Chapter

Renin-Angiotensin-Aldosterone System in Heart Failure: Focus on Nonclassical Angiotensin Pathways as Novel Upstream Targets Regulating Aldosterone

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Abstract

Aldosterone plays an important role in the regulation of blood pressure, body fluid, and electrolyte homeostasis. Overactivation of aldosterone/mineralocorticoid receptor (MR) pathway leads to hypertension, atherosclerosis, vascular damage, heart failure, and chronic kidney disease and is involved in many other diseases associated with endothelial dysfunction, inflammation, fibrosis, and metabolic disorders. Aldosterone is a final product of the renin-angiotensin-aldosterone system (RAAS), and its production is activated by angiotensin II, while angiotensin-(1–7) negatively regulates angiotensin II-mediated aldosterone production and in some experimental models inhibits aldosterone-induced damage in target tissues. In fact, the aldosterone/mineralocorticoid receptor-dependent pathway is regulated upstream by at least two major axes of RAAS: classical axis (ACE/Ang II) and nonclassical axis (ACE2/Ang-(1–7)). The relative balance between these two axes determines aldosterone production and activity. To better understand the regulation of aldosterone activity in physiology and diseases, it is important to analyze the role of aldosterone/mineralocorticoid receptor-dependent pathways in the context of upstream angiotensin pathways as some of the recently described mechanisms of RAAS represent possible novel upstream targets to inhibit aldosterone/mineralocorticoid receptor-dependent responses. In this review, we highlight the complexity of angiotensin pathways focusing on their role in various tissues in heart failure, with particular emphasis on nonclassical pathways including protective ACE2/Ang-(1–7) axis and detrimental Ang-(1–12)/chymase/Ang II axis.

Keywords: angiotensin pathways, angiotensin-converting enzyme (ACE), angiotensin-converting enzyme 2 (ACE2), chymase, aldosterone, heart failure

1. Introduction

The renin-angiotensin-aldosterone system (RAAS) includes angiotensin (poly) peptides such as angiotensinogen, angiotensin I, angiotensin II, angiotensin III,

angiotensin IV, angiotensin-(1–9), angiotensin-(1–7), alamandine and angiotensin A [1], and a number of enzymes regulating the production of particular angiotensins (renin, angiotensin-converting enzyme (ACE) and angiotensin-converting enzyme 2 (ACE2), chymase, neutral endopeptidase (NEP), prolyl endopeptidase (PEP), and others) [2–5], as well as specific receptors such as AT_1R, AT_2R, AT_4R, MasR, or MrgDR, activated in response to a given angiotensin sub-type [5]. All these elements contribute to the incredible complexity of the RAAS that is not perceived any more like a simple linear system with two major enzymes (renin and ACE) generating Ang II but rather as a network of tightly regulated peptides and enzymes endowed not only with endocrine (tissue to tissue) but also paracrine (cell-to-cell) and an intracrine (intercellular/nuclear) activities. There is also abundant evidence for the importance of tissue-based angiotensin pathways that seems to be heterogeneously organized in various organs which act independently of the RAAS in plasma. The major role of the protective ACE2/Ang-(1–7) axis counteracting classic ACE/Ang II axis has also been well documented. A simplified scheme of the angiotensin pathways with major angiotensins, enzymes, and receptors is shown in **Figure 1**.

The final RAAS product, aldosterone, plays an important role in the regulation of blood pressure, body fluid, and electrolyte homeostasis, but overactivation of aldosterone/mineralocorticoid receptor (MR) pathway leads to hypertension, atherosclerosis, vascular damage, heart failure, and chronic kidney disease and is involved in many other diseases associated with endothelial dysfunction, inflammation, fibrosis, metabolic disorders, and organ damage [6–9]. The overstimulation of AT_1R (by Ang II and its metabolites) and increased aldosterone production result among others in increased ROS production and NADPH oxidase activation [9, 10] that contribute to cardiac and vascular pathology [11].

The importance of RAAS in cardiovascular, hypertensive, and kidney diseases has been firmly established by therapeutic effects of renin inhibitors,

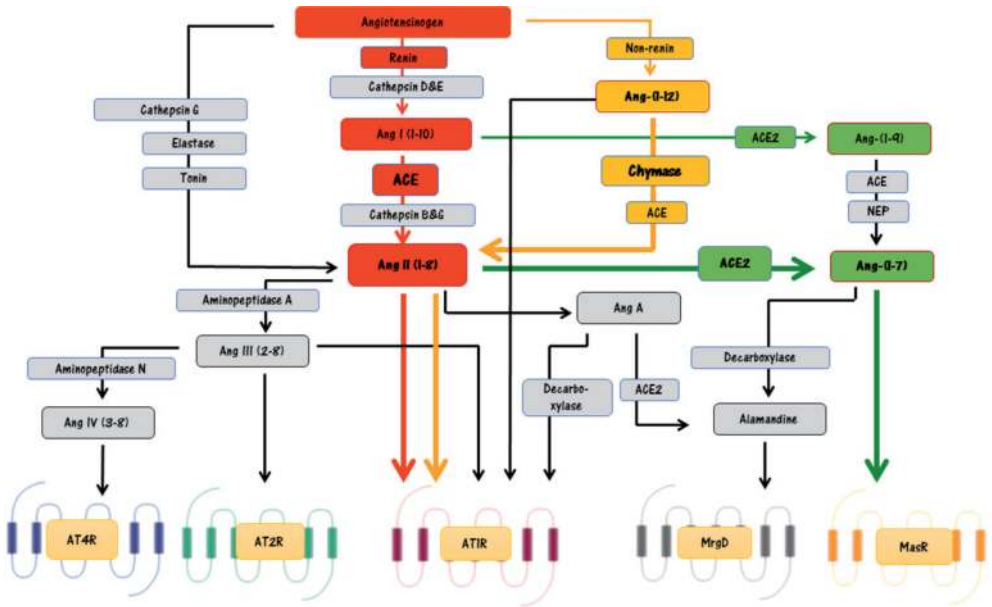

Figure 1.
Simplified scheme of major angiotensin pathways with respective enzymes and receptors. Abbreviations: Ang, angiotensin; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; NEP, neutral endopeptidase; AT1R, angiotensin receptor type 1; AT2R, angiotensin receptor type 2; AT4R, angiotensin receptor type 4; MasR, Mas receptor; MrgD, MAS-related G protein-coupled receptor member D. Colors: Red, classical axis (renin/ACE/Ang II); green, nonclassical protective axis (ACE2/Ang-(1–7); orange, non-renin non-ACE, chymase-dependent axis Ang-(1–12)/chymase/Ang II axis; gray, other elements.

angiotensin-converting enzyme inhibitors (ACE-I), angiotensin receptor blockers (ARBs), and finally mineralocorticoid receptor antagonists (MRA). Importantly, these drugs influence not only the downstream but also the upstream activity of the RAAS. This phenomenon is rather overlooked but needs to be taken into account in designing the optimal RAAS-targeted therapy for the treatment of a variety of diseases. In particular, evidence accumulated showing reciprocal regulation of major angiotensins and aldosterone/mineralocorticoid pathway (**Figure 2**).

Indeed, MRA modify upstream pathways. MRA decrease ACE activity and increase ACE2 activity, suggesting a protective role for MRA is not only mediated by the direct inhibition of MR-dependent pathways but also by increasing the expression of ACE2 and generating angiotensin-(1–7) and decreasing the formation of angiotensin II as documented in heart failure (HF) patients and in the rat model of renal dysfunction [12–14]. Noteworthy, plasma levels of Ang-(1–7) increase after treatment with ACE-I or ARB [15–20]. On the other hand, aldosterone upregulates the expression and activity of upstream ACE [21]. Furthermore, aldosterone-induced accelerated production of an angiotensin II is negatively regulated by angiotensin-(1–7) via the Mas receptor and JAK/STAT signaling in human adrenal cells [22]. Angiotensin-(1–7) may also suppress aldosterone-induced damage in target tissues. For example, angiotensin-(1–7) inhibits hypertensive kidney damage induced by infusion of aldosterone [23]. Interestingly, this effect is independent of blood pressure and mediated by the suppression of the expression of TGF, FGF, TIMP, and ROS production suggesting that the inhibition of aldosterone activity by angiotensin-(1–7) occurs locally in the kidney [23]. Angiotensin-(1–7) may inhibit angiotensin II-mediated effects on aldosterone not only by counterbalancing effects mediated by the activation of Mas receptor [23] but also by acting as natural-biased ligand for the AT_1 receptor, behaving as a natural competitive neutral antagonist for AT_1 in G protein-dependent signaling while simultaneously acting as an agonist for β-arrestin-related signaling [24].

In summary, aldosterone/mineralocorticoid receptor-dependent signaling pathways are under upstream regulation by at least two major axes of the RAAS: classical axis (ACE/Ang II) and nonclassical axis (ACE2/Ang-(1–7)). The relative ratio of these two axes determines aldosterone production and activity, and reciprocally aldosterone production might affect upstream mechanisms of RAAS. For the better understanding of the regulation of aldosterone/mineralocorticoid receptor-dependent pathways and optimal pharmacotherapy of diseases associated with aldosterone overactivation, one needs to take into account the regulatory

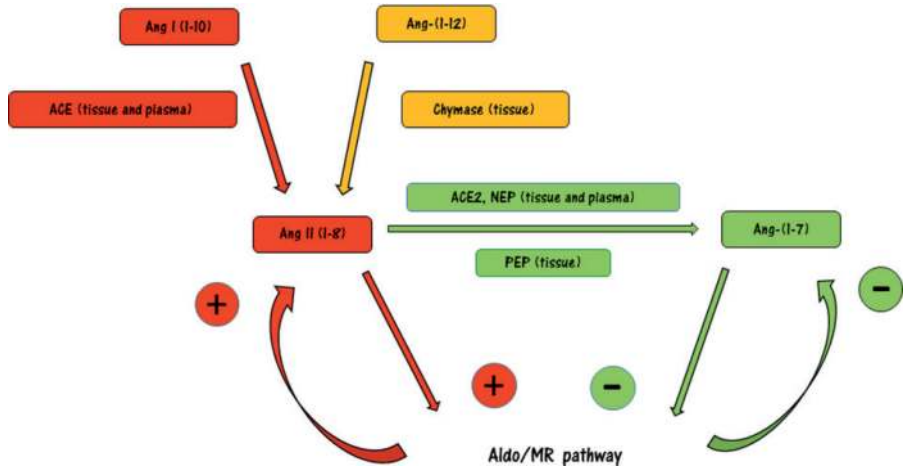

Figure 2.
*Scheme showing the reciprocal regulation of major angiotensins and aldosterone/mineralocorticoid pathway. Abbreviations: PEP, prolyl endopeptidase (see **Figure 1** for other abbreviations and colors coding).*

role of at least two major angiotensin pathways, the balance of which determines aldosterone/mineralocorticoid receptor-dependent pathways. Here, we review the complexity of local angiotensin pathways focusing on their role in various tissues in heart failure with particular emphasis on nonclassical pathways including protective ACE2/Ang-(1–7) axis and detrimental Ang-(1–12)/chymase/Ang II axis.

2. Classical (ACE/Ang II/AT_1R) and nonclassical (ACE2/Ang-(1–7)/MasR) axes of RAAS

The classical RAAS pathway involves renin secreted by the kidney to produce Ang I from angiotensinogen (derived from the liver) (**Figures 1** and **2**). Ang I is then converted mainly through ACE to Ang II which predominantly stimulates the AT_1 receptor, the major culprit receptor for Ang II-induced cardiovascular pathology. Indeed, overactivation of AT_1R contributes to the pathophysiology of heart failure inducing cardiac fibrosis, inflammation, cell proliferation, coronary vasoconstriction, and cardiomyocyte hypertrophy, as well as apoptosis [25], cardiac remodeling [25, 26], vascular stiffness and atherosclerosis [27], endothelial dysfunction, oxidative stress, or insulin resistance [28]. Ang II may also stimulate AT_2R that has a vasoprotective profile—anti-inflammatory, antifibrotic, and anti-apoptotic—involving the activation of bradykinin/NO/cGMP system [29]. AT_2R is linked also to the regulation of vascular and cardiac growth responses [30]. Activation of AT_2R after cardiac injury decreases sympathetic overstimulation and stimulates cardiac regeneration with increasing coronary vasodilation [31]. It was also shown that AT_2R stimulation may indirectly increase ACE2 activity, Ang-(1–7), and MasR expression level [32]. Additionally, in physiological conditions, AT_2R may downregulate [33] or directly inhibit AT_1R [34–36]; however, physiological AT_2R activation occurs mostly at embryonal stage (responsible for fetus development), while in adulthood AT_2R expression is low [36, 37]. Nevertheless, it may still be detectable in different organs, including the heart in particular in pathological conditions. Both AT_1R and AT_2R are located on cell surfaces or nuclear membranes [38–40].

The second dominant RAAS pathway opposing the classical axis (ACE/Ang II/AT_1R) is the ACE2-dependent pathway, leading to the generation of Ang-(1–7) acting on Mas receptors. The major player in this system, ACE2, converts Ang I to Ang-(1–9), Ang II to Ang-(1–7), and Ang A to alamandine [41]. Ang-(1–7) is the main opposing signaling peptide to Ang II with a broad range of effects in different organs. The most significant activity of Ang-(1–7) includes vasodilation and anti-proliferative and anti-inflammatory effects that are mediated by Mas receptors [42]. Alamandine (the product of Ang A and Ang-(1–7)), despite its similarity in function to Ang-(1–7), acts on different receptors identified as Mas-related G protein-coupled receptor member D (MrgDR) [43]. Both of these vasoprotective angiotensins induce endothelial-dependent vasorelaxation and central nervous system-dependent cardiovascular effects [41], but their activity is not always identical [44, 45]. Importantly, the Mas receptor was found in cardiomyocytes and cardiac endothelial cells [46–48], as well as on cardiac fibroblasts [49]. MasR genetic deletion leads to impairment of cardiac function and endothelial dysfunction pointing to the important protective role of this receptor in cardiac and vascular physiology. Although there is equivocal evidence that Ang-(1–7) has vasoprotective, cardioprotective, and anti-inflammatory effects, still it is not clear if all of the effects of ACE2 pathway are mediated by Ang-(1–7) and by MasR. Ang-(1–7), alamandine, and bradykinin could act in concert as

their concentrations increase simultaneously with decreased ACE/ACE2 ratio and Ang-(1–7)-mediated effects in some systems are inhibited by B_2 receptor antagonists [50]. Although evidence supporting the protective role of ACE2/Ang-(1–7) axis is convincing, it is still not clear if ACE2 is the only enzyme that plays a key role in Ang-(1–7) generation in various pathologies.

The major difference between ACE and ACE2 (which are quite similar in structure: 42% of amino acids are identical in the extracellular domain) is that ACE acts as dipeptidyl carboxypeptidase (removing a dipeptide from the C-terminus of substrate), while ACE2 acts as a mono-carboxypeptidase (removing a single amino acid) [2, 3]. Both enzymes are type I transmembrane proteins with an extracellular N-terminal domain containing the catalytic site and an intracellular C-terminal tail. ACE inhibitors do not act on ACE2 catalytic activity, the latter is affected by MLN 4760, a prototypic ACE2 inhibitor [51]. In the healthy heart, ACE2 is present in cardiomyocytes, fibroblasts, and coronary endothelial cells [52], while ACE is mainly found in endothelial cells [53]. ACE2 catalytic efficiency is 400-fold higher with Ang II as a substrate than with Ang I, suggesting a dominant role for ACE2 in Ang II metabolism as compared with Ang I metabolism. In this way, ACE2 counterbalances ACE activity mainly at the level of Ang II. In fact, ACE increases Ang II levels, and ACE2 decreases Ang II levels resulting in the activation of MasR instead of AT_1R. The relative significance of ACE2 converting Ang I to Ang-(1–9) and Ang A to alamandine seems to be of less importance, but further studies are needed.

The discovery of ACE2 in 2000 [3] and subsequent studies documenting opposite actions of ACE2/Ang-(1–7)/MasR axis as compared to ACE/Ang II/AT_1R axis have revealed this pathway as a major protective arm of RAAS.

3. Other angiotensin pathways, enzymes, and receptors

In addition to ACE/Ang II/AT_1 and ACE2/Ang-(1–7)/MasR axes, the couple of other angiotensin axes has been proposed as important counterparts of RAAS including the protective axes of Ang III/aminopeptidase N(APN)/Ang IV/insulin-regulated aminopeptidase (IRAP)/AT_4R and Ang II/aminopeptidase A(APA)/Ang III/AT_2R/NO/cGMP [54] (**Figure 1**). Additionally, the prorenin/renin/prorenin receptor was proposed to constitute an important vasopressor pathway in addition to the ACE/Ang II/AT_1 axis, with an emerging role for the prorenin receptor (PRR) [55] that may affect intracellular signaling pathways in an angiotensin-independent manner [56, 57]. On the other hand, the generation of Ang III stimulating directly AT_2R and AT_4R after conversion of Ang III to Ang IV represents a novel vasoprotective arm of angiotensin pathways regulating RAAS with vasodilator properties, as well as promoting endothelial cell proliferation [58, 59].

Additionally, intracellular Ang II in various tissues may be generated in a non-ACE-dependent way from Ang-(1–12) via chymase, particularly in pathological conditions [59, 60] (**Figure 1**). The detrimental effects of Ang-(1–12)/chymase/Ang II axis seem to play an important role, for example, in heart failure [61]. Ang-(1–12) when activated may lead to tissue remodeling and potentiated vascular as well as cardiac contractility [62, 63]. The independence of intracellular Ang II production from extracellular system was confirmed by studies showing that chronic administration of losartan and lisinopril did not influence cardiac Ang II content, despite antihypertensive effects of these treatments linked to circulating angiotensins [59].

Many other enzymes are also implicated in the generation of Ang II (besides ACE and chymase), including chymostatin-sensitive Ang II-generating enzyme

(CAGE), endopeptidase-2, meprin [64], cathepsins D and G, or tonin [65–67]. Various types of aminopeptidases (-A,-N,-M,-B) were suggested to take part in the generation of Ang III or Ang IV. Production of Ang-(1–9) may be mediated by carboxypeptidase A (CP-A) or cathepsin, while the generation of Ang-(1–7) can occur by activation of prolyl endopeptidase (PEP), neutral endopeptidase [68], neprilysin (NEP) [69–71], or thimet oligopeptidase (TOP) [72]. Prolyl endopeptidase has also an influence on tissue angiotensins which makes it an interesting target for pharmacotherapy [73].

The physiological and pathophysiological relevance of these multiple enzymes in the regulation of the angiotensin pathways influencing the RAAS network as well as the pathophysiological importance of prorenin/renin/prorenin receptor pathways (**Figure 1**) still needs to be delineated. Currently, among nonclassical pathways influencing angiotensin pathways, protective ACE2/Ang-(1–7)/MasR axis and detrimental Ang-(1–12)/chymase/Ang II axis are best characterized and seem to play a major role in heart failure. Both of them could influence the activity of aldosterone/mineralocorticoid receptor-dependent pathways (**Figure 2**).

4. Alterations of RAAS in heart failure

It is well known that overactivation of RAAS plays a crucial role in heart failure progression, while the inhibition of RAAS (by ACE-I, ARB, and MRA) represents a cornerstone for the current pharmacotherapy of HF [74]. It is clear that systemic RAAS and local angiotensin pathways in tissues act independently as alterations in systemic and tissue-derived angiotensins in HF progression do not coincide. Moreover, the range of concentrations of angiotensins in plasma and tissue differs, that is, cardiac Ang II concentration is about 100-fold higher than that of plasma [75]. This phenomenon may result from intrinsic cardiac Ang I production, which was estimated to represent about 90% of cardiac Ang I and about 75% cardiac Ang II [76], the rest being regulated by RAAS components taken to the tissue from the systemic circulation, for example, by plasma-derived renin [77]. Cardiac intrinsic angiotensin pathway activity gains particular importance in course of heart failure, activating additional mechanisms leading to increased Ang II production [78]. Cardiac intrinsic angiotensin pathways are upregulated in HF progression mainly through increased ACE/ACE2 ratio, leading to excessive Ang II production and through activation of intracellular chymase-dependent axis responsible for additional Ang II production [53, 79]. Both of these pathways lead to cardiac Ang II generation and AT_1R stimulation.

Indeed, apart from ACE the second major cardiac intrinsic mechanism leading to excessive Ang II production in course of HF utilizes an intracellular source of Ang-(1–12) and chymase (present in different cell types, including mast cells, cardiac fibroblasts, and vascular endothelial cells [87, 88]). In HF, chymase-dependent conversion of Ang-(1–12) to Ang II [4, 62, 89–91] was proposed to play a role of an independent intracrine pathway accounting for trophic, contractile, and pro-arrhythmic effects of Ang II in the human heart as well as in resistance arteries [92]. Interestingly, it was shown that MR antagonists decrease Ang-(1–12) production and by this may additionally decrease the detrimental effects of Ang II [14]. The combined inhibition of chymase and ACE compared to ACE inhibition alone provided an added benefit in terms of left ventricular function and adverse cardiac remodeling [93, 94]. Chymase-specific inhibitor improved cardiac function in human myocardial infarction (MI) [95] and significantly attenuated cardiac diastolic dysfunction accompanied by fibrosis in an experimental dog model of

tachycardiac-induced HF [96]. There is also evidence for local (intracellular) chymase activity that generates Ang II in the vascular wall [73, 95]. In relation to ACE, chymase is much more specific in Ang II production and does not break down bradykinin [87, 88, 97]. In contrast to ACE, chymase is not present in plasma and contributes only to tissue-based angiotensin pathways [87]. There is evidence for ACE inhibition-dependent chymase activation, which may explain a secondary increase in Ang II level in a large group of patients treated with ACE-I [93, 98].

In contrast to ACE and chymase, ACE2 has cardioprotective effects (influencing left ventricle remodeling and function) in HF [80]. In turn, loss of ACE2 leads to deterioration of cardiac function [81] and deleterious effects linked to increased Ang II production [49]. The ACE2 activity may be regulated by cardiac sheddases, which are located near ACE2 in the cellular membrane and their activation results in the secretion of a soluble form of tissue ACE2 into the circulation and decreases its activity in the heart. ADAM 17 (known as TACE) was proposed to act as a local sheddase [82, 83]. In humans, there are 21 sheddases described, among them 13 are proteolytically active [84], suggesting that besides ADAM 17 there may be other sheddases involved in ACE2 regulation. Shedding of ACE2 may be stimulated by Ang II acting through AT_1R, which induces phosphorylation and activation of ADAM 17. Circulating soluble form of ACE2 was recognized as one of the markers of worsening HF prognosis [85, 86] that, in our opinion, might reflect the increased shedding of ACE2 from the heart and dominance of ACE/Ang II/AT_1 axis in the heart.

In our recent study [99], in a unique murine model of HF that is characterized by a long-term development of end-stage HF [100], we demonstrated that changes in the profile of systemic versus tissue angiotensin pathways seem independent of each other. As shown in **Figure 3**, a significant increase in local Ang-(1–7) and alamandine content in the heart and aorta was observed at the early stage of HF and was followed by a decrease of Ang-(1–7) and alamandine in the heart and in the aorta at the late HF stage with simultaneous increase in Ang-(1–12). We concluded that HF progression in this murine model of HF was associated with a pronounced activation of the local ACE/Ang II pathway that was counterbalanced by a prominent ACE2/Ang-(1–7) activation with distinct pattern of changes in ACE/ACE2 balance in plasma. We tempted to speculate that the dominance of ACE2/Ang-(1–7) over ACE/Ang II in the adaptive phase of HF may contribute to the late onset of apparent cardiac dysfunction in this model and the balance between ACE/Ang II and ACE2/Ang-(1–7) in favor of the first axis determines the progression to the end stage of heart failure. Interestingly, the balance between ACE/Ang II and ACE2/Ang-(1–7) seems to correspond with aldosterone plasma concentration, low in the early phase and increased at the end stage of HF in this model (unpublished data).

Up to 45% of patients with reduced ejection fraction present elevated plasma angiotensin II levels despite ACE-I and MRA therapy [101, 102]. Moreover, for heart failure patients, with preserved ejection fraction and diastolic disturbance (which form up to 40% of HF patients), ACE-I are much less effective [103]. Lack of sufficient effectiveness of ACE-I and MRA therapy seems to support the notion of an ACE-independent local angiotensin pathway that may independently regulate Ang II production as well as AT_1R stimulation and may represent an important contributing mechanism to heart failure progression. Clearly, the Ang II-generating mechanisms in HF are not well-controlled by current therapy, and this is also one of the reasons why additional treatment with MRA is frequently required and highly effective in HF patients.

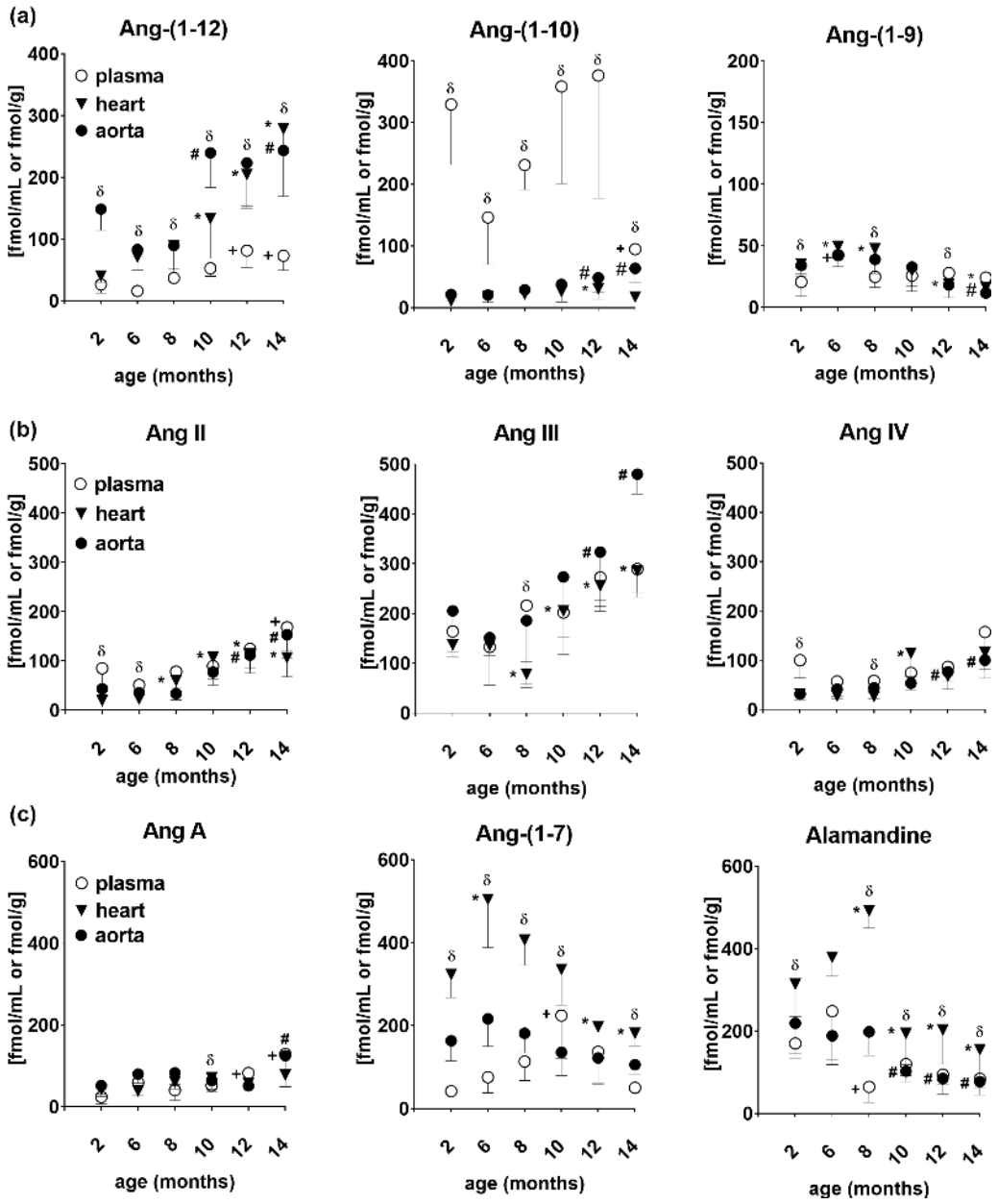

Figure 3.
*Angiotensin profile in plasma, the heart, and the aorta in Tgαq*44 mice. Concentration of Ang-(1–12), Ang-(1–10), and Ang-(1–9) (a), Ang II, Ang III and Ang IV (b), Ang A, Ang-(1–7), and alamandine (c) in plasma, aorta, and heart homogenates. *P < 0.05 for the heart tissue of a given group of Tgαq*44 mice vs. 2-month-old Tgαq*44 mice; #P < 0.05 for the aorta tissue of a given Tgαq*44 group vs. 2-month-old Tgαq*44 mice; +P < 0.05 for plasma of a given Tgαq*44 group vs. 2-month-old Tgαq*44 mice (one-way ANOVA with Tukey post hoc test or Kruskal-Wallis); δP < 0.05 hearts vs. plasma (t-test or Wilcoxon test). Reprinted with permission from [99].*

5. Quantification of angiotensin peptides and clinical needs

To better understand the regulation of angiotensin pathways and its impact on aldosterone/mineralocorticoid receptor-dependent pathways, the reliable quantification of endogenous angiotensin peptides is needed, in particular for angiotensins that are representatives of classical ACE/Ang II and nonclassical ACE2/Ang-(1–7) pathways. As the physiological levels of angiotensin peptides in biological samples are extremely low (fmol/mL in plasma or fmol/g tissue in organs), the

analytical approaches require very sensitive methods among which enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and liquid chromatography combined with RIA (LC-RIA) or mass spectrometry detection (LC-MS) are used so far (**Table 1**).

Origin	Angiotensin peptides (endogenous level in healthy subjects*)	Analytical approach	Ref.
Plasma			
Mouse	**Ang II** (24–215 fmol); **Ang-(1–7)** (ca. 142 fmol)	ELISA	[109, 110]
	Ang I (20–328 fmol); **Ang II** (15–48 fmol)	LC-RIA	[99, 111]
	Alamandine (40–263 fmol); **Ang I** (57–180 fmol) **Ang II** (28–86 fmol); **Ang III** (50–176 fmol) **Ang IV** (35–118 fmol); **Ang A** (10–50 fmol) **Ang-(1–12)** (8–75 fmol); **Ang-(1–9)** (8–46 fmol) **Ang-(1–7)** (23–72 fmol)	LC-MS	[99, 106]
Rat	**Ang II** (72–95 pmol)	ELISA	[112, 113]
	Ang I (40–137 fmol); **Ang II** (25–130 fmol)	RIA	[114, 115]
	Ang I (10–130 fmol); **Ang II** (5–30 fmol) **Ang III** (4–8 fmol); **Ang IV** (2.5–7 fmol) **Ang-(2–10)** (26–70 fmol); **Ang-(1–9)** (2–6 fmol) **Ang-(3–10)** (5–30 fmol); **Ang-(1–7)** (1.4–15 fmol) **Ang-(2–7)** (2.6–7 fmol); **Ang-(3–7)** (ca. 8 fmol) **Ang-(4–8)** (ca. 8 fmol)	LC-RIA	[116–120]
Human	**Ang-(4–10)** (ca. 16 fmol); **Ang-(5–10)** (ca. 80 fmol) **Ang-(6–10)** (ca. 12 nmol)	FLD-EIA	[121]
	Ang II (ca. 18 fmol)	LC-MS	[122]
	Ang I (ca. 20 fmol); **Ang II** (ca. 14 fmol) **Ang III** (ca. 3.0 fmol); **Ang-(1–9)** (<0.4 fmol) **Ang-(2–10)** (ca. 2.4 fmol); **Ang-(2–9)** (<2.1 fmol) **Ang-(1–7)** (1.0–9.5 fmol); **Ang-(2–7)** (<1.1 fmol)	LC-RIA	[123, 124]
Serum			
Rat	**Ang II** (42–87 fmol); **Ang-(1–7)** (2220–6310 fmol)	CZE-PDA	[125]
Urine			
Rat	**Ang I** (ca. 0.5 pmol); **Ang II** (ca. 1.25 pmol) **Ang-(1–7)** (ca. 0.5 pmol)	RIA	[115]
Human	**Ang-(1–7)** (ca. 0.11 pmol)	LC-RIA	[126]
Kidney			
Mouse	**Ang I** (60–184 fmol); **Ang II** (159–328 fmol)	LC-RIA	[111, 127]
Rat	**Ang I** (52–1050 fmol); **Ang II** (90–250 fmol) **Ang III** (ca. 50 fmol); **Ang IV** (ca. 6 fmol) **Ang-(1–9)** (ca. 64 fmol); **Ang-(2–10)** (ca. 300 fmol) **Ang-(3–10)** (ca. 90 fmol); **Ang-(1–7)** (24–120 fmol) **Ang-(2–7)** (ca. 50 fmol)	LC-RIA	[117, 118, 128]
Adrenal gland			
Mouse	**Ang I** (ca. 7 fmol); **Ang II** (ca. 300 fmol)	LC-RIA	[111]
Rat	**Ang I** (6–180 fmol); **Ang II** (545–2000 fmol) **Ang III** (ca. 150 fmol); **Ang IV** (ca. 10 fmol) **Ang-(1–9)** (<62 fmol); **Ang-(2–10)** (3–80 fmol) **Ang-(3–10)** (ca. 3 fmol); **Ang-(1–7)** (30–180 fmol) **Ang-(2–7)** (15–40 fmol); **Ang-(3–7)** (ca. 90 fmol)	LC-RIA	[117, 118, 128]

Origin	Angiotensin peptides (endogenous level in healthy subjects*)	Analytical approach	Ref.
Lungs			
Mouse	**Ang I** (ca. 5 fmol); **Ang II** (ca. 90 fmol)	LC-RIA	[111]
Rat	**Ang I** (2–3 fmol); **Ang II** (70–90 fmol) **Ang-(1–9)** (ca. 4.6 fmol); **Ang-(1–7)** (<4.4 fmol)	LC-RIA	[117, 128]
Liver			
Mouse	**Ang I** (1.9–39 fmol); **Ang II** (42–204 fmol)	LC-RIA	[127]
Heart			
Mouse	**Ang I** (5.3–36 fmol); **Ang II** (49–201fmol)	LC-RIA	[111, 127]
	Alamandine (70–320 fmol); **Ang I** (5–50 fmol) **Ang II** (10–100 fmol); **Ang III** (50–150 fmol) **Ang IV** (15–35 fmol); **Ang A** (25–55 fmol) **Ang-(1–12)** (20–280 fmol); **Ang-(1–9)** (35–50 fmol) **Ang-(1–7)** (125–330 fmol)	LC-MS	[99, 106]
Rat	**Ang I** (5–25 fmol); **Ang II** (6–20 fmol); **Ang III** (ca. 5 fmol); **Ang IV** (ca. 1 fmol); **Ang-(1–9)** (<3.8 fmol) **Ang-(2–10)** (ca. 2.5 fmol); **Ang-(3–10)** (ca. 2 fmol) **Ang-(1–7)** (3.5–8 fmol); **Ang-(2–7)** (ca. 5 fmol)	LC-RIA	[117, 120, 128]
Brain			
Mouse	**Ang I** (ca. 2 fmol); **Ang II** (ca. 5 fmol)	LC-RIA	[111]
Rat	**Ang I** (<4 fmol); **Ang II** (8–16 fmol) **Ang-(1–9)** (ca. 20 fmol); **Ang-(1–7)** (<13 fmol)	LC-RIA	[117, 128]
Rat (medulla)	**Ang I** (1.5–520 fmol); **Ang II** (3–900 fmol) **Ang III** (ca. 3 fmol); **Ang IV** (ca. 90 fmol) **Ang-(2–10)** (1.2–80 fmol); **Ang-(3–10)** (1.4–45 fmol) **Ang-(1–7)** (5–720 fmol); **Ang-(2–7)** (ca. 7 fmol) **Ang-(3–7)** (ca. 180 fmol)	LC-RIA	[116, 120]
Aorta			
Mouse	**Alamandine** (ca. 185 fmol); **Ang I** (ca. 16 fmol) **Ang II** (ca. 15 fmol); **Ang III** (ca. 122 fmol) **Ang IV** (ca. 30 fmol); **Ang A** (ca. 52 fmol) **Ang-(1–12)** (ca. 57 fmol); **Ang-(1–9)** (ca. 25 fmol) **Ang-(1–7)** (ca. 240 fmol)	LC-MS	[99]
Rat	**Ang I** (<10 fmol); **Ang II** (76–200 fmol) **Ang-(1–9)** (<19 fmol); **Ang-(1–7)** (<20 fmol)	LC-RIA	[117, 128]
Adipose			
Rat (BAT)	**Ang I** (ca. 8 fmol); **Ang II** (42–60 fmol) **Ang-(1–9)** (ca. 8 fmol); **Ang-(1–7)** (<8 fmol)	LC-RIA	[117, 128]
Rat (WAT)	**Ang II** (18–56 pmol); **Ang-(1–7)** (190–648 pmol)	CZE-PDA	[125]

**The range of Ang peptides endogenous levels in healthy subjects was roughly estimated based on published data and expressed per milliliter (mL) of plasma, per mg of creatinine excreted per day for urine, and per g of tissue for organs; BAT, brown adipose tissue; WA, white adipose tissue; LC-RI, liquid chromatography combined with radioimmunoassay; LC-MS, liquid chromatography combined with mass spectrometry; RIA, radioimmunoassay; FLD-EIA, immunofluorescence assay; CZE-PDA, capillary zone electrophoresis with PDA detection.*

Table 1.
The range of endogenous levels of angiotensin peptides in various biological matrices and the most commonly used analytical approaches for their quantification.

The immunoassay-based methods have many drawbacks, among others, being the lack of specific antibodies as the antibodies used currently in ELISA kits for Ang II quantification cross-react with Ang III (36–100%), Ang IV

(33–100%), and Ang A (100%) which leads to the overestimation of the real concentration of Ang II in measured samples and does not allow to discern the role of individual angiotensin peptides. The limitations of immunoassay-based approaches are overcome by a highly specific, sensitive LC-MS technique. As LC-MS relies on the initial identification of studied peptides based on their molecular weight followed by detection of peptide fragmentation signatures, this approach is highly specific for individual angiotensins [105, 106]. Indeed, in a number of studies including our own [99, 106–108], LC-MS enabled a comprehensive analysis of various angiotensin peptides in in vivo, in vitro, and ex vivo studies (**Table 1**).

It seems that the pattern of Ang peptides measured in plasma could be of the clinical value and LC-MS could offer adequate analytical potential to foster development of angiotensin profiling in clinical field. After optimization, introduction of such analyses into the clinic may provide fundamental information in many current clinical challenges such as treatment of resistant hypertension or reversal of pathological cardiac remodeling. At the same time, angiotensin profiling could lead to a better understanding of upstream mechanisms of classical and nonclassical pathways of RAAS in the regulation of aldosterone/mineralocorticoid receptor-dependent pathways.

6. Conclusion

The diverse role of the aldosterone/mineralocorticoid receptor-dependent pathway in physiology and pathology needs to be analyzed in the context of the increasingly complex network of angiotensins. In fact a number of noncanonical mechanisms of angiotensin pathways represent possible novel upstream targets to inhibit aldosterone/mineralocorticoid receptor-dependent pathways, for example, the ACE2/Ang-(1–7) pathway and their novel regulatory elements such as sheddases (ADAM 17) or apelin (which increases ACE2 promotor activity) [129], as well as Ang-(1–12)/chymase/Ang II pathway. As expected, interventions blocking Ang-(1–12)/chymase/Ang II as well as enhancing ACE2/Ang-(1–7) diminished aldosterone production [124, 130]. It remains to be determined, however, which of the novel pharmacotherapies, shown to be effective in experimental heart failure including chymase inhibitors [131], recombinant human ACE2 [132–134], Ang-(1–7) [135], or combined angiotensin receptor antagonist and neprilysin inhibitor (ARNI) [104], are most effective in reducing the activity of aldosterone/mineralocorticoid receptor-dependent signaling. To exploit further these novel mechanisms pharmacotherapeutically, it is important to better understand the heterogeneity of local angiotensin pathways in various organs and their effects on aldosterone/mineralocorticoid receptor-dependent pathways.

Finally, we believe that the profiling of angiotensins in clinical facilities, at least for these two angiotensins (i.e., Ang II, Ang-(1–7)) with opposite actions on MR and aldosterone production, may prove to be a good tool to optimize the pharmacotherapy of RAAS including treatment with MRA.

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References

[1] Patel S, Rauf A, Khan H, Abu-Izneid T. Renin-angiotensin-aldosterone (RAAS): The ubiquitous system for homeostasis and pathologies. Biomedicine & Pharmacotherapy. 2017;**94**:317-325

[2] 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. Journal of Biological Chemistry. 2000;**275**(43):33238-33243

[3] Donoghue M, Hsieh F, Baronas E, Godbout K, Gosselin M, Stagliano N, et al. A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. Circulation Research. 2000;**87**(5):E1-E9

[4] Ahmad S, Varagic J, Groban L, Dell'Italia LJ, Nagata S, Kon ND, et al. Angiotensin-(1-12): A chymase-mediated cellular angiotensin II substrate. Current Hypertension Reports. 2014;**16**(5):429

[5] Holappa M, Vapaatalo H, Vaajanen A. Many faces of renin-angiotensin system—Focus on eye. Open Ophthalmology Journal. 2017;**11**:122-142

[6] Milliez P, Girerd X, Plouin PF, Blacher J, Safar ME, Mourad JJ. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. Journal of the American College of Cardiology. 2005;**45**(8):1243-1248

[7] Andreozzi F, Laratta E, Sciacqua A, Perticone F, Sesti G. Angiotensin II impairs the insulin signaling pathway promoting production of nitric oxide by inducing phosphorylation of insulin receptor substrate-1 on Ser(312) and Ser(616) in human umbilical vein endothelial cells. Circulation Research. 2004;**94**(9):1211-1218

[8] Nakashima H, Suzuki H, Ohtsu H, Chao JY, Utsunomiya H, Frank GD, et al. Angiotensin II regulates vascular and endothelial dysfunction: Recent topics of angiotensin II type-1 receptor signaling in the vasculature. Current Vascular Pharmacology. 2006;**4**(1):67-78

[9] Kawai T, Forrester SJ, O'Brien S, Baggett A, Rizzo V, Eguchi S. AT1 receptor signaling pathways in the cardiovascular system. Pharmacological Research. 2017;**125**(Pt A):4-13

[10] Miyata K, Rahman M, Shokoji T, Nagai Y, Zhang GX, Sun GP, et al. Aldosterone stimulates reactive oxygen species production through activation of NADPH oxidase in rat mesangial cells. Journal of the American Society of Nephrology. 2005;**16**(10):2906-2912

[11] Munzel T, Gori T, Keaney JF Jr, Maack C, Daiber A. Pathophysiological role of oxidative stress in systolic and diastolic heart failure and its therapeutic implications. European Heart Journal. 2015;**36**(38):2555-2564

[12] Keidar S, Gamliel-Lazarovich A, Kaplan M, Pavlotzky E, Hamoud S, Hayek T, et al. Mineralocorticoid receptor blocker increases angiotensin-converting enzyme 2 activity in congestive heart failure patients. Circulation Research. 2005;**97**(9):946-953

[13] Kong EL, Zhang JM, An N, Tao Y, Yu WF, Wu FX. Spironolactone rescues renal dysfunction in obstructive jaundice rats by upregulating ACE2 expression. Journal of Cell Communication and Signaling. 2019;**13**(1):17-26

[14] Whaley-Connell A, Habibi J, Wei Y, Gutweiler A, Jellison J, Wiedmeyer CE, et al. Mineralocorticoid receptor antagonism attenuates glomerular filtration barrier remodeling in the transgenic Ren2 rat. American Journal of Physiology. Renal Physiology. 2009;**296**(5):F1013-F1022

[15] Kucharewicz I, Pawlak R, Matys T, Pawlak D, Buczko W. Antithrombotic effect of captopril and losartan is mediated by angiotensin-(1-7). Hypertension. 2002;**40**(5):774-779

[16] Kaiqiang J, Minakawa M, Fukui K, Suzuki Y, Fukuda I. Olmesartan improves left ventricular function in pressure-overload hypertrophied rat heart by blocking angiotensin II receptor with synergic effects of upregulation of angiotensin converting enzyme 2. Therapeutic Advances in Cardiovascular Disease. 2009;**3**(2):103-111

[17] Sukumaran V, Veeraveedu PT, Gurusamy N, Yamaguchi K, Lakshmanan AP, Ma ML, et al. Cardioprotective effects of telmisartan against heart failure in rats induced by experimental autoimmune myocarditis through the modulation of angiotensin-converting enzyme-2/angiotensin 1-7/Mas receptor axis. International Journal of Biological Sciences. 2011;**7**(8):1077-1092

[18] Wang XX, Ye Y, Gong H, Wu J, Yuan J, Wang SJ, et al. The effects of different angiotensin II type 1 receptor blockers on the regulation of the ACE-AngII-AT1 and ACE2-Ang(1-7)-Mas axes in pressure overload-induced cardiac remodeling in male mice. Journal of Molecular and Cellular Cardiology. 2016;**97**:180-190

[19] Zhong JC, Ye JY, Jin HY, Yu X, Yu HM, Zhu DL, et al. Telmisartan attenuates aortic hypertrophy in hypertensive rats by the modulation of ACE2 and profilin-1 expression. Regulatory Peptides. 2011;**166**(1-3):90-97

[20] Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. Hypertension. 2004;**43**(5):970-976

[21] Harada E, Yoshimura M, Yasue H, Nakagawa O, Nakagawa M, Harada M, et al. Aldosterone induces angiotensin-converting-enzyme gene expression in cultured neonatal rat cardiocytes. Circulation. 2001;**104**(2):137-139

[22] Itcho K, Oki K, Kobuke K, Ohno H, Yoneda M, Hattori N. Angiotensin 1-7 suppresses angiotensin II mediated aldosterone production via JAK/STAT signaling inhibition. The Journal of Steroid Biochemistry and Molecular Biology. 2019;**185**:137-141

[23] Chen Y, Zhao W, Liu C, Meng W, Zhao T, Bhattacharya SK, et al. Molecular and cellular effect of angiotensin 1-7 on hypertensive kidney disease. American Journal of Hypertension. 2019;**32**(5):460-467

[24] Galandrin S, Denis C, Boularan C, Marie J, M'Kadmi C, Pilette C, et al. Cardioprotective angiotensin-(1-7) peptide acts as a natural-biased ligand at the angiotensin II type 1 receptor. Hypertension. 2016;**68**(6):1365-1374

[25] Gavras H, Lever AF, Brown JJ, Macadam RF, Robertson JI. Acute renal failure, tubular necrosis, and myocardial infarction induced in the rabbit by intravenous angiotensin II. Lancet. 1971;**2**(7714):19-22

[26] Ikeda Y, Nakamura T, Takano H, Kimura H, Obata JE, Takeda S, et al. Angiotensin II-induced cardiomyocyte hypertrophy and cardiac fibrosis

in stroke-prone spontaneously hypertensive rats. The Journal of Laboratory and Clinical Medicine. 2000;**135**(4):353-359

[27] Daugherty A, Cassis L. Chronic angiotensin II infusion promotes atherogenesis in low density lipoprotein receptor −/− mice. Annals of the New York Academy of Sciences. 1999;**892**:108-118

[28] Karnik SS, Unal H, Kemp JR, Tirupula KC, Eguchi S, Vanderheyden PM, et al. International Union of Basic and Clinical Pharmacology. XCIX. Angiotensin receptors: Interpreters of pathophysiological angiotensinergic stimuli [corrected]. Pharmacological Reviews. 2015;**67**(4):754-819

[29] Kaschina E, Namsolleck P, Unger T. AT2 receptors in cardiovascular and renal diseases. Pharmacological Research. 2017;**125**(Pt A):39-47

[30] Singh KD, Karnik SS. Angiotensin receptors: Structure, function, signaling and clinical applications. Journal of Cell Signalling. 2016;**1**(2)

[31] Skorska A, von Haehling S, Ludwig M, Lux CA, Gaebel R, Kleiner G, et al. The CD4(+)AT2R(+) T cell subpopulation improves post-infarction remodelling and restores cardiac function. Journal of Cellular and Molecular Medicine. 2015;**19**(8):1975-1985

[32] Zhu LP, Carretero OA, Xu J, Harding P, Ramadurai N, Gu XS, et al. Activation of angiotensin II type 2 receptor suppresses TNF-alpha-induced ICAM-1 via NF-kappa B: Possible role of ACE2. American Journal of Physiology-Heart and Circulatory Physiology. 2015;**309**(5):H827-H834

[33] Tanaka M, Tsuchida S, Imai T, Fujii N, Miyazaki H, Ichiki T, et al. Vascular response to angiotensin II is exaggerated through an upregulation of AT1 receptor in AT2 knockout mice. Biochemical and Biophysical Research Communications. 1999;**258**(1):194-198

[34] Bedecs K, Elbaz N, Sutren M, Masson M, Susini C, Strosberg AD, et al. Angiotensin II type 2 receptors mediate inhibition of mitogen-activated protein kinase cascade and functional activation of SHP-1 tyrosine phosphatase. The Biochemical Journal. 1997;**325**(Pt 2): 449-454

[35] AbdAlla S, Lother H, Abdel-Tawab AM, Quitterer U. The angiotensin II AT2 receptor is an AT1 receptor antagonist. The Journal of Biological Chemistry. 2001;**276**(43):39721-39726

[36] de Gasparo M, Catt KJ, Inagami T, Wright JW, Unger T. International Union of Pharmacology. XXIII. The angiotensin II receptors. Pharmacological Reviews. 2000;**52**(3):415-472

[37] Jones ES, Vinh A, McCarthy CA, Gaspari TA, Widdop RE. AT2 receptors: Functional relevance in cardiovascular disease. Pharmacology & Therapeutics. 2008;**120**(3):292-316

[38] Cook JL, Mills SJ, Naquin R, Alam J, Re RN. Nuclear accumulation of the AT1 receptor in a rat vascular smooth muscle cell line: Effects upon signal transduction and cellular proliferation. Journal of Molecular and Cellular Cardiology. 2006;**40**(5):696-707

[39] Cook JL, Mills SJ, Naquin RT, Alam J, Re RN. Cleavage of the angiotensin II type 1 receptor and nuclear accumulation of the cytoplasmic carboxy-terminal fragment. American Journal of Physiology. Cell Physiology. 2007;**292**(4):C1313-C1322

[40] Cook JL, Re R, Alam J, Hart M, Zhang Z. Intracellular angiotensin

II fusion protein alters AT1 receptor fusion protein distribution and activates CREB. Journal of Molecular and Cellular Cardiology. 2004;**36**(1):75-90

[41] Villela DC, Passos-Silva DG, Santos RA. Alamandine: A new member of the angiotensin family. Current Opinion in Nephrology and Hypertension. 2014;**23**(2):130-134

[42] Keidar S, Kaplan M, Gamliel-Lazarovich A. ACE2 of the heart: From angiotensin I to angiotensin (1-7). Cardiovascular Research. 2007;**73**(3):463-469

[43] Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(14):8258-8263

[44] Menon J, Soto-Pantoja DR, Callahan MF, Cline JM, Ferrario CM, Tallant EA, et al. Angiotensin-(1-7) inhibits growth of human lung adenocarcinoma xenografts in nude mice through a reduction in cyclooxygenase-2. Cancer Research. 2007;**67**(6):2809-2815

[45] Lautner RQ, Villela DC, Fraga-Silva RA, Silva N, Verano-Braga T, Costa-Fraga F, et al. Discovery and characterization of alamandine: A novel component of the renin-angiotensin system. Circulation Research. 2013;**112**(8):1104-1111

[46] Dias-Peixoto MF, Santos RA, Gomes ER, Alves MN, Almeida PW, Greco L, et al. Molecular mechanisms involved in the angiotensin-(1-7)/Mas signaling pathway in cardiomyocytes. Hypertension. 2008;**52**(3):542-548

[47] Zhong J, Basu R, Guo D, Chow FL, Byrns S, Schuster M, et al. Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. Circulation. 2010;**122**(7):717-728, 18 p following 728

[48] Sampaio WO, Souza dos Santos RA, Faria-Silva R, da Mata Machado LT, Schiffrin EL, Touyz RM. Angiotensin-(1-7) through receptor Mas mediates endothelial nitric oxide synthase activation via Akt-dependent pathways. Hypertension. 2007;**49**(1):185-192

[49] Patel VB, Zhong JC, Grant MB, Oudit GY. Role of the ACE2/angiotensin 1-7 axis of the renin-angiotensin system in heart failure. Circulation Research. 2016;**118**(8):1313-1326

[50] Kozlovski VI, Lomnicka M, Bartus M, Sternak M, Chlopicki S. Anti-thrombotic effects of nebivolol and carvedilol: Involvement of beta2 receptors and COX-2/PGI2 pathways. Pharmacological Reports. 2015;**67**(5):1041-1047

[51] Dales NA, Gould AE, Brown JA, Calderwood EF, Guan B, Minor CA, et al. Substrate-based design of the first class of angiotensin-converting enzyme-related carboxypeptidase (ACE2) inhibitors. Journal of the American Chemical Society. 2002;**124**(40):11852-11853

[52] Gallagher PE, Ferrario CM, Tallant EA. Regulation of ACE2 in cardiac myocytes and fibroblasts. American Journal of Physiology. Heart and Circulatory Physiology. 2008;**295**(6):H2373-H2379

[53] Weber KT, Sun Y. Recruitable ACE and tissue repair in the infarcted heart. Journal of the Renin-Angiotensin-Aldosterone System. 2000;**1**(4):295-303

[54] Li XC, Zhang JF, Zhuo JL. The vasoprotective axes of the

renin-angiotensin system: Physiological relevance and therapeutic implications in cardiovascular, hypertensive and kidney diseases. Pharmacological Research. 2017;**125**:21-38

[55] Saris JJ, 't Hoen PA, Garrelds IM, Dekkers DH, den Dunnen JT, Lamers JM, et al. Prorenin induces intracellular signaling in cardiomyocytes independently of angiotensin II. Hypertension. 2006;**48**(4):564-571

[56] Zhuo JL, Ferrao FM, Zheng Y, Li XC. New frontiers in the intrarenal renin-angiotensin system: A critical review of classical and new paradigms. Frontiers in Endocrinology (Lausanne). 2013;**4**:166

[57] Blair-West JR, Carey KD, Denton DA, Madden LJ, Weisinger RS, Shade RE. Possible contribution of brain angiotensin III to ingestive behaviors in baboons. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2001;**281**(5):R1633-R1636

[58] Zulli A, Burrell LM, Buxton BF, Hare DL. ACE2 and AT4R are present in diseased human blood vessels. European Journal of Histochemistry. 2008;**52**(1):39-44

[59] Ferrario CM, Ahmad S, Varagic J, Cheng CP, Groban L, Wang H, et al. Intracrine angiotensin II functions originate from noncanonical pathways in the human heart. American Journal of Physiology. Heart and Circulatory Physiology. 2016;**311**(2):H404-H414

[60] Isa K, Garcia-Espinosa MA, Arnold AC, Pirro NT, Tommasi EN, Ganten D, et al. Chronic immunoneutralization of brain angiotensin-(1-12) lowers blood pressure in transgenic (mRen2)27 hypertensive rats. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2009;**297**(1):R111-R115

[61] Urata H, Hoffmann S, Ganten D. Tissue angiotensin II system in the human heart. European Heart Journal. 1994;**15**(Suppl D):68-78

[62] Jessup JA, Trask AJ, Chappell MC, Nagata S, Kato J, Kitamura K, et al. Localization of the novel angiotensin peptide, angiotensin-(1-12), in heart and kidney of hypertensive and normotensive rats. American Journal of Physiology. Heart and Circulatory Physiology. 2008;**294**(6):H2614-H2618

[63] McDonald JE, Padmanabhan N, Petrie MC, Hillier C, Connell JM, McMurray JJ. Vasoconstrictor effect of the angiotensin-converting enzyme-resistant, chymase-specific substrate [pro(11)(D)-Ala(12)] angiotensin I in human dorsal hand veins: in vivo demonstration of non-ace production of angiotensin II in humans. Circulation. 2001;**104**(15):1805-1808

[64] Stephenson SL, Kenny AJ. The metabolism of neuropeptides. Hydrolysis of peptides by the phosphoramidon-insensitive rat kidney enzyme 'endopeptidase-2′ and by rat microvillar membranes. Biochemical Journal. 1988;**255**(1):45-51

[65] Patil J, Stucki S, Nussberger J, Schaffner T, Gygax S, Bohlender J, et al. Angiotensinergic and noradrenergic neurons in the rat and human heart. Regulatory Peptides. 2011;**167**(1):31-41

[66] Uehara Y, Urata H, Ideishi M, Arakawa K, Saku K. Chymase inhibition suppresses high-cholesterol diet-induced lipid accumulation in the hamster aorta. Cardiovascular Research. 2002;**55**(4):870-876

[67] Borges JC, Silva JA Jr, Gomes MA, Lomez ES, Leite KM, Araujo RC, et al. Tonin in rat heart with experimental hypertrophy. American Journal of Physiology. Heart and Circulatory Physiology. 2003;**284**(6):H2263-H2268

[68] Welches WR, Brosnihan KB, Ferrario CM. A comparison of the properties and enzymatic-activities of 3 angiotensin processing enzymes—Angiotensin-converting enzyme, prolyl endopeptidase and neutral endopeptidase 24.11. Life Sciences. 1993;**52**(18):1461-1480

[69] Allred AJ, Diz DI, Ferrario CM, Chappell MC. Pathways for angiotensin-(1-7) metabolism in pulmonary and renal tissues. American Journal of Physiology-Renal Physiology. 2000;**279**(5):F841-F850

[70] Campbell DJ, Anastasopoulos F, Duncan AM, James GM, Kladis A, Briscoe TA. Effects of neutral endopeptidase inhibition and combined angiotensin converting enzyme and neutral endopeptidase inhibition on angiotensin and bradykinin peptides in rats. The Journal of Pharmacology and Experimental Therapeutics. 1998;**287**(2):567-577

[71] Chappell MC, Gomez MN, Pirro NT, Ferrario CM. Release of angiotensin-(1-7) from the rat hindlimb: Influence of angiotensin-converting enzyme inhibition. Hypertension. 2000;**35**(1 Pt 2):348-352

[72] Ferrario CM. Contribution of angiotensin-(1-7) to cardiovascular physiology and pathology. Current Hypertension Reports. 2003;**5**(2):129-134

[73] Jeong JK, Diano S. Prolyl carboxypeptidase and its inhibitors in metabolism. Trends in Endocrinology and Metabolism. 2013;**24**(2):61-67

[74] Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. R. Document, 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European Journal of Heart Failure. 2016;**18**(8):891-975

[75] Dell'Italia LJ, Meng QC, Balcells E, Wei CC, Palmer R, Hageman GR, et al. Compartmentalization of angiotensin II generation in the dog heart. Evidence for independent mechanisms in intravascular and interstitial spaces. Journal of Clinical Investigation. 1997;**100**(2):253-258

[76] de Lannoy LM, Danser AH, van Kats JP, Schoemaker RG, Saxena PR, Schalekamp MA. Renin-angiotensin system components in the interstitial fluid of the isolated perfused rat heart. Local production of angiotensin I. Hypertension. 1997;**29**(6):1240-1251

[77] Danser AHJ, Vankats JP, Admiraal PJJ, Derks FHM, Lamers JMJ, Verdouw PD, et al. Cardiac renin and angiotensins—Uptake from plasma versus in-situ synthesis. Hypertension. 1994;**24**(1):37-48

[78] Barlucchi L, Leri A, Dostal DE, Fiordaliso F, Tada H, Hintze TH, et al. Canine ventricular myocytes possess a renin-angiotensin system that is upregulated with heart failure. Circulation Research. 2001;**88**(3):298-304

[79] Ihara M, Urata H, Kinoshita A, Suzumiya J, Sasaguri M, Kikuchi M, et al. Increased chymase-dependent angiotensin II formation in human atherosclerotic aorta. Hypertension. 1999;**33**(6):1399-1405

[80] Dong B, Yu QT, Dai HY, Gao YY, Zhou ZL, Zhang L, et al. Angiotensin-converting enzyme-2 overexpression improves left ventricular remodeling and function in a rat model of diabetic cardiomyopathy. Journal of

the American College of Cardiology. 2012;**59**(8):739-747

[81] Kassiri Z, Zhong J, Guo D, Basu R, Wang X, Liu PP, et al. Loss of angiotensin-converting enzyme 2 accelerates maladaptive left ventricular remodeling in response to myocardial infarction. Circulation. Heart Failure. 2009;**2**(5):446-455

[82] Lambert DW, Yarski M, Warner FJ, Thornhill P, Parkin ET, Smith AI, et al. Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). The Journal of Biological Chemistry. 2005;**280**(34):30113-30119

[83] Patel VB, Clarke N, Wang Z, Fan D, Parajuli N, Basu R, et al. Angiotensin II induced proteolytic cleavage of myocardial ACE2 is mediated by TACE/ADAM-17: A positive feedback mechanism in the RAS. Journal of Molecular and Cellular Cardiology. 2014;**66**:167-176

[84] Edwards DR, Handsley MM, Pennington CJ. The ADAM metalloproteinases. Molecular Aspects of Medicine. 2008;**29**(5):258-289

[85] Putko BN, Wang Z, Lo J, Anderson T, Becher H, Dyck JR, et al. Circulating levels of tumor necrosis factor-alpha receptor 2 are increased in heart failure with preserved ejection fraction relative to heart failure with reduced ejection fraction: Evidence for a divergence in pathophysiology. PLoS One. 2014;**9**(6):e99495

[86] Uri K, Fagyas M, Kertesz A, Borbely A, Jenei C, Bene O, et al. Circulating ACE2 activity correlates with cardiovascular disease development. Journal of the Renin-Angiotensin-Aldosterone System. 2016;**17**(4)

[87] Urata H, Kinoshita A, Misono KS, Bumpus FM, Husain A. Identification of a highly specific chymase as the major angiotensin II-forming enzyme in the human heart. The Journal of Biological Chemistry. 1990;**265**(36):22348-22357

[88] Dell'Italia LJ, Collawn JF, Ferrario CM. Multifunctional role of chymase in acute and chronic tissue injury and remodeling. Circulation Research. 2018;**122**(2):319-336

[89] Ahmad S, Varagic J, VonCannon JL, Groban L, Collawn JF, Dell'Italia LJ, et al. Primacy of cardiac chymase over angiotensin converting enzyme as an angiotensin-(1-12) metabolizing enzyme. Biochemical and Biophysical Research Communications. 2016;**478**(2):559-564

[90] Patella V, de Crescenzo G, Lamparter-Schummert B, De Rosa G, Adt M, Marone G. Increased cardiac mast cell density and mediator release in patients with dilated cardiomyopathy. Inflammation Research. 1997;**46**(Suppl 1):S31-S32

[91] Ferrario CM, Ahmad S, Nagata S, Simington SW, Varagic J, Kon N, et al. An evolving story of angiotensin-II-forming pathways in rodents and humans. Clinical Science (London, England). 2014;**126**(7):461-469

[92] Petrie MC, Padmanabhan N, McDonald JE, Hillier C, Connell JMC, McMurray JJV. Angiotensin converting enzyme (ACE) and non-ACE dependent angiotensin II generation in resistance arteries from patients with heart failure and coronary heart disease. Journal of the American College of Cardiology. 2001;**37**(4):1056-1061

[93] Wei CC, Hase N, Inoue Y, Bradley EW, Yahiro E, Li M, et al. Mast cell chymase limits the cardiac efficacy of Ang I-converting enzyme

inhibitor therapy in rodents. The Journal of Clinical Investigation. 2010;**120**(4):1229-1239

[94] Reyes S, Varagic J, Ahmad S, VonCannon J, Kon ND, Wang H, et al. Novel cardiac intracrine mechanisms based on Ang-(1-12)/chymase axis require a revision of therapeutic approaches in human heart disease. Current Hypertension Reports. 2017;**19**(2):16

[95] Jin D, Takai S, Nonaka Y, Yamazaki S, Fujiwara M, Nakamura Y. A chymase inhibitory RNA aptamer improves cardiac function and survival after myocardial infarction. Molecular Therapy—Nucleic Acids. 2019;**14**:41-51

[96] Matsumoto T, Wada A, Tsutamoto T, Ohnishi M, Fujii M, Yamamoto T, et al. Chronic chymase inhibition prevented cardiac fibrosis and improved diastolic dysfunction in the progression of heart failure. Circulation. 2002;**106**(19):99-100

[97] Balcells E, Meng QC, Johnson WH Jr, Oparil S, Dell'Italia LJ. Angiotensin II formation from ACE and chymase in human and animal hearts: Methods and species considerations. The American Journal of Physiology. 1997;**273**(4 Pt 2): H1769-H1774

[98] Zablocki D, Sadoshima J. The one-two punch: Knocking out angiotensin II in the heart. The Journal of Clinical Investigation. 2010;**120**(4):1028-1031

[99] Tyrankiewicz U, Olkowicz M, Skorka T, Jablonska M, Orzylowska A, Bar A, et al. Activation pattern of ACE2/Ang-(1-7) and ACE/Ang II pathway in course of heart failure assessed by multiparametric MRI in vivo in Tgalphaq*44 mice. Journal of Applied Physiology. 1985, 2018;**124**(1):52-65

[100] Mackiewicz U, Czarnowska E, Brudek M, Pajak B, Duda M, Emanuel K, et al. Preserved cardiomyocyte function and altered desmin pattern in transgenic mouse model of dilated cardiomyopathy. Journal of Molecular and Cellular Cardiology. 2012;**52**(5):978-987

[101] Athyros VG, Mikhailidis DP, Kakafika AI, Tziomalos K, Karagiannis A. Angiotensin II reactivation and aldosterone escape phenomena in renin-angiotensin-aldosterone system blockade: Is oral renin inhibition the solution? Expert Opinion on Pharmacotherapy. 2007;**8**(5):529-535

[102] Juillerat L, Nussberger J, Menard J, Mooser V, Christen Y, Waeber B, et al. Determinants of angiotensin II generation during converting enzyme inhibition. Hypertension. 1990;**16**(5):564-572

[103] Fukuta H, Goto T, Wakami K, Ohte N. Effect of renin-angiotensin system inhibitors on mortality in heart failure with preserved ejection fraction: A meta-analysis of observational cohort and randomized controlled studies. Heart Failure Reviews. 2017;**22**(6):775-782

[104] Jhund PS, McMurray JJV. The neprilysin pathway in heart failure: A review and guide on the use of sacubitril/valsartan. Heart. 2016;**102**(17):1342-1347

[105] Cui L, Nithipatikom K, Campbell WB. Simultaneous analysis of angiotensin peptides by LC-MS and LC-MS/MS: Metabolism by bovine adrenal endothelial cells. Analytical Biochemistry. 2007;**369**(1):27-33

[106] Olkowicz M, Chlopicki S, Smolenski RT. A primer to angiotensin peptide isolation, stability, and analysis by nano-liquid chromatography with mass detection. Methods in Molecular Biology. 2017;**1614**:175-187

[107] Olkowicz M, Debski J, Jablonska P, Dadlez M, Smolenski RT. Application of a new procedure for liquid chromatography/mass spectrometry profiling of plasma amino acid-related metabolites and untargeted shotgun proteomics to identify mechanisms and biomarkers of calcific aortic stenosis. Journal of Chromatography. A. 2017;**1517**:66-78

[108] Olkowicz M, Radulska A, Suraj J, Kij A, Walczak M, Chlopicki S, et al. Development of a sensitive, accurate and robust liquid chromatography/mass spectrometric method for profiling of angiotensin peptides in plasma and its application for atherosclerotic mice. Journal of Chromatography. A. 2015;**1393**:37-46

[109] Chen J, Zhao Y, Chen S, Wang J, Xiao X, Ma X, et al. Neuronal over-expression of ACE2 protects brain from ischemia-induced damage. Neuropharmacology. 2014;**79**:550-558

[110] Tsukuda K, Mogi M, Iwanami J, Min LJ, Jing F, Ohshima K, et al. Influence of angiotensin II type 1 receptor-associated protein on prenatal development and adult hypertension after maternal dietary protein restriction during pregnancy. Journal of the American Society of Hypertension. 2012;**6**(5):324-330

[111] Campbell DJ, Alexiou T, Xiao HD, Fuchs S, McKinley MJ, Corvol P, et al. Effect of reduced angiotensin-converting enzyme gene expression and angiotensin-converting enzyme inhibition on angiotensin and bradykinin peptide levels in mice. Hypertension. 2004;**43**(4):854-859

[112] Waghe P, Sarath TS, Gupta P, Kandasamy K, Choudhury S, Kutty HS, et al. Arsenic causes aortic dysfunction and systemic hypertension in rats: Augmentation of angiotensin II signaling. Chemico-Biological Interactions. 2015;**237**:104-114

[113] Tufino C, Villanueva-Lopez C, Ibarra-Barajas M, Bracho-Valdes I, Bobadilla-Lugo RA. Experimental gestational diabetes mellitus induces blunted vasoconstriction and functional changes in the rat aorta. BioMed Research International. 2014;**2014**:329634

[114] Nishiyama A, Seth DM, Navar LG. Renal interstitial fluid angiotensin I and angiotensin II concentrations during local angiotensin-converting enzyme inhibition. Journal of the American Society of Nephrology. 2002;**13**(9):2207-2212

[115] Gilliam-Davis S, Payne VS, Kasper SO, Tommasi EN, Robbins ME, Diz DI. Long-term AT1 receptor blockade improves metabolic function and provides renoprotection in Fischer-344 rats. American Journal of Physiology. Heart and Circulatory Physiology. 2007;**293**(3):H1327-H1333

[116] Chappell MC, Brosnihan KB, Diz DI, Ferrario CM. Identification of angiotensin-(1-7) in rat brain. Evidence for differential processing of angiotensin peptides. Journal of Biological Chemistry. 1989;**264**(28):16518-16523

[117] Campbell DJ, Duncan AM, Kladis A, Harrap SB. Angiotensin peptides in spontaneously hypertensive and normotensive Donryu rats. Hypertension. 1995;**25**(5):928-934

[118] Senanayake PS, Smeby RR, Martins AS, Moriguchi A, Kumagai H, Ganten D, et al. Adrenal, kidney, and heart angiotensins in female murine Ren-2 transfected hypertensive rats. Peptides. 1998;**19**(10):1685-1694

[119] Ocaranza MP, Lavandero S, Jalil JE, Moya J, Pinto M, Novoa U, et al.

Angiotensin-(1-9) regulates cardiac hypertrophy in vivo and in vitro. Journal of Hypertension. 2010;**28**(5):1054-1064

[120] Senanayake PD, Moriguchi A, Kumagai H, Ganten D, Ferrario CM, Brosnihan KB. Increased expression of angiotensin peptides in the brain of transgenic hypertensive rats. Peptides. 1994;**15**(5):919-926

[121] Hildebrand D, Merkel P, Eggers LF, Schluter H. Proteolytic processing of angiotensin-I in human blood plasma. PLoS One. 2013;**8**(5):e64027

[122] Schulz A, Jankowski J, Zidek W, Jankowski V. Absolute quantification of endogenous angiotensin II levels in human plasma using ESI-LC-MS/MS. Clinical Proteomics. 2014;**11**(1):37

[123] Lawrence AC, Evin G, Kladis A, Campbell DJ. An alternative strategy for the radioimmunoassay of angiotensin peptides using amino-terminal-directed antisera: Measurement of eight angiotensin peptides in human plasma. Journal of Hypertension. 1990;**8**(8):715-724

[124] Nussberger J, Brunner DB, Nyfeler JA, Linder L, Brunner HR. Measurement of immunoreactive angiotensin-(1-7) heptapeptide in human blood. Clinical Chemistry. 2001;**47**(4):726-729

[125] Rubio-Ruiz ME, Del Valle-Mondragon L, Castrejon-Tellez V, Carreon-Torres E, Diaz-Diaz E, Guarner-Lans V. Angiotensin II and 1-7 during aging in metabolic syndrome rats. Expression of AT1, AT2 and Mas receptors in abdominal white adipose tissue. Peptides. 2014;**57**:101-108

[126] Ferrario CM, Martell N, Yunis C, Flack JM, Chappell MC, Brosnihan KB, et al. Characterization of angiotensin-(1-7) in the urine of normal and essential hypertensive subjects. American Journal of Hypertension. 1998;**11**(2):137-146

[127] Mazzolai L, Pedrazzini T, Nicoud F, Gabbiani G, Brunner HR, Nussberger J. Increased cardiac angiotensin II levels induce right and left ventricular hypertrophy in normotensive mice. Hypertension. 2000;**35**(4):985-991

[128] Campbell DJ, Kladis A, Duncan AM. Nephrectomy, converting enzyme inhibition, and angiotensin peptides. Hypertension. 1993;**22**(4):513-522

[129] Kuba K, Zhang L, Imai Y, Arab S, Chen M, Maekawa Y, et al. Impaired heart contractility in Apelin gene-deficient mice associated with aging and pressure overload. Circulation Research. 2007;**101**(4):e32-e42

[130] Shefer G, Marcus Y, Knoll E, Dolkart O, Foichtwanger S, Nevo N, et al. Angiotensin 1-7 is a negative modulator of aldosterone secretion in vitro and in vivo. Hypertension. 2016;**68**(2):378-384

[131] Kanefendt F, Thuss U, Becka M, Boxnick S, Berse M, Schultz A, et al. Pharmacokinetics, safety, and tolerability of the novel chymase inhibitor BAY 1142524 in healthy male volunteers. Clinical Pharmacology in Drug Development. 2018

[132] Basu R, Poglitsch M, Yogasundaram H, Thomas J, Rowe BH, Oudit GY. Roles of angiotensin peptides and recombinant human ACE2 in heart failure. Journal of the American College of Cardiology. 2017;**69**(7):805-819

[133] Patel VB, Lezutekong JN, Chen X, Oudit GY. Recombinant human ACE2 and the angiotensin 1-7 axis as potential new therapies for heart failure. The Canadian Journal of Cardiology. 2017;**33**(7):943-946

[134] Oudit GY, Penninger JM. Recombinant human angiotensin-converting enzyme 2 as a new renin-angiotensin system peptidase for heart failure therapy. Current Heart Failure Reports. 2011;**8**(3):176-183

[135] Luo D, Zhuang X, Luo C, Long M, Deng C, Liao X, et al. Continuous angiotensin-(1-7) infusion improves myocardial calcium transient and calcium transient alternans in ischemia-induced cardiac dysfunction rats. Biochemical and Biophysical Research Communications. 2015;**467**(4):645-650