

Angiotensin II and Aging

Manuel Martínez-Maldonado, León F. Ferder

Departments of Physiology, Pharmacology and Medicine.
Ponce School of Medicine. Ponce, Puerto Rico.

INTRODUCTION

Organ malfunction is an inevitable result of the natural process of aging which all species undergo. Modifications in organ structure directly related to aging cause functional deterioration of varying degrees in humans by, among other things, leading to replacement of functional parenchyma by fibro-connective tissues and induction of arteriosclerosis in the blood vessels of several organs. Age related changes in kidney function, including its role as an endocrine organ, have been recognized since the seventies. These changes include alterations in the regulation of the renin-angiotensin system (decreased renin production and secretion), and in the endocrine and paracrine functions of their product angiotensin II (All), both of which are dependent on angiotensin II receptors^{1,2}. We advance herein the hypothesis that aging, and possibly hypertension and its consequences, including cardiac hypertrophy, result from actions of All.

ANGIOTENSIN RECEPTORS

The AT₁ receptor belongs to the G protein-coupled receptor superfamily and has been cloned and characterized^{3,4}. The AT₁ receptor is considered to be the mediator for the cardiovascular and renal effects of All in normal and, possibly, aging subjects. In contrast, the role, if any, of the AT₂ receptor, highly expressed in growing tissues, in the cardiovascular changes in normal and aging subjects, remains unclear⁵⁻⁷. All receptors show a great variability and can be detected in a wide variety of tissues. It is difficult, therefore, to relate them to primary hypertension and their role has not been extensively explored in the aging process. Two AT₁ receptor subtypes appear to exist in rodents, the AT_{1A} and AT_{1B} which exhibit highly homologous sequences and similar binding and functional characteristics⁸. AT_{1A} expression is by far the dominant form in liver, kidney, vasculature and heart whereas the AT_{1B} is expressed predominantly in the adrenal gland, uterus and anterior pituitary gland⁹. Both are down-regulated by All.

Complementary mechanisms exist for All down regulation of the cardiac AT₁ gene *in vitro* via calcium- and cAMP-dependent mechanisms.

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Such a downregulation of the receptor gene by its agonist is consistent with other members of the G-protein coupled family of receptors. The AT_2 receptor, however, does not appear to be G-protein coupled and possibly signals through phosphotyrosine phosphatase¹⁰. All receptor subtypes have been characterized in cardiac and renal tissue. The AT_{1A} and AT_{1B} receptors presents highly homologous sequences, and similar binding and functional characteristics^{3, 8, 11}. In the rat heart, levels of both AT_1 and AT_2 receptor expression are increased during the neonatal period and decrease with maturation^{6, 12}.

This family of receptors typically responds to long-term agonist binding with a decrease in receptor number and mRNA levels¹³⁻¹⁶. All treated mesangial cells also demonstrate a dose-dependent decrease in AT_1 mRNA levels similar to that seen in cardiocytes and fibroblasts, whereas in some studies All infused *in vivo* had no effect on the AT_1 mRNA levels in the rat kidney or aorta, although mRNA levels were increased in the adrenal gland^{17, 18}. By contrast, in models of experimental ureteral obstruction^{19, 20}, which leads to high intrarenal levels of All, AT_1 mRNA levels are downregulated, and this downregulation is partially reversed by angiotensin I converting inhibitors (ACEI) and angiotensin II receptor antagonists (AIIIRA), suggesting that All participates in gene regulation and the underlying pathophysiological conditions may determine the nature of the relationship between the peptide and the expression of the gene for its receptor. Moreover, in ureteral obstruction, both glomerular, and tubular cells exhibit the changes in AT_1 mRNA levels. This tissue- and cell-specific regulation of the AT_1 gene likely speaks to the potential differences in the role of All in different segments of various tissues²¹.

The high susceptibility of cardiomyocytes to the effects of All and aging may be the result of

the density of receptors at this site. Quantitative PCR studies in the rat have found that both AT_1 receptor subtypes are present in the heart with AT_{1A} mRNA levels three-fold higher than AT_{1B} ²².

Analysis of both the number and binding affinity of specific receptors appears to be important in understanding the pathophysiological role of the renin-angiotensin system and in the potential role of All in the heart and kidney in hypertension and aging. Nevertheless, as already mentioned, variation in All receptor expression, complicates the design of studies relating it to primary hypertension, while studies of its potential role in aging are just beginning to emerge^{23, 24}.

ANGIOTENSIN II AND REACTIVE OXYGEN SPECIES IN AGING

Experimental findings indicate that hypertension and aging have similar effects on the structure and function of blood vessels, the heart and, presumably, the kidney. Both conditions also lead to endothelial dysfunction, decreased vascular compliance, left ventricular hypertrophy and stiffness. In the normal kidney, despite no change in blood pressure, All leads to the expression of genes of sclerosis-generating cytokines such as transforming growth factor beta ($TGF\beta 1$), platelet-derived growth factors (PDGF) and osteopontin (OPT), all which enhance tissue fibrosis²⁵.

Despite the fact that aging is associated with decreased production and secretion of renin, kidneys are more sensitive to the renal vasoconstrictive effects of exogenous All²⁶ suggesting an increase in receptor number, greater binding affinity of the peptide or diminished metabolism of the peptide-receptor complex. Similar changes might be expected in the aging kidney that could explain why ACEI or AIIIRA mitigate

some of the functional and architectural renal changes observed as age advances.

All has been suggested as a mediator of oxidative stress through the induction of reactive oxygen species (ROS) in aging and in normal animals²⁷. Oxidative stress is known to induce apoptosis and activation of the pro-inflammatory transcription factor nuclear factor kappa B (NFκB), whose biologic effects may play a role in the renal damage associated with arterial hypertension²⁸. Oxidative stress, fueled in part by All, up-regulates the expression of adhesion molecules, chemoattractant compounds and cytokines²⁹.

Mitochondria are a major source of ROS in aging because their mechanisms of defense from normally formed ROS and superoxide become less effective with the aging process. Moreover, excessive respiratory chain superoxide can combine with nitric oxide (NO) produced from mitochondrial nitric oxide synthase (NOS) to induce apoptosis through the formation of peroxynitrate. This potent oxidant can promote cytochrome C release by increasing the permeability of mitochondrial membranes by opening up permeability transition pores³⁰. While All contributes to ventricular remodeling by promoting both cardiac hypertrophy and apoptosis, the mechanism underlying the latter phenomenon is poorly understood. One possibility that has been advanced is that All activates NADPH oxidase, generating free radicals that trigger DNA damage and apoptosis³¹. All blockade can protect against both age-related mitochondrial dysfunction and ultrastructural alterations, underscoring the role of RAS in the aging process²⁷.

It has been suggested that the adaptor protein p66^{Shc} may also be a target for ROS³². When this protein is phosphorylated on Ser36 by oxidative stress, it markedly sensitizes cells to apoptosis, because it participates in the phos-

phorylation-induced repression of Forkhead transcription factors that regulate the expression of various antioxidant enzymes³². Furthermore, activation of a mitochondrial pool of p66^{Shc} leads to enhanced generation of ROS³³. Although the study did not examine the effect of All on this potential pathway of oxidative damage in aging, it is conceivable that the same mechanism may apply to aging tissues, including the kidney. It is of great interest that p66^{Shc} knock-out mice were resistant to the deleterious cardiac effects (remodeling) of sub-pressor doses of All³⁴. It remains to be studied whether a similar effect of p66^{Shc} occurs in renal tissue.

AGING RESPONSE TO ALL BLOCKADE

It has long been recognized that plasma renin and aldosterone level decrease with advancing age. Studies in aging animals indicate that both renal renin formation and release are reduced, and contribute to the fall in plasma renin concentration. The significance of this finding has remained obscure and its complexity is enhanced by the fact that elderly patients develop hypertension, which could be a factor in the reduced activity of the RAS. Even more intriguing are the findings reported by experiments in one of our laboratories in the attempt to explain the importance of falls in circulating levels of renin and angiotensin.

It was found that CF1 mice treated with the angiotensin I converting enzyme blocker Enalapril and Wistar rats treated with an AIIRA exhibited a reduction of age-associated cardiovascular and renal changes, and had increases in the mitochondrial number within cells. This was associated with an increase in survival and lifespan of the animals. The beneficial effect of RAS blockade occurs in spite of the fact that, as reported, plasma renin concentration and intrarenal renin

mRNA is reduced in older animals³⁵. It is, however, consistent with an increase in angiotensin II formation or action within the kidney³⁶, and supports the concept that the intrarenal RAS is regulated independently of the circulating system in normal and also in aging animals.

Our studies revealed that plasma levels of angiotensin I and angiotensin II were low in control (untreated) aged animals and that, as expected in non-aged normal animals, aged rats receiving AIIRA exhibited elevated levels of plasma renin, AI and AII, an indication that the angiotensin-renin feedback mechanism is intact in aged rats. Aging was accompanied by high glomerular angiotensin II receptor density as demonstrated by autoradiography. By contrast, receptor density was much lower in the interstitium of untreated aged animals, and was lower in both glomeruli and interstitium in the losartan-treated group³⁷.

These results strongly suggest that the renin angiotensin system plays an important role in the mechanisms by which aging affects renal function and that an abnormal regulation of angiotensin II receptors may be responsible for the functional alterations of the kidney. Moreover, AI effects through the production of reactive oxygen species and by damage of mitochondrial function can be responsible for these alterations. This hypothesis is supported by our findings that ACE inhibitors and AIIRA decrease tissue oxidative state, improve mitochondrial number and function, leading to higher animal survival and prolonged lifespan (Figure 1).

VASCULAR AND CARDIAC AII AT₁ AND AT₂ RECEPTORS

The percentages of AT₁ and AT₂ receptors vary depending on the cell type or tissues under consideration. In rat aorta, 60% of the receptors

are AT₁, whereas in total rat heart, the percentage of AT₁ reaches 90%³⁸ of the angiotensin receptors. Cardiomyocytes express exclusively the AT₁ subtype³⁹, whereas fibroblasts express AT₂ receptor subtype as well⁴⁰. Although it has been suggested that AT₂ receptor activation is involved in the control of cell differentiation, proliferation and apoptosis^{41,42}, the possible roles of AT₂ receptors *in vivo* are poorly understood. In addition, their contribution to the pathophysiology of aging remains obscure (see below). Moreover, limited knowledge exists of the state of function and genetic regulation of AT receptors and their subtypes in renal tissues of aging animals.

AI-induced hypertension and associated cardiac hypertrophy are mediated mainly via the AT₁ receptor subtype. That AI mediates vascular smooth muscle cell (VSMC) trophic effect via AT₂ receptor subtype and independently of a pressure-dependent mechanism can be inferred from the finding that AT₂ receptor blockade with PD123319 infusion in AI-treated rats had no significant effect on blood pressure but prevented the development of vascular hypertrophy of both aorta and coronary arteries. In addition, in normotensive rats, treatment with losartan alone had no effect on blood pressure but induced a medial hypertrophy that was prevented by an additional treatment with PD123319 suggesting that the increase in systemic AI concentration, as a result of AT₁ receptor blockade⁴³ activates the AT₂ receptors and, as a consequence, unmasks their trophic effect. The trophic effect of the AT₂ receptor subtype appears to be specific to VSMCs and independent of whether it is found in conductive (aorta) or resistance (coronary artery) vessels. Also, it has been shown that AT₂ receptors play a major role in myointimal formation after arterial injury⁴⁴ and that the expression of AT₂ receptors is increased

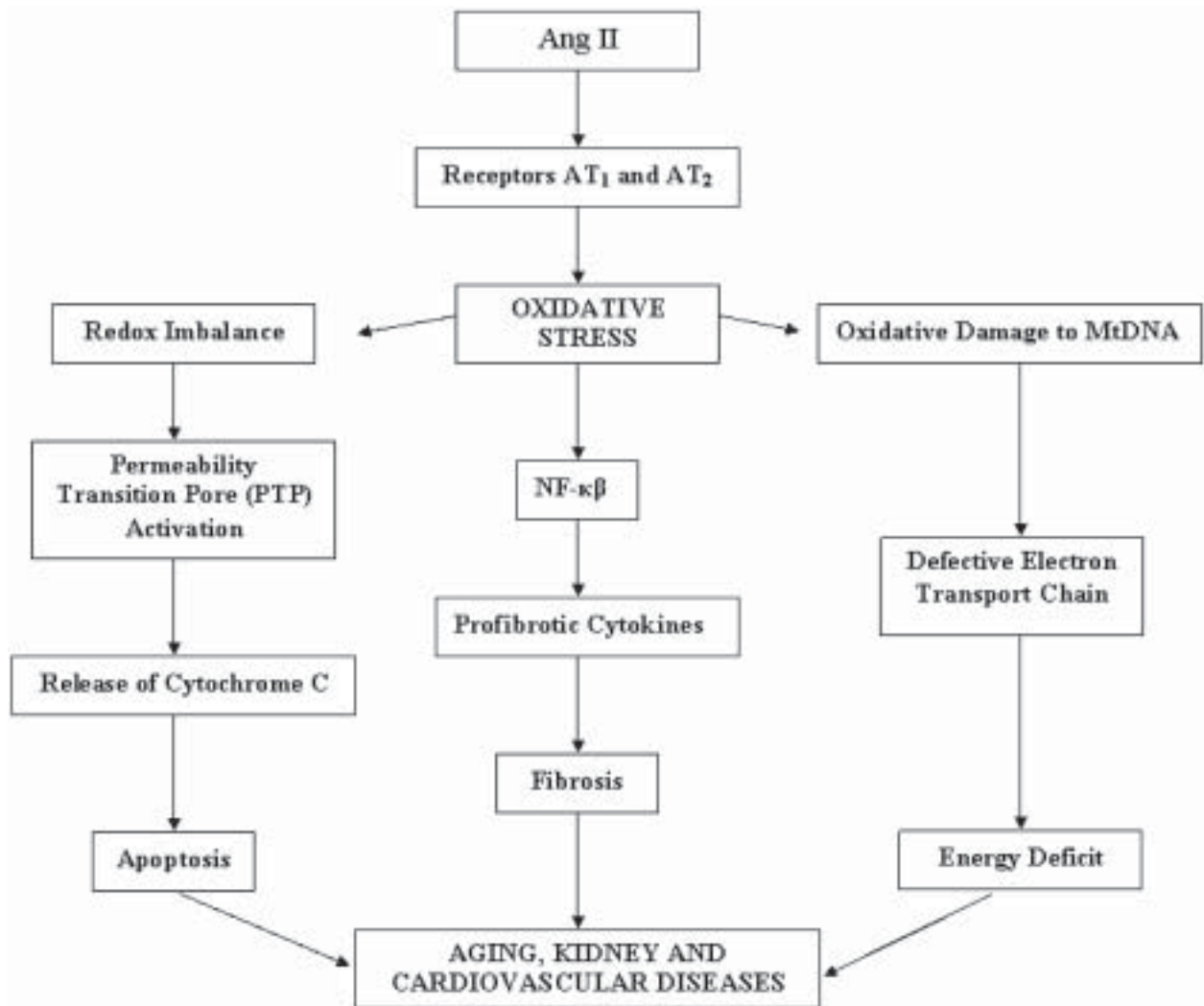


Figura 1

in hypertrophied left ventricle⁴⁵. The role, if any, of this interactive regulation between AT₁ and AT₂ receptors in aging tissues (whether accompanied or not by hypertension), particularly the kidney, remains to be established.

The relative abundance of AT₂ subtype receptors in many fetal tissues supports a role of AT₂ during development⁴⁶. Furthermore, a key role for the AT₂ subtype receptor is suggested by several studies that correlate en-

hanced AT₂ receptor expression with cardiovascular system disease states such as diabetes, hypertension and senescence⁴⁷⁻⁴⁹. The ability of a tissue to change the expression of AT₁ receptors to AT₂ has been described in experimentally induced vascular injury²¹, suggesting that Ang II may also play a role, through AT₂ receptors, in smooth muscle cell differentiation and proliferation. In addition, Brilla et al.⁴⁰ reported that in cultured adult rat cardiac fibro-

blasts All stimulates collagen synthesis by both AT₁ and AT₂ receptors, and that All inhibition of collagenase activity is specifically mediated by the AT₂ subtype receptor.

Chronic pharmacological blockade of AT₂ subtype All receptors has no systemic hemodynamic (arterial pressure, cardiac output, heart rate) either in normotensive rats or in All-induced hypertensive rats, and does not affect the hypertension-induced cardiac hypertrophy⁵⁰. Furthermore, plasma angiotensin II level and aortic reactivity to All are not affected after chronic AT₂ receptor blockade. Chronic blockade of AT₂ receptors antagonizes the vascular growing effects related to long term All injection, whereas blockade of AT₁ receptors does not. The vasotropic effect of All is at least partially mediated via AT₂ receptor subtype in some experimental model of hypertension.

Considering All's role in the induction of cardiac hypertrophy and possibly fibrosis of tissues, such a mechanism to decrease receptor number could be essential for maintaining normal cardiac structure and function. A similar proposal could be marshalled for the kidney. The protective effects of All blockade, by either diminishing its production or its actions, on fibrosis and proliferation is indirect evidence that, despite low angiotensin II production, increased sensitivity of the kidney to the effects of the hormone could mediate the development of fibrosis and tissue damage in aging, hypertension or aging accompanied by hypertension.

AGING AND ENHANCED CARDIAC EXPRESSION OF ANGIOTENSIN II RECEPTOR SUBTYPES

In the heart and the kidney, the presence of angiotensinogen (ANG), renin, and angiotensin-converting enzyme (ACE) suggests local syn-

thesis of All^{51,52}. In young adult rats, the myocardium is able to synthesize All from angiotensinogen and angiotensin-converting enzyme⁵¹, but it has been shown that a several-fold increase in both ANG and ACE mRNA occurs in the aged LV but not in the RV. This suggests that during aging, an activation of cardiac All synthesis may compensate in part for the depression of the circulating RAS. The possibility cannot be discarded that a similar mechanism exists to maintain normal intrarenal resistances and glomerular filtration fraction in the aging kidney.

Although less is known relative to the kidney, senescence is associated with marked changes in cardiac structure and morphology such as cardiomyocyte loss, hypertrophy of the remaining cells, and the development of fibrosis^{53,54}. These changes may account for the functional characteristics of the senescent myocardium such as impaired myocardial perfusion⁵⁵, altered diastolic compliance⁵³, and arrhythmias⁵⁶. Vascular structures are also modified, and aging is associated with increased arterial wall stiffness, as shown by a significant decrease in systemic and local arterial compliance and an increase in aortic impedance^{53,57}, which may induce mild left ventricular hypertrophy. There is now evidence that senescence is associated with increased cardiac ANG and ACE gene expression, suggesting increased cardiac All synthesis, whereas plasma RAS activity, as already mentioned, is largely depressed⁵⁸.

The presumed mechanistic pathways involved in the increase in cardiac All receptor gene expression are multiple. Up-regulation could be intrinsic to the developmental gene reprogramming often associated with senescence. Cardiac senescence is characterized by the reexpression of fetal proteins, such as the contractile protein isoform b-MHC and the

atrial natriuretic peptide gene^{59,60}. On the other hand, several studies in rat ventricular tissue have demonstrated developmental regulation of cardiac All receptor subtype densities and gene expression, which are abundant during the neonatal period and decrease with maturation⁶¹⁻⁶³. Secondly, humoro-hormonal status is modified during senescence, and is characterized by a large decrease in plasma All synthesis, and an increase in plasma cortisol level⁵⁸. A number of circulating factors, such as vasoactive substances, growth factors, and steroids modulate AT₁ expression via their effects on the transcriptional activity of AT₁ gene^{18,64,65}. However, the differential pattern of AT₁ gene expression observed in the LV and RV of aged rats makes a major role of these hormones in the regulation of AT₁ gene expression unlikely.

Much less is known about the hormonal regulation of AT₂mRNA level. Yet, based on the up-regulation of AT₂ gene expression in both ventricles of aged rats, hormonal and humoral factors might be one of the triggers for the increase in cardiac AT₂ gene expression during senescence. Mechanical factors are repeatedly proposed as triggers for the regulation of genetic expression during the development of cardiac hypertrophy. Activation of AT₁ and/or AT₂ gene expression has been demonstrated in ventricular tissue during haemodynamic overload^{66,67}. Mechanical stretch has also been recently shown to up-regulate AT₁ and AT₂ gene expression in neonatal rat cardiac myocytes, the increase in AT₁ gene expression being mainly due to increased transcription, whereas that of AT₂ results from stabilization of AT₂ mRNA metabolism⁶⁸. Even though cardiac output and ejection fraction of both aged human and rat hearts are unaltered, the increase in vascular stiffness and aortic impedance during aging result in a moderate increase in LV

afterload^{69,70}. These changes in LV properties might therefore account, at least in part, for the up-regulation of AT₁ gene expression in the LV of aged rats.

Heymes and collaborators⁴⁹ have demonstrated increased density of both AT₁ and AT₂ receptors in the LV myocardium of senescent rats and activation of the intracardiac RAS in aging, associated with suppression of plasma All synthesis. Such local and independent regulation of intracardiac All synthesis and receptor subtype expression could account for both autocrine and paracrine actions⁷¹ and support the concept of intracardiac All production as a regulator of cardiac hypertrophy and collagen accumulation; a condition that could also prevail in renal tissues.

A strong correlation between AT₂ gene expression and fibrosis in both ventricles, compared with the correlation of AT₁ and ACE mRNA levels with LV fibrosis only, has suggested⁴⁹ that age-associated cardiac fibrosis is more closely related to the AT₂ than the AT₁ receptor subtype. This suggestion is given credence by studies of Lorell *et al.*⁷², who demonstrated that, both AT₁ inhibition and decrease in ACE gene expression affected neither cardiac fibrosis nor hypertrophy in a pressure-overload rat model.

Up-regulation of the cardiac RAS or the intrarenal RAS may, in part, compensate for the large age-related fall in plasma All synthesis. However, activation of local RAS systems and increased expression of All receptor subtypes might have detrimental effects when other pathological manifestations often associated with senescence, such as hypertension, heart failure, tubulointerstitial or glomerular damage, are superimposed. All receptor subtype antagonists could therefore be therapeutically useful in normal elderly people or elderly people with renal disease.

KIDNEY AII RECEPTORS AS A FUNCTION OF AGE

Studies by Correa and collaborators⁷³ in immature (1-week-old) and adult (12-week-old) normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) revealed that AII receptors and ACE binding sites, measured by quantitative autoradiography for quantification of AII receptors in both neonatal and adult animals of either strain, were of AT₁ subtype. In all kidney segments of 1-week-old rats AII receptor density was higher in SHR than WKY. Binding density increased with age in WKY rats; thus, in the glomeruli and the outer stripe of the outer medulla of 12-week-old WKY, binding was significantly higher than that present in age-matched SHR. In contrast, [¹²⁵I]351A (an iodotyrosyl derivative of the ACE inhibitor lisinopril that binds specifically to the active site of ACE⁷⁴) was highest in the outer medulla and not detectable in glomeruli. In 1-week-old rats, binding to ACE was higher in WKY than in SHR strain. Differences in ACE binding between adult SHR and WKY rats were inexistent, with the exception of the inner stripe of the outer medulla, where no binding was detected in SHR. These studies suggest that the renal RAS is developmentally regulated and is involved in the genesis and maintenance of genetic hypertension in SHR as aging proceeds. The evolution of the regulation of ACE and the AT₁ and AT₂ receptor beyond 12-weeks of aging and how this evolves in relation to hypertension and renal damage remains to be studied.

An influence of age on the RAS can also be gleaned from studies in male heterozygous transgenic hypertensive rats TGR (mREN2)27 (TGR)⁷⁵. Compared to Sprague-Dawley control rats, receptor density was significantly lower in TGR. Measurement of density and affinity of AII

receptors in glomeruli of animals 11 weeks old as compared to 18-20 weeks old rats revealed that receptor number increased with aging. In renal arteries, the AII receptor mRNA of the main receptor subtype AT_{1A} was neither strain nor age dependent, AT_{1B}- and AT₂-receptor mRNAs were significantly lower in TGR than SPRD rats. This study provide evidence that an overactive renin-angiotensin system in TGR rats led to a down-regulation of glomerular angiotensin II receptors that was not accompanied by a down-regulation of the mRNA of the dominant AT_{1A}-receptor subtype, particularly as age advanced. Diminished density of AII receptor leading to enhanced sensitivity to AII could explain in part the development of nephrosclerosis and the tubulointerstitial damage seen in these rats, particularly as they age.

Correspondence to:

M. Martínez-Maldonado, MD
Ponce School of Medicine
P.O. Box 7004
Ponce, Puerto Rico 00732
mmartinez@psm.edu

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