Aging and the Renin-Angiotensin System

Sara Conti, Paola Cassis, Ariela Benigni

Angiotensin II (Ang II), the dominant effector molecule of the renin-angiotensin system (RAS), exerts its effects on binding of 2 pharmacologically distinct rhodopsin-like G protein–associated receptors, the type 1 and the type 2 (AT\textsubscript{1} and AT\textsubscript{2}) receptors.\textsuperscript{1} Human cells express a single AT\textsubscript{1} receptor, whereas 2 isoforms, AT\textsubscript{1A} and AT\textsubscript{1B} with 95% of amino acid sequence identity and similar affinities for Ang II, are present in rodents. The AT\textsubscript{1A} receptor, the closest murine homologue to the human AT\textsubscript{1} receptor, predominates in most tissues, including heart, kidney, and brain. Expression of the AT\textsubscript{2} receptor is ubiquitous in fetal tissues of rodents and humans and it decreases after birth, remaining at a low level in adulthood in adrenal medulla, uterus, ovary, vascular endothelium, and distinct brain areas.\textsuperscript{2}

Arterial blood pressure is regulated by diverse pathways that include vasomotion, regulation of sodium and water excretion by the kidney, and activity of the central and sympathetic nervous systems. Circulating Ang II through activation of the AT\textsubscript{1} receptor contributes to an increase in blood pressure and promotes renal tubular sodium and water retention.\textsuperscript{3,4}

The discovery of tissue-specific RAS components in the heart, kidney, brain, and immune system led to the definition of local RAS that exerts organ-specific functions and can operate independently or in close interaction with circulating RAS components. A role of intrarenal RAS in hypertension has been suggested by cross-transplantation experiments showing that the development of hypertension is predominantly dependent on the presence of the AT\textsubscript{1} receptor in the kidney.\textsuperscript{5} Based on the evidence that activation of the brain RAS regulates fluid balance and energy metabolism, the involvement of altered brain RAS signaling in high- and low-renin forms of hypertension has been proposed.\textsuperscript{6}

Intriguing actions of Ang II in modulating immune response and inflammation that impact both the blood pressure and target organ damage have been attributed to the activation of the AT\textsubscript{1} receptor on T cells,\textsuperscript{7,8} although a recent study has challenged this view.\textsuperscript{9}

The functions of the AT\textsubscript{2} receptor are opposite to those of the AT\textsubscript{1} receptor. Early studies indicated a secondary role for the AT\textsubscript{2} receptor in normal physiological regulation,\textsuperscript{10} whereas subsequent reports showed a protective role for the AT\textsubscript{2} receptor in different disease conditions. Deletion of the AT\textsubscript{2} receptor accelerates cardiac damage in mice with myocardial infarction,\textsuperscript{11} exacerbates atherosclerosis in apolipoprotein E–deficient mice,\textsuperscript{12} aggravates chronic disease in mice with remnant kidney promoting animal death,\textsuperscript{13} and increases cerebral infarct size in experimental ischemia-induced neuronal injury.\textsuperscript{14} On the other hand, activation of the AT\textsubscript{2} receptor protects stroke-prone spontaneously hypertensive rats from vascular injury and myocardial fibrosis\textsuperscript{15} and limits atherogenesis in apolipoprotein E (AT\textsubscript{1A}) double-knockout mice.\textsuperscript{16} Recently, a role for the AT\textsubscript{2} receptor expressed on e-kits+ progenitor cells in mediating cardiac homing and tissue repair after myocardial infarction has been proposed.\textsuperscript{17}

Finally, the discovery of the angiotensin-converting enzyme (ACE)2-angiotensin (1–7)-Mas receptor axis that exerts vasodilator, antiproliferative, and antifibrotic actions opposed to those of the ACE-Ang II-AT\textsubscript{1} receptor axis has led to the hypothesis that a decrease in the expression or activity of angiotensin (1–7) renders the cardiovascular system more susceptible to the pathological actions of Ang II\textsuperscript{18} (Figure 1).

Ang II Inhibition Protects End Organs From Damaging Insults

During the last 3 decades, experimental and clinical studies have consistently shown that pharmacological blockade of RAS, other than its antihypertensive effect, slows progressive age-related organ damage specifically in the heart, kidney, and brain. The renoprotective action of drugs that inhibit Ang II synthesis/biological activity in experimental and human nephropathies is possibly the consequence of lowering intraglomerular pressure and proteinuria. This in turn protects animals from renal disease progression, which translates in cardioprotection. Animal and human data supporting such effects of Ang II blockers in nondiabetic and diabetic renal disease are overwhelming.\textsuperscript{19–23}

Concerning the brain, studies indicate that Ang II inhibitors mitigate progressive cognitive decline associated with aging, as well as with various neurodegenerative disorders. Recent studies show that Ang II inhibitors help to preserve cognitive functions in patients with Alzheimer disease through a mechanism that is independent of the blood pressure–lowering effect.\textsuperscript{24} It has been proposed that the Ang II receptor blocker (ARB) prevents or modulates accumulation of misfolded...
proteins, including the amyloid-(Aβ) peptide responsible for oxidative and inflammatory damage that leads to energy failure and synaptic dysfunction. In a cohort of male patients with Alzheimer disease, ARB reduces the incidence and progression of the disease better than ACE inhibitor (ACEi), and combined use of the ARB and ACEi shows even greater benefits. The superior protective effect of ARB over ACEi might reside in use of the ARB and ACEi shows even greater benefits. The superior protective effect of ARB over ACEi might reside in ACEi-induced lowering of oxidative stress. The possible impact of such treatment on healthy aging is being investigated but appears plausible. Thus, reduction of oxidative stress by inhibition of the RAS system reverses advanced cardiac hypertrophy in spontaneously hypertensive aged rats, restores the prosurvival gene klotho in the kidney in a model of cyclosporine A-induced oxidative damage, and protects the brain from age-associated cognitive impairment in experimental Alzheimer disease. Additional evidence indicates that the beneficial effect of ACEi or ARB in reducing heart, kidney, and brain damage associated with aging is attributed to the drug capacity to attenuate ROS production and preserve mitochondrial function. Lending support to the role of various RAS components in oxidative injury predisposing to aging are findings that ACE2 null mice are more sensitive to Ang II–mediated oxidative stress and inflammation than wild-type littermates. Moreover, overexpression of ACE2 in the central nervous system reduces oxidative stress and improves autonomic function in mice.

A life-prolonging effect of AT1 receptor blockade has been proposed by a study using mice with targeted disruption of the gene encoding the AT1 receptor (AT1−/−). Such genetic manipulation does not cause severe postnatal mortality or structural abnormalities of the major organs. AT1−/− mice have normal physical activity and outlive their wild-type littermates by 26%, in line with previous data that long-term treatment with the ACEi enalapril results in increased life span in rats. Prolongation of life span in AT1−/− mice is compromised mitochondrial integrity and function, leading to decreased ATP generation and further production of ROS and peroxynitrite, a cytotoxic anion that inhibits mitochondrial electron transport. Ang II via the AT1 receptor activates intracellular NADPH oxidase to generate superoxide anion. Excessive superoxide anion production promotes the uncoupling of endothelial NO synthase, which in turn reduces NO availability and enhances ROS production. The capacity of Ang II to foster oxidative stress is tightly regulated under physiological conditions. At variance, uncontrolled Ang II–dependent ROS generation takes place as a consequence of age-associated activation of RAS (Figure 2). Ang II also accelerates cellular senescence by inducing the shortening of telomeres and cell cycle arrest, effects that are reversed by losartan.

Age-dependent stimulation of local RAS in the myocardium induces NADPH oxidase activity and drives cardiac hypertrophy and fibrosis, features that could be replicated in young rats chronically infused with Ang II. Examples are available in the literature of organ damage induced by ROS that is prevented by ACEi-induced lowering of oxidative stress. The possible impact of such treatment on healthy aging is being investigated but appears plausible. Thus, reduction of oxidative stress by inhibition of the RAS system reverses advanced cardiac hypertrophy in spontaneously hypertensive aged rats, restores the prosurvival gene klotho in the kidney in a model of cyclosporine A-induced oxidative damage, and protects the brain from age-associated cognitive impairment in experimental Alzheimer disease. Additional evidence indicates that the beneficial effect of ACEi or ARB in reducing heart, kidney, and brain damage associated with aging is attributed to the drug capacity to attenuate ROS production and preserve mitochondrial function. Lending support to the role of various RAS components in oxidative injury predisposing to aging are findings that ACE2 null mice are more sensitive to Ang II–mediated oxidative stress and inflammation than wild-type littermates. Moreover, overexpression of ACE2 in the central nervous system reduces oxidative stress and improves autonomic function in mice.
associated with fewer aortic atherosclerotic lesions and lower propensity to develop heart failure compared with wild-type mice, possibly as a consequence of reduced production of peroxynitrite in the heart and aorta. Lesser oxidative damage in AT1A-deficient animals is accompanied by the protection of proximal tubular cells from the loss of mitochondria during aging.46

The discovery of intramitochondrial angiotensin system provides new insights into the mechanisms contributing to senescence.48 Functional AT2 (mitochondrial AT2) receptors are expressed on the mitochondrial inner membrane and colocalize with endogenous Ang II. With age, mitochondrial AT2 receptor density decreases in renal tubular cells paralleled by increased expression of mitochondrial AT1 receptors. Of note, chronic administration of losartan prevents an age-related decrease of mitochondrial AT1 receptors.49

**Sirtuins and Longevity: Is RAS Dispensable?**

Sirtuins have been proposed to be regulators of aging and age-related diseases.49 They are nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylases highly conserved from *Escherichia coli* to humans and associated with mitochondrial and cell cycle regulation, apoptosis, DNA damage repair, and longevity.49 In mice and humans, seven different sirtuins (SIRT1-7) are described, and 3 of them are localized in the mitochondria (SIRT3–5). SIRT3 is so far the only sirtuin associated with longevity in humans, because a polymorphism in the enhancer region of the *Sirt3* gene that potentially upregulates the expression of the corresponding protein has been found in long-lived individuals.50 Under oxidative stress, SIRT3 overexpression protects cardiomyocytes against Bax-mediated apoptosis.51 SIRT3 also regulates adaptive thermogenesis and decreases mitochondrial membrane potential and ROS production, whereas increasing cellular respiration.52 For these reasons, SIRT3 is considered a sensor of ROS that could activate specific cellular signaling pathways to counteract oxidative stress through increased expression of manganese superoxide dismutase, a mitochondrial antioxidant protein.52

Cardiomyocytes from *Sirt3*-deficient mice exhibit increased levels of ROS, decreased ATP levels, and intolerance to oxidant stress.53 Hearts from *Sirt3*−/− mice, challenged with Ang II, develop more severe myocardial hypertrophy than wild-type animals.54 The role of SIRT3 in Ang II–induced cardiac hypertrophy is also supported by data showing that SIRT3 substrate NAD+ blocks hypertrophy by increasing kinase B1-AMP protein kinase signaling in the heart.55

A connection between aging and carcinogenesis has been proposed because the incidence of human malignancies increases as a function of aging. SIRT3 functions as a tumor suppressor because of its ability to preserve functional organelle integrity and to prevent the accumulation of oxidized molecules either spontaneously or after genotoxic stress. Silencing the *Sirt3* gene in tumor cell lines increases ROS production and activates hypoxia-inducible factor 1α, known to enhance tumor growth.56 In vivo, loss of SIRT3 in mice results in tumor-permissive phenotype as *Sirt3*−/− mice spontaneously develop mammary tumors at higher frequency than wild-type littermates and creates a permissive environment for in vivo carcinogenesis in the liver through the decrease of manganese superoxide dismutase.57

Among different targets, SIRT3 deacetylases and activates acetyl coenzyme A synthetase and glutamate dehydrogenase, which are required for ATP synthesis and metabolic pathways of glucose use.58 In conditions of diabetes mellitus or high-fat diet, low levels of SIRT3 in skeletal muscle and liver induce mitochondrial dysfunction and increase ROS production with consequent development of insulin resistance and obesity.59,60 Altogether, these data indicate that SIRT3 controls a regulatory network implicated in energy metabolism, which in turn is involved in life span determination (Figure 3). A direct effect of SIRT3 activation on life span prolongation remains to be elucidated, because no experimental evidence is available in genetically manipulated SIRT3 mice.

SIRT3 exerts its deacetylase action only in the presence of the substrate, NAD+, for which the concentration is
linked to cell well being. In the context of nutrient restriction, the expression of the nicotinamide phosphoribosyltransferase (Nampt) prosurvival gene is enhanced leading to the accumulation of its biosynthetic product, NAD+, which promotes cell survival via activation of SIRT3.\(^6\) These findings have stimulated studies on the role of Nampt and Sirt3 in the prolongation of life of AT1A−/− mice. Transcript levels of both Nampt and Sirt3 increase in the kidney of long-lived AT1A−/− mice compared with aged wild-type animals. Finding that candesartan prevents Ang II−induced Nampt and Sirt3 mRNA reduction in cultured tubular epithelial cells suggests that Ang II downregulates survival genes via the AT1A receptor. That silencing of the Nampt gene limits Ang II−induced reduction of Sirt3 mRNA would indicate a causative role of Nampt in modulating Sirt3 gene transcription in response to Ang II.\(^6\)

Scientists are beginning to look at the effects of other sirtuins on mammalian longevity, and the work on other members of the family is continuing apace. In this context, mice lacking SIRT6 age faster, whereas animals that overexpress SIRT6 have a prolonged life span through changes in insulin growth factor 1 signaling cascade, a key pathway in the prolongation of survival in rodents.\(^6\) Furthermore, Sirt7−deficient mice die prematurely and experience degenerative heart hypertrophy.\(^6\)

The effect of RAS activation and specifically the role of Ang II changes in SIRT6 and 7 genetically modified animals have not been addressed so far.

Concluding Remarks

Over the last few decades, a bulk of information indicates that Ang II represents a key molecule in physiological and pathological mechanisms of the heart, kidney, and brain. By promoting inflammation, cell growth, and ROS generation at cellular and mitochondrial levels, Ang II regulates energy metabolism and affects the onset and the progression of cell senescence. Chronic activation of RAS promotes end-stage organ injury associated with aging by increasing tissue and mitochondrial oxidative stress, justifying the use of ACEis and ARBs, which reduce age-associated cardiovascular, renal, and brain damage and preserve mitochondria function. Results of studies of genetically manipulated animals indicate a role for the AT receptor in aging, because targeted disruption of the gene encoding AT1A receptor in mice alleviates the aging-like phenotype of the heart and kidney by reducing the oxidative stress and upregulating SIRT3. Consistent with these experimental data are recent findings showing the presence of 2 genetic variants in the promoter region of the human AT1 receptor gene that are associated with human longevity and result in low levels of AT1 receptor on blood cells.\(^6\) The beneficial effect of ACEis or AT1 blockers on age-associated disabilities and diseases offers the rationale that such drugs could prolong life in humans. Sirtuins have intrigued scientists involved in age-associated disorders and longevity studies since the late 1990s. Increasing interest in aging has been raised by SIRT3 because of its mitochondrial localization and the ability to suppress ROS and to be modulated by Ang II. In light of the importance of mitochondrial ROS production in the onset and progression of diverse diseases associated with aging, as well as the protective role of SIRT3 on heart, kidney, and cancer development, the search for SIRT3 activators could represent a challenge for aging research. This achievement would allow us to definitively address whether the activation of SIRT3 can offer a therapeutic strategy for postponing age-associated chronic diseases, reducing the most frequent causes of death and possibly extending the human life span.

Acknowledgments

We are greatly indebted to Prof Michael Goligorsky for English language editing.

Sources of Funding

The research was partially supported by Fondazione ART per la Ricerca sui Trapianti ONLUS (ART, Milan, Italy).

Disclosures

None.

References


Aging and the Renin-Angiotensin System
Sara Conti, Paola Cassis and Ariela Benigni

*Hypertension*, published online August 27, 2012:

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2012/08/27/HYPERTENSIONAHA.110.155895.citation