(1–7). This heptapeptide has emerged as a major protective peptide in the cardiovascular system. Clinical and experimental findings have demonstrated that ACE2 and ANG-(1–7) comprise the dominant mechanism for protective regulation of the RAS in many types of HF. ANG-(1–7) generated by ACE2-dependent conversion of ANG II is a crucial mediator of the cardioprotective effects, which makes ANG-(1–7) a promising therapy for HF.

After a long period of preclinical research to understand AT2R signaling and function better, this knowledge is currently being translated into developments for a potential, future clinical use of drugs targeting the AT2R. Most of these drugs in development are AT2R agonists, with current primary indications being fibrotic diseases and diabetic nephropathy. The AT2R antagonist EMA401 is being developed for the treatment of neuropathic pain. Phase II clinical studies with EMA401 are currently being initiated and will provide information about the therapeutic potential of drugs targeting the AT2R.

The first compounds activating the Mas and AT2 axis are available, and clinical trials have been started. For alatensins, the physiological and pathophysiological importance has still to be confirmed before therapeutic approaches can be initiated. However, it is quite likely that we will soon have novel therapies for cardiovascular and other diseases based on beneficial RAS peptides. Indeed, a recent study has described the beneficial effects of an oral formulation of hydroxypropyl-β-cyclodextrin/ANG-(1–7) in volunteers submitted to isometric overload muscle damage (7). This study, which is the first to test the effects of an oral formulation of ANG-(1–7) in humans, opens new possibilities for testing the actions of ANG-(1–7) and therapeutic effects in patients.

Finally, this review highlights the potential of phosphoproteomics to investigate ANG-(1–7) and aldosterone signaling in an unbiased way, allowing the identification of unanticipated downstream effectors of these signaling networks.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

R.A.S., G.Y.O., T.V.-B., and G.C. prepared figures; R.A.S., G.Y.O., T.V.-B., G.C., U.M.S., and M.B. drafted manuscript; R.A.S., G.Y.O., T.V.-B., G.C., U.M.S., and M.B. edited and revised manuscript; R.A.S., G.Y.O., T.V.-B., G.C., U.M.S., and M.B. approved final version of manuscript.

**REFERENCES**

1. Alenina N, Xu P, Rentzsch B, Patkin EL, Bader M. Genetically altered animal models for Mas and angiotensin-(1–7). *Exp Physiol* 93: 528–537. 2008. doi: 10.1113/expphysiol.2007.040345.

2. Alghamri MS, Weir NM, Anstadt MP, Elased KM, Gurley SB, Morris M. Enhanced angiotensin II-induced cardiac and arterial remodeling in ACE2 knockout mice. *J Cardiovasc Pharmacol Ther* 18: 138–151, 2013. doi: 10.1177/1074248412460124.

3. Alfred AJ, Diz DI, Ferrario CM, Chappell MC. Pathways for angiotensin-(1–7) metabolism in pulmonary and renal tissues. *Am J Physiol Renal Physiol* 279: F841–F850, 2000. doi: 10.1152/ajprenal.2000.279.5.F841.

4. Bader M, ACE2, angiotensin-(1–7), and Mas: the other side of the coin. *Pflugers Arch* 465: 79–85, 2013. doi: 10.1007/s00424-012-1120-0.

5. Bader M, Santos RA, Unger T, Steckelings UM. New therapeutic pathways in the RAS. *J Renin Angiotensin Aldosterone Syst* 13: 505–508, 2012. doi: 10.1177/1740771912466519.

6. Basu R, Poglitisch M, Yogasundaram H, Thomas J, Rowe BH, Oudit GY. Roles of angiotensin peptides and recombinant human ACE2 in heart failure. *J Am Coll Cardiol* 69: 805–819, 2017. doi: 10.1016/j.jacc.2016.11.064.

7. Becker LK, Totou N, Moura S, Kangussu L, Millán RDS, Campagnola-Santos MJ, Coelho D, Motta-Santos D, Santos RAS. Eccentric overload muscle damage is attenuated by a novel angiotensin-(1–7) treatment. *Int J Sports Med* 39: 743–748, 2018. doi: 10.1055/a-0633-8892.

8. Bedec K, Elbaz N, Sutren M, Masson M, Susini C, Strosberg AD, Nahmias C. Angiotensin II type 2 receptors mediate inhibition of particulate guanylate cyclase. *Biochem Soc Trans* 183: 206–211, 1992. doi: 10.1002/bst.291192629-5.

9. Bottari SP, King IN, Reichlin S, Dahlstroem I, Lydon N, de Gasparo M. The angiotensin AT2 receptor stimulates protein tyrosine phosphatase activity and mediates inhibition of particulate guanylate cyclase. *Biochem Biophys Res Commun* 183: 206–211, 1992. doi: 10.1006/bisr.1991.1074.

10. Bottari SP, Taylor V, King IN, Bogdal Y, Whitebread S, de Gasparo M. Angiotensin II AT2 receptors do not interact with guanine nucleotide binding proteins. *Eur J Pharmacol* 207: 157–163, 1991. doi: 10.1016/0922-4106(91)90091-U.

11. Boudina S, Han YH, Pei S, Sidwell TJ, Henrie B, Tuinei J, Olsen C, Sena S, Abel ED. UCP3 regulates cardiac efficiency and mitochondrial coupling in high fat-fed mice but not in leptin-deficient mice. *Diabetes* 61: 3260–3269, 2012. doi: 10.2337/db12-0063.

12. Boudina S, Sena S, Theobald H, Sheng X, Wright JJ, Hu XX, Aziz S, Johnson JJ, Bugger H, Zaha VG, Abel ED. Mitochondrial energetics in the heart in obesity-related diabetes: direct evidence for increased un-
coupled respiration and activation of uncoupling proteins. *Diabetes* 56: 2457–2466, 2007. doi:10.2337/db07-0481.

13. **Braunwald E, Bristow MR**. Congestive heart failure: fifty years of progress. *Circulation* 102, Suppl 4: IV14–IV23, 2000. doi:10.1161/01.CIR.102.suppl_4.IV-14.

14. Bruce E, Shenoy V, Rathinasabapathy A, Espejo A, Horowitz A, Oswalt A, Francis J, Nair A, Unger T, Raizada MK, Steckelings UM, Summers C, Katovich MJ. Selective activation of angiotensin AT2 receptors attenuates progression of pulmonary hypertension and inhibits cardiopulmonary fibrosis. *Br J Pharmacol* 172: 2219–2231, 2013. doi: 10.1111/bph.13044.

15. Brunet A, Bonni A, Zigmond MJ, Lin MJ, Zuo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 96: 857–867, 1999. doi:10.1016/S0092-8674(00)80595-4.

16. Campagnole-Santos MJ, Miz DI, Santos RA, Khosla MC, Broshann KB, Ferrario CM. Cardiovascular effects of angiotensin-(1–7) injected into the dorsal medulla of rats. *Am J Physiol Heart Circ Physiol* 257: H324–H329, 1989. doi:10.1152/ajpheart.1989.257.1.H324.

17. Canta GN, Lautner RQ, Santos RA. AT1R blockade increases the depressive effect of alamandine in normotensive SD rats [Abstract]. *Hypertension* 68, Suppl 1: AP192, 2016.

18. Chamoux E, Breault L, Lehoux JG, Gallo-Payet N. Influence of the angiotensin II type 2 receptor in apoptosis during human fetal adrenal gland development. *J Clin Endocrinol Metab* 84: 4722–4730, 1999. doi:10.1210/jc.84.12.4722.

19. Chappell MC, Pirro NT, Sykes A, Ferrario CM. Protective angiotensin type 2 receptors attenuates progression of pulmonary hypertension and inhibits cardiopulmonary fibrosis. *J Am Coll Cardiol* 52: 750–754, 2008. doi:10.1016/j.jacc.2008.02.088.

20. Fan D, Takawale A, Shen M, Samokhvalov V, Basu R, Patel V, Wang X, Fernandez-Patron C, Seubert JM, Oudit GY, Kassiri Z. A disintegrin and metalloprotease-17 regulates pressure overload-induced myocardial hypertrophy and dysfunction through proteolytic processing of integrin β1. *Hypertension* 68: 937–948, 2016. doi:10.1161/HYPERTENSIONAHA.116.07566.

21. Fan D, Takawale A, Shen M, Wang W, Wang X, Basu R, Oudit GY, Kassiri Z. Cardiomyocyte a disintegrin and metalloprotease 17 (ADAM17) is essential in post-myocardial infarction repair by regulating angiogenesis. *Circ Heart Fail* 8: 970–979, 2015. doi:10.1161/CIRCHEARTFAILURE.114.002029.

22. Giani JF, Muñoz MC, Penninger JM, Ferrario CM, Chappell MC. Activation of central angiotensin type 2 receptor. *Am J Physiol Heart Circ Physiol* 257: H137–H152, 2000. doi:10.1152/ajpheart.00618.2009.

23. Giani JF, Muñoz MC, Penninger JM, Ferrario CM, Chappell MC. Distinct roles for angiotensin-converting enzyme 2 and carboxypeptidase A in the processing of angiotensins within the murine heart. *Exp Physiol* 93: 613–621, 2008. doi:10.1113/epjhp.2007.040246.

24. Gembardt F, Sterner-Kock A, Imboden H, Spalteholz M, Reibitz F, Zylka MJ, Arden KC, Blenis J, Greenberg ME. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ Res* 87: E1–E9, 2000. doi:10.1161/01.RES.87.5.5.e1.

25. Doobay MF, Talman LS, Obr TD, Tian X, Davison RL, Lazartigues E. Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain-renin-angiotensin system. *Am J Physiol Regul Integr Comp Physiol* 292: R373–R381, 2007. doi:10.1152/ajpregu.00292.2006.

26. Epelman S, Tang WH, Chen SY, Van Lente F, Francis GS, Sen S. Detection of soluble angiotensin-converting enzyme 2 in heart failure: insights into the endothelin-counter-regulatory pathway of the renin-angiotensin-aldosterone system. *J Am Coll Cardiol* 52: 750–754, 2008. doi:10.1016/j.jacc.2008.02.088.

27. Grundy SM, Benjamin LJ, Burke GL, Chait A, Eckel RH, Howard BV, Milich W, Smith SC JR, Sowers JR. Diabetes and cardiovascular disease: a statement for healthcare professionals from the American
Heart Association. *Circulation* 100: 1134–1146, 1999. doi: 10.1161/01.CIR.100.1134.

48. Guy JL, Jackson RM, Acharya KR, Sturrock ED, Hooper NM, Turner AJ. Angiotensin-converting enzyme-2 (ACE2): comparative modeling of the active site, specificity requirements, and chloride dependence. *Biochemistry* 42: 13185–13192, 2003. doi: 10.1021/bi035268s.

49. Guy JL, Jackson RM, Jensen HA, Hooper NM, Turner AJ. Identification of critical active-site residues in angiotensin-converting enzyme-2 (ACE2) by site-directed mutagenesis. *FEBS J* 272: 3512–3520, 2005. doi: 10.1111/j.1742-4658.2005.04576.x.

50. Gwathmey TM, Pendergrass KD, Reid SD, Rose JC, Diz DJ, Chappell MC. Angiotensin-(1–7)-angiotensin-converting enzyme 2 attenuates reactive oxygen species formation to angiotensin II within the cell nucleus. *Hypertension* 55: 166–171, 2010. doi: 10.1161/HYPERTENSIONAHA.109.146126.

51. Habiyakare B, Alsaadon H, Mathai ML, Hayes A, Zulli A. Reduction of angiotensin A and amaldine vasoactivity in the rabbit model of atherogenesis: differential effects of alamandine and Ang(1–7). *Int J Exp Pathol* 95: 290–295, 2014. doi: 10.1111/iеп.12087.

52. Hakam AC, Siddiqui AH, Hussain T. Renal angiotensin II AT2 receptors promote natriuresis in streptozotocin-induced diabetic rats. *Am J Physiol Renal Physiol* 290: F503–F508, 2006. doi: 10.1152/ajprenal.01002.2005.

53. Hammer A, Yang G, Friedlich J, Kovacs A, Lee DH, Grave K, Jörg S, Alenina N, Grosch J, Winkel J, Gold R, Bader M, Manzel A, Rump LC, Müller DN, Linker RA, Stegbauer J. Role of the receptor Mas in macrophage-mediated inflammation in vivo. *Proc Natl Acad Sci USA* 113: 14109–14114, 2016. doi: 10.1073/pnas.162661113.

54. Hamming I, Timens W, Buhluis ML, Lely AT, van Goor H. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J Pathol* 203: 631–637, 2004. doi: 10.1002/path.1570.

55. Hansen JL, Servant G, Baranski TJ, Fujita T, Iiri T, SHEIKH SP. Functional reconstitution of the angiotensin II type 2 receptor and G activation. *Circ Res* 87: 753–759, 2000. doi: 10.1161/01..RES.87.9.753.

56. Hayashi T, Takai S, Yamashita C. Impact of the renin-angiotensin-aldosterone-system on cardiovascular and renal complications in diabetes mellitus. *Curr Vasc Pharmacol* 8: 189–197, 2010. doi: 10.2174/1741670709686947.

57. Hunter JJ, Chien KR. Signaling pathways for cardiac hypertrophy and failure. *N Engl J Med* 341: 1276–1283, 1999. doi: 10.1056/NEJM19990121341106.

58. Iwata M, Cowling RT, Gurantz D, Moore C, Zhang S, Yuan JX, Infeld J, Cukon S, Jorde UP, Ennezat PV, Lisker J, Suryadevara V, Infeld J. Evidence for heterodimerization of the renin-angiotensin-system in myocardial infarction? *Circulation* 118: 2523–2532, 2008. doi: 10.1161/CIRCULATIONAHA.107.184468.

59. Kassiri Z, Oudit GY, Sanchez O, Dawood F, Mohammed FF, Nuttall RK, Edwards DR, Liu PP, Backh PH, Khokh R. Combination of tumor necrosis factor-alpha ablation and matrix metalloproteinase inhibition prevents heart failure after pressure overload in tissue inhibitor of matrix metalloproteinase-3 knock-out mice. *Circ Res* 97: 380–390, 2005. doi: 10.1161/01.RES.0000178789.16929.e1.

60. Kassiri Z, Zhong J, Guo D, Basu R, Wang X, Liu PP, Scholey JW, Penninger JM, Oudit GY. Loss of angiotensin-converting enzyme 2 accelerates maladaptive left ventricular remodeling in response to myocardial infarction. *Circ Heart Fail* 2: 446–455, 2009. doi: 10.1161/CIRCHARTFAILURE.108.340124.

61. Kenchais S, Evans JC, Levy D, Wilson PW, Benjamin EJ, Larson MG, Kannal WB, Vasan RS. Obesity and the risk of heart failure. *N Engl J Med* 347: 305–312, 2002. doi: 10.1056/NEJMoa020245.

62. Kenchais S, Sesso HD, Gaziano JM. Body mass index and vigorous physical activity and the risk of heart failure among men. *Circulation* 119: 44–52, 2009. doi: 10.1161/CIRCULATIONAHA.108.807289.

63. Kopelman PG. Obesity as a medical problem. *Nature* 404: 635–643, 2000.

64. Kuba K, Imai Y, Rao S, Jiang C, Penninger JM. Lessons from SARS: control of acute lung failure by the SARS receptor ACE2. *J Mol Med (Berl)* 84: 814–820, 2006. doi: 10.1007/s00109-006-0994-9.

65. Langlet F, Haeusler RA, Lindén D, Ericson E, Norris T, Johansson MG, Kannel WB, Ferran R. Obesity and the risk of heart failure. *Hypertension* 58: 598–603, 2006. doi: 10.1161/HYPERTENSIONAHA.109.141622.

66. Li M, Liu K, Michalicek J, Angus JA, Hunt JE, Dell'Italia LJ, Feneley MP, Graham RM, Husain A. Involvement of chymase-mediated activation of the angiotensin type 2 receptor fragment. *Am J Physiol Cell Physiol* 268: C278–C282, 1995. doi: 10.1152/ajpcell.1995.268.1.C278.

67. Kaschina E, Grzesiak A, Li J, Foryst-Ludwig A, Timm M, Rompe F, Sommerfeld M, Kemnitz UR, Curato C, Namsolleck P, Tschöpe C, Hallberg A, Alterman M, Hukoo T, Paetsch I, Dietrich T, Schnackenburg B, Graf K, Dahlöf B, Kintschers U, Unger T, Steckelings UM. Angiotensin II type 2 receptor stimulation: a novel option of therapeutic interference with the renin-angiotensin system in myocardial infarction? *Circ Res* 118: 1126–1135, 2016. doi: 10.1161/CIRCULATIONAHA.116.08814.

68. Li M, Liu K, Michalicek J, Angus JA, Hunt JE, Dell’Italia LJ, Feneley MP, Graham RM, Husain A. Involvement of chymase-mediated...
81. Mercure C, Yogi A, Callera GE, Aranha AB, Bader M, Ferreira AJ, Meffert S, Stoll M, Steckelings UM, Bottari SP, Unger T.

82. Marques FD, Ferreira AJ, Sinisterra RD, Jacoby BA, Sousa FB, Caliari AM.

83. Marins FR, Oliveira AC, Qadri F, Alenina N, Fontes MAP, Santos RA.

84. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol 292: C82–C97, 2007. doi:10.1152/ajpcell.00287.2006.

85. Mercure C, Yogi A, Callera GE, Aranha AB, Bader M, Ferreira AJ, Santos RA, Walther T, Touzy RM, Reudellhuber TL. Angiotensin-(1-7) blunts hypertensive cardiac remodeling by a direct effect on the heart. Circ Res 103: 1319–1326, 2008. doi:10.1161/CIRCRESAHA.108.184911.

86. Mori J, Patel VB, Abo Alrob O, Basu R, Altamimi T, Desauniers J, Wagg CS, Kassiri Z, Lopachuk GD, Oudit GY. Angiotensin 1-7 ameliorates diabetic cardiomyopathy and diastolic dysfunction in db/db mice by reducing lipotoxicity and inflammation. Circ Heart Fail 7: 327–339, 2014. doi:10.1161/CIRCHEARTFAILURE.113.000672.

87. Nakao YM, Teramukai S, Tanaka S, Yasuno S, Fujimoto A, Kasa-hara M, Ueshima K, Nakao K, Hirutani S, Nakao K, Kawakami K. Effects of renin-angiotensin system blockades on cardiovascular outcomes in patients with diabetes mellitus: a systematic review and meta-analysis. Diabetes Res Clin Pract 96: 68–75, 2012. doi:10.1016/j.diabres.2011.11.025.

88. Namsolleck P, Recarti C, Foulquier S, Steckelings UM, Unger T. AT2 receptor and tissue injury: therapeutic implications. Circ Res Rep 16: 416, 2014. doi:10.1161/HS1006-013-0416-0.

89. Noutet S, Amzallag N, Li JM, Louis S, Seitz I, Cui TX, Alleaume AM, Di Benedetto M, Boden C, Masson M, Strosberg AD, Horiiuchi M, Couraud PO, Nahmias C. Trans-inactivation of receptor tyrosine kinases by novel angiotensin II AT2 receptor-interacting protein, ATIP. J Biol Chem 279: 28899–28997, 2004. doi:10.1074/jbc.M403880200.

90. Ocarranza MP, Lavandero S, Jalil JE, Moyo J, Pinto M, Novoa V, Agache F, González-L, Hernandez C, Varas M, Lopez R, Godoy I, Verdejo H, Chiong M. Angiotensin-(1–9) regulates cardiomyocyte hypertrophy in vivo. J Hypertens 28: 527–528, 2010. doi:10.1097/01.HYP.0000240382.04903.45.

91. Ocarranza MP, Lavandero S, Jalil JE, Moyo J, Pinto M, González-L, Chiong M, Lavandero S, Jalil JE. Rho kinase inhibition activates the homologous angiotensin-converting enzyme-angiotensin-(1–9) axis in experimental hypertension. J Hypertens 29: 706–715, 2011. doi:10.1097/HIJ.0b013e3283496665.

92. Oliveira AC, Melo MB, Motta-Santos D, Peluso AA, Souza-Neto F, da Silva RF, Almeida JFP, Canta G, Reis AM, Gonçalves G, Cerri G, Coutinho D, Guedes de Jesus IC, Guatimosim S, Linhares ND, Alenina N, Bader M, Campagnole-Santos MJ, Santos RAS. Genetic deletion of the alamandine receptor MRGD leads to dilated cardiomyopathy in mice. Am J Physiol Heart Circ Physiol 316: H123–H133, 2019. doi:10.1152/ajpheart.00075.2018.

93. Oudit GY, Penninger JM. Recombinant human angiotensin-converting enzyme 2 as a new renin-angiotensin system peptidase for heart failure therapy. Curr Heart Fail Rep 8: 176–183, 2011. doi:10.1007/s11897-011-0063-7.

94. Owman TE, Hodge DO, Herzges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. N Engl J Med 355: 251–259, 2006. doi:10.1056/NEJMoa052256.

95. Païgis G, Tilkellis C, Cooper ME, Schembri JM, Lew RA, Smith AI, Shaw T, Warner FJ, Zauli A, Burrell LM, Angus PW. Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. Gut 54: 1790–1796, 2005. doi:10.1136/gut.2004.062398.

96. Parajuli N, Patel VB, Wang W, Basu R, Oudit GY. Loss of NOX2 (gp91phox) prevents oxidative stress and progression to advanced heart failure. Clin Sci (Lond) 127: 331–340, 2014. doi:10.1042/CS20130787.

97. Patel SN, Ali Q, Samuel P, Steckelings UM, Hussain T. Angiotensin II type 2 receptor and receptor mas are colocalized and functionally interdependent in obese Zucker rat kidney. Hypertension: 70: 831–838, 2017. doi:10.1161/HYPERTENSIONAHA.117.096405.

98. Patel VB, Bodiga S, Fan D, Das SK, Wang Z, Wang W, Basu R, Zhong J, Kassiri Z, Oudit GY. 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, 2012. doi:10.1161/HYPERTENSIONAHA.112.191690.

99. Patel VB, Clarke N, Wang Z, Fan D, Parajuli N, Basu R, Putko B, Kassiri Z, Turner AJ, Oudit GY. Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: a positive feedback mechanism in the RAS. J Mol Cell Cardiol 66: 167–174, 2014. doi:10.1016/j.yjmcc.2013.11.017.

100. Patel VB, Lezutokeng JN, Chen X, Oudit GY. Recombinant human ACE2 and the angiotensin 1–7 axis as potential new therapies for heart failure. Can J Cardiol 33: 943–946, 2017. doi:10.1016/j.cja.2016.12.012.

101. Patel VB, Mori J, McLean BA, Basu R, Das SK, Ramprashat T, Parajuli N, Penninger JM, Grant MB, Lopachuk GD, Oudit GY. ACE2 deficiency worsens epididymal adipose tissue inflammation and cardiac dysfunction in response to diet-induced obesity. Diabetes 65: 85–95, 2016. doi:10.2337/db15-0399.

102. Patel VB, Shah S, Verma S, Oudit GY. Epididymal adipose tissue as a metabolic transducer: role in heart failure and coronary artery disease. Heart Fail Rev 22: 889–902, 2017. doi:10.1007/s10741-017-9464-1.

103. Patel VB, Takawale A, Ramprashat T, Das SK, Basu R, Grant MB, Hall DA, Kassiri Z, Oudit GY. Antagonism of angiotensin 1–7 prevents the therapeutic effects of recombinant human ACE2. J Mol Med (Berl) 93: 1003–1013, 2015. doi:10.1007/s00109-015-1285-z.

104. Patel VB, Zhong JC, Fan D, Basu R, Morton JS, Parajuli N, McMurtry MS, Davidge ST, Kassiri Z, Oudit GY. Angiotensin-converting enzyme 2 is a critical determinant of angiotensin II-induced loss of vascular smooth muscle cells and adverse vascular remodeling. Hypertension 64: 157–164, 2014. doi:10.1161/HYPERTENSIONAHA.114.03388.

105. Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ACE2/angiotensin-1–7 axis of the renin-angiotensin system in heart failure. Circ Res 118: 1313–1326, 2016. doi:10.1161/CIRCRESAHA.116.307708.

106. Paulos WJ, Tschipe C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol 62: 263–271, 2013. doi:10.1016/j.jacc.2013.02.092.
Elevated plasma angiotensin converting enzyme 2 activity is an independent predictor of major adverse cardiac events in patients with obstructive coronary artery disease. PLOS One 13: e0198144, 2018. doi:10.1371/journal.pone.0198144.

Rice ASC, Dworkin RH, McCarthy TD, Anand P, Bountra C, McCloud PI, Hill J, Cutter G, Kitson D, Gesem N, Raff M; EMA01-003 study group. EMA01, an orally administered highly selective angiotensin-(1–7) receptor antagonist, as a novel treatment for heart failure with preserved ejection fraction relative to heart failure with reduced ejection fraction: evidence for a divergence in pathophysiology. PLoS One 9: e99495, 2014. doi:10.1371/journal.pone.0099495.

Qaradaki T, Matsoukas MT, Hayes A, Rybalka E, Caprnda M, Rimarova K, Sepi M, Büsselberg D, Matozak M, Apostolopoulos V, Zulii A. Alamadine reverses hyperhomocysteineemia induced vascular dysfunction via PKA-dependent mechanisms. Cardiovasc Ther 35: e1236, 2017. doi:10.1111/1755-5222.12306.

Qi Y, Shenoy V, Wong F, Li H, Afzal A, Mocoo J, Sumners C, Raizada MK, Katovich MJ. Linivertus-mediated overexpression of angiotensin-(1–7) attenuated ischaemia-induced cardiac pathophysiology. Exp Physiol 96: 863–874, 2011. doi:10.1113/exphysphysioll.2011.056994.

Indrand P, Patel SK, Srivastava PM, Farouque D, Burrell LM. Impairment of in vitro and in vivo heart function in angiotensin-(1–7) receptor MAS knockout mice. Hypertension 47: 996–1002, 2006. doi:10.1161/01.HYP.0000213529.51180.5c.

Santos RA, Simoes e Silva AC, Marie 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. Angiotensin-(1–7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci USA 100: 8258–8263, 2003. doi:10.1073/pnas.1432869100.

Santos RA, Campanoa WO, Alzamora AC, Motta-Santos D, Alenina N, Bader M, Campagnole-Santos MJ. The ACE2/angiotensin-(1–7) MAS axis of the renin-angiotensin system: focus on angiotensin-(1–7). Physiol Rev 98: 505–553, 2018. doi:10.1152/physrev.00023.2016.

Sato M, Iwasaka J, Nakamura M, Akatsu T, Shimoda Y, Hiramori K. Increased expression of tumor necrosis factor-alpha converting enzyme and tumor necrosis factor-alpha in peripheral blood mononuclear cells in patients with advanced congestive heart failure. Eur J Heart Fail 6: 869–875, 2004. doi:10.1016/j.ejheart.2002.02.007.

Sato M, Nakamura M, Sato H, Saitoh H, Segawa I, Hiramori K. Expression of tumor necrosis factor-alpha-converting enzyme and tumor necrosis factor-alpha in human myocarditis. J Am Coll Cardiol 36: 1288–1294, 2000. doi:10.1016/S0735-1097(00)00827-5.

Schiavote MT, Santos RA, Brosnihan KB, Khosla MC, Ferrario CM. Release of vaso.pressin from the rat hypothalamo-neurohypophyseal system by angiotensin-(1–7) heptapeptide. Proc Natl Acad Sci USA 85: 185–188, 1988. doi:10.1073/pnas.85.1.185.

Schiavone F, Tesouro M, Veneziani A, Mores N, Di Daniele N, Cardillo C. Favorable vascular actions of angiotensin-(1–7) in human obesity. Hypertension 71: 185–198, 2015. doi:10.1161/HYPERTESNAHA.117.10280.

Seguin LR, Villarreal RS, Ciuffo GM. AT2 receptors recruit c-Src, SHP-1 and FAK upon activation by Ang II in PND15 rat hindbrain. Neurochem Int 60: 199–207, 2012. doi:10.1016/j.neuint.2011.11.008.

Shaltout HA, Westwood BM, Avillar DB, Ferrario CM, Figueroa JP, Diz DI, Rose JC, Chappell MC. Angiotensin metabolism in renal proximal tubules, urine, and serum of sheep: evidence for ACE2-dependent processing of angiotensin II. Am J Physiol Renal Physiol 292: F82–F91, 2007. doi:10.1152/ajprenal.00139.2006.

Shen M, Hu M, Fedak PWM, Oudit GY, Kassiri Z. Cell-specific functions of ADAM17 regulate the progression of thoracic aortic aneurysm. Circ Res 123: 372–388, 2018. doi:10.1161/CIRCRESAHA.118.313181.

Shen M, Morton J, Davidge ST, Kassiri Z. Loss of smooth muscle cell disintegrin and metalloproteinase 17 transiently suppresses angiotensin II-induced hypertension and end-organ damage. J Mol Cell Cardiol 103: 11–21, 2017. doi:10.1016/j.yjmcc.2016.12.001.

Shen YH, Chen XR, Yang CX, Liu BX, Li P. Mas-related G protein-coupled receptor-like 2 receptor antagonist as a novel treatment for postherpetic neuralgia: a randomised, double-blind, placebo-controlled phase 2 clinical trial. Lancet 383: 1637–1647, 2014. doi:10.1016/S0140-6736(13)62337-5.

Rome F, Unger T, Steckelings UM. The angiotensin AT1 receptor in inflammation. Drug News Perspect 23: 104–111, 2010. doi:10.1585/dnp.2010.23.2.1475901.

Sampaio WO, Henrique de Castro C, Santos RA, Schiffrin EL, Touyz RM. Angiotensin-(1–7) counterregulates angiotensin II signaling in human endothelial cells. Hypertension 50: 1093–1098, 2007. doi:10.1161/HYPERTEHSNAHA.106.084848.

Santos RA, Campanoa WO, Souza do Santos RA, Faria-Silva R, de Mata Machado LT, Schiffrin EL, Touyz RM. Angiotensin-(1–7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. Hypertension 49: 185–192, 2007. doi:10.1161/01.HYP.0000251865.35728.2f.

Santos RA, Brosnihan KB, Chappell MC, Pesquero J, Chernicky CL, Greene LJ, Ferrario CM. Converting enzyme activity and angiotensin metabolism in the dog brainstem. Hypertension 11: 1153–1157, 1988. doi:10.1161/01.HYP.11.2.P_2.1153.

Santos RA, Castro CH, Gava E, Pinheiro SV, Almeida AP, de Paula RD, Cruz JS, Ramos AS, Rosa KT, Irigoyen MC, Bader M, Alenina N, Kitten GT, Ferreira AJ. Impairment of in vitro and in vivo heart function in angiotensin-(1–7) receptor MAS knockout mice. Hypertension 47: 996–1002, 2006. doi:10.1161/01.HYP.0000213529.51180.5c.

Santos RA, Brosnihan KB, Chappell MC, Pesquero J, Chernicky CL, Greene LJ, Ferrario CM. Converting enzyme activity and angiotensin metabolism in the dog brainstem. Hypertension 11: 1153–1157, 1988. doi:10.1161/01.HYP.11.2.P_2.1153.

Santos RA, Castro CH, Gava E, Pinheiro SV, Almeida AP, de Paula RD, Cruz JS, Ramos AS, Rosa KT, Irigoyen MC, Bader M, Alenina N, Kitten GT, Ferreira AJ. Impairment of in vitro and in vivo heart function in angiotensin-(1–7) receptor MAS knockout mice. Hypertension 47: 996–1002, 2006. doi:10.1161/01.HYP.0000213529.51180.5c.
144. Steckelings UM, Paulis L, Namskoleck P, Unger T. AT2 receptor agonists: hypertension and beyond. Curr Opin Nephrol Hypertens 21: 142–146, 2012. doi:10.1097/MNH.0b013e328350261b.

145. Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1–7) induces growth of cardiac myocytes through activation of the mas receptor. Am J Physiol Heart Circ Physiol 289: H1560–H1566, 2005. doi:10.1152/ajpheart.00941.2004.

146. te Riet L, van Esch JH, Roks AJ, van den Meiracker AH, Danse AH. Hypertension: renin-angiotensin-aldosterone system alterations. Circ Res 116: 960–975, 2015. doi:10.1161/CIRCRESAHA.116.303587.

147. Tetzner A, Gebolys K, Meintert C, Uhlich A, Trebicka J, Villacádas Ó, Walther T. G-protein-coupled receptor MgD is a receptor for angiotensin-(1–7) involving adenylyl cyclase, cAMP, and phosphokinase A. Hypertension 68: 185–194, 2016. doi:10.1161/HYPERTENSIONAHA.116.05752.

148. Tigerstedt R, Bergman PG. Niere und kreislauf. Skand Arch Physiol 8: 223–271, 1898. doi:10.1111/j.1744-1718.1898.tb00272.x.

149. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem 275: 33238–33243, 2000. doi:10.1074/jbc.M002615200.

150. Uchiyama Y, Okajima F, Mogi G, Tobo A, Tomono S, Sato K. Alamandine reduces leptin expression through the c-Src/p38 MAP kinase pathway in adipose tissue. PLoS One 12: e0178769, 2017. doi:10.1371/journal.pone.0178769.

151. Unger T, Steckelings UM, Santos RA. The Protective Arm of the Renin Angiotensin System (RAS): Functional Aspects and Therapeutic Implications. Cambridge, MA: Academic, 2015, p. 316.

152. Verano-Braga T, Schwämme V, Sylvester M, Passos-Silva DG, Peluso AA, Eletvino GM, Santos RA, Roepstorff P. Time-resolved quantitative phosphoproteomics: new insights into angiotensin-(1–7) signaling networks in human endothelial cells. J Proteome Res 11: 3370–3381, 2012. doi:10.1021/pr3001755.

153. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout T, Kassiri Z, Penninger JM, Oudit GY. Heterozygote loss of ACE2 is sufficient to increase the susceptibility to heart disease. Circ Res 116: 1165–1175, 2017. doi:10.1161/CIRCRESAHA.116.05752.

154. Wang W, Patel VB, Parajuli N, Fan D, Basu R, Wang Z, Ramprasath V, Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, Godbout T, Kassiri Z, Penninger JM, Batlle D. Targeting the degradation of angiotensin II with recombinant angiotensin-converting enzyme 2: prevention of angiotensin II-dependent hypertension. Hypertension 55: 90–98, 2010. doi:10.1161/HYPERTENSIONAHA.109.138420.

155. Xu J, Sirtanumula S, Xu Y, Taverna-Walton L, Cucích F, Domenig O, Poglitsch M, Lazartigues E. Clinical relevance and role of neuronal AT1 receptors in ADAM17-mediated ACE2 shedding in neurogenic hypertension. Circ Res 121: 43–55, 2017. doi:10.1161/CIRCRESAHA.116.310509.

156. Xu P, Costa-Goncalves AC, Todiras M, Rabelo LA, Sampaio WO, Moura MM, Santos SS, Luft FC, Bader M, Gross V, Alenina N, Santos RA. Endothelial dysfunction and elevated blood pressure in MAS gene deficient mice. Hypertension 51: 574–580, 2008. doi:10.1161/HYPERTENSIONAHA.107.102764.

157. Yamada T, Horiiuchi M, Dzau VJ. Angiotensin II type 2 receptor mediates programmed cell death. Proc Natl Acad Sci USA 93: 156–160, 1996. doi:10.1073/pnas.93.1.156.

158. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE JR, Drzewiecki M, Jararov GC, Garnezi S, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBridge PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American College of Cardiology Foundation/Quantitative Heart Association Task force on practice guidelines. Circulation: 128: 1810–1852, 2013. doi:10.1161/CIR.0b013e3182f9a007.

159. Yang R, Smolders I, Vanderheyden P, Demaegdt H, Van Eeckhaut A, Vauquelin G, Lukaszuk A, Tourwé D, Chai SY, Albiston AL, Nahmias C, Walther T, Dupont AG. Pressor and renal hemodynamic effects of the novel angiotensin A peptide: angiotensin II type 1A receptor dependent. Hypertension 57: 956–961, 2011. doi:10.1161/HYPERTENSIONAHA.110.161836.

160. Yayama K, Iiyoshi H, Imazu D, Okamoto H. Angiotensin II stimulates endothelial NO synthase phosphorylation in thoracic aorta of mice with abdominal aortic banding via type 2 receptor. Hypertension 48: 958–964, 2006. doi:10.1161/HYPERTENSIONAHA.105.05092.

161. Ye M, Wysocki J, Gonzalez-Pacheco FR, Salem M, Evora K, Garcia-Halpin L, Poglitsch M, Schuster M, Battle D. Murine recombinant angiotensin-converting enzyme 2: effect on angiotensin II-dependent hypertension and distinctive angiotensin-converting enzyme 2 inhibitor characteristics on rodent and human angiotensin-converting enzyme 2. Hypertension 60: 730–740, 2012. doi:10.1161/HYPERTENSIONAHA.112.198622.

162. Zhang H, Han GW, Batyuik A, Ishchenko A, White KL, Patel N, Sadybekov A, Zamlynnob B, Rudd MT, Holenstein K, Tolstikova A, White TA, Hunter MS, Weierstall U, Liu W, Babbage K, Moore EL, Katz RD, Shipman JM, Garcia-Cevalo M, Sharma S, Sheth P, Soisson SM, Stevens RC, Katritch V, Cherezov V. Structural basis for selectivity and diversity in angiotensin II receptors. Nature 544: 327–332, 2017. doi:10.1038/nature22035.

163. Zhang Y, Li B, Wang B, Zhang J, Wu J, Morgan T. Alteration of cardiac ACE2/Mas expression and cardiac remodelling in rats with aortic constriction. Clin J Physiol 70: 335–342, 2014. doi:10.4077/CJP.2014. BAD268.

164. Zhang ZZ, Wang W, Jin HY, Chen X, Cheng YW, Yu YL, Song B, Pennington JM, Oudit GY, Zhong HC. Apelin is a negative regulator of angiotensin II-mediated adverse myocardial remodelling and dysfunction. Hypertension 70: 1165–1175, 2017. doi:10.1161/HYPERTENSIONAHA.117.10156.

165. Zhong J, Basu R, Guo D, Chow FL, Byrnes S, Schuster M, Loibner A, Wang XH, Pennington JM, Kassiri Z, Oudit GY. Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction in rat hypertension. Circulation 122: 717–728, 2010. doi:10.1161/CIRCULATIONAHA.110.055369.

166. Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. Nature 414: 782–787, 2001. doi:10.1038/414782a.