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,

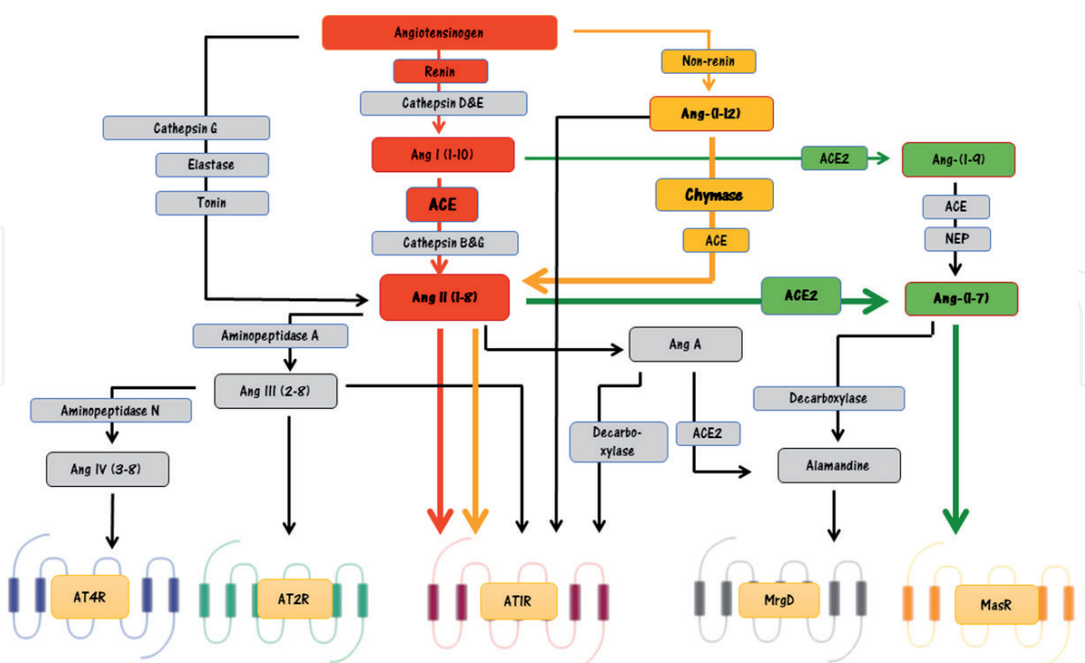

Figure 1.
Simplified scheme of major angiotensin pathways with respective enzymes and receptors. Abbreviations: Ang, angiotensin; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; NEP, neutral endopeptidase; AT1R, angiotensin receptor type 1; AT2R, angiotensin receptor type 2; 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

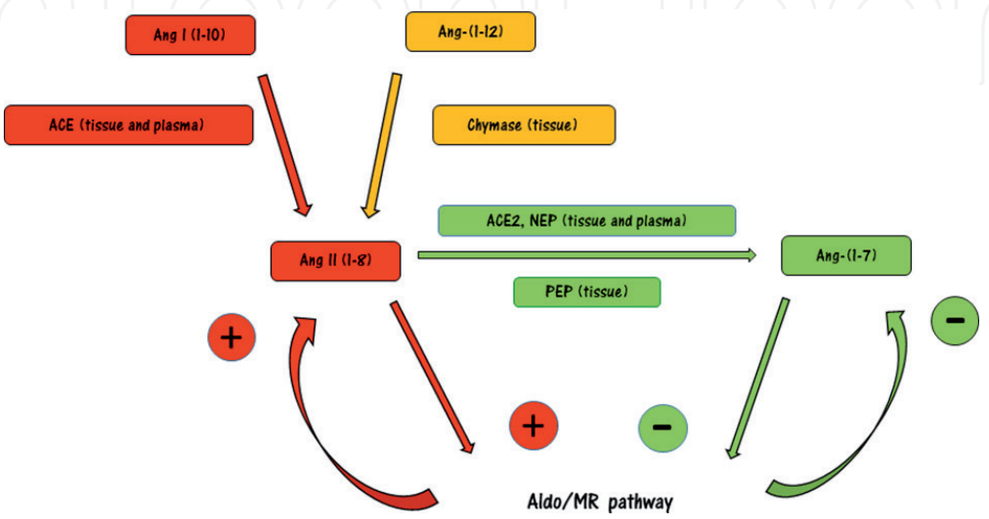

Figure 2.
*Scheme showing the reciprocal regulation of major angiotensins and aldosterone/mineralocorticoid pathway. Abbreviations: PEP, prolyl endopeptidase (see **Figure 1** for other abbreviations and colors coding).*

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.

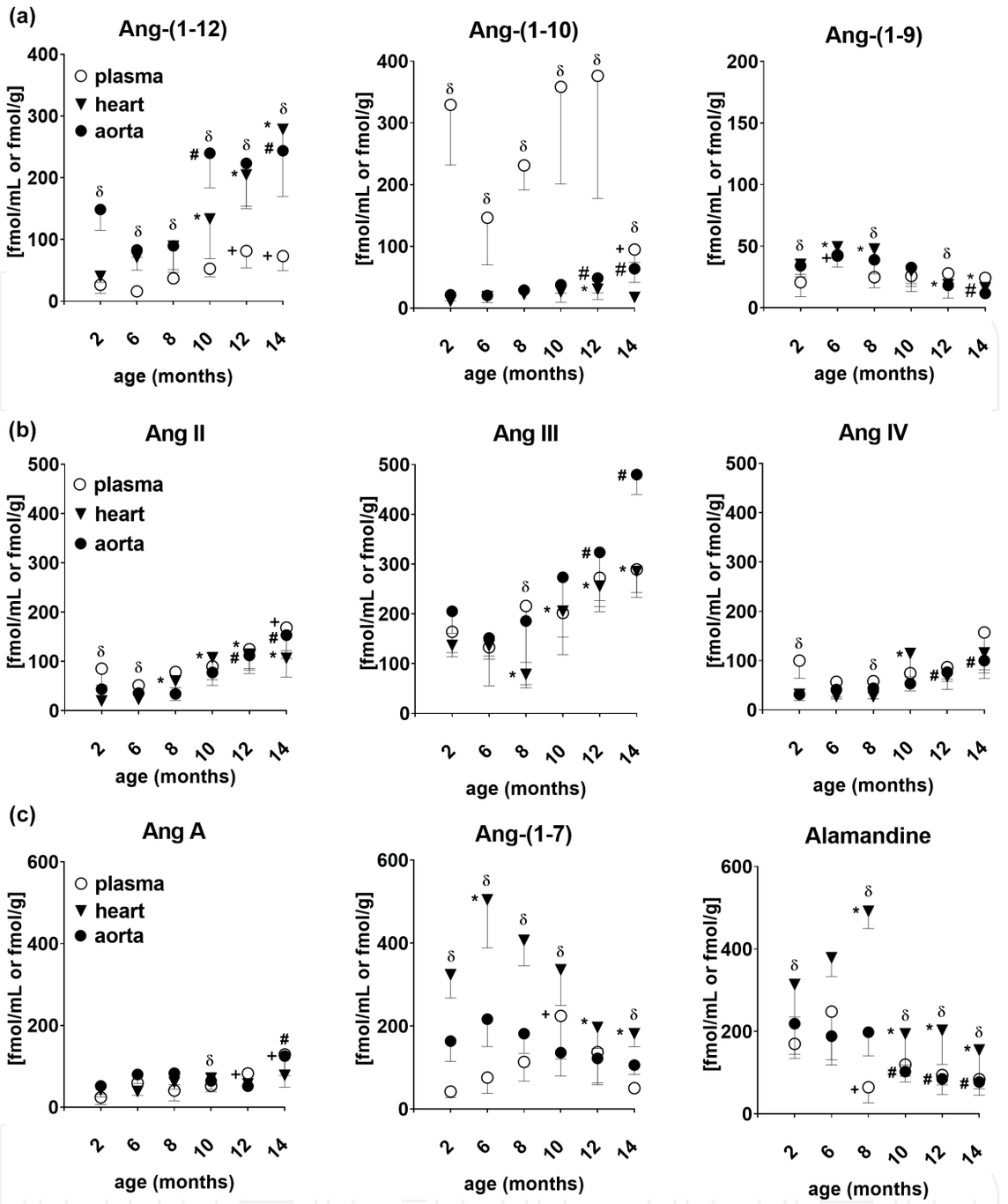

Figure 3.
*Angiotensin profile in plasma, the heart, and the aorta in Tgαq*44 mice. Concentration of Ang-(1–12), Ang-(1–10), and Ang-(1–9) (a), Ang II, Ang III and Ang IV (b), Ang A, Ang-(1–7), and alamandine (c) in plasma, aorta, and heart homogenates. *P < 0.05 for the heart tissue of a given group 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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