

DEBRA I. DIZ, PhD*

Hypertension and Vascular Disease Center
Wake Forest University School of Medicine
Winston-Salem, NC

SHERRY O. KASPER, PhD*

Hypertension and Vascular Disease Center
Wake Forest University School of Medicine
Winston-Salem, NC

ATSUSHI SAKIMA, MD*

Hypertension and Vascular Disease Center
Wake Forest University School of Medicine
Winston-Salem, NC

CARLOS M. FERRARIO, MD*

Hypertension and Vascular Disease Center
Wake Forest University School of Medicine
Winston-Salem, NC

Aging and the brain renin-angiotensin system: Insights from studies in transgenic rats

Aging is characterized by increased systolic blood pressure resulting from activation of the sympathetic nervous system, reduced vagal activity, and reduced vascular distensibility. Imbalances in sympathetic and parasympathetic outflow to vessels, heart, kidney, and other organs contribute to the increase in systolic blood pressure as well as the associated impairment in the gain of the baroreceptor reflex. The reduced heart rate variability linked to increased mortality in patients with cardiovascular disorders could be attributed in part to impaired reflex function. Alterations in reflex control of autonomic outflow may also contribute to the constellation of cardiovascular and metabolic changes known to accompany hypertension, especially during aging. Understanding the factors that regulate the function of the brainstem areas controlling autonomic outflow during aging is critical as the elderly proportion of the population continues to increase.

■ PROTECTIVE EFFECTS OF RENIN-ANGIOTENSIN SYSTEM BLOCKADE DURING AGING

One factor with a close anatomic association with both the sympathetic and parasympathetic limbs of the autonomic nervous system is the renin-angiotensin system (RAS).¹ Abundant evidence of functional interactions between the RAS and the autonomic nervous system at sites in the brain and periphery provides a strong rationale for therapeutic interventions involving RAS blockade during aging. Indeed, RAS blockade extends lifespan and improves or prevents age-related deficits in cardiovascular and metabolic function in rats.^{2,3} Multiple benefits are derived from long-term inhibition of angiotensin-converting enzyme (ACE) or angiotensin II type 1 (AT₁) receptor blockade in normotensive rats at doses

that do not lower blood pressure at the outset of treatment in early adulthood: attenuation of the age-related decline in cognitive function, the increase in body weight gain, and the decline in mitochondrial function, as well as preservation of renal function.²⁻⁸

However, during aging there is a decrease in plasma renin activity and circulating angiotensin (Ang) peptide levels,⁹⁻¹¹ which raises questions about whether the beneficial actions of RAS blockade may signal a role of excess Ang II in tissue rather than plasma Ang II. It is well known that local tissue renin-angiotensin systems exist in a variety of tissues and organs, including the brain, kidney, heart, pancreas, and adrenal gland.^{12,13} The brain cardiovascular nuclei influencing many of the age-related changes in the autonomic system exhibit high levels of AT₁ receptors and other RAS components.¹⁴⁻¹⁷

■ THE BRAIN RENIN-ANGIOTENSIN SYSTEM AND AGING IN THE RAT

To understand whether the brain RAS plays a role in the aging process with respect to metabolic and cardiovascular function, our recent studies compared Sprague-Dawley (SD) rats with transgenic rats that have a deficiency in brain angiotensinogen (ASrAogen rats). The ASrAogen transgenic rats have a glial fibrillary acidic protein (GFAP) promoter-linked angiotensinogen antisense sequence overexpressed in brain glia.¹⁸ Since glia are the major source of angiotensinogen in the brain, this “knockdown” reduces cerebral levels of angiotensinogen to less than 10% of normal.^{18,19} ASrAogen rats have slightly lower values of resting arterial pressure but otherwise exhibit similar circulating levels of leptin, insulin, and glucose in early adulthood.²⁰ SD rats demonstrate an increase in systolic blood pressure during aging that is associated with increased circulating insulin and leptin levels.²⁰ There is also activation of the intrarenal RAS in SD rats during aging, as demonstrated by increases in excretion of urinary Ang peptides, preceding the increase in systolic pressure in these animals.¹⁰

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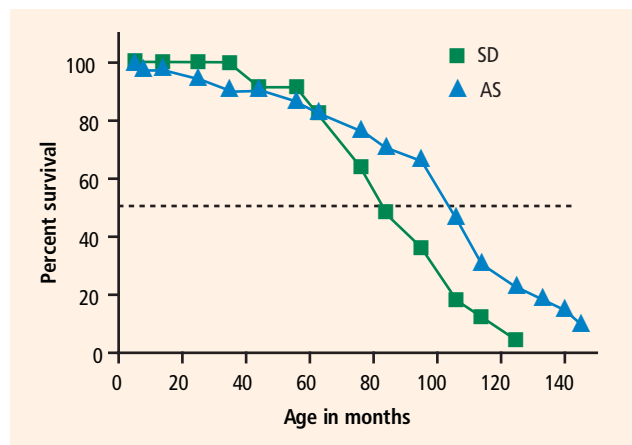


FIGURE 1. Comparative lifespans of Hannover Sprague-Dawley (SD) and ASrAogen transgenic (AS) rats.

Renin-angiotensin system blockade prolongs survival

That the brain RAS plays a key role in these age-related changes is supported by the remarkable finding that ASrAogen rats do not exhibit increases in systolic pressure and maintain a lower body weight relative to SD rats over the same time span of aging.²⁰ Moreover, ASrAogen rats experience no increase in insulin or leptin during the aging process²⁰ and no activation of the intrarenal RAS, as evidenced by maintenance of a low level of excretion of Ang peptides.¹⁰ Lifespan is also increased by approximately 30% in ASrAogen rats relative to the SD control strain (**Figure 1**), to a length comparable to that seen in rats receiving lifetime treatment with an ACE inhibitor or an AT₁ receptor blocker begun in early adulthood.^{2,3} These findings—that selective knockout of the glial angiotensinogen source has minimal effects at an early age but mitigates many age-related pathologies and mimics almost completely the effects of long-term RAS blockade in humans and rats—strongly argue that it is blockade of the brain RAS that makes a major contribution to the beneficial effects.

Ang II and Ang-(1-7) in baroreflex regulation

Impairment in the gain of the baroreflex to levels comparable to those seen in overt hypertension accompanies the increase in systolic pressure in older humans, which is similar to observations in the older SD rats in our studies (**Figure 2**). At the level of the nucleus tractus solitarius (nTS) in the dorsal medulla oblongata, Ang II is known to provide tonic inhibition of the sensitivity of the baroreceptor reflex control of heart rate, a vagally mediated component of the reflex control of arterial pressure.^{15,16} This effect of

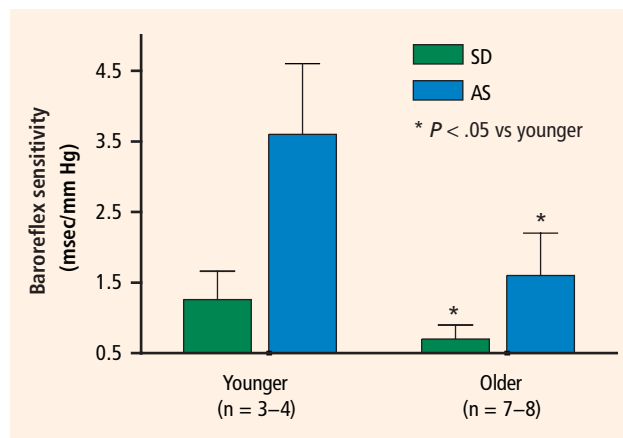


FIGURE 2. Baroreflex sensitivity in conscious Hannover Sprague-Dawley (SD) and ASrAogen (AS) transgenic rats at younger (~15 weeks) and older (~65 weeks) ages.

Ang II is counteracted within the nTS, in part, by Ang-(1-7) (**Figure 3**). Ang-(1-7) is another active component of the RAS that opposes many of the actions of Ang II in the brain and systemic circulation, and increases in Ang-(1-7) contribute to the beneficial actions of ACE inhibition and AT₁ receptor blockade.^{16,21-23} A deficit in endogenous Ang-(1-7) at the level of the nTS may be one mechanism for the impairment in the reflex in the SD rats during aging.²⁴

Figure 4, adapted from data reported by Sakima et al.,²⁴ demonstrates that in older SD rats, blockade of endogenous Ang-(1-7) by the receptor antagonist D-Ala⁷-Ang-(1-7) does not inhibit the sensitivity of the baroreflex, in contrast to what is seen in younger SD rats. These data reveal that facilitation of the reflex by endogenous Ang-(1-7) is reduced or absent in older animals. On the other hand, there is no difference between older and younger rats in the response to blockade of endogenous Ang II by an AT₁ receptor antagonist (**Figure 4**).

Whether these observations on blockade of Ang II and Ang-(1-7) hold true for older ASrAogen rats is not known, but there is a decline in reflex function in the transgenic animals at the older age (**Figure 2**). However, note in **Figure 2** that the reflex sensitivity in the older ASrAogen rats, while lower than that in their younger counterparts, is comparable to that seen in the younger normotensive SD rats. Therefore, the functional impact of the decline in reflex sensitivity in the ASrAogen rats is hard to assess at this stage of our investigations.

As shown above, Ang-(1-7) and Ang II initiate opposing actions in baroreceptor reflex regulation of heart rate at the level of the nTS. If an imbalance in

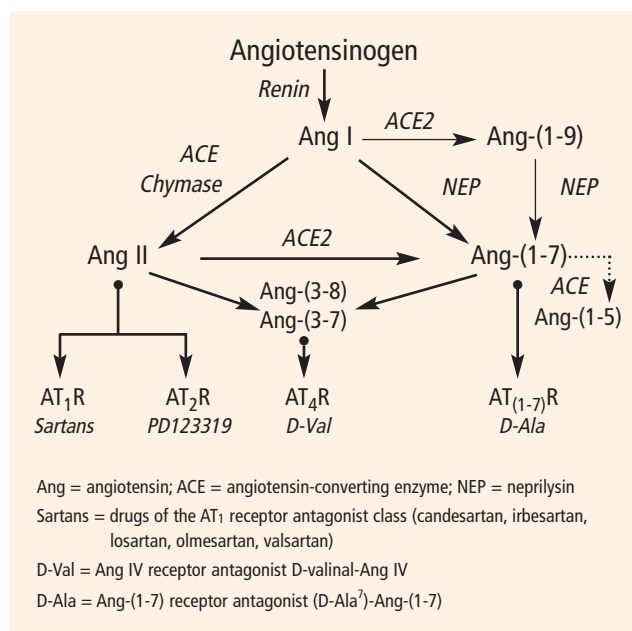


FIGURE 3. Diagram of renin-angiotensin system processing pathways and receptors for active peptides.

these two peptides contributes to the changes in reflex function seen during aging, then an understanding of the factors that regulate the levels of these peptides and their receptors during aging in brainstem areas controlling sympathetic outflow is of considerable importance. Little is known about the regulation of the local RAS within each tissue during aging. There may be overt elevation of components of the system, as reported for Ang II in the heart during aging,¹¹ consistent with observations on the intrarenal RAS.¹⁰ Preliminary studies from our laboratory reveal that reductions in enzymes in the dorsal medulla oblongata, such as neprilysin and ACE2, may be responsible for reduced formation of Ang-(1-7) in older animals. The mRNA for neprilysin and ACE2 is lower in older SD and ASrAogen rats, respectively, than in younger animals of the same strains.²⁵ This may indicate that rather than frank elevation of the early precursor substrate or initial enzymes of the system, a shift in the processing favoring Ang II at the expense of Ang-(1-7) could underlie the development of age-related pathologies. This hypothesis is currently under investigation.

■ A LINK WITH AGING-RELATED METABOLIC CHANGES

The above studies focused on the age-related changes that occur in nTS pathways involved in autonomic function directed toward baroreflex function. It must be emphasized that these same brain areas are

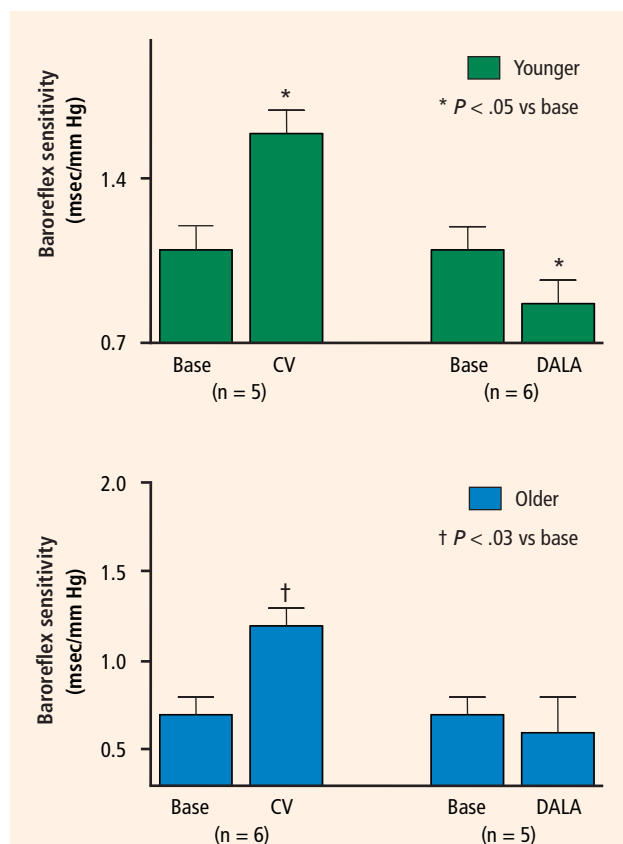


FIGURE 4. Baroreflex sensitivity in anesthetized Hannover Sprague-Dawley rats at younger (~15 weeks) and older (~70 weeks) ages before (Base) and after microinjection of either the AT₁ receptor antagonist candesartan (CV) or the Ang-(1-7) receptor antagonist (D-Ala⁷)-Ang-(1-7) (DALA) into the nucleus tractus solitarius. Adapted from data published by Sakima et al.²⁴

involved in control of appetite and body energy metabolism. A variety of peptide transmitters, including those identified as part of the gut-brain connection, as well as insulin and leptin, act at receptors on vagal sensory and motor fibers in the periphery as well as within the nTS and other dorsal medullary sites to influence food intake and satiety and contribute to regulation of autonomic outflow.²⁶⁻³⁰ Of particular significance is the report that the forms of the leptin receptor and melanocortin receptor present in the dorsal medulla are similar to those in the hypothalamic centers known for regulation of temperature, ingestive behaviors, and energy metabolism.^{30,31} Our early studies showed that the distribution of Ang II receptors in brainstem nuclei clearly overlapped the distribution of the entire vagal sensory and motor pathways, and was not confined to vagal fibers associated solely with the cardiopulmonary system.^{16,32,33} As reviewed previously, the anatomic distribution pat-

tern suggested a widespread influence of the RAS on autonomic function.^{1,34} Thus, we propose that the alterations in the brain RAS during the aging process, including changes in the balance of actions between Ang II and Ang-(1-7) in brain nuclei of the dorsal medulla, may provide a link between impairments in autonomic reflex function and the metabolic changes of aging.

REFERENCES

1. Diz DI, Averill DB. Angiotensin II/autonomic interactions. In: Robertson D, Biaggioni I, Burnstock G, Law PA, eds. *Primer on the Autonomic Nervous System*. San Diego, CA: Elsevier Academic Press; 2004:168–171.
2. Basso N, Paglia N, Stella I, et al. Protective effect of the inhibition of the renin-angiotensin system on aging. *Regul Pept* 2005; 128:247–252.
3. Linz W, Jessen T, Becker RHA, Scholkens BA, Wiemer G. Long-term ACE inhibition doubles lifespan of hypertensive rats. *Circulation* 1997; 96:3164–3172.
4. Ferder L, Inzeria F, Romano L, Ercole L, Pszeny V. Effects of angiotensin-converting enzyme inhibition on mitochondrial number in the aging mouse. *Am J Physiol Cell Physiol* 1993; 34:C15–C18.
5. de Cavanagh EMV, Piotrkowski B, Basso N, et al. Enalapril and losartan attenuate mitochondrial dysfunction in aged rats. *FASEB J* 2003; 17:1096–1098.
6. Gilliam-Davis S, Payne VS, Kasper SO, Robbins ME, Diz DI. Differential regulation of circulating and intra-renal renin-angiotensin systems and improved metabolic function in Fisher 344 rats after long-term AT1 receptor blockade. *Hypertension* 2005; 46:818. Abstract.
7. Kasper SO, Basso N, Kurnjek ML, et al. Divergent regulation of circulating and intrarenal renin-angiotensin systems in response to long-term blockade. *Am J Nephrol* 2005; 25:335–341.
8. Carter CS, Cesari M, Ambrosius WT, et al. Angiotensin-converting enzyme inhibition, body composition, and physical performance in aged rats. *J Gerontol A Biol Sci Med Sci* 2004; 59:416–423.
9. Anderson S. Ageing and the renin-angiotensin system. *Nephrol Dial Transplant* 1997; 12:1093–1094.
10. Oden SD, Ganten D, Ferrario CM, Chappell MC, Diz DI. Rats with low brain angiotensinogen maintain normal levels of insulin and components of the circulating and intra-renal renin-angiotensin systems during aging. *FASEB J* 2004; 18:A738. Abstract.
11. Groban L, Pailles NA, Bennett CDL, et al. Growth hormone replacement attenuates diastolic dysfunction and cardiac angiotensin II expression in senescent rats. *J Gerontol A Biol Sci Med Sci* 2006; 61:28–35.
12. Chappell MC, Diz DI, Gallagher PE. The renin-angiotensin system and the exocrine pancreas. *JOP (online)* 2001; 2:33–39.
13. Bader M, Peters J, Baltatu O, Muller DN, Luft FC, Ganten D. Tissue renin-angiotensin systems: new insights from experimental animal models in hypertension research. *J Mol Med* 2001; 79:76–102.
14. Brosnihan KB, Diz DI, Schiavone MT, Averill DB, Ferrario CM. Approaches to establish angiotensin II as a neurotransmitter. In: Buckley JP, Ferrario CM, eds. *Brain Peptides and Catecholamines in Cardiovascular Regulation*. New York, NY: Raven Press; 1987:313–328.
15. Averill DB, Diz DI. Angiotensin peptides and baroreflex control of sympathetic outflow: pathways and mechanisms of the medulla oblongata. *Brain Res Bull* 2000; 51:119–128.
16. Diz DI, Jessup JA, Westwood BM, et al. Angiotensin peptides as neurotransmitters/neuromodulators in the dorsomedial medulla. *Clin Exper Pharmacol Physiol* 2002; 29:473–482.
17. Diz DI. Approaches to establishing angiotensin II as a neurotransmitter revisited. *Hypertension* 2006; 47:334–336.
18. Schinke M, Baltatu O, Bohm M, et al. Blood pressure reduction and diabetes insipidus in transgenic rats deficient in brain angiotensinogen. *Proc Natl Acad Sci U S A* 1999; 96:3975–3980.
19. Huang BS, Ganten D, Leenen FH. Responses to central Na(+) and ouabain are attenuated in transgenic rats deficient in brain angiotensinogen. *Hypertension* 2001; 37:683–686.
20. Kasper SO, Carter CS, Ferrario CM, et al. Growth, metabolism, and blood pressure disturbances during aging in transgenic rats with altered brain renin-angiotensin systems. *Physiol Genomics* 2005; 23:311–317.
21. Ferrario CM, Chappell MC, Tallant EA, Brosnihan KB, Diz DI. Counter-regulatory actions of angiotensin-(1-7). *Hypertension* 1997; 30:535–541.
22. Chappell MC, Modrall JG, Diz DI, Ferrario CM. Novel aspects of the renal renin-angiotensin system: angiotensin-(1-7), ACE2 and blood pressure regulation. In: Suzuki H, Saruta T, eds. *Kidney and Blood Pressure Regulation*. Basel, Switzerland: Karger; 2004:77–89.
23. Iyer SN, Chappell MC, Averill DB, Diz DI, Ferrario CM. Vasodepressor actions of angiotensin-(1-7) unmasked during combined treatment with lisinopril and losartan. *Hypertension* 1998; 31:699–705.
24. Sakima A, Averill DB, Gallagher PE, et al. Impaired heart rate baroreflex in older rats. Role of endogenous angiotensin-(1-7) at the nucleus tractus solitarius. *Hypertension* 2005; 46:333–340.
25. Gegick S, Garcia-Espinosa MA, Gallagher PE, et al. Reduced formation of Ang-(1-7) by ACE2 in dorsal medulla oblongata of Sprague-Dawley (SD) and ASrAogen rats during aging [abstract]. *FASEB J* 2006; 20:A1209.
26. Giza BK, Scott TR, Vanderweele DA. Administration of satiety factors and gustatory responsiveness in the nucleus tractus solitarius of the rat. *Brain Res Bull* 1992; 28:637–639.
27. Elmquist JK, Ahima RS, Maratos-Flier E, Flier JS, Saper CB. Leptin activates neurons in ventrobasal hypothalamus and brainstem. *Endocrinology* 1997; 138:839–842.
28. Diz DI, Barnes KL, Ferrario CM. Functional characteristics of neuropeptides in the dorsal medulla oblongata and vagus nerve. *Fed Proc* 1987; 46:30–35.
29. Berthoud HR. A new role for leptin as a direct satiety signal from the stomach. *Am J Physiol Regul Integr Comp Physiol* 2005; 288: R796–R797.
30. Schwartz GJ, Moran TH. Leptin and neuropeptide Y have opposing modulatory effects on nucleus of the solitary tract neurophysiological responses to gastric loads; implications for the control of food intake. *Endocrinology* 2002; 143:3779–3784.
31. Ellacott KLJ, Cone RD. The central melanocortin system and the integration of short- and long-term regulators of energy homeostasis. *Recent Prog Horm Res* 2004; 59:395–408.
32. Diz DI, Barnes KL, Ferrario CM. Contribution of the vagus nerve to angiotensin II binding in the canine medulla. *Brain Res Bull* 1986; 17:497–505.
33. Diz DI, Block CH, Barnes KL, Ferrario CM. Correlation between angiotensin II binding sites and substance P in the canine brain stem. *J Hypertension* 1986; 4(Suppl 6):S468–S471.
34. Diz DI. Commentary: angiotensin II receptors in central nervous system physiology. In: Husain A, Graham R, eds. *Drugs, Enzymes and Receptors of the Renin Angiotensin System: Celebrating a Century of Discovery*. Amsterdam: Harwood Academic Publishers; 1999:45–51.

Address: Debra I. Diz, PhD, Hypertension and Vascular Disease Center, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157; ddiz@wfubmc.edu.