Curr Hypertens Rep (2014) 16:433
DOI 10.1007/s11906-014-0433-0

HYPERTENSION AND THE KIDNEY (R CAREY, SECTION EDITOR)

# New Components of the Renin-Angiotensin System: Alamandine and the Mas-Related G Protein-Coupled Receptor D

**Gisele Maia Etelvino · Antônio Augusto Bastos Peluso · Robson Augusto Souza Santos**

Published online: 24 April 2014
© Springer Science+Business Media New York 2014

**Abstract** The renin-angiotensin system is an important component of the central and humoral mechanisms of blood pressure and hydro-electrolytic balance control. Angiotensin II is a key player of this system. Twenty-five years ago the first manuscripts describing the formation and actions of another peptide of the RAS, angiotensin-(1-7), were published. Since then several publications have shown that angiotensin-(1-7) is as pleiotropic as angiotensin II, influencing the functions of many organs and systems. The identification of the ACE homologue ACE2 and, a few years later, Mas, as a receptor for angiotensin-(1-7) contributed a great deal to establish this peptide as a key player of the RAS. Most of the actions of angiotensin-(1-7) are opposite to those described for angiotensin II. This has led to the concept of two arms of the RAS: one comprising ACE/AngII/AT1R and the other ACE2/Ang-(1-7)/Mas. More recently, we have described the identification of a novel component of the RAS, alamandine, which binds to the Mas-related G protein coupled receptor D. This peptide is formed by decarboxylation of the Asp residue of angiotensin-(1-7), leading to the formation of Ala as the N-terminal amino acid. Alternatively, it can be formed by hydrolysis of Ang A, by ACE2. Its effects include vasorelaxation, central effects similar to those produced by angiotensin-(1-7), blunting of isoproterenol-induced heart fibrosis, and anti-hypertensive action in SHR. The putative enzyme responsible for alamandine formation from angiotensin-(1-7) is under investigation. The identification of this novel component of the RAS opens new venues for understanding its physiological role and opens new putative therapeutic possibilities for treating cardiovascular diseases.

**Keywords** Mas1 · Angiotensin II · Angiotensin-(1-7) · Hypertension · Angiotensin-(1-12) · Angiotensin A

This article is part of the Topical Collection on *Hypertension and the Kidney*

G. M. Etelvino · A. A. B. Peluso · R. A. S. Santos (✉)
National Institute of Science and Technology in Nanobiopharmaceutics, Department of Physiology and Biophysics, Federal University of Minas Gerais, Av. Antonio Carlos, 6627 – ICB – UFMG, 31270-901 Belo Horizonte, MG, Brazil
e-mail: robsonsant@gmail.com

## Introduction

The renin angiotensin system (RAS) has been described as an endocrine and tissular system involved in cardiovascular and renal control. Classically, it is comprised by angiotensinogen, an α-2-globulin produced mainly by the liver, which is cleaved in the amino-terminal portion by renin, an aspartyl protease produced in the kidney and secreted into the bloodstream, forming angiotensin I (Ang I), a decapeptide with no known biological function.

The monocarboxypeptidase angiotensin converting enzyme (ACE) removes the dipeptide His-Leu from the C-terminal portion of Ang I, converting it into angiotensin II (Ang II), the major peptide of this classical axis. Other enzymes can also make this conversion, such as tonin, chymase, and cathepsin G [1]. The main effects of Ang II include vasoconstriction, increasing blood pressure, sodium retention, and aldosterone release by the adrenal, as well as fibrotic, hypertrophic, and proliferative effects trough the $AT_1$ receptor [2, 3]. However, Ang II can also bind to $AT_2$ receptors producing, usually, opposite effects from those produced by $AT_1$ activation, such as vasodilation, anti-fibrosis, and anti-hypertrophic and anti-proliferative effects [4•, 5]. Both $AT_1$ and $AT_2$ are described as seven-transmembrane domain G-protein-coupled receptors, which can be blocked by specific antagonists [6]. While losartan and other sartans block $AT_1$ activation, $AT_2$ can be blocked by PD123319 and PD123177 [7, 8].

New peptides, enzymes, and receptors with biological activity have been recently reported as components of the RAS. Several of these compounds are described as counter-regulatory against the classical ACE/AngII/$AT_1$ axis. Angiotensin-(1-7) [Ang-(1-7)] is a heptapeptide which can be formed directly from Ang II by angiotensin converting enzyme 2 (ACE2) or other peptidases, including prolyl-endopeptidase (PEP) and prolyl-carboxipeptidase (PCP). ACE2 can also convert Ang I into angiotensin-(1-9) [Ang-(1-9)] which can be hydrolyzed by ACE or neutral endopeptidase (NEP), forming Ang-(1-7). In addition, Ang-(1-7) can be formed directly from Ang I by endopeptidases including neprilysin, PEP, and NEP [9–12] (Fig. 1). The Ang-(1-7) actions include nitric oxide-dependent vasodilation, anti-arrhythmogenesis, anti-thrombogenesis, anti-fibrogenesis, and improvement in glucose and lipid metabolism [13–17]. Ang-(1-7) is currently classified as an endogenous hormone with anti-proliferative properties, and it is being tested in clinical trials phases I and II [18, 19]. The actions of Ang-(1-7) are strictly related to Mas activation, another seven-transmembrane domain G-protein-coupled receptor [20].

Here we briefly review the properties of novel peptide components of RAS angiotensin (1-12) [Ang-(1–12)] and angiotensin A (Ang A). In addition, we focused on the new angiotensin, alamandine, and its putative receptor, MrgD.

## Angiotensin-(1-12)

Ang-(1–12) was identified in rodents by Nagata et al. in 2006 [21] as a peptide composed of the first 12 N-terminal amino acids of rat angiotensinogen (Asp-Arg-Val-Ttyr-Ile-His-Pro-Phe-His-Leu-Leu-Tyr) [21]. Among other tissues, this peptide is expressed in the kidney and the heart and serves as a substrate for Ang I and Ang II formation. The Ang-(1-12) effects can be blocked by ACE inhibitors or $AT_1$ receptor antagonists, suggesting its role as an Ang II precursor [21, 22]. However, it can also be a precursor for Ang-(1-7) [23]. Cardiac tissue levels of Ang-(1–12) are higher in spontaneously hypertensive rats (SHR) compared with normotensive rats and is associated with higher tissue levels of Ang I and Ang II in SHR [22]. Moreover, rat nephrectomy produced an increase of Ang I, Ang II, and $AT_1$ density in cardiac tissue, suggesting Ang-(1-12) as a precursor for angiotensin peptides independent of circulating renin [24]. Using rat neonatal cardiomyocytes and human atrial tissue, the same group confirmed that Ang-(1-12) is converted into smaller peptides mostly by ACE, but also by neprilysin and chymase actions as well, suggesting the importance of this peptide as a substrate for the formation of the active peptides Ang II and Ang-(1-7) [25, 26].

Recently, Ang-(1-12) was reported to bind $AT_1$ receptors, suggesting that in some conditions its effects could be independent of its role as a substrate for the formation of smaller angiotensin peptides [27•]. One important aspect related to Ang-(1-12) is whether the peptide exists in human tissues. As can be deduced from the human angiotensinogen sequence, the answer to this question is no. In mice and rats, Ang-(1-12) ends with $Leu^{11}$-$Tyr^{12}$, while in humans it would be $Val^{11}$-$Iso^{12}$. Nevertheless, using an in silico cleavage analysis of human angiotensinogen, we noted that the putative fragment Ang-(1-13), derived from a chymotripsin-like activity, was formed, whereas when using the rat and mice angiotensinogen

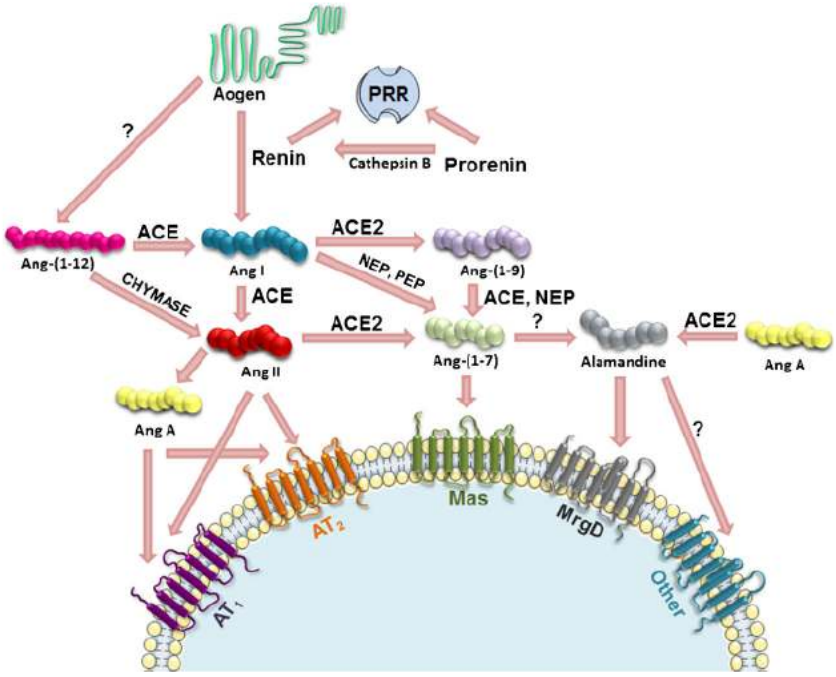


**Fig. 1** Simplified view of the Renin-Angiotensin System Cascade. Aogen indicates angiotensinogen; PRR, (Pro)renin receptor; Ang, angiotensin; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; NEP, neutral-endopeptidase; PEP, prolyl-endopeptidase; $AT_1$, angiotensin type-1 receptor; $AT_2$, angiotensin type 2 receptor; Mas, Ang-(1-7) receptor Mas; MrgD, MAS-related G-protein coupled receptor D. (This figure was made using graphic components obtained from the website: www.servier.com/powerpoint-image-bank)

sequence, the same software gave the expected Ang-(1-12) fragment (Peptide cutter software <www.expasy.org>). Therefore, additional studies are needed to clarify whether the putative Ang-(1-12) and/or Ang-(1-13) fragments of human angiotensinogen would have the same biochemical and enzymatic profile of the corresponding rodent sequences.

## Angiotensin A

Angiotensin A (Ang A) is an octapeptide first identified in human plasma using mass spectrometry by Jankowski et al. (2006). This peptide has the same affinity of Ang II for $AT_1$ receptors, although it has a slightly higher affinity for $AT_2$ receptors [28]. Ang A only differs from Ang II by having an $Ala^1$ instead of $Asp^1$ due to decarboxylation of $Asp^1$ residue. Ang A produces $AT_1$-dependent effects such as vasoconstriction in isolated perfused rat kidney, pressor effect and renal vasoconstriction in anesthetized normotensive and hypertensive rats [29, 30]. In general, these $AT_1$-mediated effects of Ang A are less pronounced than those elicited by Ang II. Strikingly, in isolated cardiomyocytes, Ang A, in contrast to Ang II, did not increase the amplitude of $Ca^{+2}$ transient, suggesting that it may act as a bias agonist of $AT_1$ receptors, at least, in the heart [30].

## Alamandine

Recently, a new component of the RAS was described by our group and was named alamandine [$Ala^1$-Ang-(1-7)] [31••]. The Ang A peptide only differs from Ang II in the N-terminal portion where the Asp1 is decarboxylated into Ala1. This indicated that Ang A could be a substrate for ACE2 to convert it into alamandine (Ala-Arg-Val-Tyr-Ile-His-Pro). Indeed, human ACE2 was able to hydrolyze the C-terminal amino acid of Ang II, producing alamandine. Moreover, this peptide can be produced directly from Ang-(1-7) through decarboxylation of N-terminal aspartate amino acid residue [31••]. Our group recently showed that alamandine is an endogenous peptide in both rat cardiac tissue and human plasma. In addition, patients with nephropathy presented high plasma levels of alamandine, suggesting participation of this peptide in pathological conditions [31••].

Little is known about alamandine degradation. However, by its sequence it is possible to suppose a critical role for aminopeptidases, because removal of $Ala^1$ will lead to the formation of Ang-(2-7) considered an inactive peptide, although it displays ACE inhibitory activity [32]. As observed for Ang-(1-7), alamandine is also an ACE inhibitor, probably because it is an ACE substrate (Paula and Santos, unpublished results). The likely contribution of other Ang-(1-7) degrading enzymes such as NEP and nephrilysin for the degradation of alamandine was not tested yet.

## Biological Action of Alamandine

Because of the high similarity between alamandine and Ang-(1-7), the biological actions of these peptides seem to be closely related to each other, although each acts through different receptors (see below). Alamandine produced endothelial-dependent vasodilation in rat and mice aortic rings. Further, alamandine was able to modulate the baroreflex sensibility after intra-cerebro ventricular (ICV) infusion, facilitating a selective increase in phenylephrine-evoked bradycardia. Administration of alamandine into the RVLM and CVLM also produced blood pressure increase and decrease, respectively. Then, both Ang-(1-7) and alamandine seem to act similarly in medullary areas of the brain [31••]. An oral formulation of alamandine, obtained by its inclusion in HP-β cyclodextrin, produced effects resembling those already observed for Ang-(1-7) such as a long-lasting antihypertension in SHR and a decrease of collagens I, III, and fibronectin deposition in isoproterenol-treated rats [33] (Fig. 2). However, not all effects produced by alamandine are the same as those of Ang-(1-7). No anti-proliferative effect of alamandine was observed in two types of human tumor cells. In the same cells Ang-(1-7) produces an anti-proliferative effect [15, 16].

## The MrgD Receptor as a Bind Site for Alamandine

Mas-related genes (Mrgs) are a large family of G protein-coupled receptors (GPCRs) that are related to MAS [34–44].

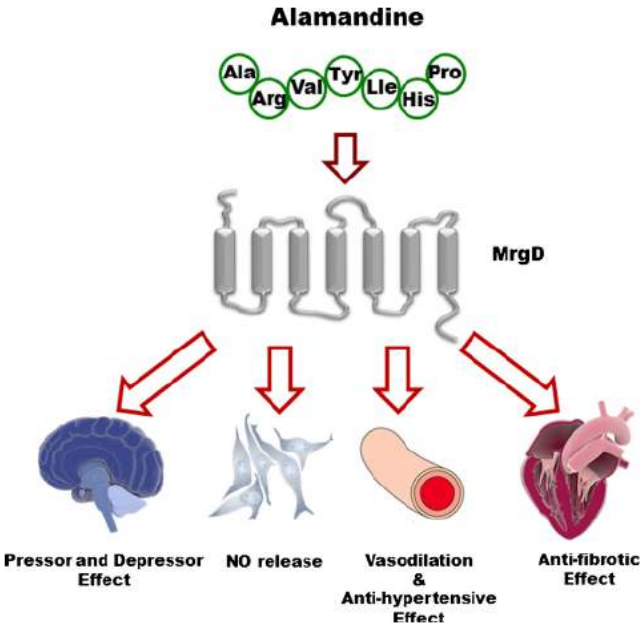


**Fig. 2** Cardiovascular actions of alamandine (This figure was made using graphic components obtained from the website: www.servier.com/powerpoint-image-bank; http://www.somersault1824.com/science-illustrations)

The Mrgs have been divided into subfamilies established by sequence homology (MrgA, MrgB, and MrgC) and some more single genes (MrgD, MrgE, MrgF, MrgG, MrgH, and Mas 1) [37]. These receptors, likewise GPCRs, are constituted of seven transmembrane loops, an N-terminal extracellular domain, and a C-terminal intracellular domain [45]. Their transmembrane and intracellular domains are highly conserved, indicating that they can share some functions [34] and also form heterodimers [38].

The Mrg family is localized in chromosome 1 in the rat [35] and chromosome 11 in humans [34, 46]. This group of receptors shows evident orthology across species [37, 40]. There are many orphan receptors in the Mrg family. Gembart et al. (2008) have shown that Mrg receptors can interact with peptides of the angiotensin system. Among the Mrgs tested, angiotensin-(1-7) has proved to be a weak agonist of the MrgD receptor [41].

MrgD is widely expressed in sensory neurons in the dorsal root spinal ganglia (DRG) [34–37, 39, 40, 43], but it can be located in other organs in lower levels: testis, urinary bladder, arteries, uterus, brain, cerebellum, eyeball, spinal cord, trachea, thymus, heart, lung, diaphragm, peritoneum, gastrointestinal tract, skeletal muscle, prostate, seminal vesicle, and white and brown adipose tissue [35, 37; http://www.ebi.ac.uk/gxa/experiment/E-GEOD-24940/ENSMUS-G00000051207?ef=organism_part]. Shinohara et al. (2004) found that a small amino acid, β-alanine was able to internalize MrgD, inducing intracellular calcium influx and inhibiting cAMP production in Chinese hamster ovary (CHO) cells that expressed rat, mouse, or human MrgD. The effect in calcium influx can be explained by connection of the MrgD with the G-protein α subunit ($G_q$), and the suppression of cAMP suggested an interaction between MrgD and an inhibitory regulative G-protein ($G_i$) [36]. Further, activation of MrgD by β-alanine also suppressed KCNQ/M-type potassium channels, increasing neuron excitability by the $G_q$ and phospholipase C (PLC) pathway [40]. It has been suggested that there are many important functions for the MrgD receptor since it is involved in pain pathways [34, 36, 38–40, 43], sensitivity to thermal and mechanical stimuli [42], and tumorigenic activity [44, 47].

Considering that alamandine is different from Angiotensin-(1-7) only by the presence of an amino acid alanine instead of aspartate in the N-terminal domain, the possibility that alamandine could bind to the Ang-(1-7) receptor Mas or to the MrgD receptor was tested. Alamandine produced vasorelaxation in blood vessels of Mas-deficient mice, and the Mas antagonist A-779 could not block the effect of alamandine in aortic rings of FVBN mice. In addition alamandine did not stimulate NO release in Mas-transfected CHO cells [31••]. These observations ruled out Mas as a primary binding site for alamandine. Therefore, experiments were performed to investigate the possible interaction between alamandine and MrgD.

Binding studies with human MrgD-transfected CHO cells indicated that fluorescent-labeled alamandine (FAM-Alamandine) binds to MrgD, and this binding was prevented by β-alanine and D-Pro$^7$-Ang-(1-7), but not by A-779. Moreover, incubation of MrgD-transfected CHO cells with alamandine produced NO release, indicating a functional response resulting from the interaction of the peptide with the receptor. In keeping with the blood vessel experiments, alamandine did not bind to Mas-transfected cells. Interestingly, the endothelial-dependent vasorelaxation induced by alamandine in aortic rings of mice was not observed with the MrgD ligand β-alanine. However, pre-incubation (20 min) with this amino acid blocked the alamandine vasorelaxation. These observations suggest that alamandine and β-alanine might act as MrgD bias agonists.

In addition to β-alanine, the Ang-(1-7) antagonist D-Pro$^7$-Ang-(1-7) also blocked the alamandine-induced vasorelaxation and its effects on the RVLM and CVLM. Therefore, this peptide appears to act as a dual Ang-(1-7)/alamandine antagonist. This may explain reports describing blockade of some Ang-(1-7) effects with D-Pro$^7$-Ang-(1-7) but not with A-779 [48–50]. On the other hand, the fact that PD-123319 blocked the alamandine-induced vasorelaxation in aorta taken from WT and AT$_2$-KO mice [31••] and also the binding of FAM-alamandine to human MrgD-CHO-transfected cells further support the relative non-specificity of this compound and suggest that some early observations of blockade of Ang-(1-7) effects by PD-123319 [51–53] were in fact due to blockade of Ang-(1-7)-derived alamandine. Whether this is true awaits clarification. It is important to point out that the identification of MrgD as a receptor for alamandine does not preclude the possibility of the existence of other binding sites for this novel angiotensin, such as the MrgE receptor [34].

## Concluding Remarks

In recent years new components of the renin-angiotensin system were discovered, increasing the complexity of this vital hormonal system. The identification of alamandine and its receptor MrgD might contribute to a new understanding of the RAS physiology and also open new possibilities for the development of novel therapeutic strategies for the treatment of cardiovascular-related diseases.

**Acknowledgments** This work was supported by Conselho Nacional de Pesquisas (CNPq), Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG), and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

**Compliance with Ethics Guidelines**

**Conflict of Interest** Gisele Maia Etelvino, Antônio Augusto Bastos Peluso, and Robson Augusto Souza Santos declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** This article does not contain any studies with human or animal subjects performed by any of the authors.

## References

Papers of particular interest, published recently, have been highlighted as:

- • Of importance
- •• Of major importance


1. Belova LA. Angiotensin II-generating enzymes. Biochemistry. 2000;65(12):1337–45.
2. Neves LA, Almeida AP, Khosla MC, Campagnole-Santos MJ, Santos RA. Effect of angiotensin-(1-7) on reperfusion arrhythmias in isolated rat hearts. Braz J Med Biol Res. 1997;30:801–9.
3. Timmermans PB, Benfield P, Chiu AT, Herblin WF, Wong PC, Smith RD. Angiotensin II receptors and functional correlates. Am J Hypertens. 1992;5:221S–35.
4. • Carey RM. Newly discovered components and actions of the renin-angiotensin system. Hypertension. 2013;62(5):818–22. *This brief review describes the main characteristics of novel components of the renin-angiotensin system*.
5. Batenburg WW, Garrelds IM, Bernasconi CC, Juillerat-Jeanneret L, van Kats JP, Saxena PR, et al. Angiotensin II type 2 receptor-mediated vasodilation in human coronary microarteries. Circulation. 2004;109:2296–301.
6. Kim S, Iwao H. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. Pharmacol Rev. 2000;52:11–34.
7. Rompe F, Artuc M, Hallberg A, Alterman M, Ströder K, Reichenbach A, et al. Direct angiotensin II type 2 receptor stimulation acts anti-inflammatory through epoxyeicosatrienoic acid and inhibition of nuclear factor kB. Hypertension. 2010;55:924–31.
8. Wong C, Hart SD, Timmermans PBMWM, Smith D. Functional studies of nonpeptide angiotensin II receptor subtype-specific ligands: DuP 753 (All-i) and PD1 231 77 (All-2). J Pharmacol Exp Ther. 1976;584–92.
9. Vickers C, Hales P, Kaushik V, Dick L, Gavin J, Tang J, et al. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J Biol Chem. 2002;277:14838–43.
10. Chappell MC, Pirro NT, Sykes A, Ferrario CM. Metabolism of angiotensin-(1-7) by angiotensin-converting enzyme. Hypertension. 1998;31:362–7.
11. Welches WR, Brosnihan KB, Ferrario CM. A comparison of the properties and enzymatic activities of three angiotensin processing enzymes: angiotensin converting enzyme, prolyl endopeptidase and neutral endopeptidase 24.11. Life Sci. 1993;52:1461–80.
12. Santos RA, Campagnole-Santos MJ, Andrade SP. Angiotensin-(1-7): an update. Regul Pept. 2000;91:45–62.
13. Ferreira AJ, Santos RA, Almeida AP. Angiotensin-(1-7): cardioprotective effect in myocardial ischemia/reperfusion. Hypertension. 2001;38:665–8.
14. Ferreira AJ, Santos RAS. Cardiovascular actions of angiotensin-(1-7). Braz J Med Biol Res. 2005;38:499–507.
15. Grobe JL, Mecca AP, Mao H, Katovich MJ. Chronic angiotensin-(1-7) prevents cardiac fibrosis in DOCA-salt model of hypertension. Am J Physiol Heart Circ Physiol. 2006;290:H2417–23.
16. Giani JF, Mayer MA, Muñoz MC, Silberman EA, Höcht C, Taira CA, et al. Chronic infusion of angiotensin-(1-7) improves insulin resistance and hypertension induced by a high-fructose diet in rats. Am J Physiol Endocrinol Metab. 2009;296:E262–71.
17. Santos SHS, Fernandes LR, Mario EG, Ferreira AVM, Pôrto LCJ, Alvarez-Leite JI, et al. Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism. Diabetes. 2008;57:340–7.
18. 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 Res. 2007;67:2809–15.
19. Krishnan B, Smith TL, Dubey P, Zapadka ME, Torti FM, Willingham MC, et al. Angiotensin-(1-7) attenuates metastatic prostate cancer and reduces osteoclastogenesis. Prostate. 2013;73:71–82.
20. Santos RAS, Simoes e Silva AC, Maric C, Silva DMR, Machado RP, de Buhr I, et al. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci U S A. 2003;100:8258–63.
21. Nagata S, Kato J, Sasaki K, Minamino N, Eto T, Kitamura K. Isolation and identification of proangiotensin-12, a possible component of the renin-angiotensin system. Biochem Biophys Res Commun. 2006;350:1026–31.
22. 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. Am J Physiol Heart Circ Physiol. 2008;294:H2614–8.
23. Trask AJ, Jessup JA, Chappell MC, Ferrario CM. Angiotensin-(1-12) is an alternate substrate for angiotensin peptide production in the heart. Am J Physiol Heart Circ Physiol. 2008;27157:2242–7.
24. Ferrario CM, Varagic J, Habibi J, Nagata S, Kato J, Chappell MC, et al. Differential regulation of angiotensin-(1-12) in plasma and cardiac tissue in response to bilateral nephrectomy. Am J Physiol Heart Circ Physiol. 2009;H1184–92.
25. Ahmad S, Simmons T, Varagic J, Moniwa N, Chappell MC, Ferrario CM. Chymase-dependent generation of angiotensin II from angiotensin-(1-12) in human atrial tissue. PLoS ONE. 2011;6:e28501.
26. Ahmad S, Varagic J, Westwood BM, Chappell MC, Ferrario CM. Uptake and metabolism of the novel peptide angiotensin-(1-12) by neonatal cardiac myocytes. PLoS ONE. 2011;6:e15759.
27. • Chan KH, Chen YH, Zhang Y, Wong YH, Dun NJ. Angiotensin-[1-12] interacts with angiotensin type I receptors. Neuropharmacology. Elsevier Ltd; 2013;1–7. *This report suggests that some biological actions of the new member of renin-angiotensin system, angiotensin-(1-12), can be mediated by a direct interaction with AT1R*.
28. Jankowski V, Vanholder R, van der Giet M, Tölle M, Karadogan S, Gobom J, et al. Mass-spectrometric identification of a novel angiotensin peptide in human plasma. Arterioscler Thromb Vasc Biol. 2007;27:297–302.
29. Yang R, Smolders I, Vanderheyden P, Demaegdt H, Van Eeckhaut A, Vauquelin G, et al. Pressor and renal hemodynamic effects of the novel angiotensin A peptide are angiotensin II type 1A receptor dependent. Hypertension. 2011;57:956–64.
30. Coutinho DC, Foureaux G, Rodrigues KD, Salles RLA, Moraes PL, Murça TM, et al. Cardiovascular effects of angiotensin A: a novel peptide of the renin-angiotensin system. J Renin Angiotensin Aldosterone Syst. 2013;1–7.
31. •• 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. Circ Res.

2013;112:1104–11. *This study reported the discovery of a novel peptide of the renin-angiotensin system, alamandine. Evidence that the Mas-related G protein-coupled receptor D (MrgD) is a receptor for alamandine was also provided*.
32. Paula RD, Lima CV, Britto RR, Santos MJC, Khosla MC, Santos RAS. Potentiation of the hypotensive effect of bradykinin by. Peptides. 1999;20:493–500.
33. Marques FD, Ferreira AJ, Sinisterra RDM, Jacoby BA, Sousa FB, Caliari MV, et al. An oral formulation of angiotensin-(1-7) produces cardioprotective effects in infarcted and isoproterenol-treated rats. Hypertension. 2011;477–83.
34. Dong X, Han S, Zylka MJ, Simon MI, Anderson DJ. A diverse family of GPCRs expressed in specific subsets of nociceptive sensory neurons. Cell. 2001;106:619–32.
35. Zylka MJ, Dong X, Southwell AL, Anderson DJ. Atypical expansion in mice of the sensory neuron-specific Mrg G protein-coupled receptor family. Proc Natl Acad Sci U S A. 2003;100:10043–8.
36. Shinohara T, Harada M, Ogi K, Maruyama M, Fujii R, Tanaka H, et al. Identification of a G protein-coupled receptor specifically responsive to beta-alanine. J Biol Chem. 2004;279:23559–64.
37. Zhang L, Taylor N, Xie Y, Ford R, Johnson J, Paulsen JE, et al. Cloning and expression of MRG receptors in macaque, mouse, and human. Brain Res Mol Brain Res. 2005;133:187–97.
38. Milasta S, Pediani J, Appelbe S, Trim S, Wyatt M, Cox P, et al. Interactions between the Mas-related receptors MrgD and MrgE alter signalling and trafficking of MrgD. Mol Pharmacol. 2006;69(2):479–91.
39. Zhang R, Yan P-K, Zhou C-H, Liao J-Y, Wang M-W. Development of a homogeneous calcium mobilization assay for high throughput screening of mas-related gene receptor agonists. Acta Pharmacol Sin. 2007;28:125–31.
40. Crozier RA, Ajit SK, Kaftan EJ, Pausch MH. MrgD activation inhibits KCNQ/M-currents and contributes to enhanced neuronal excitability. J Neurosci. 2007;27:4492–6.
41. Gembardt F, Grajewski S, Vahl M, Schultheiss H-P, Walther T. Angiotensin metabolites can stimulate receptors of the Mas-related genes family. Mol Cell Biochem. 2008;319:115–23.
42. Rau KK, McIlwrath SL, Wang H, Lawson JJ, Jankowski MP, Zylka MJ, et al. Mrgprd enhances excitability in specific populations of cutaneous murine polymodal nociceptors. J Neurosci. 2009;29:8612–9.
43. Wang H, Zylka MJ. Mrgprd-expressing polymodal nociceptive neurons innervate most known classes of substantia gelatinosa neurons. J Neurosci. 2009;29:13202–9.
44. Nishimura S, Uno M, Kaneta Y, Fukuchi K, Nishigohri H, Hasegawa J, et al. MRGD, a MAS-related G-protein coupled receptor, promotes tumorigenisis and is highly expressed in lung cancer. PLoS ONE. 2012;7:e38618.
45. Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: an evolutionary success. EMBO J. 1999;18(7):1723–9.
46. Glusman G, Yanai I, Rubin I, Lancet D. The complete human olfactory subgenome. Genome Res. 2001;11:685–702.
47. Uno M, Nishimura S, Fukuchi K, Kaneta Y, Oda Y, Komori H, et al. Identification of physiologically active substances as novel ligands for MRGPRD. J Biomed Biotechnol. 2012;2012:816159.
48. Silva DMR, Vianna HR, Cortes SF, Campagnole-Santos MJ, Santos RAS, Lemos VS. Evidence for a new angiotensin-(1-7) receptor subtype in the aorta of Sprague–Dawley rats. Peptides. 2007;28:702–7.
49. Herath CB, Mak K, Burrell LM, Angus PW. Angiotensin-(1-7) reduces the perfusion pressure response to angiotensin II and methoxamine via an endothelial nitric oxide-mediated pathway in cirrhotic rat liver. Am J Physiol Gastrointest Liver Physiol. 2013;304:G99–108.
50. Kluskens LD, Nelemans SA, Rink R, de Vries L, Meter-Arkema A, Wang Y, et al. Angiotensin-(1-7) with thioether bridge: an angiotensin-converting enzyme-resistant, potent angiotensin-(1-7) analog. J Pharmacol Exp Ther. 2009;328(3):849–54.
51. Dudley DT, Panek RL, Major TC, Lu GH, Bruns RF, Klinkefus BA, et al. Subclasses of angiotensin II binding sites and their functional significance. Mol Pharmacol. 1990;38(3):370–7.
52. Nakajima M, Hutchinson HG, Fujinaga M, Hayashida W, Morishita R, Zhang L, et al. The angiotensin II type 2 (AT2) receptor antagonizes the growth effects of the AT1 receptor: gain-of-function study using gene transfer. Proc Natl Acad Sci U S A. 1995;92:10663–7.
53. Kaschina E, Unger T. Angiotensin AT1/AT2 receptors: regulation, signalling and function. Blood Press. 2003;12:70–88.