

## REVIEW

# Inhibition of the renin–angiotensin system and target organ protection

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The renin–angiotensin system (RAS) is involved in the pathological mechanisms of target organ damage, as well as in the induction of hypertension. RAS inhibition by angiotensin converting enzyme (ACE) inhibitors and angiotensin (Ang) II receptor blockers can prevent tissue damage by inhibition of Ang II type 1 receptor signaling. A beneficial effect of RAS inhibition on the heart, vasculature and kidney in cardiovascular disease has been reported. However, RAS inhibition can also prevent fibroproliferative diseases and damage of other tissues, such as brain, adipose tissue and muscle, because local RAS has an important role in tissue damage compared with circulating RAS. Moreover, other players, such as Ang II type 2 receptor signaling, aldosterone and ACE2 have been highlighted. Furthermore, there has also been a focus on the emerging concept of regulation of RAS, such as receptor-interacting proteins and receptor modifications, in the new discovery of therapeutic agents for tissue protection. The RAS has a pivotal role in various target organ damage, with complicated mechanisms; therefore, blockade of RAS may be therapeutically effective in preventing organ damage, as well as in having an antihypertensive effect. *Hypertension Research* (2009) 32, 229–237; doi:10.1038/hr.2009.5; published online 27 February 2009

**Keywords:** organ damage; local RAS; oxidative stress; inflammation; fibroproliferative disease

## INTRODUCTION

Hypertension is one of the most common chronic diseases, being observed in one-fourth of the world's population,<sup>1</sup> and contributes substantially to the burden of cardiovascular (CV) disease and end organ damage. Although blood pressure lowering with antihypertensive agents prevents CV events, such as stroke, myocardial infarction, heart failure and kidney disease,<sup>2</sup> accumulating evidence from large clinical trials suggests that blockade of the renin–angiotensin system (RAS) is more effective in the prevention of CV and target organ damage compared with other hypertensive agents. Many tissues are considered to be capable of local angiotensin (Ang) II production through the tissue-specific local RAS. In pathological conditions, the local RAS is activated in various tissues, such as endothelium, vascular smooth muscle and renal mesangium, leading to organ damage. RAS is involved in all stages of the CV continuum,<sup>3</sup> because the major effector of RAS, Ang II, has a direct pathobiological effect on the heart, brain, vessel wall, kidney and adipose tissue. Moreover, new evidence has recently accumulated showing the existence of several novel receptor-interacting proteins and various Ang II receptor activation mechanisms beyond the classical actions of receptors for Ang II. Ang II exerts its important physiological functions through two distinct receptor subtypes: type 1 (AT<sub>1</sub>) and type 2 (AT<sub>2</sub>) receptors. These associated proteins could contribute not only to Ang II receptors' functions but also to influencing pathophysiological states. Moreover, other players in RAS have been highlighted in recent CV research.

In this review, the major basic mechanisms of target organ damage induced by RAS in each organ and the effect of blockade of RAS, such as with angiotensin converting enzyme (ACE) inhibitors (ACEIs) and Ang II receptor blockers (ARBs), on target organ damage are discussed, together with the recent paradigm of RAS.

## NEW PARADIGM SHIFT OF RENIN–ANGIOTENSIN SYSTEM

Ang II is the principal vasoactive substance of RAS, having a variety of physiological actions including vasoconstriction, aldosterone release and cell growth.<sup>4</sup> Ang II binds two major receptors: the AT<sub>1</sub> receptor and the AT<sub>2</sub> receptor. The majority of well-known Ang II actions are mediated through AT<sub>1</sub> receptor stimulation, and RAS inhibition by ACEIs and ARBs is expected to protect against CV disease. AT<sub>2</sub> receptor stimulation by unbound Ang II could also be expected during treatment with ARBs. Recent accumulating evidence has suggested that the AT<sub>2</sub> receptor not only opposes the AT<sub>1</sub> receptor but also has unique effects beyond an interaction with AT<sub>1</sub> receptor signaling. On the other hand, recent experimental studies have shown the existence of proteins interacting with Ang II receptors, screened by the yeast-based two-hybrid protein–protein interaction assay technique, and have also revealed their functions.<sup>5–12</sup> For the AT<sub>1</sub> receptor, AT<sub>1</sub> receptor-associated protein could act as a negative regulator in AT<sub>1</sub> receptor-mediated cell proliferation and vascular remodeling, at least in part by the enhancement of AT<sub>1</sub> receptor internalization.<sup>13,14</sup> On the other hand, the AT<sub>2</sub> receptor-interacting protein seems to act

as a novel early component of the growth inhibitory signaling cascade of the  $AT_2$  receptor.<sup>12</sup> Moreover, the  $AT_1$  receptor is also reported to form homodimers or heterodimers, and undergo complex formation with the  $AT_2$  receptor<sup>15</sup> and other GPCRs, such as the bradykinin B2 receptor in patients with preeclampsia,<sup>16</sup> the EGF receptor,<sup>17</sup> dopamine receptors,<sup>18–21</sup>  $AT_1$ -endothelin receptor type B<sup>22</sup> and  $AT_1$ -Mas receptor.<sup>23</sup> These dimerizations of Ang II receptors could possibly indicate unknown effects of Ang II receptors' functional aspects in CV biology.

The ligand-independent activation of GPCRs has been highlighted, especially in the potential new discovery of drug targets.<sup>24</sup> Mechanical stress activates the  $AT_1$  receptor independently of Ang II.<sup>25</sup> This activation can be inhibited by an inverse agonist of the  $AT_1$  receptor; therefore, ARBs can be classified into competitive antagonists and inverse agonists.<sup>26</sup> Moreover, agonistic antibodies against the second extracellular  $AT_1$  receptor loop have been shown in women with preeclampsia<sup>27</sup> and in renal transplant recipients during an episode of rejection.<sup>28,29</sup>

Moreover, another recent topic is new players in RAS (Figure 1). The counter-regulatory axis of the RAS, such as ACE2, Ang-(1–7) and its receptor, Mas, is potentially important for promoting vasoprotective effects. The balance between the ACE–Ang II– $AT_1$  receptor and the ACE2–Ang-(1–7)–Mas axis is considered to play an important role in organ damage, related directly to hypertension and associated diseases; therefore, the regulation of ACE2 offers a novel target for CVD therapeutics.<sup>30</sup> On the other hand, brain Ang III, which is converted *in vivo* from Ang II by aminopeptidase A, controls vasopressin release and increases blood pressure. Although the true effector of Ang III is totally unknown, Ang III could constitute a putative central therapeutic target for the treatment of hypertension.<sup>31</sup> Ang (1–12), an intermediate precursor derived directly from angiotensinogen,

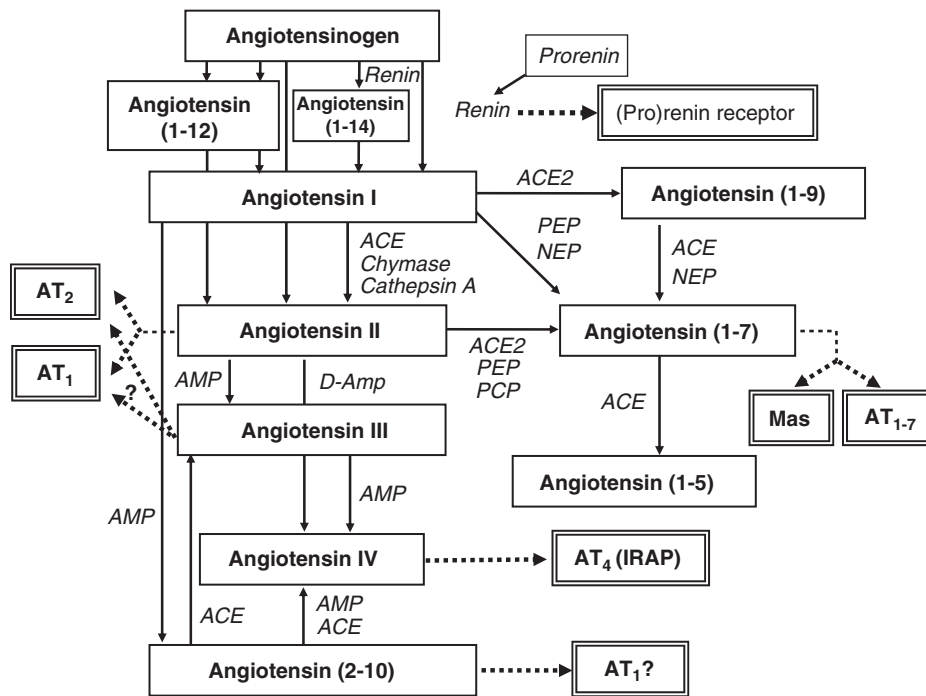
was identified recently by Nagata *et al.*<sup>32</sup> Although Ang (1–12) may be an alternate precursor substrate for the formation of bioactive angiotensin peptides, its detailed function is an enigma. Furthermore, cross-talk of Ang II and aldosterone has also received attention. We earlier reported the interaction between Ang II and aldosterone on vascular smooth muscle cell proliferation, and the interaction between Ang II and Aldo on vascular smooth muscle cell proliferation<sup>33</sup> and senescence.<sup>34</sup> These results provide evidence that blockade of both Ang II and aldosterone could be of therapeutic benefit for vascular disorders.

Although the correlation between the emerging concept of RAS and pathophysiological conditions has not been elucidated in detail, further investigation of the functional regulation of Ang II receptor-interacting proteins, receptor modification and new players in RAS could be useful for new drug discovery for ameliorating the enhanced tissue RAS.

## TISSUE RENIN-ANGIOTENSIN SYSTEM

### Vascular remodeling, atherosclerosis and senescence

Ang II is generated by ACE secreted from endothelial cells, and constricts blood vessels through  $AT_1$  receptors that are expressed in them.<sup>35,36</sup> In blood vessels, RAS induces pathophysiological disorders after an increase in oxidative stress, mediated mainly by NAD(P)H oxidase.<sup>37–39</sup> In endothelial cells, the NAD(P)H oxidase induces production of superoxide ( $\cdot O_2^-$ ), which oxidizes tetrahydrobiopterin ( $BH_4$ ), a co-factor of the enzyme endothelial nitric oxide (NO) synthase. In the presence of a high  $BH_4$  concentration, endothelial NO synthase is dimerized and produces NO. Reduced availability of  $BH_4$  causes uncoupling of endothelial NO synthase, leading to production of  $\cdot O_2^-$ , reduced availability of NO and generation of peroxynitrate ( $\cdot ONOO^-$ ) as a result of the action of  $\cdot O_2^-$  on NO.<sup>40</sup> Production of increased reactive oxygen species also leads to the



**Figure 1** Emerging concept of the renin–angiotensin system. Metabolism of angiotensinogen and major players in renin–angiotensin system. ACE, angiotensin converting enzyme; ACE2, angiotensin converting enzyme-2; AMP, aminopeptidase; D-Amp, dipeptidyl-aminopeptidase; IRAP, insulin-regulated aminopeptidase; NEP, neutral-endopeptidase; PCP, prolyl-carboxypeptidase; PEP, prolyl-endopeptidase.

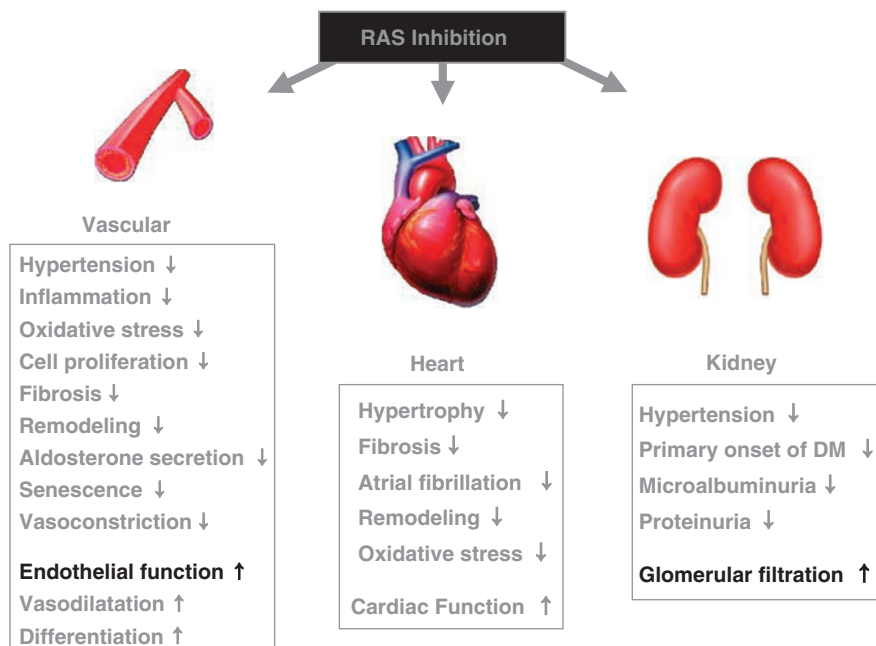
activation of redox-sensitive pro-inflammatory transcription factors, such as NF (nuclear factor)- $\kappa$ B and Ets-1, which trigger and/or potentiate the inflammatory cascade.<sup>41–43</sup> Reactive oxygen also promotes the expression of VCAM-1 (vascular cell adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1) in endothelial cells, and that of VCAM-1, MCP-1 (monocyte chemoattractant protein-1) and IL-6 (interleukin-1) in smooth muscle cells.<sup>44,45</sup> Therefore, AT<sub>1</sub>-receptor-induced oxidative stress may cause NO inactivation, lipid oxidation and activation of redox-sensitive genes, such as chemotaxis and adhesion molecules, pro-inflammatory cytokines and matrix metalloproteinases, all of which are involved in the initiation and progression of endothelial dysfunction and which manifest atherosclerosis. Although apolipoprotein E-deficient mice with a high-cholesterol diet show marked arteriosclerosis, administration of an ACEI or ARB to these mice suppresses the development of arteriosclerosis.<sup>46,47</sup> Moreover, activation of RAS is also related to the formation of thrombus, because Ang II activates plasminogen activator inhibitor type 1 (PAI-1) in endothelial cells.<sup>48</sup> Thus, when the vascular RAS is activated by hypertension, not only the development of coronary artery plaque but also the formation of thrombus is accelerated, and this is considered to be involved in the destabilization of arteriosclerotic plaque. Therefore, the HOPE (Heart Outcomes Prevention Evaluation) study proved clinically that RAS inhibition not only decreases blood pressure but is also vasoprotective<sup>49</sup> (Figure 2).

On the other hand, vascular senescence mediated by AT<sub>1</sub> receptor stimulation has been highlighted recently,<sup>50</sup> and ARBs have been shown to prevent vascular disorders associated with aging.<sup>51,52</sup> Recently, it was reported that the senescence of smooth muscle cells was increased by persistent Ang II stimulation.<sup>34</sup> This senescence was suppressed by an ARB. A similar result was also obtained by the administration of aldosterone. Although senescence was not seen in smooth muscle cells treated with low-concentration Ang II or aldosterone added individually, senescence was induced by adding low-concentration Ang II and aldosterone simultaneously. This result suggests that Ang II and aldosterone have an interaction in vascular smooth muscle cells.

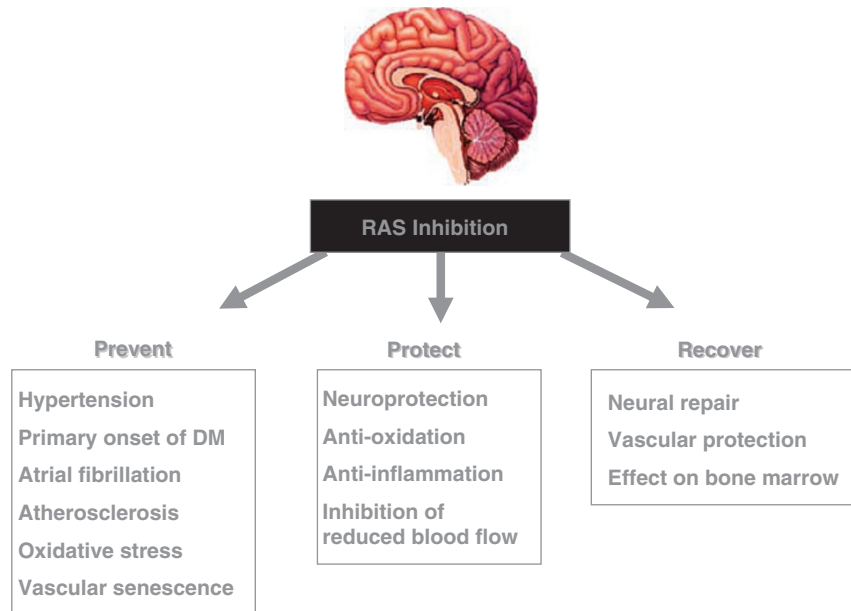
## Heart

Although diuretics and cardiotoxic agents were used mainly for the medical treatment of heart failure till 1980, they were not associated with a marked improvement in prognosis.<sup>53–58</sup> Entering the 1980s, research on neurohumoral factors, such as the sympathetic nerve RAS progressed, and the beneficial effects of ACEIs in heart failure were proved by large clinical trials, such as the CONSENSUS (Cooperative North Scandinavian Enalapril Survival Study)<sup>59</sup> and the SOLVD (Studies of Left Ventricular Dysfunction),<sup>60</sup> and they were used as the first-line drug. In the Valsartan Heart Failure Trial (Val-HeFT),<sup>61</sup> together with the standard treatment, an ARB was proved to reduce CV events in heart failure patients. Moreover, the CHARM (Candesartan in Heart Failure Assessment of Reduction in Mortality and Morbidity) study also showed that the ARB candesartan was generally well tolerated and significantly reduced CV deaths and hospital admissions for heart failure.<sup>62</sup>

Ang II receptors are expressed in the human heart,<sup>63</sup> and their expression is changed in the failing heart. Haywood *et al.*<sup>64</sup> showed that AT<sub>1</sub> receptor expression decreased in the failing human ventricle, whereas AT<sub>2</sub> receptor expression was unaffected. Moreover, especially in human primary pulmonary hypertension hearts, the AT<sub>1</sub> receptor was downregulated only in the failing right ventricle,<sup>65</sup> suggesting that Ang II receptors may be regulated differentially in the failing heart. Basic research has also shown the role of RAS in the heart. The mice have two AT<sub>1</sub> receptor subtypes: AT<sub>1a</sub> and AT<sub>1b</sub>. Ang II acts mainly through AT<sub>1a</sub> receptors. In AT<sub>1a</sub> receptor-deficient mice with myocardial infarction, cardiac muscle remodeling was less marked and the survival rate was higher compared with those in wild-type mice.<sup>66</sup> In contrast, overexpression of the AT<sub>1</sub> receptor in cardiomyocytes induced significant cardiac hypertrophy and remodeling, with increased expressions of ventricular atrial natriuretic factor and interstitial collagen deposition, and the mice died prematurely of heart failure<sup>67</sup> showing upregulation of gene expression, such as c-Jun N-terminal kinase and c-fos.<sup>68</sup> Upregulation of c-fos in cardiomyocytes by Ang II administration activates protein kinase C and



**Figure 2** Inhibition of the renin–angiotensin system in heart, kidney and vasculature. DM, diabetes.



**Figure 3** Inhibition of the renin–angiotensin system in brain. DM, diabetes.

extracellular signal-regulated kinase, and results in cardiac hypertrophy.<sup>69,70</sup> Moreover, cardiac myocytes have the ability to sense mechanical stretch and convert it into intracellular growth signals, resulting in cardiac hypertrophy. Mechanical stretch stimulates the rapid secretion of Ang II from neonatal rat cardiac myocytes.<sup>71</sup> Furthermore, recently, mechanical stretch was reported to activate the AT<sub>1</sub> receptor independently of Ang II.<sup>25</sup> This activation can be inhibited by some ARBs, which are inverse agonists of the AT<sub>1</sub> receptor, indicating a therapeutic effect of RAS inhibition through multiple mechanisms dependent and independent of the AT<sub>1</sub> receptor (Figure 2).

Aldosterone has recently been implicated as playing a major role in the progression of heart failure. Major clinical trials designed to analyze clinical outcomes of heart failure using an aldosterone antagonist, such as spironolactone or eplerenone, have been conducted. The first, the RALES (Randomized Aldactone Evaluation Study), showed that an aldosterone antagonist, spironolactone, significantly reduced mortality in symptomatic chronic advanced heart failure patients compared with that in placebo.<sup>72</sup> The second was the EPHEUS (Eplerenone Post myocardial infarction Heart failure Efficacy and SURvival Study), which showed a significant reduction in mortality and hospitalization in post-myocardial infarction patients with heart failure by a selective mineralocorticoid receptor blocker, eplerenone.<sup>73</sup> These trials showed a beneficial effect of an aldosterone antagonist in chronic advanced heart failure patients, as well as post-myocardial infarction heart failure patients with reduced ejection fraction.

### Kidney

The RAS has been developed for maintaining the sodium and electrolyte balance so that land animals can maintain body fluid. In recent times, however, the excess intake of sodium chloride has contributed to the development of hypertension, the metabolic syndrome and other lifestyle-related diseases.

All components of RAS are expressed in the kidney, and it appears that most renal AT<sub>1</sub> receptors are exposed to locally generated Ang II rather than to Ang II from circulation.<sup>74</sup> Ang II acts on the renal tubules, promotes the reabsorption of sodium, and has an effect that

increases blood pressure. Gene-modification mice, which are double transgenic with human angiotensinogen and human renin in the proximal tubule, have significantly increased blood pressure, suggesting that activation of RAS in the kidney influences blood pressure. Moreover, as Ang II has a greater constrictive effect on the efferent arteriole than the afferent arteriole through AT<sub>1</sub> receptor stimulation, excess pressure in the glomerular endothelial cells and the mesangial cells leads to nephropathy and renal dysfunction. Therefore, RAS inhibition increases renal blood flow beyond antihypertensive effects. Other beneficial roles of RAS inhibition in the kidney have been reported (Figure 2). For example, administration of an ARB in a diabetic nephropathy rat model prevents the development of nephropathy.<sup>75</sup> Also, RAS inhibition prevents renal fibrosis.<sup>76–78</sup> In clinical trials, the risk of onset of end-stage renal failure was inhibited by ARB treatment in the RENAL (Reduction of Endpoints in non-insulin-dependent diabetes mellitus (NIDDM) with the Angiotensin II Antagonist Losartan) and the IDNT (Irbesartan Diabetic Nephropathy Trial) studies.<sup>79,80</sup> Moreover, IRMA2 (Irbesartan in patients with type II diabetes and Micro-Albuminuria Study) showed that the ARB irbesartan exerts renal protection without a decrease in blood pressure.<sup>81</sup>

The renal protective effects of aldosterone blockade independent of Ang II blockade have been reported in animal models, and an additional effect of aldosterone blockade with Ang II inhibition has also been shown in clinical studies.<sup>82</sup> For example, blockade of aldosterone, independent of RAS blockade, reduces proteinuria and nephrosclerosis in the SHRSP (spontaneously hypertensive, stroke-prone) rat.<sup>83</sup> Clinically, co-administration of eplerenone with an ACEI, enalapril, significantly reduced albuminuria in patients with diabetes, compared with enalapril treatment alone.<sup>84</sup> Moreover, the plasma concentration of aldosterone is reported to begin to rise after long-term RAS blockade in some patients.<sup>85</sup> These ‘aldosterone breakthrough’ effects reflect incomplete blockade of RAS, and may promote renal injury even in patients with RAS blockade.

### Brain

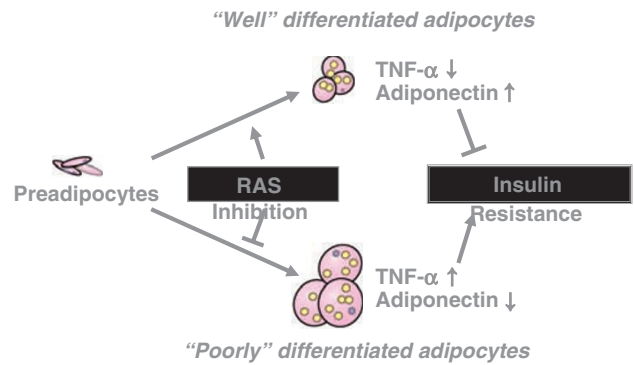
Recently, there has been a focus on the local RAS in the brain.<sup>86</sup> Although it is thought that Ang II does not cross the blood–brain

barrier, it is reported that all components of RAS exist in the central nervous system, suggesting that Ang II is produced and functions in the central nervous system (Figure 3).

Recent large clinical trials, such as the LIFE (Losartan Intervention For Endpoint) reduction in hypertension<sup>87</sup> and the MOSES (MOrbidity and mortality after Stroke, Eprosartan compared with nitrendipine for Secondary prevention)<sup>88</sup> studies, indicated that the blockade of RAS is effective in preventing a first or recurrent stroke beyond its blood pressure-lowering effects. The ACCESS (Acute Candesartan Cilexetil Therapy in Stroke Survivors) study showed lower mortality at 12 months after stroke in the candesartan-treated group compared with that in the placebo group even with similar blood pressure in the 7 days after stroke in the two groups, suggesting that RAS inhibition in the acute phase of stroke leads to brain protection without a hypotensive effect.<sup>89</sup> Moreover, the MOSES study showed that the primary end point, a composite of total mortality and all CV and cerebrovascular events, was significantly lower for treatment with an ARB, eprosartan without a change of blood pressure.<sup>88</sup> An experimental brain injury model with middle cerebral artery occlusion, using genetically modified mice, revealed that AT<sub>1</sub> receptor signaling enhances brain damage partly because of an increase in oxidative stress in the ischemic brain and a decrease in cerebral blood flow in the penumbral region of the middle cerebral artery territory. In contrast, the activation of the AT<sub>2</sub> receptor-attenuated brain injury,<sup>90</sup> with counter-regulatory effects on the AT<sub>1</sub> receptor and the enhancement of neural differentiation and the repair of damaged DNA by the induction of a neural differentiating factor, MMS2, which is one of the ubiquitin conjugating enzyme variants.<sup>86</sup> Recent studies have also shown the possibility that stimulation of AT<sub>2</sub> receptors may promote cell differentiation and regeneration in neuronal tissue.<sup>4,91</sup> Li *et al.*<sup>92</sup> reported that AT<sub>2</sub> receptor stimulation supported neuronal survival and neurite outgrowth in response to ischemia-induced neuronal injury. Moreover, Gallo-Payet *et al.*<sup>93</sup> showed that Ang II induces neural differentiation and neurite outgrowth through mitogen-activated protein kinase or produces NO<sup>94</sup> through AT<sub>2</sub> receptor activation and is involved in brain development.<sup>95</sup> This accumulating evidence indicates that AT<sub>2</sub> receptor signaling acts as a crucial cerebroprotective factor after stroke.

Moreover, we also showed that a non-hypotensive dose of a mineralocorticoid receptor antagonist, eplerenone, reduced the stroke size after middle cerebral artery occlusion in mice.<sup>96</sup> These inhibitory effects of eplerenone on stroke size in the brain were at least partly owing to an improvement in the early phase of cerebral blood flow in the peripheral region of the ischemic area and to the prevention of superoxide production in the injured brain. Furthermore, spironolactone improves the structure and increases the tone in the cerebral vasculature of male SHRSP,<sup>97</sup> indicating that long-term administration of spironolactone is effective in the prevention of stroke onset. These results indicate that aldosterone is involved in stroke onset and in the expansion of brain damage after ischemic stroke.

Although an improvement of the cognitive function by RAS inhibition has not been confirmed clinically, RAS inhibition is expected to prevent a cognitive decline in Alzheimer's disease<sup>98</sup> and in the metabolic syndrome,<sup>99</sup> based on animal studies. Recently, it has been proven that RAS inhibition by an ARB is expected to prevent the onset of Alzheimer's disease. An ARB, valsartan, was able to attenuate oligomerization of amyloid  $\beta$  peptides into high-molecular-weight oligomeric peptides.<sup>100</sup> Moreover, treatment with valsartan also attenuated the development of amyloid  $\beta$ -mediated cognitive impairment in Tg2576 mice, an Alzheimer's disease mouse model. On the other hand, ACE is concerned with the decomposition of amyloid  $\beta$ .<sup>101</sup> In a



**Figure 4** Inhibition of the renin-angiotensin system in adipose tissue differentiation.

clinical investigation of the relationship between antihypertensive medication use and the onset of Alzheimer's disease,<sup>102</sup> the onset of Alzheimer's disease was attenuated by a diuretic agent or calcium channel blocker; however, an ACEI failed to prevent Alzheimer's disease, indicating the involvement of ACE in amyloid  $\beta$  deposition. However, amyloid  $\beta$  level in the brain was not changed in ACE-deficient mice, suggesting that further investigations are required to determine whether ACE is actually involved in the decomposition of amyloid  $\beta$ .<sup>103</sup>

Moreover, potassium-sparing diuretics, which include spironolactone and eplerenone, are associated with the reduced incidence of Alzheimer's disease according to the examination of the relationship of antihypertensive medication with Alzheimer's disease onset in the elderly population aged 65 years and older in Cache County, Utah, USA.<sup>102</sup> Although further investigation is needed, these results suggest that aldosterone may affect the incidence of Alzheimer's disease.

### Metabolic syndrome

The VALUE (Valsartan Antihypertensive Long-term Use Evaluation) study<sup>104</sup> and other large clinical studies showed that a new onset of diabetes is suppressed by administration of an ARB compared with other antihypertensive agents, such as calcium channel blockers and  $\beta$ -blockers. Thus, local actions of RAS in adipose tissue,<sup>105</sup> skeletal muscle and the pancreas have also been highlighted recently. Obesity is one of the major risks for the metabolic syndrome with hypertension and glucose intolerance. However, there have been inconsistent reports on the role of the adipose tissue RAS. There are at least two differentiation steps in adipogenesis involving RAS; from mesenchymal stem cell (MSC) to adipocytes and from preadipocytes to adipocytes. The inhibitory effects of Ang II seem to differ between MSC and preadipocyte differentiation. Janke *et al.*<sup>106</sup> reported that Ang II inhibits differentiation of human adipocyte progenitor cells, and inhibiting this function by an ARB, thereby results in large insulin-resistant adipocytes with an increased storage of lipid.<sup>105</sup> In contrast, blockade of RAS promotes the recruitment of preadipocytes, thereby increasing the number of small insulin-sensitive adipocytes. This hypothesis is supported by the observation by Shimamoto *et al.*<sup>107</sup> that an ARB, olmesartan, significantly reduced adipocyte size in fructose-fed rats, with an improvement in glucose intolerance. In association with preadipocyte differentiation, an ARB increased well-differentiated adipocytes, which can secrete inflammatory adipocytokines, such as TNF- $\alpha$ , and more beneficial adipocytokines, such as adiponectin. Therefore, the effect of an ARB on preadipocyte differentiation is to increase 'well-differentiated adipocytes' rather than

'poorly differentiated adipocytes'. On the other hand, in MSC differentiation, relative stimulation of AT<sub>2</sub> receptors by an ARB increased less-differentiated adipocytes, such as progenitors with adipocyte characteristics, which secrete less adipocytokines and may have transdifferentiation potential.<sup>108</sup> Thus, ARBs have two different possible beneficial effects on adipocyte differentiation, resulting in an improvement in the metabolic syndrome (Figure 4).

Moreover, we earlier reported that blockade of RAS by an ARB, valsartan, increased insulin uptake into skeletal muscle and attenuated the increase in plasma glucose concentration.<sup>109</sup> Insulin-induced phosphorylation of insulin receptor substrate-1 (IRS-1), the association of IRS-1 with the p85 regulatory subunit of phosphoinositide 3 kinase (PI 3-K), PI 3-K activity and the translocation of glucose transporter type 4 (GLUT4) to the plasma membrane are exaggerated by valsartan treatment. Furthermore, Ang II receptors are expressed in the pancreatic tissue.<sup>110</sup> An increase in Ang II suppressed the secretion of insulin dose-dependently in hyperglycemic mice.<sup>111</sup> Moreover, administration of an ACEI or ARB attenuated inflammation and fibrosis in the pancreas.<sup>112,113</sup> These results indicate that RAS inhibition prevents the onset of diabetes and the metabolic syndrome.

#### Other tissues

Fibroproliferative diseases, including liver cirrhosis, pulmonary fibrosis, aortic aneurysm and macular degeneration, are also target-organ diseases and are the leading causes of morbidity and mortality. CV disease and progressive kidney disease also involve these pathological disorders. Fibrosis is characterized by the accumulation of matrix molecules, such as collagen and fibronectin, owing to overexpression and decreased clearance and degradation of these matrix components. Epithelial–mesenchymal transition, a process through which an epithelial cell changes its phenotype to become more like a mesenchymal cell, activates local tissue fibroblasts. Ang II induces an epithelial–mesenchymal transition through increased expression of vimentin and  $\alpha$ -smooth muscle actin and downregulation of E-cadherin, followed by phosphorylation of Smad2/3 by TGF- $\beta$  (transforming growth factor- $\beta$ ) activation.<sup>114</sup> Recently, an association of fibroproliferative diseases with upregulation of TGF- $\beta$  by Ang II has been reported.

Hepatocytes are the main source of angiotensinogen.<sup>115,116</sup> Recently, the hepatic RAS has been reported to be related to the pathogenesis of chronic liver disease. Hepatic RAS is upregulated in chronic liver injury, and contributes to oxidative stress,<sup>117</sup> recruitment of inflammatory cells and the development of fibrosis. In AT<sub>1a</sub>-deficient mice, inflammation and fibrosis were attenuated after treatment with either CCl<sub>4</sub> or bile duct ligation.<sup>118,119</sup> Although large randomized clinical trials have not been conducted to date, on the basis of the results of clinical<sup>120</sup> and experimental studies *in vivo*<sup>121,122</sup> and *in vitro*,<sup>123</sup> RAS inhibition has been expected to become a potential new therapeutic strategy against the progression of chronic liver disease, for preventing liver fibrosis.

An elevated ACE concentration in bronchoalveolar lavage fluid and/or serum has been reported in many potentially fibrotic lung diseases, including sarcoidosis,<sup>124</sup> idiopathic pulmonary fibrosis<sup>125</sup> and in the acute respiratory distress syndrome.<sup>126</sup> Ang II has been identified as a proapoptotic and profibrotic factor in experimental lung fibrosis models, and patients with the insertion/deletion (ID)/deletion/deletion (DD) polymorphism of ACE, which confers higher levels of ACE, are predisposed to pulmonary fibrosis.<sup>127</sup> ACEI and ARB prevented experimental lung fibrosis through inhibition of the angiotensin/TGF- $\beta$  'autocrine loop',<sup>128</sup> indicating that RAS inhibition may prevent the fibrotic change in the lung.

On the other hand, excessive TGF- $\beta$  signaling enlarges the aortic root and results in aortic aneurysm. Treatment with an ARB attenuated aortic root dilatation in a mouse model<sup>129</sup> and in patients with Marfan's syndrome.<sup>130</sup> Marfan's syndrome is a disorder of connective tissue resulting from mutations in the gene for fibrillin-1, which leads to skeletal muscle weakness and CV abnormalities. A similar group also reported that treatment with the ARB losartan normalized muscle architecture observed in fibrillin-1 deficient mice, a mouse model of Marfan's syndrome.<sup>131</sup> Interestingly, losartan treatment improved the muscle function in a dystrophin-deficient mouse model of Duchenne's muscular dystrophy.<sup>131</sup>

Further clinical investigation should be carried out to prove the actual beneficial effect of RAS inhibition in such fibroproliferative diseases in the future.

#### CONCLUSIONS

Recent clinical studies indicate multiple preventive effects of the blockade of RAS on target organ damage. As the TROPHY (Trial Of Preventing Hypertension) showed recently that the treatment of prehypertension with an ARB, candesartan, reduced the incidence of hypertension even after the cessation of ARB administration,<sup>132</sup> RAS inhibition may have to be initiated as soon as possible for resetting RAS. Moreover, in some patients, long-term treatment with an ACEI or ARB induces an increase in serum aldosterone level, which is called 'aldosterone breakthrough'.<sup>133</sup> Therefore, in the next stage, further investigation is necessary to determine when to start and what doses of ACEI and ARB and other RAS inhibition agents to use through a comparison of the long-term therapeutic effects of RAS inhibition with single and combined agents for the optimal prevention of target organ damage.

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