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REVIEW

Devil and angel in the renin–angiotensin system: ACE–angiotensin II–AT_1 receptor axis *vs.* ACE2–angiotensin-(1–7)–Mas receptor axis

Masaru Iwai and Masatsugu Horiuchi

Recent studies have established a new regulatory axis in the renin–angiotensin system (RAS). In this axis, angiotensin (Ang)-(1–7) is finally produced from Ang I or Ang II by the catalytic activity of angiotensin-converting enzyme 2 (ACE2). Ang-(1–7) shows actions different from those of AT_1 receptor stimulation, such as vasodilatation, natriuresis, anti-proliferation and an increase in the bradykinin–NO (nitric oxide) system. As the catalytic efficiency of ACE2 is approximately 400-fold higher with Ang II as a substrate than with Ang I, this axis is possibly acting as a counter-regulatory system against the ACE/Ang II/AT_1 receptor axis. The signaling pathway of the ACE2–Ang-(1–7) axis has not yet been totally and clearly understood. However, a recent report suggests that the Mas oncogene acts as a receptor for Ang-(1–7). Intracellular signaling through Mas is not clear yet. Several factors such as Akt phosphorylation, protein kinase C activation and mitogen-activated protein (MAP) kinase inhibition seem to be involved in this signaling pathway. Further investigations are needed to clarify the regulation and mechanism of action of ACE2 and Ang-(1–7). However, this second axis through ACE2 and Ang-(1–7) in RAS can be an important target for the therapy of cardiovascular and metabolic disorders.
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INTRODUCTION

The renin–angiotensin system (RAS) has a critical role in the cardiovascular system. Recent studies on RAS have found various components and have proposed new axes of signaling pathways. In a classical system, angiotensin (Ang) II produced from Ang I by an angiotensin-converting enzyme (ACE) is a strong bioactive substance. Ang II binds to two major types of receptors, namely, Ang II type 1 (AT_1) and Ang II type 2 (AT_2) receptors,[1] the signaling pathways of which have been well studied, including in the identification of new signaling-related molecules, such as AT_1 receptor-associated protein (ATRAP)[2] and AT_2 receptor-interacting protein (ATIP).[3] In cardiovascular diseases, AT_2-receptor stimulation seems to antagonize the signaling associated with AT_1-receptor stimulation. As the binding affinity of Ang II for the AT_2 receptor does not differ from that for the AT_1 receptor, it is considered that AT_2-receptor stimulation contributes to the beneficial actions of AT_1-receptor blockers (ARBs).[4] In this context, AT_2-receptor agonists, such as compound 21, have been newly developed and are expected to function as useful agents against pathological disorders in the future.[5]

Recently, a new axis of RAS has been established. In this axis, Ang I is finally converted to Ang-(1–7) by the catalytic activity of ACE2.[6,7] The mechanisms of action of Ang-(1–7) are still being investigated by many research groups. Santos *et al.*[7] reported that the Mas oncogene is a receptor for Ang-(1–7). There are an increasing number of reports on the role of ACE2, Ang-(1–7) and Mas in the cardiovascular system. In this review, an outline of the role and function of ACE2 with the Ang-(1–7) pathway is discussed in comparison with the classical ACE–Ang II–AT_1 receptor axis.

ACE, ANG II AND ANGIOTENSIN-II RECEPTORS—CLASSICAL RAS AXIS

Ang II is a well-known bioactive substance involved in the regulation of blood pressure. Ang II is involved in the exaggeration of cardiovascular disease.[4] Recent studies indicate that Ang II is also involved in the onset of diabetes and metabolic disorders.[8] In classical RAS, Ang II is produced from Ang I by the action of ACE. This classical axis can be called as the ACE–AngII–AT_1 receptor axis. Such findings are closely related to the development of RAS inhibitors, such as the ACE inhibitor and ARB. The major receptor subtypes for Ang II are the AT_1 and AT_2 receptors. The AT_1 receptor was cloned in 1991, and this finding has contributed to the development of ARBs and accelerated basic and clinical researches.[9] The distribution of the AT_1 receptor covers most organs, whereas AT_2-receptor expression is observed in only a few organs after birth and is upregulated in pathological states.[1]

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AT_1-receptor stimulation mediates the classical major actions of Ang II, and large clinical trials and basic researches have established the concept of the cardiovascular continuum proposed by Dzau *et al.*[10,11] AT_1-receptor stimulation is in fact known to exert hypertension, stroke, cardiovascular events and renal diseases (Figure 1). It has also been reported that the blockade of AT_1 receptor promotes longevity.[12]

In recent years, efforts have been carried out to produce the inhibitor of RAS. The first trial to produce a renin inhibitor did not succeed for clinical use because of chemical and technical difficulties. However, these efforts produced the ACE inhibitor. This success triggered the development of new RAS inhibitors. ACE inhibitors have some adverse effects, such as coughing. In addition, chronic administration of an ACE inhibitor showed an escape phenomenon, in which the inhibitory action of the ACE inhibitor is strongly attenuated. To solve such problems, ARB has been developed as the second RAS inhibitor. As most actions of Ang II are mediated through the AT_1 receptor, the non-peptide ARB is available and widely used in the treatment of hypertension, and in cardiovascular and renal diseases.

Ang II acts mainly through AT_1 receptors, using various signaling mechanisms. For example, AT_1-receptor stimulation increases the influx of extracellular Ca^{2+} and mobilization of intracellular Ca^{2+}. An increase in the intracellular Ca^{2+} level activates acute responses, such as vascular smooth-muscle contraction, and also activates various kinases, including the mitogen-activated protein (MAP) kinase pathway, to induce cell-proliferation signaling. AT_1-receptor stimulation seems to activate the EGF receptor in the plasma membrane. Therefore, a part of the action caused by AT_1-receptor stimulation is similar to that caused by Ca^{2+}-mobilizing hormones and by the EGF family. In contrast, signaling mediated by AT_2-receptor stimulation is transferred mainly by phosphatases.[1] Therefore, the action of AT_2-receptor stimulation is considered to be antagonized against AT_1-receptor-mediated signaling. In fact, an antagonizing action or the counter-regulation of AT_2-receptor stimulation has been reported previously.[13–15]

ROLES OF ACE2, ANG-(1–7) AND THE MAS AXIS—A NEW RAS PATHWAY

Possible effects such as vasodilation mediated by Ang-(1–7) have been reported; however, the mechanisms leading to Ang-(1–7) production and its receptor were unclear, resulting in the delay of Ang-(1–7)-based researches. Recent reports show that Ang-(1–7) is produced by ACE2 activity.[16,17] ACE2 is reported to be highly expressed in the heart, kidney and testis. ACE2 is a membrane-associated hydrolase and it hydrolyzes Ang I to Ang-(1–9) and Ang II to Ang-(1–7) (Figure 2).[18] As Ang-(1–9) can be converted to Ang-(1–7) by ACE or by other peptidases, ACE2 facilitates Ang-(1–7) production by two separate pathways.[19] Ang-(1–7) loses only one amino acid from Ang II. It might be possible that Ang-(1–7) is a degradation product of Ang II and one of the inactivation mechanisms of Ang II. However, recent studies indicate that Ang-(1–7) has more active roles in RAS. Ang-(1–7) causes vasodilation, which antagonizes AT_1-receptor stimulation-mediated vasoconstriction. This effect seemed to be mediated by the bradykinin–NO (nitric oxide) pathway.[18,20] Accordingly, these results suggest that Ang-(1–7) antagonized the pressor effect of AT_1-receptor stimulation, suggesting that it may act as a kind of AT_1-receptor antagonist, thereby resulting in a blood-pressure-lowering effect and an organ-protective effect, such as a reduction of cardiac hypertrophy and fibrosis and renal damage (Figure 3). It is reported that a lack of ACE2 accelerates the pressure-overload-induced cardiac dysfunction.[21] On the other hand, the Ang-(1–7) agonist,

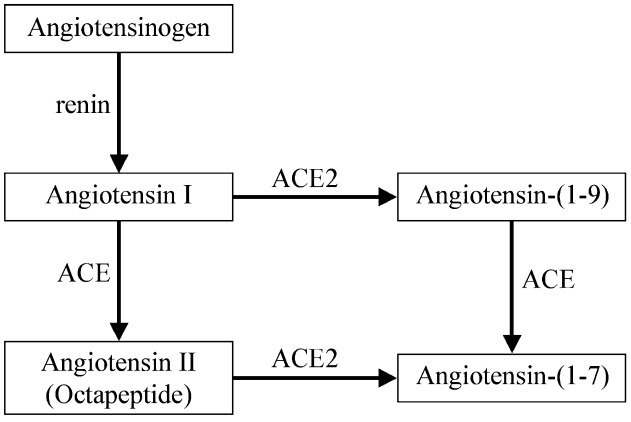

Figure 2 The production of angiotensin-(1–7) by the angiotensin-converting enzyme 2 (ACE2).

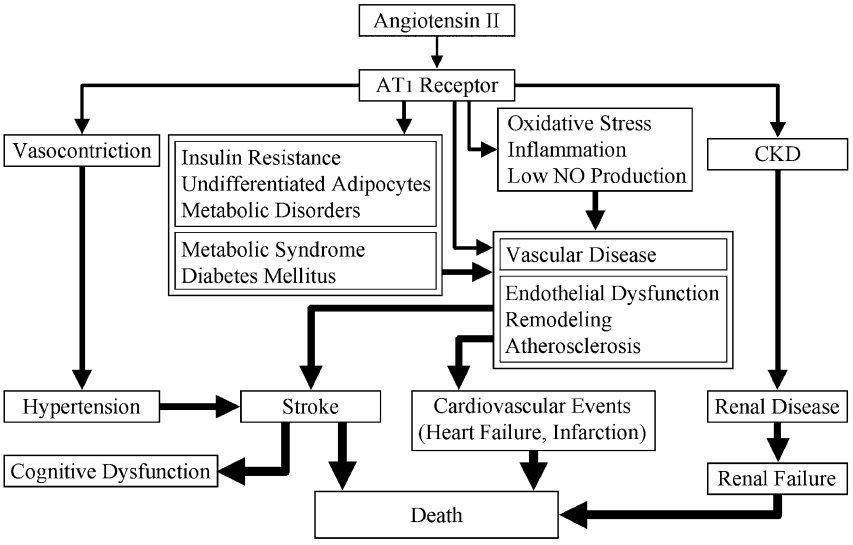

Figure 1 The role of AT_1 receptor stimulation in hypertension and organ damage. NO, nitric oxide; CKD, chronic kidney disease.

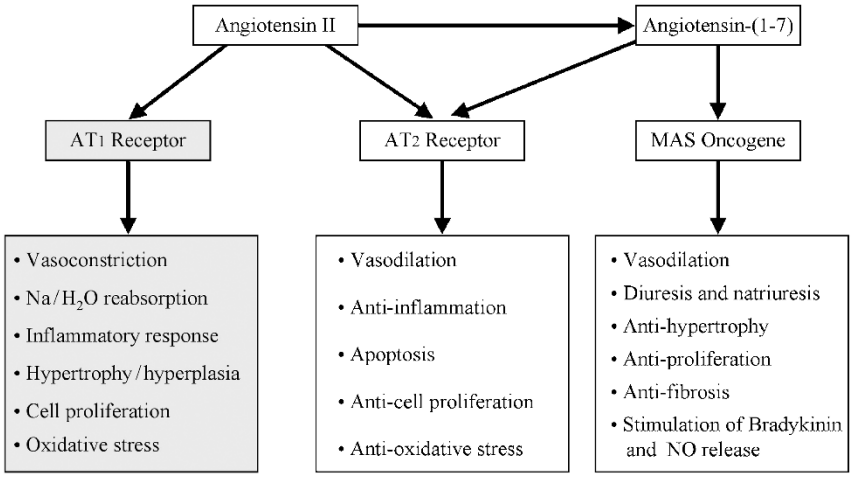

Figure 3 Action of AT_1 and AT_2 receptors and Mas-mediated signaling. AT_1, angiotensin-II type-1 receptor; AT_2, angiotensin-II type-2 receptor; NO, nitric oxide.

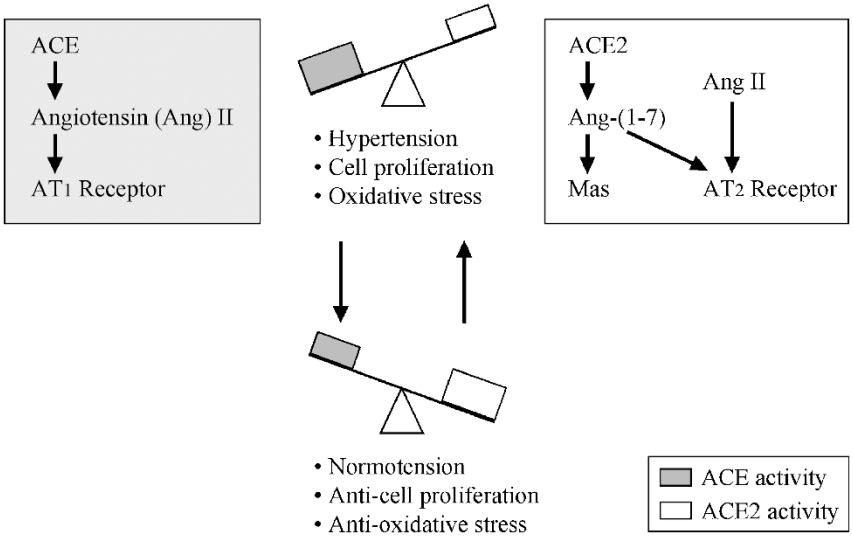

Figure 4 The balance between ACE and ACE2 activities affects cardiovascular disorders. ACE, angiotensin-converting enzyme; Ang, angiotensin; AT_1, angiotensin-II type-1 receptor; AT_2, angiotensin-II type-2 receptor.

AVE0991, was cardioprotective in diabetic rats.[22] In addition, Ang-(1–7) potentiates bradykinin, either through an AT_2-receptor-dependent mechanism or through the inhibition of ACE.[23–25] Recent studies have also shown that Ang-(1–7), similar to ACE inhibitors, potentiates the effect of bradykinin by inhibiting the desensitization of its receptor.[26,27] It has been discovered that the catalytic efficiency of ACE2 is approximately 400-fold higher with Ang II as a substrate than with Ang I,[17] suggesting that this second arm of the system acts as a counter-regulator of the first arm. Previous reports suggest that olmesartan, an ARB, increased the plasma concentration of Ang-(1–7) and the cardiac ACE2-expression level.[28,29] Another ARB, losartan, also induced similar changes; however, its dose was 100-fold higher than that of olmesartan,[28] suggesting that this effect of ARB is not a class effect. A more detailed analysis could show the regulation of ACE2 and Mas, which could contribute toward a new drug discovery for regulating RAS.

Recent studies on the function of ACE2 suggest that ACE2 has an important role in the severity of lung failure, such as in acute respiratory distress syndrome (ARDS) or in acute lung injury.[30] Moreover, it has been shown that the severe acute respiratory syndrome (SARS) corona virus utilizes ACE2 as an essential receptor for cell fusion and for *in vivo* infections, suggesting that ACE2 contributes to SARS pathogenesis.[31] These results indicate that ACE2 activity has an important role not only in cardiovascular diseases but also in damages or dysfunctions of other organs. We can expect the further studies on ACE2 functions to contribute toward a new therapy for organ damages that target this enzyme.

A recent report suggests that the *Mas* oncogene acts as a receptor for Ang-(1–7).[32,33] Mas is a class of G-protein-coupled receptor containing 325 amino-acid residues. The expression of Mas is abundantly expressed in the brain and testis. Low levels of Mas expression were also observed in other tissues, such as in the heart, kidney, lung, liver, spleen, tongue and in the skeletal muscle.[34,35] The studies using

Mas-deficient mice showed an impairment of cardiac functions in these mice.[36,37] Recent studies suggest that Rac 1, c-Jun NH_2-terminal kinase (JNK), p38 MAP kinase and the activation of phospholipase C might be involved in Mas-mediated signaling. One of the major pathways of Mas signaling in the cardiovascular system is the phosphorylation of Akt. Moreover, in cardiomyocytes, an inhibition of MAP kinase activation by Ang-(1–7) can be blocked by antisense oligonucleotides against Mas.[38] More detailed research works can show the Mas functions more clearly.

PERSPECTIVES OF THE ACE2–ANG-(1–7)–MAS AXIS IN CLINICAL FIELD

In recent years, several classes of RAS inhibitors have been developed and these inhibitors are effective in hypertensive patients and in hypertension-associated pathological disorders. ACE inhibitors, ARBs and renin inhibitors are already available for hypertensive patients. Moreover, the AT_2-receptor agonists, such as compound 21, could be useful in the future. In previous studies, the detailed mechanism of the regulation of the ACE2–Ang-(1–7)–Mas axis was not clearly stated. However, it has been clarified that this pathway acts as a counter-regulation system against the ACE–Ang II–AT_1 receptor pathway. Therefore, it is suggested that the ACE2–Ang-(1–7)–Mas axis could become a new target for the therapy of circulatory disorders and adult diseases, including that of the metabolic syndrome. The total effects of RAS seem to stand on the balance between ACE and ACE2 activities (Figure 4). In this respect, it could be considered that the classical ACE–Ang II–AT_1 receptor axis plays as a 'devil' and the 'ACE2–Ang-(1–7)–Mas axis' plays as an angel for fruitful aging in RAS through blood pressure lowering and organ protection.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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