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Young Scholars Award Lecture:

Intratubular Angiotensinogen in Hypertension and Kidney Diseases

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Abstract

Recent findings related to the renin-angiotensin system have provided a more elaborated understanding of the pathophysiology of hypertension and kidney diseases. These findings have led to unique concepts and issues regarding the intrarenal renin-angiotensin system. Angiotensinogen is the only known substrate for renin that is the rate-limiting enzyme of the renin-angiotensin system. Because the level of angiotensinogen in human beings is close to the Michaelis-Menten constant value for renin, changes in angiotensinogen levels can control the activity of the renin-angiotensin system, and its upregulation may lead to elevated angiotensin peptide levels and increases in blood pressure. Enhanced intrarenal angiotensinogen mRNA or protein levels or both have been observed in multiple models of hypertension including angiotensin II-dependent hypertensive rats, Dahl salt-sensitive hypertensive rats, and spontaneously hypertensive rats, as well as in kidney diseases including diabetic nephropathy, immunoglobulin A (IgA) nephropathy, and radiation nephropathy. Renal angiotensinogen is formed primarily in proximal tubular cells and is secreted into the tubular fluid. Urinary angiotensinogen excretion rates show a clear relationship to kidney angiotensin II contents and kidney angiotensinogen levels, suggesting that urinary angiotensinogen may serve as an index of the intrarenal renin-angiotensin system status. Establishment of concise and accurate methods to measure human angiotensinogen may allow clinical studies that would provide important information regarding the roles of intrarenal angiotensinogen in the development and progression of hypertension and kidney diseases.

Keywords

Angiotensinogen; kidney; hypertension; diabetic nephropathy; immunoglobulin A nephropathy; radiation nephropathy

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It is a great honor to be selected to present the American Society of Hypertension 2005 Young Scholars Awards Lecture, and I greatly appreciate this recognition that has been given to our research program.

Uncontrolled hypertension induces structural and functional alterations in the kidney that can eventually lead to end-stage renal diseases.1 Effective control of blood pressure (BP) retards the progression of renal failure and reduces the morbidity and mortality rates

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associated with hypertensive vascular disease.[2–4] Recent findings related to the renin-angiotensin system (RAS), which is one of the most important regulatory mechanisms for BP regulation and electrolyte homeostasis,[5] have provided us with an improved understanding of the pathophysiology of hypertension.[6–9]

Angiotensinogen is the only known substrate for renin that is the rate-limiting enzyme of the RAS. Because the level of angiotensinogen is close to the Michaelis-Menten constant for renin,[10,11] angiotensinogen levels can control the activity of the RAS, and its upregulation may lead to elevated angiotensin peptide levels and increases in BP. Recent studies on experimental animal models and transgenic mice have documented the involvement of angiotensinogen in the activation of the RAS and development of hypertension.[12–18] Genetic manipulations that lead to overexpression of angiotensinogen have consistently been shown to cause hypertension.[17] In human genetic studies, a linkage has been established between the angiotensinogen gene and hypertension.[19,20] Thus angiotensinogen plays an important role in BP regulation.

Renin-Angiotensin System in the Kidney

In situ hybridization studies have demonstrated that the angiotensinogen gene is specifically present in the proximal tubules of the kidneys.[21] Angiotensinogen mRNA is expressed largely in proximal convoluted tubules and proximal straight tubules, and only small amounts are present in glomeruli and vasa recta as revealed by reverse transcription-polymerase chain reaction.[22] Renal angiotensinogen protein is specifically located in the proximal convoluted tubules by immunohistochemistry.[23–25] There is strong positive immunostaining for angiotensinogen protein in proximal convoluted tubules and proximal straight tubules, and there is weak positive staining in glomeruli and vasa recta; however there is no staining in distal tubules or collecting ducts.[26] The synthesized angiotensinogen in the kidney is secreted into the lumen, leading to angiotensin I generation and subsequent formation of angiotensin II. Renin mRNA and renin-like activity are also present in cultured proximal tubular cells.[27–29] In addition low but measurable renin concentrations in proximal tubule fluid have been reported in rats.[30] Abundant expression of angiotensin converting enzyme mRNA[31] and protein[32] have also been shown to be present in brush borders of proximal tubules of human kidneys. Angiotensin-converting enzyme has also been measured in proximal and distal tubular fluid but is more abundant in proximal tubule fluid.[33] Thus conditions are present in proximal tubules for angiotensin II generation.

There are two major types of angiotensin II receptor: type 1 (AT_1) receptors and type 2 (AT_2) receptors. However there is much less AT_2 receptor expression in adult kidneys.[34,35] It has been reported that AT_1 receptor mRNA has been localized to proximal convoluted and straight tubules, thick ascending limbs of the loop of Henle, cortical and medullary collecting duct cells, glomeruli, arterial vasculature, vasa recta, and juxtaglomerular cells.[22] In rodents, both subtypes of AT_{1a} receptor and AT_{1b} receptor mRNA have been demonstrated in the vasculature and glomerulus and in all nephron segments.[35] The AT_{1a} receptor mRNA is the predominant subtype in nephron segments, whereas the AT_{1b} receptor is more abundant than AT_{1a} receptor in the glomerulus.[36] Studies using polyclonal and monoclonal antibodies to the AT_1 receptor demonstrated that AT_1 receptor protein is on vascular smooth muscle cells throughout the vasculature, including the afferent and efferent arterioles and mesangial cells.[37] In addition AT_1 receptors are present on proximal tubule brush border and basolateral membranes, thick ascending limb epithelia, distal tubules, collecting ducts, glomerular podocytes, and macula densa cells.[34,35,37] These findings suggest that the RAS in the kidney works independently of the systemic RAS.

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Regulation of angiotensinogen has been extensively investigated in the liver and summarized in review articles.38,39 For example the hepatic biosynthesis of angiotensinogen is regulated by many different hormonal factors including glucocorticoid, estrogen, thyroid hormone, and insulin.38 However very little is known about intrarenal regulation of angiotensinogen.40 High-salt diet (HS) has been shown to suppress intrarenal expression of angiotensinogen in Sprague-Dawley rats41,42 and Wistar-Kyoto rats (WKY).43 In contrast a paradoxical enhancement of kidney angiotensinogen levels by HS was observed in Dahl salt-sensitive (DS) rats, but not in Dahl salt-resistant (DR) rats.44,45

Angiotensin II-Dependent Hypertension

Angiotensinogen

Angiotensin II, an extensively characterized peptide produced by successive proteolytic cleavages of its prohormone angiotensinogen, plays a critically important role in the regulation of renal hemodynamics and electrolyte homeostasis.46 It is also recognized that the tissue RAS exerts particularly important roles in several pathophysiologic conditions.47 The intrarenal RAS may be particularly significant because all components of RAS coexist in the kidney as described above and influence sodium excretion. Chronic infusion of low doses of angiotensin II provides a useful experimental model of angiotensin II-dependent hypertension and develops in association with progressive enhancement of intrarenal angiotensin II.5

Angiotensin II-infused rats have increases in renal angiotensinogen mRNA26,48 and protein49 and an enhancement of urinary excretion rate of angiotensinogen.50 Chronic angiotensin II infusion to normal rats significantly increased urinary excretion rate of angiotensinogen in a time- and dose-dependent manner. Urinary excretion rate of angiotensinogen was closely correlated with systolic BP and kidney angiotensin II content but not with plasma angiotensin II concentration. Urinary protein excretion in volume-dependent hypertensive rats was significantly increased more than in angiotensin II-dependent hypertensive rats; however urinary angiotensinogen excretion was significantly lower in volume-dependent hypertensive rats than in angiotensin II-dependent hypertensive rats.51 To determine whether circulating angiotensinogen is a source of urinary angiotensinogen, human angiotensinogen was infused in both control and hypertensive rats. Rat angiotensinogen was detected in plasma and urine before and after an acute injection of exogenous human angiotensinogen. Human angiotensinogen was detected only in the plasma collected after the acute administration of human angiotensinogen but was not detected in the urine in angiotensin II-dependent hypertensive or sham-operated normotensive rats. The failure to detect human angiotensinogen in the urine indicates limited glomerular permeability or tubular degradation or both. These findings support the hypothesis that urinary angiotensinogen originates from the angiotensinogen that is formed and secreted by the proximal tubules and not from plasma in rats.51 Moreover it was recently reported that AT_1 receptor blockade prevented the enhancement of intrarenal angiotensinogen that occurs in angiotensin II-infused hypertensive rats (Fig. 1). These data suggest that the augmentation of intrarenal angiotensinogen in angiotensin II-dependent hypertension is dependent on activation of AT_1 receptors and that the enhanced urinary excretion rate of angiotensinogen during angiotensin II infusion is blocked by AT_1 receptor blockade.52

In angiotensin II-dependent hypertension, AT_1 receptor blockade increased plasma angiotensin II concentrations; however it markedly limited the enhanced kidney angiotensin II contents elicited by chronic angiotensin II infusions.52 This dissociation between plasma angiotensin II and intrarenal angiotensin II may suggest a differential regulation of angiotensin II in the kidney and in the circulation. This dissociation between plasma

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angiotensin II and intrarenal angiotensin II has also been observed in other hypertensive models. In the Page cellophane-wrapped kidney model, it was reported that although the cellophane-wrapped group had progressive increment in BP and angiotensin II content in the kidney, the plasma levels of angiotensin II were similar in the cellophane-wrapped group and the sham-operated animals and were unchanged from baseline.53

Renin

Renin is synthesized primarily by the juxtaglomerular apparatus (JGA).54 However renin mRNA and protein have been detected in proximal and connecting tubules and in collecting duct cells of human, rat, and mouse kidneys as well as in extrarenal tissues.27,28,55,56 Although regulation of renin synthesis and secretion from JGA cells has been extensively studied,54 very little is known about the regulation of tubular renin.29,56,57 Recently it was demonstrated that chronic angiotensin II infusions to normal rats significantly increased renin mRNA and protein levels in principal cells of connecting ducts and collecting tubules.58 Moreover it was reported that this augmentation is dependent on activation of AT_1 receptors.59 Although plasma renin activity and JGA renin are markedly suppressed in angiotensin II-induced hypertension, increased distal nephron renin associated with an increased proximal tubular angiotensinogen production and spillover into the distal nephron segments may collectively contribute to elevated and sustained intratubular angiotensin I and angiotensin II formation in this hypertensive model.52,59

Intratubular Renin-Angiotensin System in Hypertension

The above-mentioned experiments established that there is a quantitative relationship between urinary angiotensinogen and intrarenal angiotensinogen or angiotensin II production, and that there is both augmented angiotensinogen and distal nephron renin leading to an increased angiotensin II-mediated sodium reabsorption in distal nephron segments of angiotensin II-infused hypertension.26,49–52,58,59 Recent studies showed that angiotensin II directly stimulates epithelial sodium channel activity in cortical collecting duct cells60 and that there is intraluminal conversion of angiotensin I to angiotensin II in cortical collecting ducts.61 Thus renin in distal nephron segments may synergistically contribute to the angiotensin II-stimulatory effect on distal tubular renin and could help to explain the marked stimulation of sodium reabsorption and suppression of the pressure-natriuresis relationship observed in angiotensin II-infused hypertensive rats.62 Therefore the concomitant increases in proximal tubular angiotensinogen and distal nephron renin may play a crucial role in the sustained high intrarenal angiotensin II levels and hence may contribute to the progressive high BP observed in angiotensin II-dependent hypertension.

Importance of angiotensinogen and renin in the tubular cells to induce systemic hypertension was also reported in a transgenic mouse model.63 Lavoie et al generated mice that express human renin under the control of the kidney-specific androgen-regulated protein promoter, which is androgen responsive. One of the lines expressed the human renin transgene primarily in the kidney. Renal expression of the transgene was undetectable in females but could be induced by testosterone treatment. Because the RAS is species-specific, these investigators bred these human renin-transgenic mice with the mice expressing human angiotensinogen under the same promoter to produce offspring that expressed both transgenes. They measured mean arterial BP in the carotid artery of double-transgenic and control mice using radiotelemetry. Double-transgenic female mice had a normal baseline mean arterial BP, which increased by 15 mm Hg after 2 weeks of testosterone treatment and returned to baseline after discontinuation of the testosterone pellet. The change in arterial pressure paralleled the change in plasma testosterone. There was no mean arterial BP change in testosterone-treated control littermates. The investigators concluded that dual production of angiotensinogen and renin in the renal proximal tubules

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can result in a systemic increase in arterial pressure. These data support a role for a tissue-specific RAS in the renal proximal tubules that contributes to the regulation of systemic BP.

Salt-Sensitive Hypertension

Clinical studies indicate a clear linkage between salt-sensitive hypertension and a polymorphism of the angiotensinogen gene.[64-66] Various epidemiologic studies have showed a correlation of dietary salt intake with the prevalence and progression of hypertension.[67] Although the degree of salt sensitivity is variable, some individuals are particularly prone to develop hypertension in response to an increased dietary salt intake. Subjects with essential hypertension have a higher frequency of salt sensitivity than is found in the normotensive population.[68] There is some evidence that salt sensitivity is associated with low plasma renin activity and impaired renal sodium excretion. However the mechanisms underlying this phenomenon are poorly understood.[69]

The DS rats have been used as a model of human salt-sensitive hypertension because salt loading exaggerates the development of hypertension in strains that are genetically predisposed to hypertension.[70] Mature DS rats are reported to have low plasma renin activity, which has been interpreted as being indicative of an overall suppression of the RAS[70]; however few studies of angiotensinogen have been carried out in these rats. Although the animals are generally considered to be characterized by a low activity of circulating RAS, recent studies indicate that treatment with angiotensin-converting enzyme inhibitors or AT_1 receptor antagonists reduces cardiac or renal dysfunction or both in DS rats fed HS.[71-76] These findings suggest that the local RAS may be inappropriately activated and contribute to the development of hypertension in this animal model.

Recent studies support the concept that there is an inappropriate regulation of intrarenal angiotensinogen in DS rats fed HS. Both DR rats and DS rats were maintained on a HS or low-salt diet (LS). Systolic BP was unaltered in DR rats; however systolic BP was significantly increased in DS rats fed HS compared with DS rats fed LS. The HS suppressed plasma renin activity in both strains. Plasma angiotensinogen levels were also suppressed by HS in both strains. However kidney angiotensinogen levels were significantly increased in DS rats fed HS compared with DS rats fed LS, DR rats fed HS, and DR rats fed LS. These data indicate that DS rats fed HS experience inappropriate and paradoxical augmentation of intrarenal angiotensinogen.[44]

Recent studies indicate that the inappropriate augmentation of intrarenal angiotensinogen in DS by HS is caused by augmented production of reactive oxygen species. Systolic BP was significantly increased in the DS+HS group compared with the DS+LS group. Treatment with a superoxide dismutase mimetic, Tempol, or treatment with a nonspecific vasodilator, hydralazine, attenuated the hypertension to an equivalent extent. Urinary excretion of thiobarbituric acid-reactive substances, a marker of oxidative stress, was significantly increased in the DS+HS group compared with the DS+LS group. Tempol treatment prevented this effect, but hydralazine treatment only partially prevented the effect. Kidney angiotensinogen levels were significantly increased in the DS+HS group compared with the DS+LS group. Tempol but not hydralazine treatment prevented the intrarenal angiotensinogen augmentation (Fig. 2). The evidence suggests that reactive oxygen species-dependent activation of intrarenal angiotensinogen plays an important role in the development of the hypertension in DS rats fed HS.[45]

Genetic Hypertension

Clinical studies also indicate a clear linkage between genetic hypertension and a polymorphism of the angiotensinogen gene.[77,78]

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Spontaneously hypertensive rats (SHR) have been used as a model of genetic hypertension.79 Although the animals are generally considered to be characterized by a low activity of circulating RAS,[80,81] recent studies indicate that treatment with angiotensin-converting enzyme inhibitors or AT_1 receptor blockers or both reduces cardiac or renal dysfunction or both of these dysfunctions in SHR,[82–84] suggesting that the intrarenal RAS may be inappropriately activated and in turn may contribute to the development of hypertension and hypertension-induced renal damages in this animal model.

A recent study was performed to determine whether augmented intrarenal angiotensinogen may contribute to the enhanced renal angiotensin II and associated tissue injury in SHR. Both SHR and WKY were maintained on a normal diet before being killed at 7 or 14 weeks of age. Two groups of SHR received either an AT_1 receptor blocker or a triple therapy, hydralazine, reserpine, and hydrochlorothiazide during weeks 7 through 14. Systolic BP and renal angiotensin II were significantly increased in SHR-14 compared with WKY-7, WKY-14, and SHR-7, and treatment with AT_1 receptor blockers prevented these increases. However, although triple therapy prevented the development of hypertension in SHR, this combination therapy failed to decrease renal angiotensin II. Using urine samples or fixed renal sections, the degree of renal injury was quantified using the following parameters: urinary excretion rate of total protein, glomerular sclerosis, interstitial expansion, monocyte/macrophage infiltration in interstitium or glomeruli, and renal arterial proliferation. Angiotensinogen mRNA and protein levels in kidney cortex and all parameters of renal damage were changed in parallel, and AT_1 receptor blocker treatment also prevented these increases. However triple therapy failed to prevent these increases (Fig. 3). These results indicate that SHR have enhanced intrarenal angiotensinogen production that contributes to increased angiotensin II levels, leading to the development of hypertension and renal injury in this strain.85

Diabetic Nephropathy

Diabetic nephropathy is one of the most common causes of end-stage renal failure in patients starting dialysis in developed countries.86 Clinical trials have demonstrated that the elevated glucose levels are closely associated to the principal cause of renal damage in both type 187 and type 288 diabetes. Until now the detailed mechanisms regarding the sequence of events leading to the development of diabetic nephropathy have remained uncertain.

High glucose induces de novo synthesis of diacylglycerol both in vivo and in vitro.89 Diacylglycerol activates the protein kinase C pathway.90 Activation of protein kinase C is one of the major mechanisms involved in high glucose-induced glomerular injury91 and produces reactive oxygen species and subsequent lipid peroxidation.[92–95] High glucose generates reactive oxygen species as a result of glucose auto-oxidation, metabolism, and formation of advanced glycosylation end products.95

Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-β1 expression in rat glomerular cells, and this induction occurs via AT_1 receptor-dependent mechanism.96 The cytokine transforming growth factor-β1 is known to be an important mediator of hypertrophy and fibrosis in kidney diseases via multiple pathways such as the G1 phase arrest, cell size enlargement, protein synthesis induction, inhibitory effect on proteinase activity, and extracellular matrix enhancement.[97,98]

Therefore high glucose-induced reactive oxygen species pathway and intrarenal RAS-dependent transforming growth factor-β1 pathway are shown to play key roles in diabetic nephropathy. Interestingly it was recently demonstrated that high glucose augments angiotensinogen gene expression in proximal tubular cells.[99–105] However no in vivo

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studies have been performed to examine the linkage between reactive oxygen species and angiotensinogen in the kidneys of diabetic animals.

It was recently reported that temporary blockade of the RAS at the prediabetic stage attenuates renal injury in a rat model of type 2 diabetes later in life, suggesting an activated renal RAS in type 2 diabetes.106 The Zucker Diabetic Fatty (ZDF) obese rat, another model of type 2 diabetes, is well known to show progressive nephropathy; however the detailed mechanisms have remained unclear. A study was recently performed to examine the possible involvement of angiotensinogen in diabetic nephropathy of ZDF obese rats. Genetic pairs of male ZDF obese rats and ZDF lean rats were maintained on a diet containing high fat from 12 to 17 weeks of age. At the end of the protocol, ZDF obese rats showed an increased body mass compared with ZDF lean rats. Fasting blood glucose levels were also significantly higher in ZDF obese rats compared with ZDF lean rats. Urinary levels of 8-isoprostane, a marker of oxidative stress, were significantly increased in ZDF obese rats compared with ZDF lean rats. Kidney angiotensinogen protein levels were significantly increased in ZDF obese rats compared with ZDF lean rats. Considering that reactive oxygen species-associated angiotensinogen enhancement plays an important role in renal damage of salt-sensitive hypertension, as described previously here, these data may suggest that reactive oxygen species are partly involved in intrarenal angiotensinogen augmentation, leading to the development of diabetic nephropathy in ZDF obese rats.107

IgA Nephropathy

Clinical and experimental studies have demonstrated that the blockade of the RAS is successful in mitigation and therapy of IgA nephropathy,108 suggesting that the RAS is activated in the development and progression of IgA nephropathy. A clinical study was recently performed to determine whether immunoreactivity of intrarenal angiotensinogen is increased in IgA nephropathy patients. An antibody against human angiotensinogen was raised in a chicken using highly purified angiotensinogen from human plasma. The immunoreactivity of angiotensinogen was then determined by an established, semiautomatic quantification system with an immunohistochemistry robot and a computerized digital image-handling system in renal specimens from 39 patients (18 male and 21 female) with IgA nephropathy. Normal portions of surgically resected kidney served as control (four male and one female). Patients with IgA nephropathy showed higher systolic BP and lower creatinine clearance compared with the control group. The IgA nephropathy patients also showed moderate proteinuria, but the control group did not show any proteinuria. Angiotensinogen was localized pre-dominantly in proximal tubular cells, and the immunoreactivity of intrarenal angiotensinogen in IgA nephropathy was significantly increased compared with normal kidneys (Fig. 4). The IgA immunoreactivity was not correlated with clinical data including BP, creatinine clearance, or urinary protein excretion. Although these IgA nephropathy patients did not show massive renal damage, angiotensinogen immunoreactivity was increased in these patients at this point. These data suggest that the activated intrarenal angiotensinogen plays some roles in the development of IgA nephropathy patients at the early stage and may provide a supportive foundation of the effectiveness of the RAS blockade in IgA nephropathy patients.109

A clear linkage between the intrarenal RAS and IgA nephropathy was also recently reported using in vitro models. It was found that the glomerular AT_1 receptor was reduced in IgA nephropathy, whereas there was no change in the expression of glomerular AT_2 receptor.110 More recently it was demonstrated that there is constitutive expression of AT_1 receptor and AT_2 receptor in renal tubules with increased expression in IgA nephropathy.111

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Radiation Nephropathy

Clinical radiation nephropathy is an acknowledged complication of blood stem-cell transplantation and internal radionuclide therapies. Excessive renal irradiation leads to progressive renal failure. Fractionated external beam doses of >20 Gy over 4 weeks, single doses of 10 Gy, and internal radionuclide doses of 7 Gy may cause chronic renal failure.112

Antagonists of the RAS are successful in mitigation and therapy of experimental radiation nephropathy.113 An animal model of radiation nephropathy was created in barrier-maintained rats, and angiotensinogen expression was evaluated to search for evidence of activation of the RAS in the animal. Barrier-maintained rats underwent total body irradiation in six equal fractions over 3 days and then underwent transplantation of blood stem cells from a syngeneic littermate. Control rats were not irradiated. Rats were killed on days 1, 22, 41, and 63 after total body irradiation. The apparent increase in angiotensinogen protein abundance occurred at a time when there is little or no renal injury in this model (Fig. 5). It is possible that the increase in angiotensinogen is mechanistically important and is relevant to the benefits of angiotensin-converting enzyme inhibitors or AT_1 receptor blockers used in this model.114

Human Angiotensinogen ELISA

As described in a previous section, urinary excretion rates of angiotensinogen provide a specific index of intrarenal RAS status in angiotensin II-dependent hypertensive rats.[26,49-52] When this is shown to be applicable to human subjects, a diagnostic test to identify those hypertensive patients most likely to respond to blockade of the RAS could provide useful information to allow a mechanistic rationale for selecting an optimized approach to the treatment of hypertensive subjects. However concise and accurate methods to measure human angiotensinogen are unavailable at this time. To perform future human subject studies, two antibodies and a sensitive and specific quantification system using a novel microtiterplate-based sandwich enzyme-linked immunoassay (ELISA) for the measurement of human angiotensinogen have been developed. This ELISA is able to detect human angiotensinogen at range of 0.01 μg/well to 1 μg/well (R^2 = 0.9945).115

Conclusion

Enhanced levels of intrarenal angiotensinogen mRNA or protein or both have been observed in multiple models of hypertension as well as in kidney diseases including diabetic nephropathy, IgA nephropathy, and radiation nephropathy.

A series of previous studies imply an augmentation of angiotensinogen by angiotensin II via reactive oxygen species pathways. Griendling et al showed that angiotensin II stimulates production of reactive oxygen species in cultured vascular smooth muscle cells.116 Nishiyama et al. also presented evidence in vivo that angiotensin II enhances oxidative stress in kidneys of rats.117 The association of renal oxidative stress, increased renal angiotensin II activity, and renal inflammation in hypertension has been emphasized recently in a review article.118 Interestingly reactive oxygen species was reported to activate angiotensinogen expression. Hsieh et al found that angiotensinogen gene expression is activated via reactive oxygen species in a proximal tubular cell line.101 In addition Kobori and Nishiyama presented in vivo evidence that reactive oxygen species stimulates angiotensinogen gene expression in kidneys of DS challenged by HS.45 These data support the concept that the enhanced expression of intrarenal angiotensinogen by angiotensin II is mediated via reactive oxygen species pathways.

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Urinary angiotensinogen excretion rates show a clear relationship to kidney angiotensin II contents and kidney angiotensinogen levels, suggesting that urinary angiotensinogen may serve as an index of intrarenal RAS status. Interestingly it was recently shown that urinary angiotensinogen is a strong predictor of hypertension in women with low plasma renin and aldosterone; in contrast men did not show this correlation. Higher sodium intake may account, in part, for the lack of a similar relationship in men.119 Establishment of concise and accurate methods to measure human angiotensinogen may provide useful information regarding the roles of intrarenal angiotensinogen in the development and progression of hypertension and kidney diseases.

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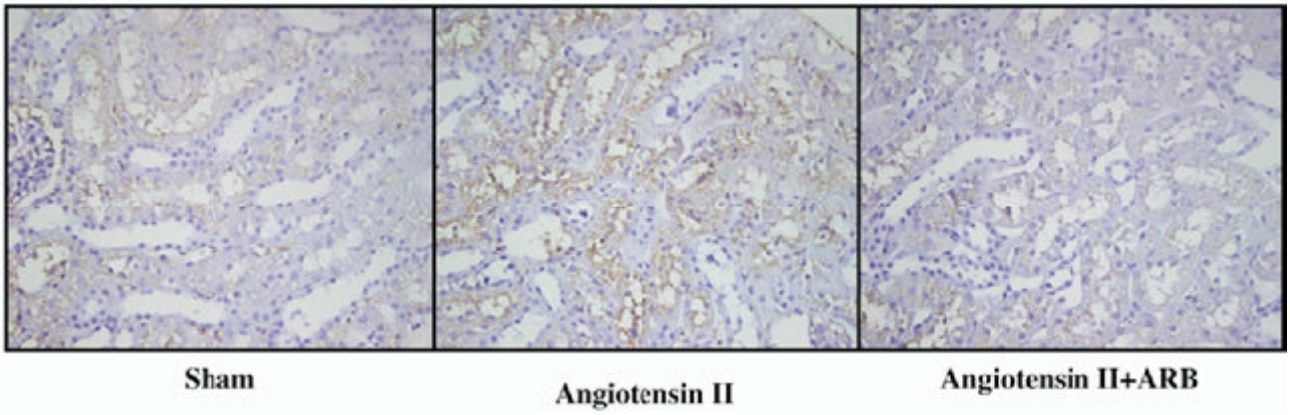

FIG. 1.

Kidney angiotensinogen immunostaining showed a significant enhancement in angiotensin II-infused rats (**center panel**) compared with sham-operated rats (**left panel**). Use of AT_1 receptor blockade (ARB) prevented this augmentation (**right panel**). Kidney angiotensinogen immunohistochemistry was performed as previously described using an automatic robotic system (Dako autostainer) to apply the exactly same condition on all slides.

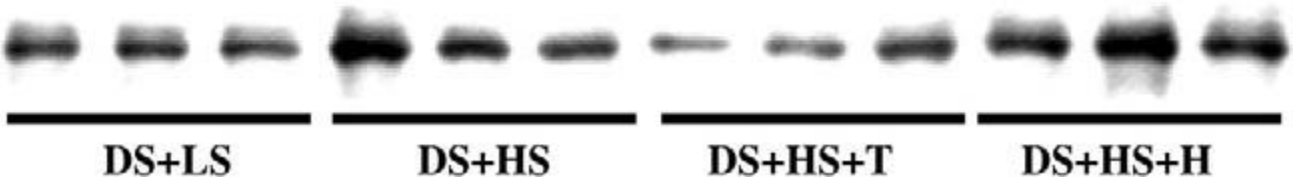

FIG. 2.

Kidney angiotensinogen protein levels were significantly increased in Dahl salt-sensitive rats (DS) on a high-salt diet (HS) compared with DS on a low-salt diet (LS). Tempol (T) but not hydralazine (H) treatment prevented the intrarenal angiotensinogen augmentation.

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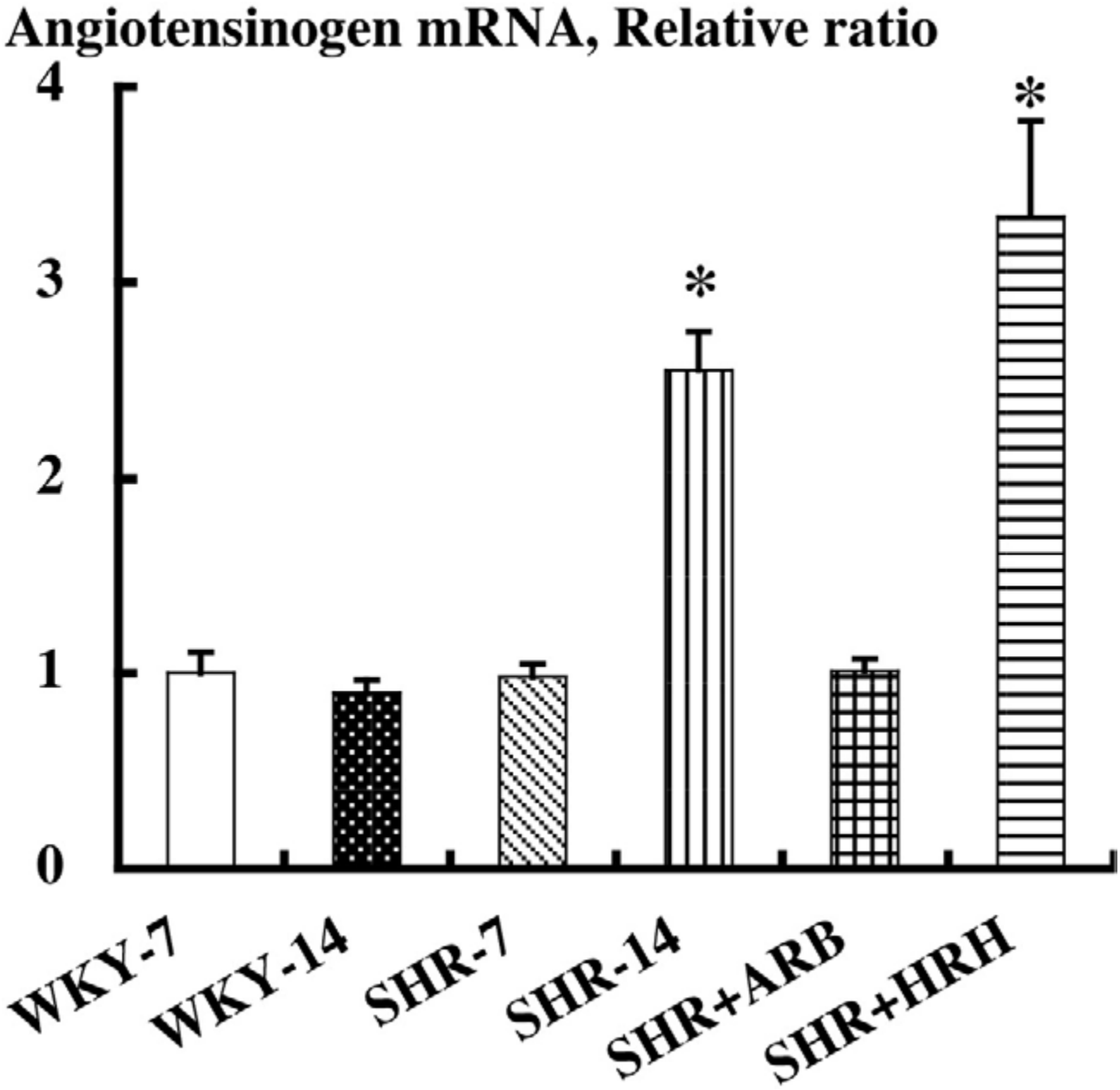

FIG. 3.

Kidney angiotensinogen mRNA levels were not changed in Wistar-Kyoto rats (WKY) at 7 weeks of age or WKY at 14 weeks of age. However angiotensinogen mRNA levels were significantly increased in spontaneously hypertensive rats (SHR) at 14 weeks of age compared with SHR at 7 weeks of age and the age-matched WKY. Treatment with AT_1 receptor blockers (ARB) prevented the augmentation of angiotensinogen mRNA. However, a triple therapy of hydralazine, reserpine, and hydrochlorothiazide (HRH) failed to prevent this augmentation.

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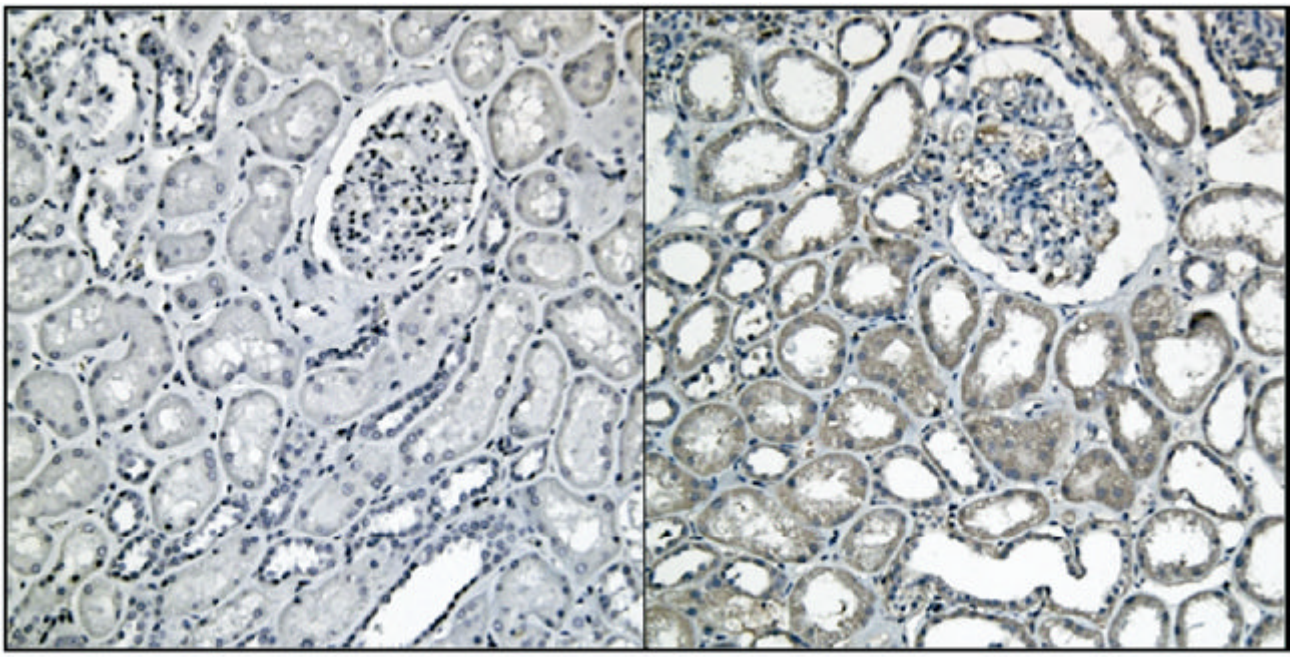

FIG. 4.

Enhanced intrarenal angiotensinogen immunoreactivity in immunoglobulin-A (IgA) nephropathy patients. Immunohistochemistry robotic system was used to apply a specimen in the exact same condition on each slide. Immunoreactivity of human angiotensinogen was significantly increased in kidneys of IgA nephropathy patients (**right panel**) compared with kidneys of normal subjects (**left panel**).

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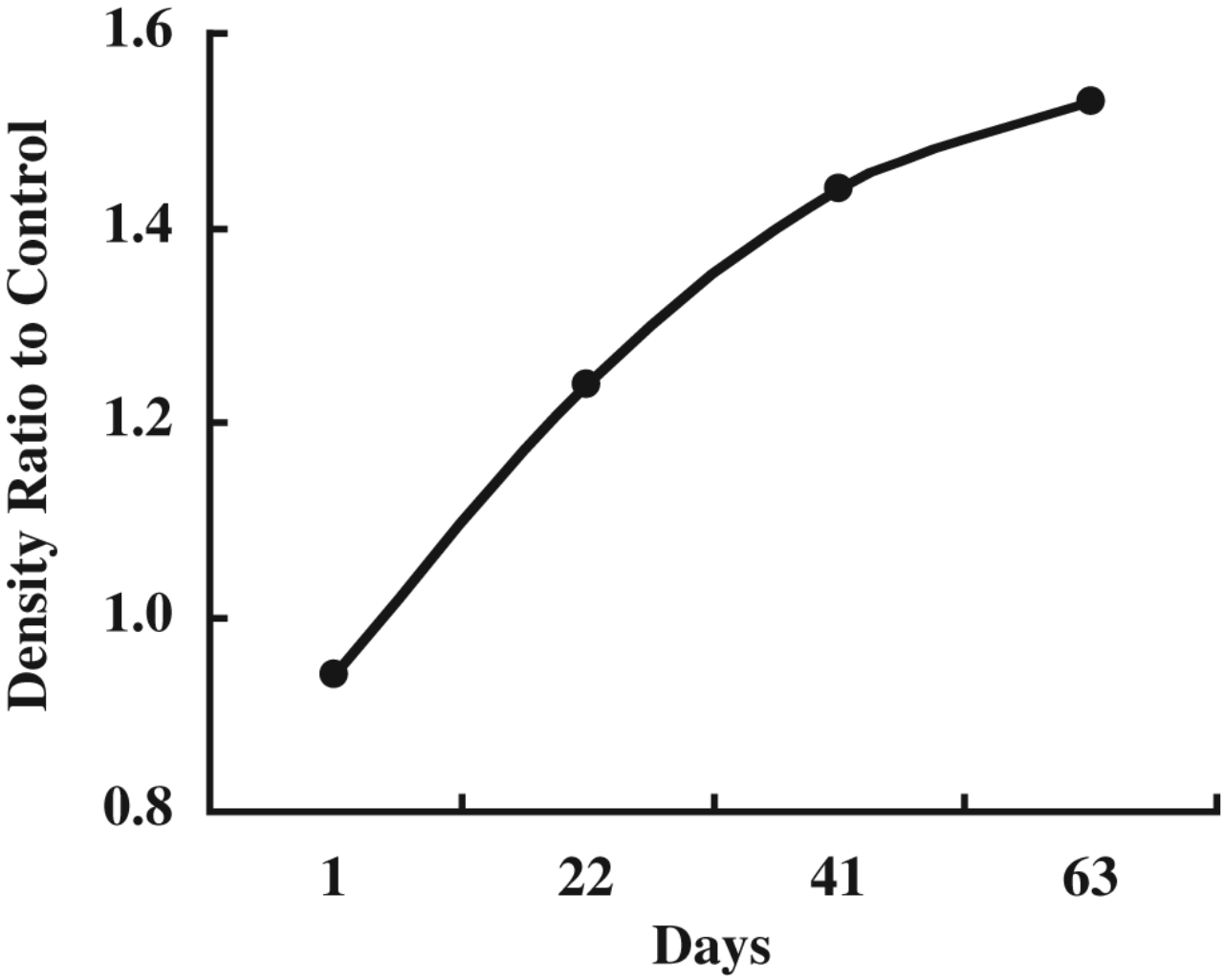

FIG. 5.

Enhanced intrarenal angiotensinogen protein levels in experimental radiation nephropathy in rats. Western blot analysis indicates that angiotensinogen protein levels significantly increased in a time-dependent manner after total body irradiation.