Review

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Renin-angiotensin system revisited

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New components and functions of the renin-angiotensin system (RAS) are still being unravelled. The classical RAS as it looked in the middle 1970s consisted of circulating renin, acting on angiotensinogen to produce angiotensin I, which in turn was converted into angiotensin II (Ang II) by angiotensin-converting enzyme (ACE). Ang II, still considered the main effector of RAS was believed to act only as a circulating hormone via angiotensin receptors, AT1 and AT2. Since then, an expanded view of RAS has gradually emerged. Local tissue RAS systems have been identified in most organs. Recently, evidence for an intracellular RAS has been reported. The new expanded view of RAS therefore covers both endocrine, paracrine and intracrine functions. Other peptides of RAS have been shown to have biological actions; angiotensin 2–8 heptapeptide (Ang III) has actions similar to those of Ang II. Further, the angiotensin 3–8 hexapeptide (Ang IV) exerts its actions via insulin-regulated amino peptidase receptors. Finally, angiotensin 1–7 (Ang 1–7) acts via mas receptors. The discovery of another ACE2 was an important complement to this picture. The recent discovery of renin receptors has made our view of RAS unexpectedly complex and multilayered. The importance of RAS in cardiovascular disease has been demonstrated by the clinical benefits of ACE inhibitors and AT1 receptor blockers. Great expectations are now generated by the introduction of renin inhibitors. Indeed, RAS regulates much more and diverse physiological functions than previously believed.

Keywords: angiotensin, angiotensin-converting enzyme, angiotensin receptor, renin.

Discovery of renin

The classical paper on the discovery of renin by the Finnish physiologist Robert Tigerstedt and his Swedish student Per Bergman in 1898 [1] was based on experiments performed 1896–97 at the Karolinska Institute. Saline extracts of rabbit kidney were shown to slowly raise the blood pressure (BP) when injected into rabbits. The principle causing this was present in kidney cortex but not in medulla and was destroyed by heating. The authors concluded that the substance was a protein and they named it renin. They speculated that '*renin might in some direct or indirect way be associated with hypertrophy of the heart found in renal disease and high BP'*. However, these early results could not be repeated in other laboratories, and it was not until late 1930s when renin was 'rediscovered'.

Circulating 'classical RAS'

An immense amount of early research on the renin-angiotensin system (RAS) paved the way for improved understanding of its physiology and pathophysiology. In the early 1970s, the major components of 'classical' circulating RAS (Fig. 1) were identified and there was compelling evidence to indicate important roles for RAS in the regulation of fluid balance and BP. At that time, however, there was widespread skepticism

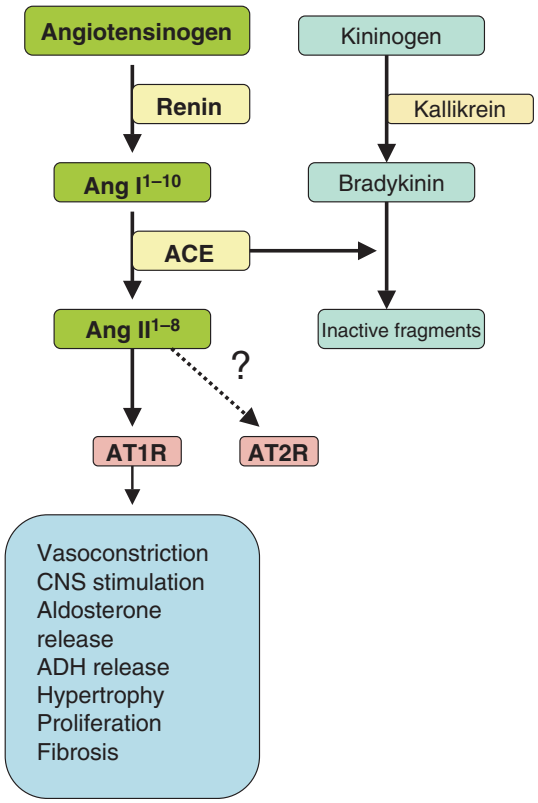

Fig. 1 A simplified view of the 'classic' circulating renin-angiotensin system. Whole arrows (⟶) indicate pathways; the clinical significance of which has been demonstrated. Dashed arrow (⇢) indicates pathway deduced from animal or cell culture experiments, not yet conclusively shown to be clinically relevant.

regarding the role of RAS in cardiovascular disease. It was not until the discovery of orally effective angiotensin-converting enzyme (ACE) inhibitors, the first of which was captopril [2], that the paramount importance of RAS in cardiovascular homeostasis and disease was being appreciated. The introduction of losartan, the first orally active and effective angiotensin receptor type 1 blocker [3] further strengthened this concept.

New expanded view of RAS

The relatively simple 'classical' concept of the 'circulating RAS', (Fig. 1) with angiotensinogen (AGT) generated by the liver, renin by the kidneys and the main effector peptide, angiotensin II (Ang II) generated by ACE in the vasculature was complete with the cloning of the AT1 and AT2 receptors [3]. However, the physiological implications of the RAS have continued to expand (Fig. 2) and we have not seen the entire picture yet. It has gradually become evident that in addition to the 'circulating RAS,' there is a local 'tissue RAS' in most organs and tissues studied. In fact, even intracellular generation of Ang II has been reported [4]. This makes RAS not only an *endocrine*, but also a *paracrine* and an *intracrine* system. Moreover, both the heptaptide angiotensin 2–8 (Ang III) and the hexapeptide 3–8 (Ang IV) have been shown to be biologically active. The angiotensin 1–7 heptapeptide (Ang 1–7) appears to play an important role by counterbalancing many actions of Ang II. Angiotensin II and Ang III actions are mediated by AT1 and AT2 receptors only. Recent discoveries have revealed specific functional receptors for Ang IV, Ang 1–7, and perhaps most surprisingly, even for renin/prorenin. Therefore, our present expanded view of RAS (Fig. 2) is quite complex and multilayered.

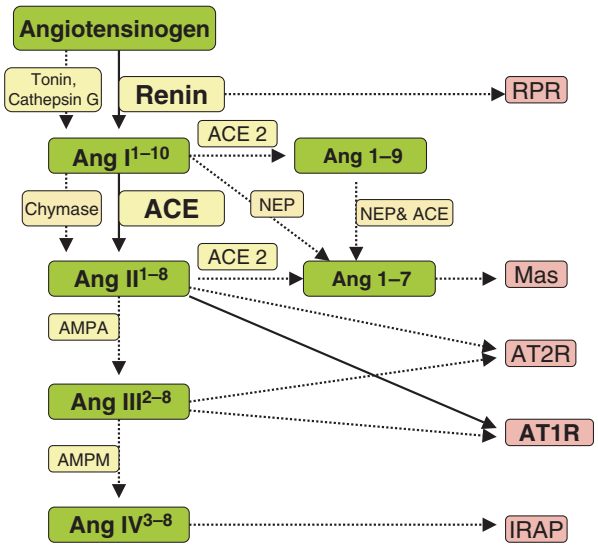

Fig. 2 The present view of the expanded renin-angiotensin system. RPR, renin/prorenin receptor; Mas, mas oncogene, receptor for Ang 1–7; AT2R, angiotensin type 2 receptor; AT1R, angiotensin type 1 receptor, IRAP, insulin-regulated aminopeptidase; Ang IV receptor AMPA, aminopeptidase A; AMPM, aminopeptidase M; ACE, angiotensin-converting enzyme; ACE2, angiotensin-converting enzyme 2; NEP, neutral endopeptidase.

Established roles of Ang II

Angiotensin II exerts its actions via AT1 and AT2 receptors which in principle, but not invariably, mediate opposite functions. AT1 receptors mediate actions with potentially harmful consequences, if not properly counterbalanced. AT2 receptors are thought to mediate protective actions, the clinical relevance of which has not yet been clearly established.

Angiotensin II is a major regulator of fluid and sodium balance and haemodynamics, but also of cellular growth and cardiovascular remodelling. Thus, AT1 receptors mediate vasoconstriction, thirst and release of vasopressin and aldosterone, fibrosis, cellular growth and migration. More recently, Ang II has been shown to cause generation of oxidative radicals via AT1 receptors and to be involved in inflammatory processes including atherosclerosis and vascular ageing. AT2 receptors mediate vasodilation, release of nitric oxide (NO) and usually inhibition of growth.

Novel functions mediated by AT1 receptors

Angiotensin II infusion caused decrease of plasma adiponectin, an insulin sensitizer, apparently via AT1 receptors in the rat [5]. Suppression of adiponectin may represent a mechanism whereby Ang II causes impaired glucose tolerance. Other metabolic actions of Ang II include pro-inflammatory modulation [6], increased insulin secretion [7], β-cell apoptosis [8], reduction of gluconeogenesis and hepatic glucose output [9] and increased plasma triglycerides [10]. We decided not to enter the complex field of intracellular AT1 and AT2 receptor signalling and therefore refer the reader to recent reviews [11, 12].

Novel functions mediated by AT2 receptors

Angiotensin receptor type 2 is generally reported to mediate effects opposing and counterbalancing those mediated by AT1 receptors (Fig. 1), e.g. vasodilatation, NO release and inhibition of proliferation and growth. However, AT2 receptors may also mediate neurotrophic effects in the central nervous system [13]. Moreover, upregulated AT2 receptors in the peri-ischaemic brain may exert protection against ischaemic damage [14]. The authors speculate that such a protective effect mediated by AT2 receptors might in part explain superior protection against stroke in patients treated with losartan vs. treatment with atenolol in the LIFE study [15]. Another explanation may be that losartan lowers central BP more effectively than atenolol [16].

In rat kidney, Ang III but not Ang II was recently reported to induce natriuresis mediated by AT2 receptors [17]. This natriuresis was augmented by blockade of aminopeptidase N, an enzyme metabolizing Ang III to Ang IV (Fig. 2) [18]. The authors speculated that blockers of aminopeptidase N might be developed to treat diseases characterized by sodium and fluid retention such as hypertension and heart failure. In theory, such inhibitors may also exert beneficial actions via reduced tissue levels of Ang IV (see below). Interestingly, renal interstitial fluid has been shown to contain roughly 1000-fold higher concentrations of Ang II and Ang III than found in the plasma [19].

Effects of unopposed stimulation of AT2 receptors are slightly controversial [20]. Thus, beneficial effects include bradykinin-NO vasodilatory effects, natriuretic and antifibrotic effects. Potentially detrimental effects are apoptosis, nuclear factor-kappa B (NF-κB) signal transduction and induction of chemokines. Despite a plethora of promising experimental results strongly suggesting beneficial actions of AT2 stimulation [20] the final clinical proof is lacking. Though treatment with AT1 receptor blockers (ARBs) substantially increase plasma Ang II levels and presumably cause increased stimulation of AT2 receptors, there is no conclusive evidence to prove the clinical relevance of increased AT2 receptor activity.

Alternative pathways of Ang II generation

Angiotensin II may be enzymatically generated from Ang I by chymase (Fig. 2) in certain pathological conditions. Chymase is stored in macromolecular complex with heparin proteoglycan in secretory granules of mast cells [21]. To become enzymatically active, complexed chymase must be released from

mast cell granules, e.g. by vascular damage caused by ballooning or other damage. Therefore, chymase is enzymatically inactive in normal vascular tissue and may produce Ang II only in damaged or atherosclerotic arterial walls. It is of note that endogenous serine protease inhibitors present in interstitial fluid [22] are potent inhibitors of chymase.

Chymase inhibitors reportedly prevent neointimal lesions following vein grafting or arterial ballooning in dogs, whereas ACE inhibitors are ineffective [23]. However, the effects of chymase inhibitors may depend on other effects of these compounds such as decrease in transforming growth factor-β (TGF-β) generation and stabilization of mast cell granules and not on decreased Ang II formation. In addition, ARBs which block Ang II actions irrespective of generating enzyme(s) have not proven superior to ACE inhibitors in large clinical trials [24, 25]. Though animal experimental results with chymase inhibitors are promising [23], the possible importance of Ang II generation by chymase is unclear and chymase inhibitors that are safe and useful for human trials have not yet been developed.

Angiotensin 2–8 heptapeptide

Angiotensin III has been known since the 1970s to cause vasoconstriction and release of aldosterone. It is generated from Ang II by aminopeptidase A (Fig. 2). Ang III exerts its actions, in principle similar to those of Ang II, via AT1 and AT2 receptors. While Ang II is considered the main effector of RAS, Ang III may be equally or even more important in some actions mediated by AT1 receptors, e.g. release of vasopressin [26].

Systemic infusion of Ang II or Ang III to conscious dogs was recently shown to result in equipotent effects at the same plasma concentration on BP, aldosterone secretion, sodium excretion and plasma renin activity; all effects inhibited by candesartan. However, the metabolic clearance rate of Ang III was five times that of Ang II [27]. This study indicated that Ang II plays a dominant role as an effector of the 'classical circulating RAS'.

Angiotensin 3–8 hexapeptide

Angiotensin IV may be generated from Ang III by aminopeptidase M (Fig. 2). This biologically active peptide has caught increasing interest following the discovery and cloning of insulin-regulated amino peptidase receptors (IRAP), [28, 29], a binding site and a probable receptor (AT4) of Ang IV. Actions of Ang IV mediated by IRAP (Fig. 3) include renal vasodilation, hypertrophy and activation of NF-κB leading to increased expression of platelet activator inhibitor-I (PAI-1), monocyte chemoattractant protein (MCP-1) interleukin-6 and tumour necrosis factor-α [30, 31]. Several studies suggest that Ang IV has

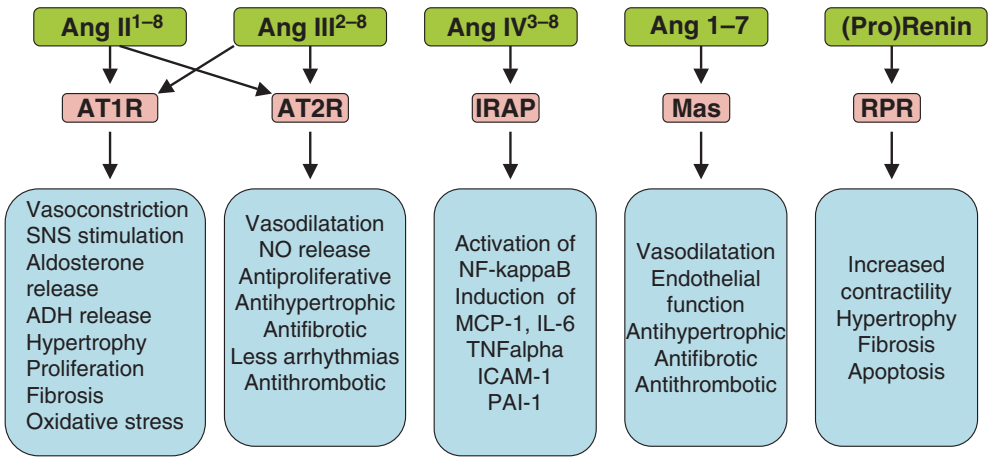

Fig. 3 Effects of angiotensin peptides and renin/prorenin mediated by their their corresponding receptors.

important regulatory functions in cognition, renal metabolism and cardiovascular damage [32, 33]. Ang IV regulates cell growth in cardiac fibroblasts, endothelial cells and vascular smooth muscle cells. It appears that Ang IV is involved in the vascular inflammatory response and could therefore play a role in cardiovascular pathophysiology.

Angiotensin 1–7 heptapeptide

Angiotensin 1–7 heptapeptide was thought for a long time to be devoid of biological actions, in spite of early reports on biological effects [34]. The importance of Ang 1–7 was emphasized by the relatively recent discovery of a 'new' ACE2. This enzyme generates Ang 1–7 from Ang II. Ang 1–7 may also be generated from Ang I or Ang II by other peptidases. Already back in 1988, Ang (1–7) was shown to release vasopressin as effectively as Ang II from neurohypophyseal explants [34]. Ang (1–7) was found to have actions opposing those of Ang II (Fig. 3), namely vasodilation and antitrophic effects and amplification of vasodilation caused by bradykinin [35–37]. Numerous experiments suggest an important interaction between Ang (1–7) and prostaglandin–bradykinin–NO systems. Ang (1–7) appears to counterbalance several actions of Ang II. Ang (1–7) binds to the mas receptor (Figs 2 and 3) which mediates vasodilating and antiproliferative actions of the heptapeptide.

Angiotensin-converting enzyme 2

Angiotensin-converting enzyme 2 was discovered and cloned rather recently [38, 39]. This discovery brought both ACE2 and its main product, Ang 1–7 into the focus of intense research. ACE2 is a carboxypeptidase which cleaves one residue from Ang I to generate angiotensin 1–9 and a single residue from Ang II to generate Ang 1–7 (Fig. 2). ACE2 is most abundant in vascular endothelium of kidney, heart, hypothalamus and aortic wall. ACE2 is also found in testis [40].

Regulation of ACE2 expression has not yet been fully clarified [35]. Neither ACE inhibitors nor ARBs do inhibit ACE2 activity, but they both appear to upregulate ACE2 expression in rat myocardium and renal cortex [35]. Expression of ACE2 in the heart is increased by myocardial infarction [41].

Disruption of the ACE2 gene in mice was reported to result in a severe cardiac contractility defect, increased Ang II plasma levels and upregulation of cardiac hypoxia-induced genes [42]. The authors concluded that ACE2 is an 'essential regulator of heart function'. Genetic ablation of ACE in ACE2 null mice completely normalized the cardiac phenotype, which fits the proposed mutually counterbalancing roles for the ACE/Ang II and ACE2/Ang 1–7 arms of RAS. The authors interpreted these findings as evidence for ACE2 being an essential regulator of heart function.

This interpretation [42] has recently been challenged by a study also on ACE2 null mice, showing 'no detectable effect on cardiac dimensions or ejection fraction in conscious mice under basal conditions' [43]. These investigators reported increased pressor sensitivity to Ang II infusion and higher plasma levels and renal concentrations of Ang II during infusion in ACE2 null mice. This study suggested an important role for ACE2 in degrading Ang II and regulation of vascular responses to Ang II. The abundance of ACE2 in kidneys notably in the proximal tubule is of particular note. ACE2 may be critical in regulating the balance between renal effects of Ang II and ang (1–7) and may therefore become a target for future therapeutic approaches [44]. ACE2 seems to have a protective role in the kidney [45].

Angiotensin-converting enzyme 2 and Ang 1–7 may play an important role in cardiovascular physiology and pathophysiology, e.g. by modulating or counterbalancing excess activity of the 'classical' RAS [45, 46]. The expression and activity of ACE2 in heart [47] and kidney is differently increased by treatment with ACE inhibitors. This leads to organ-wise regulated increase of local production of Ang 1–7, as demonstrated in rats [48–50]. This would offer an additional beneficial effect of ACE inhibitors, possibly explaining in part why ACE inhibitors and ARBs

remain effective despite increased plasma renin activity and angiotensin peptide concentrations [48–50].

Renin/prorenin receptors

Renin receptors were identified and cloned rather recently and shown to be functional [51]. Renin receptors bind both prorenin and renin [52]. Two receptors have been characterized; the M6P/insulin-like growth factor II receptor, a clearance receptor and the specific renin receptor, which activates intracellular signalling and enhances receptor-bound renin catalytic activity on the cell surface (Fig. 4). Renin receptors are abundant in heart, brain and placenta, lower levels being found in kidney and liver [52]. Visceral adipose tissue also expresses renin receptor, whereas subcutaneous adipose tissue expressed less renin receptors [53]. It appears that renin receptors participate in local production of Ang II and may contribute to systemic Ang II levels as well. Binding of prorenin to the renin receptor (Fig. 4) leads to activation of prorenin to active renin, activation of mitogen-activated protein kinases p44/42 and TGF-β, ultimately increasing contractility, hypertrophy and fibrosis [51, 52]. It has been suggested that blocking renin receptors may be a new target for tissue protection.

The 'handle and gate' hypothesis of activation of receptor-bound renin offers exciting perspectives [52]. A pentapeptide reproducing the 'handle' region of the prosegment of prorenin (Fig. 4) that covers the active site has provided promising results in the treatment of diabetic nephropathy [54] and in the prevention of hypertensive glomerulopathy in mice [55]. If

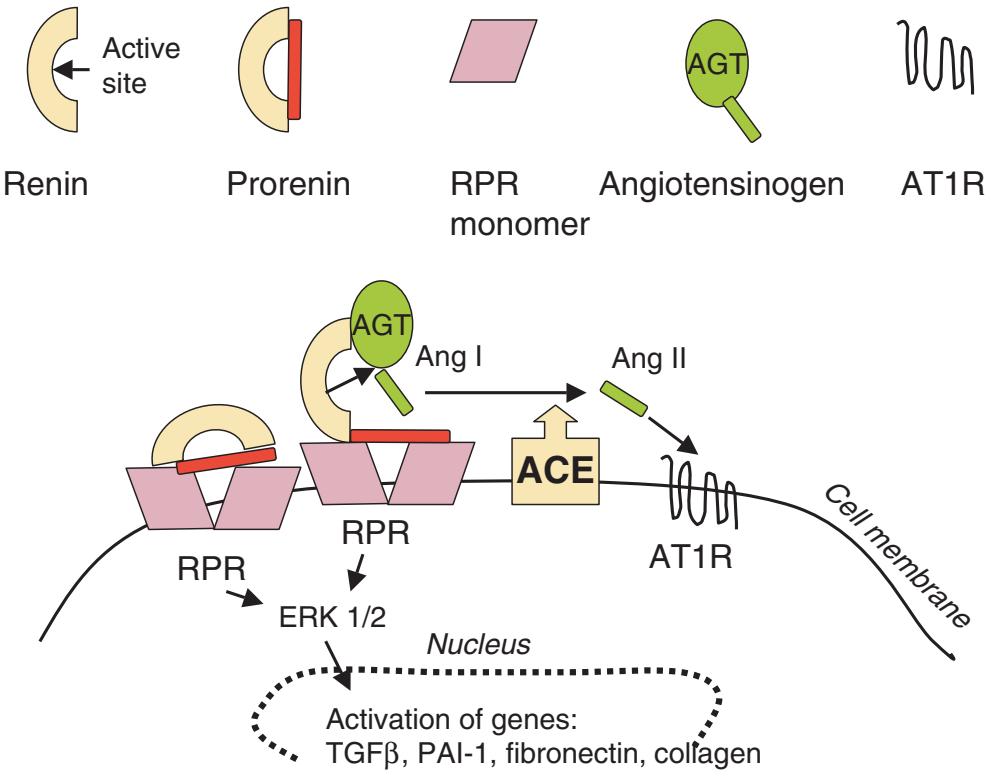

Fig. 4 Schematic presentation of prorenin interacting with renin/prorenin receptor (RPR, in dimeric form), leading to activation of prorenin by displacement of the prosegment ( ). Activated renin then generates Ang I from angiotensinogen (AGT) and angiotensin-convering enzyme generates Ang II from Ang I, and Ang II can then activate AT1 receptors on the same cell membrane. Signalling via extracellular signal-regulated kinase (ERK) 1/2 leads to activation of genes. Modified from Nguyen 2007 [52].

translated into human pathology, these observations may profoundly change our view of prorenin and the (pro) renin receptor as an actor within the tissue RAS and a potential target of treatment.

Tissue (local) RAS

Local RAS systems have been identified in most organs and tissues investigated as recently reviewed [11, 56]. They contain all components necessary for the production of Ang II and other angiotensin peptides and their respective receptors, and in addition, renin/prorenin receptors. The majority of studies indicate that most if not all renin found in local RAS systems is derived from renal renin.

Tissue RAS systems exert diverse actions in many organs. In some organs, they operate independently of the 'circulating RAS', e.g. the adrenal glands and brain. Other local RAS systems, e.g. heart and kidney operate in close interaction with the 'circulating' RAS. Thus, circulating components of RAS like renin and AGT may be taken up by tissues. Circulating RAS and local tissue RAS are thought to operate in a complementary fashion [56], not opposing each other. A proper balance between regulating and counter-regulating factors of tissue RAS appears important in maintaining normal physiological functions of many organs.

The circulating RAS is seen as a regulator of systemic volume and electrolyte balance and of BP homeostasis, whilst 'local' RAS systems have local tissue effects involving proliferation, growth, protein synthesis and organ functions, e.g. in kidney, heart, brain, reproductive organs and pancreas [56, 57].

Some recent discoveries concerning local RAS systems may deserve particular interest, namely those of the heart, brain and adipose tissue. Thus, ACE2 expression is increased in the heart following myocardial infarction [41], in heart failure [47] and during treatment with ACE inhibitors or ARBs [50]. ACE2 is the main generator of Ang 1–7 from Ang II in the heart, and the amount of Ang 1–7 is increased in the peri-ischaemic area following myocardial infarction [47].

Brain RAS components mediate a large variety of neurobiological activities [58] which are gradually being understood. For instance, neuronal AT1 receptors mediate Ang II effects on BP, salt and water intake and secretion of vasopressin whereas AT2 receptors mediate, e.g. apoptosis and possibly neural regeneration after neural injury [59]. In addition to Ang II and Ang III, Ang IV and Ang 1–7 appear to be involved in modulation of brain functions, including learning and memory responses.

Adipose tissue also contains all components of RAS including functional renin receptors co-localizing with renin and may be involved in the regulation of visceral adipose tissue accumulation [53]. Thus, visceral RAS may play a role in the pathophysiology of the metabolic syndrome [60]. Adipose tissue was shown to be an important source of both local and circulating AGT [61] and might thereby participate in systemic BP regulation. However, this has not been shown in humans.

Testicular ACE (ca 100 kDa), a smaller isoform of ACE (150–180 kDa) was recently shown to play a crucial role in fertilization by releasing a glycosylphosphatidylinositol (GPI)-anchored protein from sperm cells. ACE knock-out sperm cells showed deficient binding of egg cells [62]. The impact of this observation awaits further clarification. However, treatment with ACE inhibitors is not reported to interfere with male fertility.

Intracellular RAS

Evidence suggesting the existence a complete, functional *intracellular* RAS within cells has been provided recently [63, 64]. Intracellular RAS is reported to mediate changes in Ca^{2+} fluxes and activation of genes [65]. Intracellular Ang II reportedly caused cardiac hypertrophy *in vivo* in mice [66]. In these experiments, a plasmid construct under the control of an α-myosin promoter caused increased intracellular Ang II and 68% increase in relative heart weight. The mechanisms by which intracellular Ang II exerts its actions are not fully understood. Thus, intracellularly applied ARBs can only partly block intracellular Ang II. The role of intracellular RAS is presently unclear.

Involvement of ACE2 in avian influenza

Surprisingly, ACE2 has been shown to function as a receptor of severe acute respiratory syndrome (SARS) coronavirus [67, 68]. ACE is thought to contribute to pulmonary tissue damage and oedema by generating Ang II. ACE2 is believed to normally counteract these harmful effects, but following attachment of SARS virus to ACE2 and replication, ACE2 expression is diminished, less Ang 1–7 is formed from Ang II and AT1 receptor activation is intensified. In support of this contention, injection of recombinant ACE2 into mice protected these mice from acute lung injury caused by sepsis or acid aspiration [69]. Thus, functioning ACE2 may protect against the potentially lethal lung injury associated with SARS [69].

RAS and arterial ageing

Ageing is associated with alterations of several structural and functional properties of large arteries. Increased wall thickness and stiffness, pulse wave velocity and pulse wave augmentation and deterioration of endothelial function are hallmarks of arterial ageing [70]. These alterations form fertile soil for age-associated cardiovascular disease. Conversely, cardiovascular disease causes acceleration of these detrimental changes. Several lines of evidence support an important role of RAS in arterial ageing as well as in cardiovascular disease.

Arterial components of the Ang II-signalling cascade increase with ageing [70]. Ang II signalling via AT1 receptors increases collagen production within the arterial wall, promotes reduced forms of nicotinamide-adenine dinucleotide phosphate oxidase activity and enhances migration of vascular smooth muscle cells. Increased formation of reactive oxygen species (ROS) leads to activation of metalloprotease, less NO bioavailability and endothelial dysfunction. Formation of ROS induced by Ang II may contribute to tissue ageing and age-related cardiovascular disease [71]. Ang II also causes activation of NF-κB pathway and proinflammatory cytokines [6, 33]. Thus, judging by mechanistic criteria, Ang II appears to play a central role in many stimuli that govern arterial ageing and its functional responses.

A recent study [72] showed that treatment of male Wistar rats with an ACE inhibitor (enalapril 10 mg $\times$ kg $\times$ day) or an ARB (losartan 30 mg $\times$ kg $\times$ day) for 18 months or life-long resulted in prolongation of life span by 21% (enalapril) or 19% (losartan) compared with untreated control rats. The difference in life span could not be explained by cardiovascular protection. The authors speculate that prolongation of life span could be explained by reduction by RAS inhibitors of ROS formation and reduction of oxidative burden.

Genetics of RAS

In accordance with the generally acknowledged importance of RAS in pathophysiology of cardiovascular disease, many mutations in RAS component genes are associated with hypertension and cardiovascular diseases [73].

The AGT gene has been associated with hypertension [74], but attempts to predict responses to antihypertensive drugs based on AGT polymorphisms have yielded inconsistent results [75]. Variants of the AT1 and the AT2 receptor genes are reportedly associated with hypertension, but they are also inconsistently associated with response to antihypertensive therapy [76–79]. In a Chinese cohort of hypertensive patients, the association with combined AGT and AT1 receptor single nucleotide polymorphisms (SNP) haplotypes was modest (13% for systolic, 9% for diastolic BP reduction with ACE inhibitor [73].

In a prospective study comprising 2579 UK men, the AT1 receptor genotype 1166CC was associated with increased cardiovascular risk irrespective of BP [80] whereas the AT II receptor 1675A allele was associated with increased risk only at high systolic BP (>165 mm Hg). In general, the magnitude of predictive power of RAS gene, SNPs has been rather modest.

A possible role for the AT2 receptor in the central nervous system was first suggested by attenuated

exploratory behaviour in AT2 receptor-deficient (knockout) mice [81–83]. In humans, the absence of or mutations in the AT2 receptor gene was shown to be associated with severe X-chromosome linked mental retardation [84], showing a link between a RAS component and development of cognitive functions. Interestingly, a unique mutation of the renin receptor gene was later shown to be present in patients with X-linked mental retardation and epilepsy [85]. Functional analysis revealed that the mutated renin receptor could bind renin and increase renin catalytic activity. 'This finding confirmed the importance of the RAS in cognitive processes and indicated a novel specific role for the renin receptor in cognitive functions and brain development' [85].

Considerable widening and deepening of our understanding of RAS' physiology and pathopysiology has been achieved by genetical manipulation of experimental animals, e.g. rats [86] and in mice [87]. For instance, 'ACE 1/3 mice', compound heterozygotes for the ACE genes [88] have no endothelial ACE, but are nevertheless capable of maintaining normal physiology. The explanation for this appeared to be a compensatory increase in renal renin production followed by increased Ang II generation by nonendothelial ACE, showing the plasticity of RAS. This is just one example of fascinating gene manipulation revealing secrets of RAS physiology that would otherwise have remained enigmatic.

RAS inhibition, achievements and expectations

Angiotensin-converting enzyme inhibitors and ARBs (Fig. 5) are well established corner stones in the prevention and treatment of hypertension and cardiovascular disease, as demonstrated by numerous clinical trials and world wide clinical practice. According to a recent meta-analysis [89], ACE inhibitors and ARBs have similar BP-dependent effects for the risks of stroke, coronary heart disease and heart failure. For ACE inhibitors, but not for ARBs, there is evidence for BP-independent effects on the risk of coronary disease events [89]. The benefits of ACE inhibitors or ARBs on renal outcomes probably result from BP-lowering effects [90] whereas renoprotective benefits

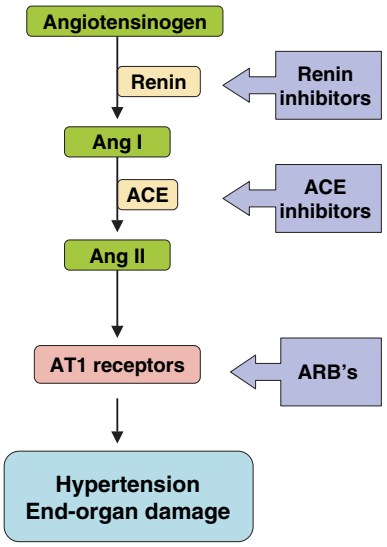

Fig. 5 Scheme of presently available renin-angiotensin system inhibitors.

in diabetic patients may partly depend on factors beyond BP lowering [15]. In fact, several studies suggest that both ACE inhibitors and ARBs offer benefits in addition to those mediated by BP lowering only.

Several trials have demonstrated a 15–30% reduction of new onset diabetes during treatment with ACE inhibitors or ARBs [91]. The mechanism behind this protective effect of RAS inhibition is not clear, but it offers a significant advantage for RAS inhibitors as we are experiencing a global epidemy of increasing incidence of diabetes.

Combined blockade of the RAS by ACE inhibitors and ARBs has been shown to provide additional benefits compared with either drug class [92–94]. However, these expectations were not confirmed by the recently published ONTARGET study [95] which compared treatment with telmisartan, ramipril or both drugs combined in a megatrial comprising 25620 patients with high cardiovascular risk profile. Of particular interest is the use in ONTARGET of telmisartan, by far, the most prominent activator of peroxisome proliferator activated receptor-γ, a mediator of a host of favourable metabolic actions [33, 96]. In the ONTARGET study, mean BP was lower

in both the telmisartan group (0.9/0.6 mm Hg greater reduction) and the combination therapy group (2.4/1.4 mm Hg greater reduction) than in the ramipril group. Telmisartan was equivalent to ramipril in terms of primary outcomes in patients with vascular disease or diabetes. The combination of telmisartan and ramipril was associated with more adverse events (hypotension, renal dysfunction) without an increase in benefit. The recent introduction of the first orally effective renin inhibitor, aliskiren, has raised additional interest in new possibilities of almost complete blockade of RAS as a tool (Fig. 5), perhaps, more effective than earlier, in the prevention and treatment of cardiovascular disease [92, 93, 97, 98]. Early reports on the use of aliskiren are promising, showing at least, an antihypertensive effect of aliskiren potent as those of other antihypertensive drugs [93]. In particular, the combination of renin inhibitors with ACE inhibitors and ARBs may offer a solution to the 'renin escape' phenomenon [93], which implies that ACE inhibitors or ARBs may lose part of their effect during long time treatment. Many questions are also raised, for instance, what would the consequences be if the 'beneficial' angiotensin peptide, Ang 1–7 was not generated at all or 'benefical' AT2 receptor mediated effects disappeared completely? Only well conducted experiments and trials may answer such questions. We may look forward to interesting years ahead whilst waiting for results and answers.

Concluding remarks

Having witnessed an amazing plethora of RAS discoveries from renin, in 1898, to mutations in the AT2 receptor and of renin receptor genes associated with X-chromosome linked mental retardation, we cannot avoid concluding that the physiology of RAS is by far more complex and multilayered than one would have thought of. It appears quite unlikely that we have seen the whole picture yet. This challenging complexity and the central position of RAS in the pathophysiology of cardiovascular disease will continue to inspire research and drug trials aiming at creating optimal pharmacological tools for RAS modulation or blockade.

Conflict of interest statement

No conflict of interest was declared.

References

1 Tigerstedt R, Bergman P. Niere und Kreislauf. *Scand Arch Physiol* (Germany) 1898; 8: 223–71.
2 Ondetti MA, Rubin B, Cushman DW. Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. *Science* 1977; 196: 441–4.
3 Timmermans PB, Wong PC, Chiu AT *et al.* Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev* 1993; 45: 205–51.
4 Kumar R, Singh VP, Baker KM. The intracellular renin-angiotensin system: a new paradigm. *Trends Endocrinol Metab* 2007; 18: 208–14.
5 Ran J, Hirano T, Fukui T *et al.* Angiotensin II infusion decreases plasma adiponectin level via its type 1 receptor in rats: an implication for hypertension-related insulin resistance. *Metabolism* 2006; 55: 478–88.
6 Ruiz-Ortega M, Esteban V, Ruperez M *et al.* Renal and vascular hypertension-induced inflammation: role of angiotensin II. *Curr Opin Nephrol Hypertens* 2006; 15: 159–66.
7 Ramracheya RD, Muller DS, Wu Y *et al.* Direct regulation of insulin secretion by angiotensin II in human islets of Langerhans. *Diabetologia* 2006; 49: 321–31.
8 Chu KY, Lau T, Carlsson PO, Leung PS. Angiotensin II type 1 receptor blockade improves beta-cell function and glucose tolerance in a mouse model of type 2 diabetes. *Diabetes* 2006; 55: 367–74.
9 Assimacopoulos-Jeannet FD, Blackmore PF, Exton JH. Studies of the interaction between glucagon and alpha-adrenergic agonists in the control of hepatic glucose output. *J Biol Chem* 1982; 257: 3759–65.
10 Ran J, Hirano T, Adachi M. Angiotensin II type 1 receptor blocker ameliorates overproduction and accumulation of triglyceride in the liver of Zucker fatty rats. *Am J Physiol Endocrinol Metab* 2004; 287: E227–32.
11 Fleming I, Kohlstedt K, Busse R. The tissue renin-angiotensin system and intracellular signalling. *Curr Opin Nephrol Hypertens* 2006; 15: 8–13.
12 Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol* 2007; 292: C82–97.
13 Culman J, Baulmann J, Blume A, Unger T. The renin-angiotensin system in the brain: an update. *J Renin Angiotensin Aldosterone Syst* 2001; 2: 96–102.
14 Li J, Culman J, Hortnagl H *et al.* Angiotensin AT2 receptor protects against cerebral ischemia-induced neuronal injury. *FASEB J* 2005; 19: 617–9.
15 Dahlof B, Devereux RB, Kjeldsen SE *et al.* Cardiovascular morbidity and mortality in the Losartan intervention for endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet* 2002; 359: 995–1003.

16 Safar ME. Systolic hypertension in the elderly: arterial wall mechanical properties and the renin-angiotensin-aldosterone system. *J Hypertens* 2005; 23: 673–81.
17 Padia SH, Howell NL, Siragy HM, Carey RM. Renal angiotensin type 2 receptors mediate natriuresis via angiotensin III in the angiotensin II type 1 receptor blocked rat. *Hypertension* 2006; 47: 537–44.
18 Padia SH, Kemp BA, Howell NL *et al.* Intrarenal aminopeptidase N inhibition augments natriuretic responses to angiotensin III in angiotensin type 1 receptor blocked rats. *Hypertension* 2007; 49: 625–30.
19 Seikaly MG, Arant BS Jr, Seney FD Jr. Endogenous angiotensin concentrations in specific intrarenal fluid compartments of the rat. *J Clin Invest* 1990; 86: 1352–7.
20 Carey RM. Update on the role of the AT2 receptor. *Curr Opin Nephrol Hypertens* 2005; 14: 67–71.
21 Takai S, Jin D, Muramatsu M, Miyazaki M. Chymase as a novel target for the prevention of vascular diseases. *Trends Pharmacol Sci* 2004; 25: 518–22.
22 Kokkonen JO, Lindstedt KA, Kovanen PT. Role for chymase in heart failure: angiotensin II-dependent or -independent mechanisms? *Circulation* 2003; 107: 2522–4.
23 Miyazaki M, Takai S, Jin D, Muramatsu M. Pathological roles of angiotensin II produced by mast cell chymase and the effects of chymase inhibition in animal models. *Pharmacol Ther* 2006; 112: 668–76.
24 Pitt B, Poole-Wilson PA, Segal R *et al.* Effect of Losartan compared with Captopril on mortality in patients with symptomatic heart failure: randomised trial – the Losartan Heart Failure Survival Study ELITE II. *Lancet* 2000; 355: 1582–7.
25 Dickstein K, Kjekshus J, OPTIMAAL Steering Committee of the OPTIMAAL Study Group. Effects of losartan and captopril on mortality and morbidity in high-risk patients after acute myocardial infarction: the OPTIMAAL randomised trial in myocardial infarction with angiotensin II antagonist Losartan. *Lancet* 2002; 360: 752–60.
26 Zini S, Fournie-Zaluski MC, Chauvel E, Roques BP, Corvol P, Llorens-Cortes C. Identification of metabolic pathways of brain angiotensin II and III using specific aminopeptidase inhibitors: predominant role of angiotensin III in the control of vasopressin release. *Proc Natl Acad Sci USA* 1996; 93: 11968–73.
27 Gammelgaard I, Wamberg S, Bie P. Systemic effects of angiotensin III in conscious dogs during acute double blockade of the renin-angiotensin-aldosterone-system. *Acta Physiol (Oxf)* 2006; 188: 129–38.
28 Albiston AL, McDowall SG, Matsacos D *et al.* Evidence that the angiotensin IV (AT(4)) receptor is the enzyme insulin-regulated aminopeptidase. *J Biol Chem* 2001; 276: 48623–6.
29 Keller SR, Scott HM, Mastick CC, Aebersold R, Lienhard GE. Cloning and characterization of a novel insulin-regulated membrane aminopeptidase from Glut4 vesicles. *J Biol Chem* 1995; 270: 23612–8.
30 Kerins DM, Hao Q, Vaughan DE. Angiotensin induction of PAI-1 expression in endothelial cells is mediated by the hexapeptide angiotensin IV. *J Clin Invest* 1995; 96: 2515–20.
31 Chai SY, Fernando R, Peck G *et al.* The angiotensin IV/AT4 receptor. *Cell Mol Life Sci* 2004; 61: 2728–37.
32 Ruiz-Ortega M, Esteban V, Egido J. The regulation of the inflammatory response through nuclear factor-kappab pathway by angiotensin IV extends the role of the renin angiotensin system in cardiovascular diseases. *Trends Cardiovasc Med* 2007; 17: 19–25.
33 Savoia C, Schiffrin EL. Inflammation in hypertension. *Curr Opin Nephrol Hypertens* 2006; 15: 152–8.
34 Schiavone MT, Santos RA, Brosnihan KB, Khosla MC, Ferrario CM. Release of vasopressin from the rat hypothalamo-neurohypophysial system by angiotensin-(1–7) heptapeptide. *Proc Natl Acad Sci USA* 1988; 85: 4095–8.
35 Ferrario CM. Angiotensin-converting enzyme 2 and angiotensin-(1–7): an evolving story in cardiovascular regulation. *Hypertension* 2006; 47: 515–21.
36 Watanabe T, Barker TA, Berk BC. Angiotensin II and the endothelium: diverse signals and effects. *Hypertension* 2005; 45: 163–9.
37 Santos RA, Castro CH, Gava E *et al.* Impairment of *in vitro* and *in vivo* heart function in angiotensin-(1–7) receptor MAS knockout mice. *Hypertension* 2006; 47: 996–1002.
38 Donoghue M, Hsieh F, Baronas E *et al.* A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. *Circ Res* 2000; 87: E1–9.
39 Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J Biol Chem* 2000; 275: 33238–43.
40 Santos RA, Ferreira AJ. Angiotensin-(1–7) and the renin-angiotensin system. *Curr Opin Nephrol Hypertens* 2007; 16: 122–8.
41 Burrell LM, Risvanis J, Kubota E *et al.* Myocardial infarction increases ACE2 expression in rat and humans. *Eur Heart J* 2005;26:369–75; Discussion 322-4.
42 Crackower MA, Sarao R, Oudit GY *et al.* Angiotensin-converting enzyme 2 is an essential regulator of heart function. *Nature* 2002; 417: 822–8.
43 Gurley SB, Allred A, Le TH *et al.* Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. *J Clin Invest* 2006; 116: 2218–25.
44 Burns KD. The emerging role of angiotensin-converting enzyme-2 in the kidney. *Curr Opin Nephrol Hypertens* 2007; 16: 116–21.
45 Danilczyk U, Penninger JM. Angiotensin-converting enzyme II in the heart and the kidney. *Circ Res* 2006; 98: 463–71.
46 Der Sarkissian S, Huentelman MJ, Stewart J, Katovich MJ, Raizada MK. ACE2: a novel therapeutic target for cardiovascular diseases. *Prog Biophys Mol Biol* 2006; 91: 163–98.
47 Keidar S, Kaplan M, Gamliel-Lazarovich A. ACE2 of the heart: from angiotensin I to angiotensin (1–7). *Cardiovasc Res* 2007; 73: 463–9.
48 Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension* 2004; 43: 970–6.

49 Ferrario CM, Jessup J, Chappell MC *et al.* Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 2005; 111: 2605–10.

50 Ferrario CM, Jessup J, Gallagher PE *et al.* Effects of renin-angiotensin system blockade on renal angiotensin-(1–7) forming enzymes and receptors. *Kidney Int* 2005; 68: 2189–96.

51 Nguyen G, Delarue F, Burckle C, Bouzhir L, Giller T, Sraer JD. Pivotal role of the renin/prorenin receptor in angiotensin II production and cellular responses to renin. *J Clin Invest* 2002; 109: 1417–27.

52 Nguyen G. The (pro)renin receptor: pathophysiological roles in cardiovascular and renal pathology. *Curr Opin Nephrol Hypertens* 2007; 16: 129–33.

53 Achard V, Boullu-Ciocca S, Desbriere R, Nguyen G, Grino M. Renin receptor expression in human adipose tissue. *Am J Physiol Regul Integr Comp Physiol* 2007; 292: R274–82.

54 Ichihara A, Hayashi M, Kaneshiro Y *et al.* Inhibition of diabetic nephropathy by a decoy peptide corresponding to the "handle" region for nonproteolytic activation of prorenin. *J Clin Invest* 2004; 114: 1128–35.

55 Ichihara A, Suzuki F, Nakagawa T *et al.* Prorenin receptor blockade inhibits development of glomerulosclerosis in diabetic angiotensin II type 1a receptor-deficient mice. *J Am Soc Nephrol* 2006; 17: 1950–61.

56 Paul M, Poyan Mehr A, Kreutz R. Physiology of local renin-angiotensin systems. *Physiol Rev* 2006; 86: 747–803.

57 Leung PS. The physiology of a local renin-angiotensin system in the pancreas. *J Physiol* 2007; 580: 31–7.

58 von Bohlen und Halbach O, Albrecht D. The CNS renin-angiotensin system. *Cell Tissue Res* 2006; 326: 599–616.

59 Thone-Reineke C, Steckelings UM, Unger T. Angiotensin receptor blockers and cerebral protection in stroke. *J Hypertens Suppl* 2006; 24: S115–21.

60 Ernsberger P, Koletsky RJ. Metabolic actions of angiotensin receptor antagonists: PPAR-gamma agonist actions or a class effect? *Curr Opin Pharmacol* 2007; 7: 140–5.

61 Engeli S, Schling P, Gorzelniak K *et al.* The adipose-tissue renin-angiotensin-aldosterone system: role in the metabolic syndrome? *Int J Biochem Cell Biol* 2003; 35: 807–25.

62 Kondoh G, Tojo H, Nakatani Y *et al.* Angiotensin-converting enzyme is a GPI-anchored protein releasing factor crucial for fertilization. *Nat Med* 2005; 11: 160–6.

63 De Mello WC. Cardiac intracrine renin angiotensin system. Part of genetic reprogramming? *Regul Pept* 2006; 133: 10–2.

64 Re RN, Cook JL. The intracrine hypothesis: an update. *Regul Pept* 2006; 133: 1–9.

65 Haller H, Lindschau C, Erdmann B, Quass P, Luft FC. Effects of intracellular angiotensin II in vascular smooth muscle cells. *Circ Res* 1996; 79: 765–72.

66 Baker KM, Chernin MI, Schreiber T *et al.* Evidence of a novel intracrine mechanism in angiotensin II-induced cardiac hypertrophy. *Regul Pept* 2004; 120: 5–13.

67 Kuba K, Imai Y, Rao S *et al.* A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus-induced lung injury. *Nat Med* 2005; 11: 875–9.

68 Kuba K, Imai Y, Rao S, Jiang C, Penninger JM. Lessons from SARS: control of acute lung failure by the SARS receptor ACE2. *J Mol Med* 2006; 84: 814–20.

69 Imai Y, Kuba K, Rao S *et al.* Angiotensin-converting enzyme 2 protects from severe acute lung failure. *Nature* 2005; 436: 112–6.

70 Najjar SS, Scuteri A, Lakatta EG. Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension* 2005; 46: 454–62.

71 de Cavanagh EM, Piotrkowski B, Fraga CG. Concerted action of the renin-angiotensin system, mitochondria, and antioxidant defenses in aging. *Mol Aspects Med* 2004; 25: 27–36.

72 Basso N, Cini R, Pietrelli A, Ferder L, Terragno NA, Inserra F. Protective effect of long-term angiotensin II inhibition. *Am J Physiol Heart Circ Physiol* 2007; 293: H1351–8.

73 Su X, Lee L, Li X *et al.* Association between angiotensinogen, angiotensin II receptor genes, and blood pressure response to an angiotensin-converting enzyme inhibitor. *Circulation* 2007; 115: 725–32.

74 Jeunemaitre X, Soubrier F, Kotelevtsev YV *et al.* Molecular basis of human hypertension: role of angiotensinogen. *Cell* 1992; 71: 169–80.

75 Mellen PB, Herrington DM. Pharmacogenomics of blood pressure response to antihypertensive treatment [see comment]. *J Hypertens* 2005; 23: 1311–25.

76 Frazier L, Turner ST, Schwartz GL, Chapman AB, Boerwinkle E. Multilocus effects of the renin-angiotensin-aldosterone system genes on blood pressure response to a thiazide diuretic. *Pharmacogenomics J* 2004; 4: 17–23.

77 Hingorani AD, Jia H, Stevens PA, Hopper R, Dickerson JE, Brown MJ. Renin-angiotensin system gene polymorphisms influence blood pressure and the response to angiotensin converting enzyme inhibition. *J Hypertens* 1995; 13: 1602–9.

78 Kurland L, Melhus H, Karlsson J *et al.* Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHI-A) Trial. Angiotensin converting enzyme gene polymorphism predicts blood pressure response to angiotensin II receptor type 1 antagonist treatment in hypertensive patients. *J Hypertens* 2001; 19: 1783–7.

79 Miller JA, Thai K, Scholey JW. Angiotensin II type 1 receptor gene polymorphism predicts response to losartan and angiotensin II. *Kidney Int* 1999; 56: 2173–80.

80 Jones A, Dhamrait SS, Payne JR *et al.* Genetic variants of angiotensin II receptors and cardiovascular risk in hypertension. *Hypertension* 2003; 42: 500–6.

81 Hein L, Barsh GS, Pratt RE, Dzau VJ, Kobilka BK. Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor in mice. *Nature* 1995; 377: 744–7.

82 Ichiki T, Labosky PA, Shiota C *et al.* Effects on blood pressure and exploratory behaviour of mice lacking angiotensin II type-2 receptor. *Nature* 1995; 377: 748–50.

83 Okuyama S, Sakagawa T, Chaki S, Imagawa Y, Ichiki T, Inagami T. Anxiety-like behavior in mice lacking the angiotensin II type-2 receptor. *Brain Res* 1999; 821: 150–9.

84 Vervoort VS, Beachem MA, Edwards PS *et al.* AGTR2 mutations in X-linked mental retardation. *Science* 2002; 296: 2401–3.

85 Ramser J, Abidi FE, Burckle CA *et al.* A unique exonic splice enhancer mutation in a family with X-linked mental retardation

and epilepsy points to a novel role of the renin receptor. *Hum Mol Genet* 2005; 14: 1019–27.
86 Mullins JJ, Peters J, Ganten D. Fulminant hypertension in transgenic rats harbouring the mouse Ren-2 gene. *Nature* 1990; 344: 541–4.
87 Bernstein KE, Xiao HD, Frenzel K *et al.* Six truisms concerning ACE and the renin-angiotensin system deduced from the genetic analysis of mice. *Circ Res* 2005; 96: 1135–44.
88 Cole JM, Khokhlova N, Sutliff RL *et al.* Mice lacking endothelial ACE: normal blood pressure with elevated angiotensin II. *Hypertension* 2003; 41: 313–21.
89 Blood Pressure Lowering Treatment Trialists' Collaboration, Turnbull F, Neal B *et al.* Blood pressure-dependent and independent effects of agents that inhibit the renin-angiotensin system. *J Hypertens* 2007; 25: 951–8.
90 Casas JP, Chua W, Loukogeorgakis S *et al.* Effect of inhibitors of the renin-angiotensin system and other antihypertensive drugs on renal outcomes: systematic review and meta-analysis. *Lancet* 2005; 366: 2026–33.
91 Aguilar D, Solomon SD. ACE inhibitors and angiotensin receptor antagonists and the incidence of new onset diabetes mellitus: an emerging theme. *Drugs* 2006; 66: 1169–77.
92 Krum H, Gilbert RE. Novel therapies blocking the renin-angiotensin-aldosterone system in the management of hypertension and related disorders. *J Hypertens* 2007; 25: 25–35.
93 Leibovitz E, Schiffrin EL. Novel drugs targeting hypertension: renin inhibitors and beyond. *J Cardiovasc Pharmacol* 2007; 50: 3–8.
94 Song JH, Cha SH, Hong SB, Kim DH. Dual blockade of the renin-angiotensin system with angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers in chronic kidney disease. *J Hypertens Suppl* 2006; 24: S101–6.
95 The ONTARGET Investigators. Telmisartan, ramipril or both in patients at high risk for vascular events. *New Engl J Med* 2008; 358: 1547–9.
96 Kurtz TW, Pravenec M. Antidiabetic mechanisms of angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists: beyond the renin-angiotensin system. *J Hypertens* 2004; 22: 2253–61.
97 Azizi M, Webb R, Nussberger J, Hollenberg NK. Renin inhibition with aliskiren: where are we now and where are we going? *J Hypertens* 2006; 24: 243–56.
98 Staessen JA, Li Y, Richart T. Oral renin inhibitors. *Lancet* 2006; 368: 1449–56.

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